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Original article

Synthesis and structure–activity relationships of guaiane-type sesquiterpene lactone derivatives with respect to inhibiting NO production in lipopolysaccharide-induced RAW 264.7 macrophages

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ABSTRACT

A guaiane framework was scaffolded by photochemical rearrangement reactions using α-santonin **1** as a starting material. Then, using a series of reactions, we synthesized the guaiane-type sesquiterpene lactone **5** in high yield. The inhibitory activities of compound **5** and of a series of derivatives on nitric oxide (NO) release were evaluated in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Compounds **6g**, **7h**, **7i**, **7k** and **8g**, exhibited significant inhibitory effects on NO production, with IC_{50} values of **14.8**, **22.3**, **18.3**, **17.4** and **7.0** μM, respectively. Their cytotoxicities were also estimated using an MTT assay. The structure–activity relationships of these compounds were also discussed.

1. Introduction

As one of the largest groups of secondary plant metabolites, sesquiterpene lactones (SQLs) have been reported to be the active components of many medicinal plants from the Asteraceae family [1]. Modern pharmacological studies have revealed their diverse biological activities, including antitumoural, anti-inflammatory, antifeedant, antimicrobial and antiprotozoal effects, among others [2–6]. In particular, their potent anti-inflammatory properties have received considerable attention. Furthermore, in traditional medicine, SQLs are always the active constituents for the treatment of inflammatory diseases [7].

Inflammation is clinically defined as a pathophysiological process characterized by redness, edema and an increase in body temperature, pain and loss of function. It is a hallmark of many diseases, and the persistence of this process may lead to various diseases associated with acute or chronic inflammation, including sepsis, arthritis, atherosclerosis, diabetes and even cancer [8]. The inflammatory response involves many categories of tissues and cells. The common modulators produced by many of these cells are eicosanoids, cytokines, reactive oxygen species and nitrogen intermediates [9]. Nitric oxide (NO) is a short-lived radical gas, which is catalysed by nitric oxide synthase (NOS) [10]. Inducible NOS (iNOS) produces a high level of NO that modulates inflammation and plays an important role in immunoregulation reactions. However, the overproduction of NO can result in tissue destruction and immunological and inflammatory diseases [10]. Therefore, substances that inhibit NO release have potential therapeutic effects for inflammatory diseases.

Due to the side effects of currently available anti-inflammatory drugs, there is a pressing need for the development of novel anti-inflammatory drugs [11]. In a previous study [1], some SQLs had good activities against NO production. Compound **5**, which was synthesized starting from α-santonin, is a guaiane-type sesquiterpene lactone belonging to the SQL family. To the best of our knowledge, there are no existing reports focused on the inhibitory effects of NO production by **5** and its derivatives. Therefore, it is important to investigate the anti-inflammatory effects of these new

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compounds that are derived from compound **5**. The present study is an attempt to investigate the correlation between the structures of the derivatives of compound **5** and their activities with respect to inhibiting NO release. Guaiane-type sesquiterpene lactone **5** and its derivatives (**6a**–**6p**, **7a**–**7l** and **8a**–**8i**) were synthesized, and then evaluated in LPS-induced RAW 264.7 macrophages in vitro. The corresponding cytotoxicities were also evaluated using an MTT assay.

2. Results and discussion

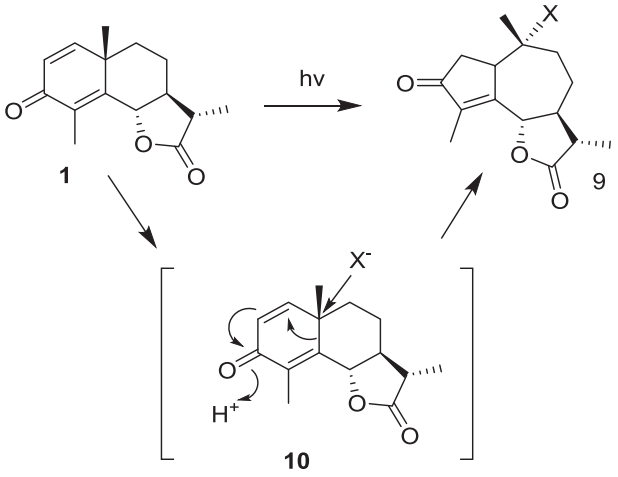
2.1. Chemistry

A convenient method for synthesizing guaiane-type sesquiterpene lactone **5** and its derivatives is shown in Scheme 2.

To prepare compounds **2** and **4**, we utilized the photochemical reaction of α-santonin (**1**) previously described in the literature [12–16].The transformation of the eudesmane framework present in α-santonin (**1**) into the guaiane framework is known to be promoted by UV irradiation; specifically, **1** is photoactivated, resulting in a sufficiently energised molecule for the occurrence of the acid-catalysed rearrangement shown in **10** [17]. The anion X^- that is added to C10 can either be a hydroxyl from water or an acetate from glacial acetic acid [16,18,19] (Scheme 1).

