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Synthesis and anti-inflammatory activities of mono-carbonyl analogues of curcumin

Guang Liang,[a,b] Xiaokun Li,[a,c,d,*] Li Chen,[b] Shulin Yang,[c] Xudong Wu,[b] Elaine Studer,[b] Emily Gurley,[b] Phillip B. Hylemon,[b] Faqing Ye,[a] Yueru Li[d] and Huiping Zhou[a,b,*]

[a]*College of Pharmacy, Wenzhou Medical College, Wenzhou, Zhejiang 325035, PR China*
[b]*Department of Microbiology and Immunology, School of Medicine, Virginia Commonwealth University, Richmond, VA 23298, USA*
[c]*College of Chemistry, Nanjing University of Science and Technology, Nanjing 210094, PR China*
[d]*Ministry of Education's Engineering Research Center of Bioreactor and Pharmaceutical Development, Jilin Agricultural University, Changchun 130118, PR China*

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Abstract—Curcumin has been extensively studied for its anti-inflammatory activities. However, its potential beneficial effects on various disease preventions and treatments are limited by its unstable structure. The β-diketone moiety renders curcumin to be rapidly metabolized by aldo–keto reductase in liver. In the present study, a series of curcumin analogues with more stable chemical structures were synthesized and several compounds showed an enhanced ability to inhibit lipopolysaccharide (LPS)-induced TNF-α and IL-6 synthesis in macrophages.

Curcumin [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, Fig. 1] is the major active constituent of turmeric, a yellow compound originally isolated from the plant *Curcuma longa* L. Tumeric has been widely used for centuries as a dietary spice and pigment.[1] In addition to its unique flavor and color, tumeric has been extensively used in traditional medicine in China and India, particularly as an anti-inflammatory agent.[2] During the last two decades, numerous studies have shown that curcumin has a variety of biological and pharmacological activities such as anti-carcinogen, immuno-modulation, anti-oxidant, anti-angiogenesis, and chemo-prevention.[3–9]

Recent studies have demonstrated that inflammation is implicated in the pathogenesis of various diseases including cancer, atherosclerosis, diabetes, fatty liver, rheumatoid arthritis, and inflammatory bowel disease.[10–13] Anti-inflammation is the major focus of current drug development.[14] Cytokines are local mediators produced by lymphocytes and macrophages as well as by epithelial and mesenchymal cells.[15] It has

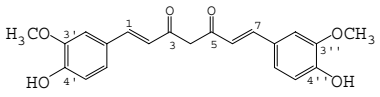

Figure 1. Chemical structure of curcumin.

been demonstrated that cytokines are involved in a variety of biological processes and play a central role in the development of inflammation and immunity. TNF-α is a multifunctional cytokine produced primarily by activated monocytes/macrophages and plays a critical role in the initiation and continuation of inflammation and immunity.[16] It is well known that TNF-α is a key proinflammatory cytokine in the pathogenesis of various inflammatory diseases and cancer.[13,17] In addition to directly inducing inflammatory response, TNF-α is also able to induce other proinflammatory cytokines including IL-6, IL-1β, IL-8 and itself by activating NF-kB and MAP kinase signaling pathways.[16,18] It has been shown that curcumin is able to inhibit the production of proinflammatory cytokines TNF-α and IL-6 in macrophages and various cancer cells.[18,19] It also can inhibit the expression of other inflammatory mediators such as COX-2 and iNOS.[20] However, preclinical and clinical studies have found that the potential beneficial effects of curcumin on various disease preventions and

Keywords: Curcumin; Anti-inflammation; TNF-α; IL-6.
* Corresponding authors. Tel.: +1 804 828 6817; fax: +1 804 828 0676 (H.Z.); e-mail addresses: xiaokunli@163.net; hzhou@vcu.edu

