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Synthesis and evaluation of 2-cyano-3, 12-dioxooleana-1, 9(11)-en-28-oate-13*β*, 28-olide as a potent anti-inflammatory agent for intervention of LPS-induced acute lung injury

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[ABSTRACT] The present study was designed to synthesize 2-Cyano-3, 12-dioxooleana-1, 9(11)-en-28-oate-13*β*, 28-olide (**1**), a lactone derivative of oleanolic acid (OA) and evaluate its anti-inflammatory activity. Compound **1** significantly diminished nitric oxide (NO) production and down-regulated the mRNA expression of iNOS, COX-2, IL-6, IL-1*β*, and TNF-*α* in lipopolysaccharide (LPS)-stimulated RAW264.7 cells. Further *in vivo* studies in murine model of LPS-induced acute lung injury (ALI) showed that **1** possessed more potent protective effects than the well-known anti-inflammatory drug dexamethasone by inhibiting myeloperoxidase (MPO) activity, reducing total cells and neutrophils, and suppressing inflammatory cytokines expression, and thus ameliorating the histopathological conditions of the injured lung tissue. In conclusion, compound **1** could be developed as a promising anti-inflammatory agent for intervention of LPS-induced ALI.

[KEY WORDS] Oleanolic acid; Lactone; Anti-inflammation; Acute lung injury

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Introduction

Acute lung injury (ALI), which is responsible for high morbidity and mortality, can be initiated by variety of stimuli such as mechanical ventilation, hyperoxia, ischemia/reperfusion, infection, and polytrauma [1-2]. The manifestations of ALI mainly include activation of pulmonary endothelium and macrophages (alveolar and interstitial), up-regulation of adhesion molecules, and production of cytokines and chemokines that induce a massive sequestration of neutrophils within the pulmonary microvasculature [3-4]. These inflammatory conditions further lead to an infinite vicious loop by recruiting additional inflammatory cells and producing more cytotoxic mediators in turn, ultimately resulting in severe injury to alveolo-capillary membrane and respiratory failure [5]. Due to the complicated germination mechanism, serious condition, and fast course of ALI, clinic treatments by respiratory support technology and drug intervention mainly aim at the amelioration of its symptoms [6]. Pharmacologic therapy strategies can be divided into four groups: (1) anti-platelet therapy; (2) fluid management; (3) neuromuscular blocking agents; and (4) anti-inflammatory drugs such as corticosteroids [7]. Corticosteroids are the mostly used drugs in clinic, thanks to their powerful anti-inflammatory effects [8]. Unfortunately, the role of corticosteroids in ALI remains controversial. Treatment with these drugs is unable to decrease the mortality of ALI patients, and even causes some side effects, *e.g.*, hyperglycemia, increased risk of secondary infections, poor wound healing, and prolonged muscle weakness during the therapy, which are the typical characteristics after treatment with corticosteroids [9]. In this context, it is of interest to search for novel nonsteroidal agents with potent therapeutic efficacy to cure the disease.

Oleanolic acid (OA) is a natural triterpenoid with multiple moderate pharmacological activities. To improve its biological potency, many synthetic OA derivatives (SOADs) have been prepared, including 2-cyano-3, 12-dioxooleana-1, 9(11)-dien-

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28-oicacid (CDDO) and its ester (CDDO-Me) and amide (CDDO-Im) analogs, among others (Fig. 1) [10]. Our group has also developed a variety of SOADs such as CDDO-amino acid-nitric oxide donor trihybrids and olean-28, 13*β*-lactams [11-12]. Compared with OA, these SOADs possess much stronger anti-inflammatory and antitumor activities [13]. CDDO-Me directly inhibits nuclear factor-*κ*B (NF-*κ*B) signaling, a key pathway that regulates the production of a number of inflammatory mediators and their signaling cascades (e.g. TNF, IL-1*β*, IL-6) [14-15]. More importantly, CDDO-Me activates the transcription factor, nuclear-factor-E2-related factor (Nrf2), which can increase the levels of antioxidant enzymes and reduce the cellular levels of ROS, thereby further attenuating NF-*κ*B signaling and transcription of pro-inflammatory genes such as iNOS and TNF [16-17].

