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A novel synthetic dibenzocyclooctadiene lignan analog XLYF-104-6 attenuates lipopolysaccharide-induced inflammatory response in RAW264.7 macrophage cells and protects BALB/c mice from sepsis

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ABSTRACT

The wide range of inflammation mechanisms under control by NF-κB makes this pathway as an attractive target for new anti-inflammatory drugs. Herein, we showed that a new dibenzocyclooctadiene lignan analog XLYF-104-6, with a chemical name of 1,2,3,10,11-pentamethoxydibenzocycloocta-6,7-[c] pyrrole-1,3-dione, inhibited lipopolysaccharide (LPS)-induced NF-κB activation in RAW264.7 cells through preventing IκBα degradation and p65 nuclear translocation. The inhibitory activity of this compound on NF-κB activation contributes to the reduction of LPS-induced TNF-α and IL-6 productions. Notably, XLYF-104-6 suppressed LPS-induced iNOS expression and NO production in a NF-κB independent manner, since IKK inhibitor BAY 11-7082 has failed to exert similar inhibitory effect on iNOS expression and NO production. In addition, XLFY-104-6 also exerted anti-inflammatory action in endotoxemic mice by decreasing plasma LPS-induced TNF-α and IL-1β levels as well as increasing plasma LPS-induced IL-10 concentrations. These findings suggest XLYF-104-6 could act as a leading compound for developing a potential anti-inflammatory drug.

1. Introduction

Stimulation of macrophages with lipopolysaccharide (LPS) elicits a variety of different signaling events, including the production of cytokines, chemokines and other communication signals important for the coordination of the inflammatory response (Joseph et al., 2003). These inflammatory responses promote the secretion of inflammatory-related cytokines including tumor necrosis factor-α (TNF-α), interleukin-1β, IL-6, IL-10, nitric oxide (NO) and so on (Bode et al., 2012). It was reported that the production of inflammatory cytokines in response to LPS-treatment involved the activation of the IκB/NF-κB pathway as well as of the p38 and the c-jun N-terminal kinase (JNK) members of the mitogen activated protein kinase (MAPK) family (Bode et al., 2012). Noticeably, excessively and chronically produced pro-inflammatory mediators could cause serious inflammatory diseases, and might even contribute to the initiation, promotion and progression of cancer and other malignant diseases (Balkwill et al., 2005; Lin and Karin, 2007).

The nuclear factor-κB (NF-κB) plays a central role in the chronic inflammatory diseases development (Hayden and Ghosh, 2008). It has served as a standard for inducible transcription factors for more than 20 years (Ghosh and Karin, 2002). As previously discovered, the NF-κB family of transcription factors is composed of NF-κB 1 (p50/p105), NF-κB 2 (p52/p100), RelA (p65), RelB and c-Rel, with the most common NF-κB dimmer composed of the RelA (p65) and p50 subunit (Demchenko and Kuehl, 2010). Normally, NF-κB is maintained in an inactive cytoplasmic state with IκBα inhibitory protein family. However, upon stimulation with lipopolysaccharide (LPS),

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IκBα is phosphorylated, ubiquitinated, and rapidly degraded, resulting in the translocation of NF-κB to the nucleus, where it binds specific DNA elements (κB motifs) in the promoter/enhancer region of target genes to initiate, enhance, or suppress the transcriptional process (Verma et al., 1995; Yamaoka et al., 1998). Toll-like receptor 4 (TLR-4), which functions as the signal-transducing receptor for LPS, can mediate the inflammatory response of host macrophages, and further lead to the production of several different pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, IL-10, major histocompatibility complex (MHC) and co-stimulatory molecules (Medzhitov, 2001; Hoshino et al., 2012). Of particular interest is TNF-α, a pivotal cytokine in inflammatory reactions. High dose of regional TNF-α can cause hemorrhagic necrosis and stimulate antitumor immunity (Luo et al., 2004; Hussain et al., 2003). TNF-α has also been proposed to stimulate the production of genotoxic molecules, such as nitric oxide (NO) and reactive oxygen species, which can lead to DNA damage and mutations. TNF-α, IL-1β or endotoxins, such as LPS, can stimulate the transcription of iNOS by activation of NF-κB which binds to κB element in the NOS promoter. The enzyme iNOS is essentially expressed in every cell type and can locally generate high output quantities of NO at micromolar range for prolonged period of time (Singh and Gupta, 2011; Knowles and Moncada, 1994).

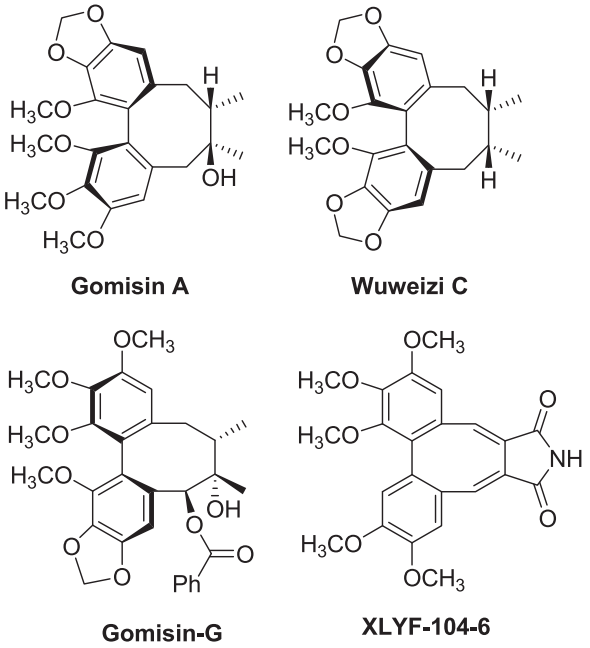


Fig. 1. Dibenzocyclooctadiene lignans with biological activities and synthetic analog XLYF-104-6.