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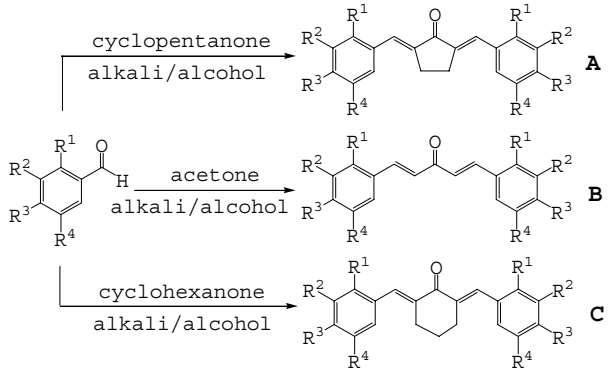
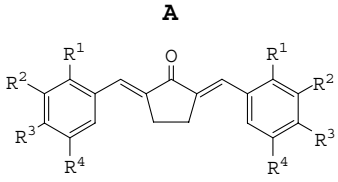
treatments are limited by its poor pharmacokinetic properties.[3,21,22] It is believed that the presence of the active methylene group and β-diketone moiety contributes to the instability of curcumin under physiological conditions, poor absorption, and fast metabolism.[23] Recently, synthetic modifications of curcumin have been studied intensively in order to develop a molecule with enhanced properties and stability.[23–26] In the present study, a series of curcumin analogues with more stable structures were synthesized and tested for anti-inflammatory activity in vitro. Several analogues showed an enhanced ability to inhibit lipopolysaccharide (LPS)-induced TNF-α and IL-6 in macrophages.

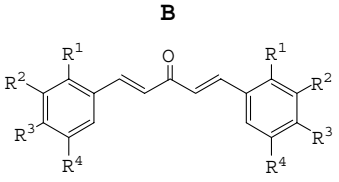
In order to identify the crucial structural motifs leading to anti-inflammatory activity and gain insight into future directions for designing new analogues with better activity, three series of mono-carbonyl curcumin analogues, 1,5-diaryl-1,4-pentadiene-3-ones (**B**), together with cyclopentanone (**A**) and cyclohexanone (**C**) analogues, were designed by deleting the methylene group and one carbonyl group. These compounds were also designed to examine the role of different substituents on the benzene ring and the influence of the structure of 5-C linker on inflammatory activities when the unstable methylene was absent. The structure and general synthesis of analogues designed are shown in Table 1, and Schemes 1 and 2, respectively.

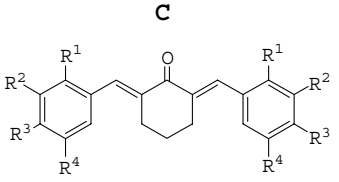


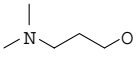
Scheme 1. General synthesis of 1,5-diphenyl-1,4-pentadiene-3-ones and cyclic analogues.

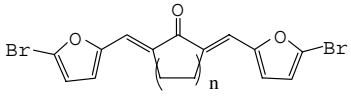
Table 1. Structure of curcumin analogues



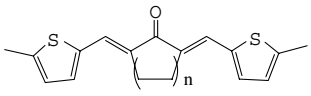




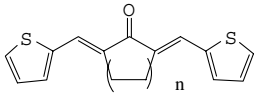
	R^1	R^2	R^3	R^4
1	H	H	OH	H
2	H	OCH_3	OH	H
3	Br	H	H	H
4	F	CF_3	H	H
5	H	Br	H	H
6	H	H	F	H
7	H	H	OCH_2CH_3	H
8	H	OCH_3	OCH_3	OCH_3
9	H	H		H
10	H	H	N $(CH_3)_2$	H
11	H	OCH_3		H
12	H	H	$OCH_2CH{=}CH_2$	H
13	H	OCH_3	$OCH_2CH{=}CH_2$	H
14	H	H		H



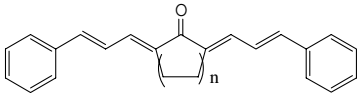
A15: n = 2
B15: n = 0
C15: n = 3



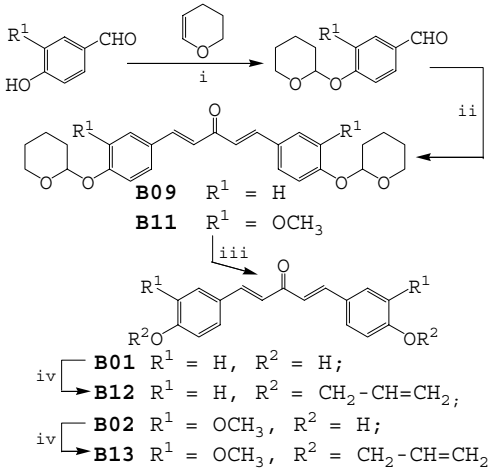
A16: n = 2
C16: n = 3



A17: n = 2
B17: n = 0
C17: n = 3



A18: n = 2
B18: n = 0
C18: n = 3



Scheme 2. Synthesis of presented compounds. Reagents and conditions: (i) pyridine–PTSA, CH_2Cl_2, rt; (ii) CH_3COCH_3, NaOH/EtOH, rt; (iii) PTSA, MeOH, rt; (iv) $CH_2{=}CH{-}CH_2Br$, K_2CO_3/acetone, reflux.

