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Selective cytotoxicity, inhibition of cell cycle progression, and induction of apoptosis in human breast cancer cells by sesquiterpenoids from *Inula linearifolia* Turcz.

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ABSTRACT

Four new sesquiterpenoid dimers (linearifolianoids E–H, **1**–**4**), five new sesquiterpenoids (**5**–**9**), and seven known sesquiterpenoids (**10**–**16**) were isolated from the aerial parts of *Inula linearifolia* Turcz. Their structures were determined by spectroscopic data analysis and X-ray diffraction studies. The compounds were then evaluated for their *in vitro* cytotoxicity against two human breast cancer cell lines (MCF-7 and MDA-MB-231) and one normal breast cell line (MCF-10A). Linearifolianoid E (**1**) showed IC_{50} values of 1.56 μM and 2.75 μM against MCF-7 and MDA-MB-231, respectively. However, linearifolianoid E demonstrated low toxicity to MCF-10A cells, which indicated a selective cytotoxicity for tumor cells. Further studies also presented that linearifolianoid E had significant, dose-dependent effects on cell cycle progression and apoptosis in breast cancer cells.

1. Introduction

Inula, a medically important genus in the family Asteraceae, encompasses about 100 species and is widely distributed throughout the world, especially in Mediterranean countries of Europe, Africa, and Asia [1]. Some of species from this genus have long been used as folk medicine for anti-inflammatory and anti-cancer activities [2]. The extracts of *Inula* plants also display high diverse biological activities, such as anti-inflammatory [3–10], anti-tumor [11–13], antimicrobial [14–16] and hepatoprotective effects [17,18]. However, the main components in these plants have been reported as sesquiterpenoids [2,4–16,19–20]. Sesquiterpenoids are well known for the high diversity in their structures and biological activities [21]. The α-methylene-γ-lactone ring, a common functional group in guaiane sesquiterpenoids, as well as other chemical properties, lead to their promising anticancer effects, and also make them reach cancer clinical trials, such as artemisinin, thapsigargin, parthenolide and many of their synthetic derivatives [21,22].

Inula linearifolia Turcz. is widely distributed in China and its aerial parts are used in the traditional Chinese medicine "JinFeiCao" [1,6,19]. Previously, fourteen sesquiterpenoids and four sesquiterpenoid dimers with anti-inflammatory effects have been reported from the aerial parts of *I. linearifolia* [6,19]. This study was designed to investigate the anticancer components in this medicinal plant and resulted in the isolation and identification of four new sesquiterpenoid dimers (linearifolianoids E–H, **1**–**4**), five new sesquiterpenoids (**5**–**9**), and seven known sesquiterpenoids (**10**–**16**) (Fig. 1). The *in vitro* cytotoxicity of the compounds were evaluated in two human breast cancer cell lines (MCF-7 and MDA-MB-231) and one normal breast cell line (MCF-10A). The most effective compound, linearifolianoid E (**1**) showed selective cytotoxicity against breast cancer cells. Further investigation indicated that linearifolianoid E induced cell cycle arrest and apoptosis in the