Irradiation of α-santonin (**1**) in anhydrous acetic acid with a high-pressure mercury lamp (500 W), afforded (3S,3αS,6R,9βS)-3,6,9-trimethyl-2,8-dioxo-2,3,3α,4,5,6,6α,7,8,9β-decahydroazuleno [4,5-β]furan-6-yl acetate (**2**) in 30% yield. Additionally, using acetic acid/H_2O (1:1) as the solvent, the (3S,3αS,6R,9βS)-6-hydroxy-3,6,9-trimethyl-3α,5,6,6α,7,9β-hexahydroazuleno[4,5-β] furan-2,8 (3H,4H)-dione (**4**) was obtained in 28% yield. Compound **4** was also afforded from compound **2** by removal of the acetyl group using KOH in methanol.

Compounds **2** and **4** were further reduced with sodium borohydride, affording (3S,3αS,6R,8S,9βS)-8-hydroxy-3,6,9-trimethyl-2-oxo-2,3,3α,4,5,6,6α,7,8,9β-decahydroazuleno[4,5-β]furan-6-yl acetate (**3**) and (3S,3αS,6R,8S,9βS)-6,8-dihy-droxy-3,6,9-trimethyl-3α,4,5,6,6α,7,8,9β-octahydroazuleno[4,5-β]furan-2(3H)-one (**5**) in good yields. Alternatively, compound **5** was also obtained from compound **4** by hydrolysing with methanolic KOH. To explore the absolute stereochemistry of compound **5**, its single-crystal X-ray structure was obtained, as shown in Fig. 1, which indicated that the stereochemistry of the resulting alcohols was a preferred attack of the hydride from the less hindered side of the carbonyl group [14].

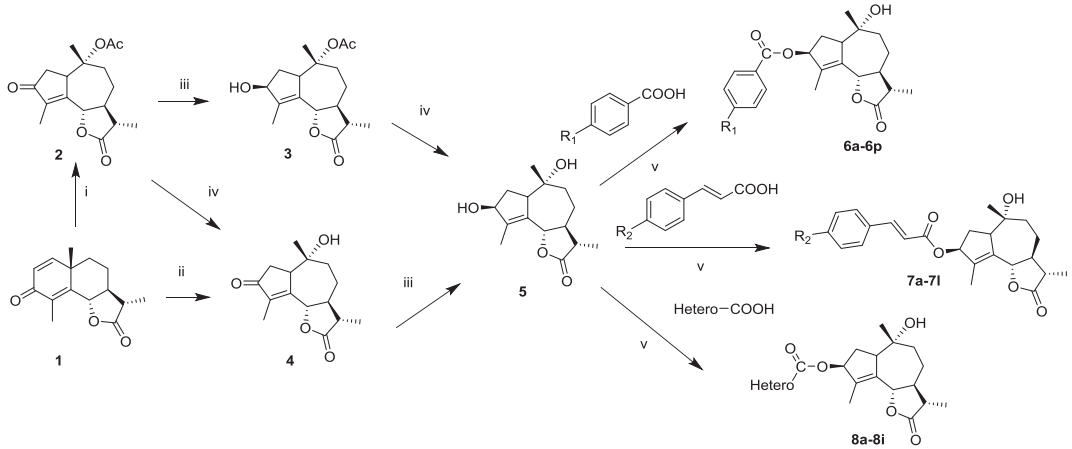


Scheme 2. The rearrangement of α-santonin (**1**).

All the final derivatives **6a**–**6p**, **7a**–**7l** and **8a**–**8i** were fully characterized using ^1H and ^{13}C NMR spectrometry, high-resolution mass and IR spectrometry. For example, compound **6c** had the composition $C_{25}H_{32}O_5$, which was determined by HR-ESI-MS at *m*/*z* 413.2346 $[M+H]^+$. The IR of **6c** indicated the existence of a hydroxyl group (3485 cm^{-1}) and two ester carbonyls (1769 and 1753 cm^{-1}). The ^{13}C NMR spectrum displayed 25 carbon resonances. Two carbonyls were located at δ 178.0 and 166.3. Signals for three carbons bearing oxygen were observed at δ 81.4, 80.3 and 74.5. The ^1H NMR spectra showed four methyls and four protons of benzene ring [δ 7.94 (d, J = 8.2 Hz, 2H), 7.23 (d, J = 8.2 Hz, 2H)]. Similarly the spectral values for all the derivatives are given in the experimental section.

2.2. Pharmacology and structure–activity relationships

The inhibition of NO production in LPS-induced RAW 264.7 macrophages was tested at 25 μM following a previously described procedure [20]. Aminoguanidine was used as the positive control [21,22]. The corresponding effects on cell viability were determined using an MTT assay as previously reported [23]. The results are shown in Table 1. Most of the tested compounds exhibited little to no cytotoxic effect on cells up to a concentration of 25 μM, except for compounds **6c**, **6d**, **6e**, **6f**, **6g**, **8g**, **7d** and **7e**. The relative cell



Scheme 1. Synthesis of guaiane-type sesquiterpene lactone **5** and its derivatives. Reagents and conditions: (i) hυ (Hg-AP/RB, 500 W), anhydrous AcOH, 12 h, 30%; (ii) hυ (Hg-AP/RB, 500 W), AcOH/H2O (1:1), 28%; (iii) NaBH4, MeOH, 0 °C, 92%; (iv) 5% KOH, EtOH, 15 h, 90%; (v) EDCI, DMAP, DCM, room temperature, overnight, 80–98%.