Most of the compounds were synthesized by coupling the appropriate aromatic aldehyde with cyclohexanone, cyclopentanone or acetone in an alkaline solution, respectively. Details of the reaction routes, yields, melting points, NMR, and ESI-MS analysis are described in Online supplemental data. All reactions were carried out with a ratio of 2:1 of substituted arylaldehydes to ketones. Incorporation of two aryl-rings was confirmed using ^{1}H NMR analysis by detecting the absence of methyl (**B** analogues) or methylene (**A** and **C** analogues) next to the carbonyl group.

For the synthesis of analogues **1** and **2** in alkaline conditions, the hydroxyls of 4-hydroxybenzaldehyde and 3-methoxy-4-hydroxybenzaldehyde were protected with an easily removable group, 3,4-dihydro-α-pyran. Scheme 2 illustrates a representative example of the preparation of the tetrahydropyran-2-yl-protected derivative and its reaction with acetone to obtain compounds **9** and **11**. Analogues **1** and **2** were prepared by deprotection with a catalytic amount of *p*-toluenesulfonic acid, and compounds **12** and **13** were obtained by further etherification with allyl bromide.

The anti-inflammatory activities of curcumin and its analogues were measured as the ability of these compounds to inhibit LPS-induced TNF-α and IL-6 expression in mouse J774A.1 macrophages using Enzyme-linked immunosorbent assays (ELISA).[27]

Briefly, cells were pre-treated with 10 μM of curcumin, each analogue or vehicle control for 2 h, then treated with LPS (0.5 μg/ml) for 24 h. At the end of treatment, the culture media were collected and centrifuged at

