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Simple synthesis and anti-inflammatory activities of Spanrstolonin B derivatives

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ABSTRACT

Toll-like receptors are identified as an important factor in regulation of expression of pro-inflammatory cytokines and used as an important target in the field of anti-inflammation. SpanrstoloninB (SsnB), a new isocoumarin compound isolated from the tuber of *Scirpus yagara*, is a Toll-like receptor 2 (TLR2) and TLR4 antagonist and can selectively block TLR2- and TLR4- mediated macrophages inflammatory responses. In this study, ten derivatives (**A1**–**A6**, **B1**–**B2**, and **C1**–**C2**) were synthesized by the structural modification of compounds **1** and **2**, of which nine derivatives are new compounds. The *n*-octanol/water partition coefficients (K_{ow}) of these derivatives were determined by high performance liquid chromatography method, and their anti-inflammatory activities were evaluated of inhibiting the secretion of TNF-α and IL-6 in LPS- or Pam3csk4- induced RAW264.7 cells. Preliminary structure-activity relationship study revealed that methylated, acetylated and butyrylated derivatives (**A1**–**A6**) exhibited weaker activities of anti-inflammatory than compound **1** and **2**. However, hydrolyzed and isoquinolone derivatives (**B1**–**B2** and **C1**–**C2**) had better potential to decrease the levels of TNF-α and IL-6.

1. Introduction

Inflammation, which is defined as the complex and essential biological response to tissue injures induced by different harmful stimuli, including pathogens, damaged cell, or irritants, represents a hot topic in biomedical research (Mauriz et al., 2013). An enormous proportion of the global burden of chronic disease involves inflammation, including atherosclerosis, type 2 diabetes, Alzheimer's disease, and some cancers (Ding and Nathan, 2010; Tabas and Glass, 2013). Cytokines are major mediators of local, intercellular communication required for an integrated response to a variety of stimuli during immune and inflammatory processes. Different cytokines are associated with inflammatory diseases, with the clinical outcome partly determined by the balance between pro-inflammatory (*i.e.*, IL-1β, IL-2, IL-6, IL-8, IFN-γ and TNF-α et al.) and anti-inflammatory molecules (*i.e.*, IL-10 and TGF-β et al.) (Santangelo et al., 2007). Currently, antibiotics are the main treatment agent for inflammation (Khan et al., 2011; Thakkinstian et al., 2012). However, their usage is limited due to low response rate, serious side effects or drug resistance. Therefore, the exploration of new drugs strongly attracts many researchers (Altenburg et al., 2011).

Natural products with various skeletons and diverse biological activities are considered as important sources in drug discovery. Spanrstolonin B (SsnB) (**1**, Fig. 1) is an isocoumarin compound isolated from the plant of *Scirpus yagara*, whose tubers have long been used in traditional Chinese medicine (TCM) for the treatment of several inflammatory diseases and as an anti-spasmodic and anti-tumor agent (Chinese Pharmacopoeia Commission, 2000; Liu et al., 2015; Liang et al., 2013a,b). Recent researches have illustrated that SsnB is a Toll-like receptor 2 (TLR2) and TLR4 antagonist, which can selectively block TLR2- and TLR4- mediated macrophages inflammatory responses (Liang et al., 2011), attenuate hypoxia-reoxygenation-induced cardiomyocyte inflammation (Liu et al., 2014), suppress lipopolysaccharide-induced inflammation in human umbilical vein endothelial cells (Liang et al., 2013a,b), and inhibit pro-angiogenic functions and blocks cell cycle progression in endothelial cells (Bateman et al., 2013). In addition, further studies have indicated that SsnB can protect mice against endotoxin shock by inhibiting production of multiple cytokines in serum, lung and liver, and alleviating lung tissue dysfunction (Liang et al., 2015). Due to its potential anti-inflammatory effects, now SsnB is being further investigated as a therapeutic agent for inflammatory cardiovascular disease.

In this study, the interest in bioactive anti-inflammatory compounds, prompted us to synthesize a series of derivatives from SsnB and its analogue SanLeng diphenyllactone (**2**, Fig. 1). The *n*-octanol/water partition coefficients (K_{ow}) of these derivatives were determined by the high performance liquid chromatography method; and anti-

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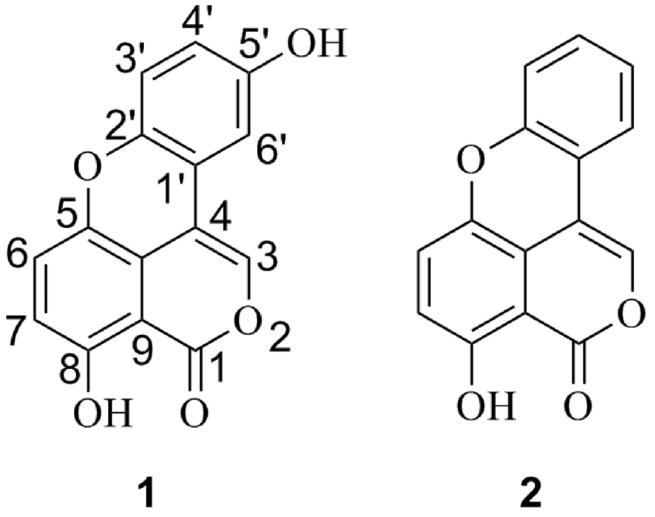

Fig. 1. Structures of compounds 1 (SsnB) and 2 (SanLeng diphenyllactone).

inflammatory activities of all compounds were evaluated on inhibiting the secretion of TNF-α and IL-6 in LPS- or Pam3csk4-induced RAW264.7 cells.