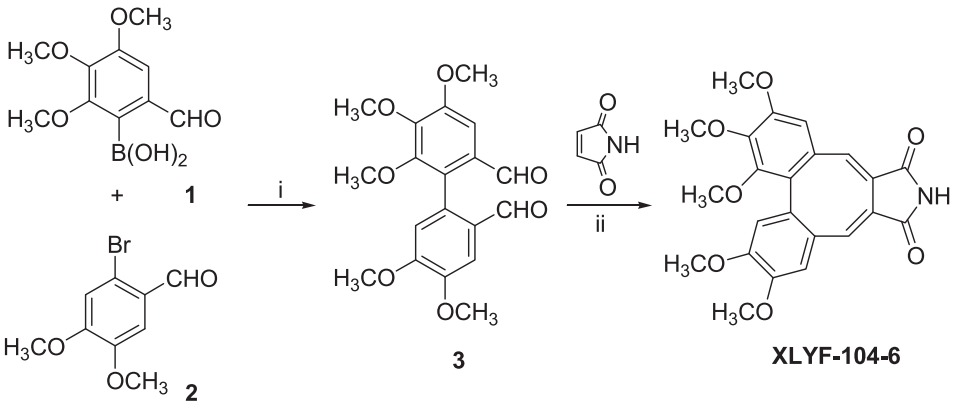
Inhibition of NF-κB has potent and broad-spectrum anti-inflammatory activity in a number of human disorders (Tak and Firestein, 2001; Karin, 2006; Chen et al., 1999). With the failure of conventional vaccines or antimicrobials, people largely focus on those strategies that directly target the host inflammatory response, specifically that result in the activation of the NF-κB (Ruan and Chen, 2012). Until now, there are many biochemical and immunological methods employed to screen compounds with NF-κB inhibitory activity (Phillips et al., 2011), such as the reporter luciferase assay and immunofluorescence assay. The inhibitory potency and kinase selective properties lead to the selection of BAY11-7082 as a tool compound to confirm the involvement of IκBα/NF-κB in LPS-stimulated NF-κB activation.

Dibenzocyclooctadiene lignans isolated from the traditional Chinese medicinal plant *Schizandra Chinese*, such as Gomisin A, Wuweizi C and Gomisin-G (Fig. 1), are known as major bioactive components with a wide variety of interesting biological activities, including hepatoprotective, antiviral, anticancer, and anti-inflammatory (Yu et al., 2011; Lee, 2010). Although their complex structures with multiple chiral centers make them non-ideal drug candidates, structural modification and simplification on this kind of natural lignans have been a practical approach to discover and develop new potential drug candidates with a novel scaffold. In our prior efforts for structural modification and simplification on active dibenzocyclooctadiene-type natural products, a series of unsymmetrical biphenyls with potent cytotoxic activity against human cancer cell lines were reported (Wu et al., 2008). As our continued studies, a dibenzocyclooctadiene lignan analog XLYF-104-6, with a chemical name of (5Z,7Z)-1,2,3,10,11-pentameth- oxydibenzocyclooctatetraene-6,7-succinimide, was synthesized as shown in Scheme 1 and exhibited significant inhibitory activity of NF-κB activation with an IC_{50} value of 0.98 μM. Current study was focused on exploring how XLYF-104-6 regulated anti-inflammatory cytokines and iNOS expression as well as NO release in LPS-stimulated RAW264.7 cells *via* NF-κB inhibition. Furthermore, the *in vivo* effects of XLYF-104-6 on cytokine production after the induction of endotoxemia in mice were also determined.

2. Materials and methods

2.1. Chemicals and drugs

XLYF-104-6 was synthesized and identified by the following methods. A mixture of 6-formyl-2,3,4-trimethoxyphenyl boronic acid (**1**, 310 mg, 1.29 mmol) and 2-bromoveratraldehyde (**2**, 200 mg, 0.82 mmol) in the presence of Pd (dppf) Cl2 (5% mol) and Cs_2CO_3 (2.0 equiv.) in 1,2-dimethoxyethane (DME, 10 mL) was heated to 80 °C for 8 h under N_2 protection. After cooling to room temperature, the mixture was diluted with EtOAc and filtered



Scheme 1. Synthesis of XLYF-104-6. (i) $Pd(dppf)Cl_2$, Cs_2CO_3, 1,2-dimethoxyethane (DME), reflux, 8 h; (ii) (a) Bu_3P, THF, reflux, overnight; (b) piperidine, HOAc, 100 °C, 4–6 h.