It has been documented that CDDO-Im exerts potent protection against hyperoxic ALI in mice [18] and that CDDO-Me has entered a phase II clinic trial for the treatment of patients with pulmonary arterial hypertension. In addition, our group has recently uncovered that CDDO-Me possesses the protective effect on lipopolysaccharide (LPS)-induced ALI in mice [19]. These findings led us to hypothesize that a *γ*-lactone compound, 2-cyano-3, 12-dioxooleana-1, 9(11)-en-28-oate-13*β*, 28-olide (**1**), isolated in improving the synthesis of CDDO from OA by our group [20], may also have the protective activity against ALI. Accordingly, we synthesized **1** through a new and convenient route, and evaluated its protective effects on LPS-induced ALI.

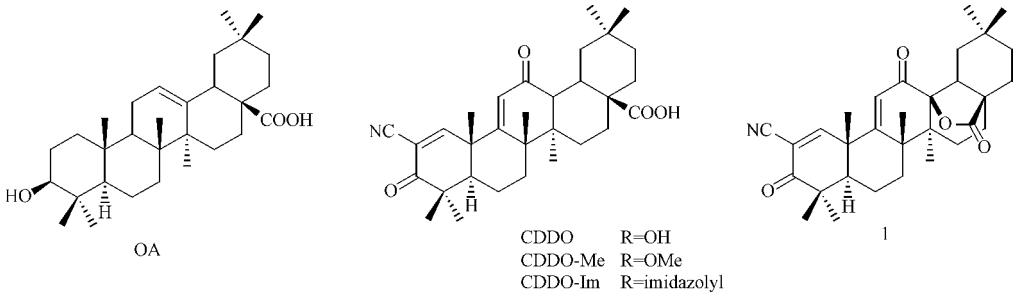

Fig. 1 Structures of OA and several SOADs

Results

Chemistry

Previously, the reported synthesis of CDDO started with methylation of OA, followed by a ten-step reaction sequence with an 18% of overall yield [21]. Unfortunately, since the reactivity of the C28-carboxyl of OA is greatly decreased due to steric hindrance from rings D and E, the methylation of C28-carboxyl requires an excess of either expensive methyl iodide or explosive diazomethane, which is not suitable for large scale preparation. Furthermore, in the last step, hydrolysis of the C28-carboxyl methyl ester proceeds in refluxing anhydrous DMF in the presence of excess costly anhydrous lithium iodide. Moreover, the crude product needs flash column chromatography and crystallization several times for purification. This synthetic procedure greatly limits the development of CDDO and its derivatives such as CDDO-Me and CDDO-Im [22]. In continuation of our studies on the synthesis and biological activities of oleanane derivatives, we developed a novel approach to synthesizing lactam derivatives of CDDO starting from benzylation of OA [12].

In the present study, we synthesized the lactone compound **1** from compound **3** which was prepared via benzylation of OA as we reported previously [12]. Debenzylation of **3** with H_2 over Pd/C gave acid **4** in 87% yield. The Kemp elimination of **4** in the presence of NaOMe led to enol **5** in 89% yield. Finally, dehydrogenation of **5** using two equivalent of 2, 3-dichloro-5, 6-dicyano-1, 4-benzoquinone (DDQ) provided **1** in 58% yield (Scheme 1). The overall yield of **1** was up to 22% based on the benzylation of OA.

*Inhibitory effects of **1** on NO production in LPS-induced macrophage RAW264.7 cells*

LPS, the major stimulus for the release of inflammatory mediators, has been widely used to induce pulmonary inflammation in animal models of ALI [23]. Nitric oxide (NO) generation induced by LPS or cytokines plays an important role in inflammatory conditions. In order to examine the anti-inflammatory activity of **1**, we first determined its inhibitory effect on NO production in RAW 264.7 cells by Griess assay, using dexamethasone as a positive control. The results indicated that **1** (IC_{50} 3.860 nmol·L^{-1}) was comparable to CDDO-Me (IC_{50} 5.083 nmol·L^{-1}), while much more potent than dexamethasone (IC_{50} 5.083 μmol·L^{-1}) in inhibition of NO production (Fig. 2).

*Inhibitory effects of **1** on the mRNA expressions of inflammatory cytokines in LPS-treated macrophage RAW264.7 cells*

To further determine the anti-inflammatory effects of **1** *in vitro*, the mRNA expressions of inflammatory cytokines, including iNOS, COX-2, IL-6, IL-1*β,* and TNF-*α* were examined. As shown in Fig. 3, relative to the control group, the mRNA expressions of iNOS, COX-2, IL-6, IL-1*β,* and TNF-*α* were evidently up-regulated after LPS-induced inflammation in RAW246.7 cells, while **1** significantly suppressed the mRNA expressions of iNOS, COX-2, IL-6, IL-1*β,* and TNF-*α*