2. Material and methods

2.1. Bioactivities assay

2.1.1. Cell culture

The mouse macrophage cell line RAW264.7 was obtained from ATCC (Manassas, VA). Cells were cultured in DMEM medium (Gibco, USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, USA), 100 U/ml penicillin, and 100 μg/mL streptomycin at 37 °C in an atmosphere of 5% CO_2 in an air.

2.1.2. Cell viability

Cell viability was determined using the 3-[4,5-dimethylthiazol-2-yl]- 2,5-diphenyltetrazolium bromide (MTT) assay. Cells were seeded at 1×10^5 cells/well in 96-well plates and stimulated for 24 h at different concentrations of compounds. MTT (5 mg/mL in the medium) were then added to the medium, and the cells were incubation for an additional 4 h. The formazan crystal in each well were solubilized in dimethyl sulfoxide (DMSO), and the absorbance was measured at 490 nm on a microplate reader.

2.1.3. Enzyme-linked immunosorbent assay (ELISA)

RAW264.7 cells (density 1×10^5 cells/well) were cultured in serum-free DMEM for 16 h before treatment was started. The cells were then stimulated with LPS (1 μg/mL) or Pam3csk4 (500 ng/mL) in the presence or absence of different concentrations of all compounds for 16 h in serum-free DMEM. The levels of TNF-α and IL-6 in the medium were determined by ELISA (eBiosciences, San Diego, CA) according to the manufacturer's instruction.

2.2. Statistical analysis

GraphPad Prism 5 software was used to carry out all statistical analysis. One-way analysis of variance was used for multiple group comparison; when only two groups were compared, Student's test was performed. The mice survival rate was analyzed using Kaplan–Meier. *P* values of < 0.05 were considered statistically significant.

3. Results

3.1. Synthesis of derivatives of SsnB

As shown in Fig. 2, with two new natural products SsnB and SanLeng diphenyllactone as the starting substrate, ten new derivatives *via* chemical modification on hydroxyl groups at C-5′, 8-position and O-2 were synthesized and screened for their anti-infammatory activities in order to further study the structure-activities relationships. Of these ten products, there are nine new compounds have not been reported except compound **B2** (Oleinik and Adamskaya, 1983).

The presence of free hydroxyl groups allowed us to prepare ether and ester derivatives of compounds **1** and **2** in order to evaluate the influence of ether and ester side chain on their anti-inflammatory activities. Compounds **1** and **2** were treated with CH_3I in the presence of Na_2CO_3 and acetone to afford methylated compounds **A1** and **A2**. Treatment of compounds **1** and **2** with various anhydrides in pyridine at r.t. gave mono- or di- acylated derivatives **A3–A6**. Alkaline hydrolysis of compounds **1** and **2** with 5% KOH in ethyl alcohol refluxed yielded derivatives **B1** and **B2** without affecting the hydroxyl groups. Synthesis of isoquinolone from isocoumarin **1** and **2** with 25–28% NH_4OH at r.t. gave derivatives **C1** and **C2**.

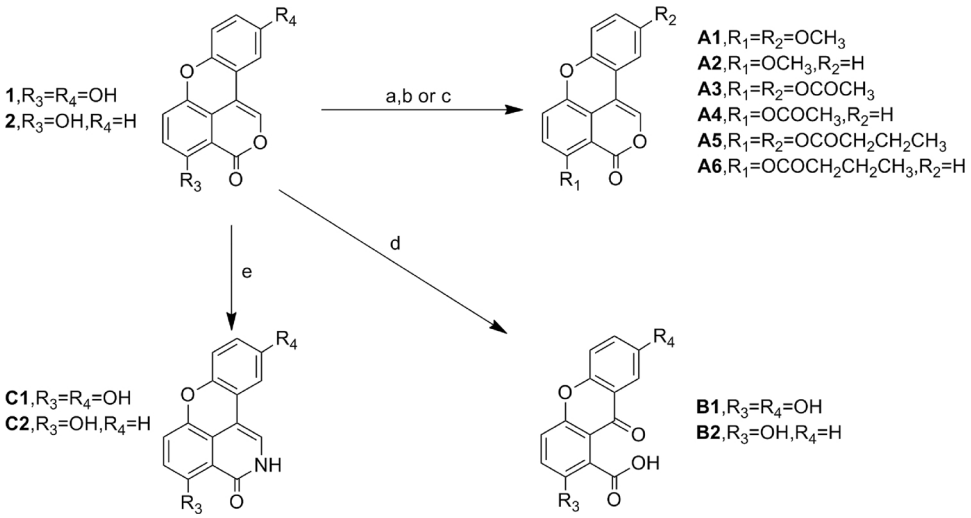

Fig. 2. Structures of the derivatives from compounds 1 and 2.
Reagents and conditions: (**a**) for **A1–A2**: CH_3I, CH_3COCH_3, K_2CO_3, 30 °C. (**b**) for **A3–A4**: $(CH_3CO)_2O$, pyridine, 80 °C. (**c**) for **A5–A6**: $(CH_3CH_2CH_2CO)_2O$, pyridine, 80 °C. (**d**) for **B1–B2**: 5% KOH, EtOH, reflux. (**e**) for **C1–C2**: NH_4OH(25–28%), DMF, r.t., 3 days, to 80 °C, 2 h.