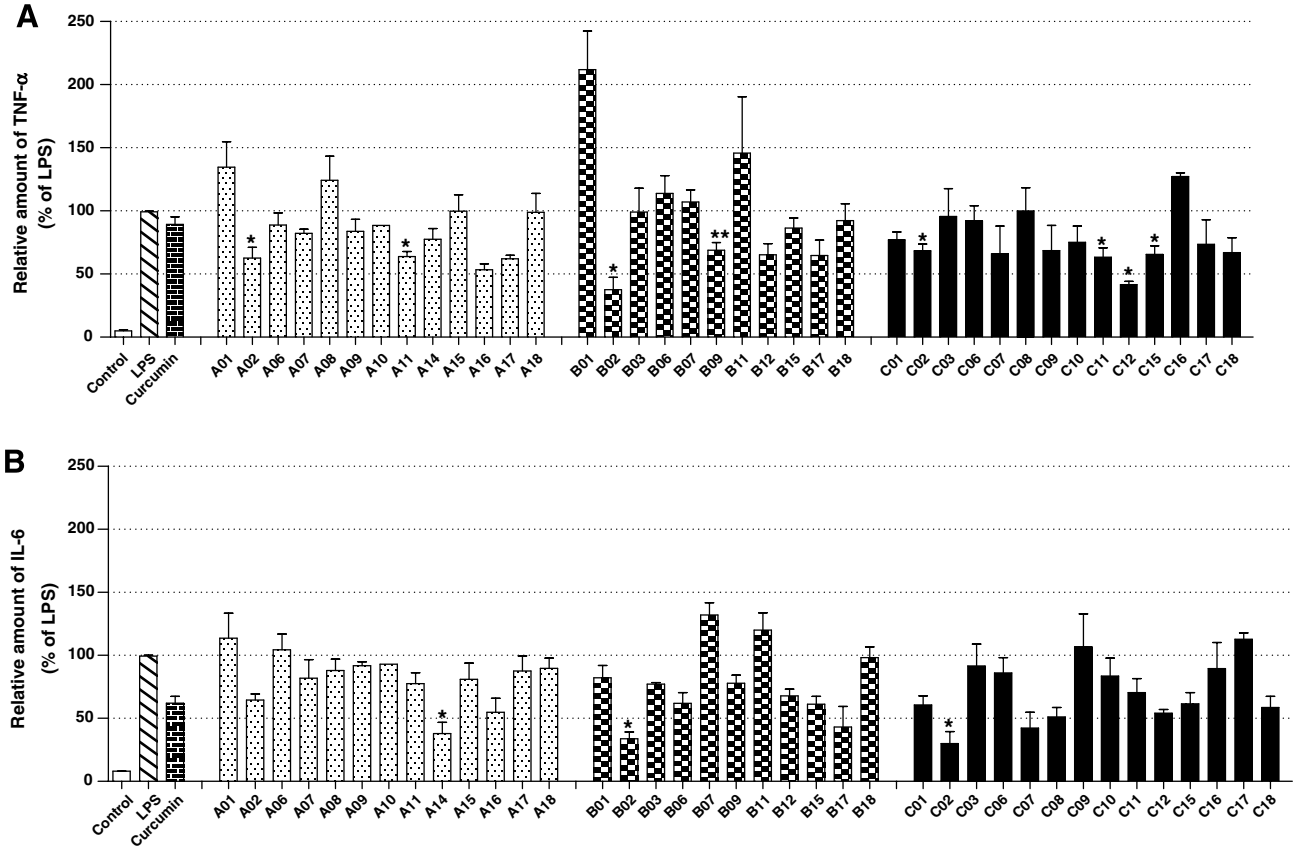


Figure 2. Inhibition of LPS-induced TNF-α and IL-6 by curcumin and its analogues in J774A.1 macrophages. Cells were pretreated with curcumin or its analogues (10 μM) for 2 h, then treated with LPS (0.5 μg/ml) for 24 h. TNF-α (A) and IL-6 (B) levels in the culture media were measured by ELISA. The results are expressed as percent of LPS control. Each bar represents mean ± SE of five independent experiments. Statistical significance relative to LPS is indicated, $^*p < 0.05$.

14,000 rpm for 5 min. TNF-α and IL-6 levels in the media were determined by ELISA using mouse TNF-α and mouse IL-6 ELISA Max™ Set Deluxe Kits (Biolegend, USA). The total protein concentrations of the viable cell pellets were determined using Bio-Rad protein assay reagents. Total amounts of the TNF-α and IL-6 in the media were normalized to the total protein amount in the viable cell pellets.

The results indicate that curcumin and its analogues inhibited LPS-induced TNF-α and IL-6 expression to various degrees. The cyclohexanone-derived **C** compounds were more effective than acetone-derived **A** and cyclopentanone-derived **B** compounds. Among these compounds, **A02**, **A11**, **B02**, **B09**, **B12**, **C02**, **C11**, **C12**, and **C15** are more potent than curcumin in inhibiting LPS-induced TNF-α expression (Fig. 2A). Compounds **B02**, **C02**, and **A14** showed better inhibitory effect than curcumin on LPS-induced IL-6 expression (Fig. 2B). **A14**, a novel compound with a long chain substituent group of 3-(dimethylamino) propoxyl, showed a similar inhibitory effect on LPS-induced TNF-α expression as curcumin, but has more potent inhibitory effect on LPS-induced IL-6 expression than curcumin. However, the similar dimethylamino-substituted compounds (**A10**, **B10**, and **C10**) showed similar or less inhibitory effects on LPS-induced TNF-α and IL-6 expression as curcumin, indicating that nitrogenous substitution by itself does not enhance the anti-inflammatory activity. On the other hand, **B12** and **C12** with a long chain allyloxyl substituent group showed stronger inhibitory effect on LPS-induced TNF-α indicating that the length and flexibility of the substituent groups may be favorable to the anti-inflammatory activity. Among eight heterocyclo-substituted compounds **15**–**17**, **A16**, **A17**, and **C15** exhibited moderate activity on inhibiting LPS-induced TNF-α secretion and **B17** displayed a strong inhibition on IL-6 expression (43.1%). Among curcumin-like compounds, **A02**, **B02**, and **C02** showed the best inhibition activities (Fig. 2) while **A01**, **B01**, and **C01** have less or opposite activities, suggesting that the presence of a 3-methyoxy group is critical to the activity.

In summary, three series of mono-carbonyl analogues of curcumin were synthesized. Their structures were identified by NMR analysis and anti-inflammatory activities were examined by measuring the inhibitory effect on LPS-induced TNF-α and IL-6 expression in macrophages using ELISA. Although the synthesis of several analogues has been reported previously, the inhibitory activities on LPS-induced TNF-α and IL-6 expression have not been explored. In the present study, several novel analogues with better inhibitory effect than curcumin were identified. The results suggest that the properties and position of the substituent and the space of the linking chain determine the anti-inflammatory activities. However, the underlying mechanisms by which curcumin and its derivatives inhibited LPS-induced TNF-α and IL-6 expression remain unknown and are the focus of our current research. These new compounds would be useful for development of new anti-inflammatory drugs to treat various inflammatory diseases.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.068.

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