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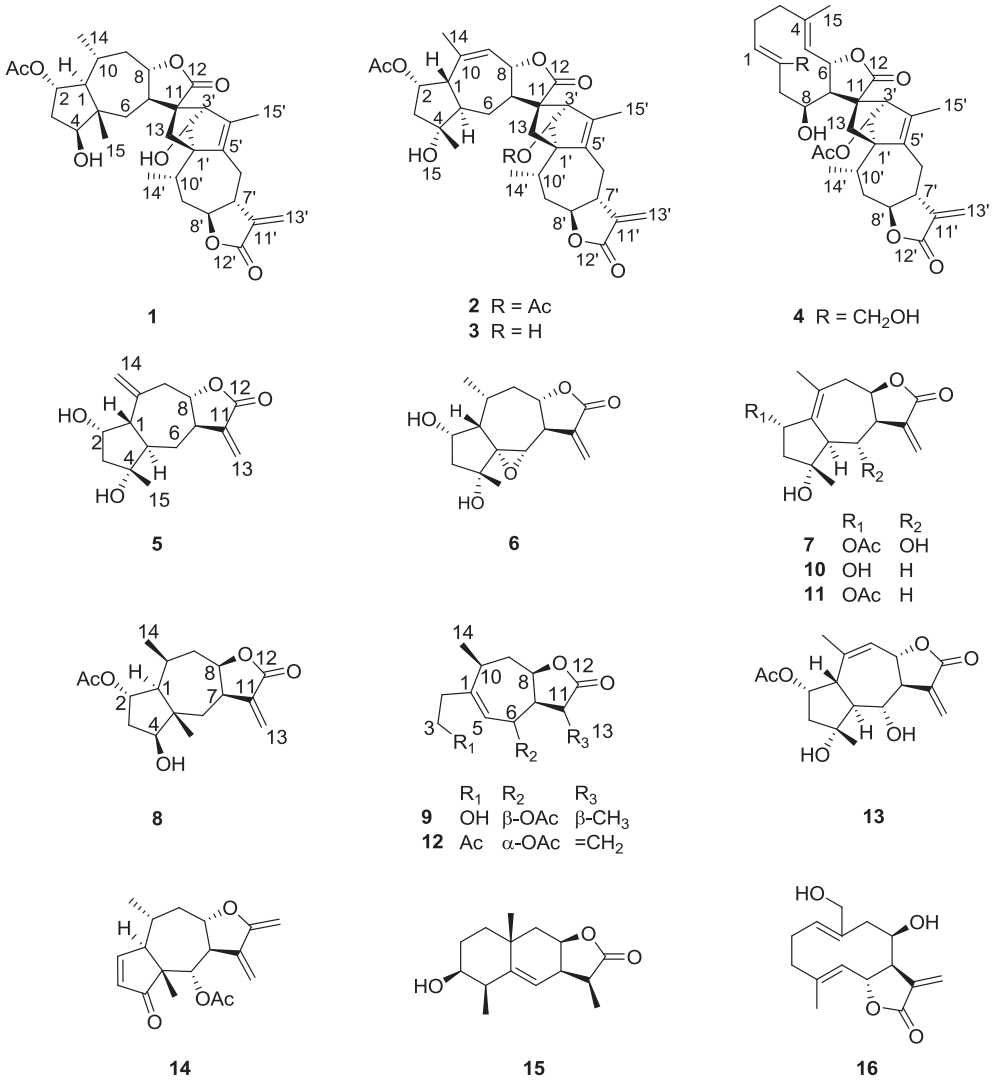

Fig. 1. Structures of compounds **1**–**16**.

breast cancer cells. The present study may provide a basis for the future development of this class of sesquiterpenoid dimers as novel anti-breast cancer agents.

2. Results and discussion

2.1. Isolation and structure elucidation

The CH_2Cl_2 soluble part of the EtOH extract from the aerial parts of *I. linearifolia* was fractionated by silica gel column chromatography, followed by Sephadex LH-20 and preparative HPLC to afford four new sesquiterpenoid dimers (linearifolianoids E–H, **1**–**4**), five new sesquiterpenoids (**5**–**9**), and seven known sesquiterpenoids: deacetylinuchinenolide B (**10**) [23,24], inuchinenolide B (**11**) [23], britanlin G (**12**) [23,25], 2α-acetoxy-4α,6α-dihydroxy-1β,5αH-guai-9(10),11(13)-dien-12,8α-olide (**13**) [9], bigelovin (**14**) [9], 3β-hydroxy-11α,13-dihydroalantolactone (**15**) [26], and deacetylovatifolin (**16**) [27] (Fig. 1).

Linearifolianoid E (**1**) was obtained as white amorphous powder and shown to have the molecular formula $C_{32}H_{42}O_8$ from HRESIMS (*m*/*z* 577.2774 for $[M + Na]^+$, calcd *m*/*z* 577.2772), indicating 12 degrees of unsaturation. The IR absorptions showed the presence of hydroxyl (3424 cm^{-1}), carbonyl (1767 and 1722 cm^{-1}), and olefinic groups (1631 cm^{-1}). The ^{13}C, DEPT, and HSQC NMR spectra of **1** (Table 1) exhibited 32 carbon signals, which revealed the presence of five methyls, seven methylenes, eleven methines, and nine quaternary carbons including three carbonyl groups. The ^1H and ^{13}C NMR spectra also suggested the presence of an acetoxy group (δ_H 2.01, δ_C 170.7 and 21.1), and its position was determined by HMBC experiment (Fig. 2). Detailed analysis of 1D and 2D NMR data of the remaining 30 carbon signals revealed that they belonged to two different sesquiterpenoid moieties, A_1 and B_1. Upon comparison with the spectral data of other sesquiterpenoid dimers isolated from genus *Inula* [5,11,19], an identical guaianolide skeleton moiety (moiety A_1) (Fig. 2) with a characteristic α-methylene lactone functionality can be authenticated by olefinic carbons C-11′ (δ_C 139.7) and C-13′ (δ_C 118.9), and exocyclic olefinic protons H-13′a (δ_H 6.25) and H-13′b (δ_H 5.60). ^1H–^1H COSY and HMBC correlations (Fig. 2) also established the other moiety (moiety B_1), similar to 2-*O*-acetyl-4-epipulchellin [28], which was previously isolated from this plant [6]. Compared with 2-*O*-acetyl-4-epipulchellin, the spectroscopic data of moiety B_1 mainly differed in the chemical shifts of C-11 and C-13, suggesting that the two moieties were connected through these units. The key HMBC