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Cytotoxic and anti-angiogenic effects of lanostane triterpenoids from *Ganoderma lucidum*

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ABSTRACT

Two new lanostane triterpenes, 3α,12β,15α-triacetoxy-5α-lanosta-7,9(11),24-trien-26-oic acid (**1**) and 5α-lanosta-8,24-diene-26,27-dihydroxy-3,7-dione (**2**), together with sixteen known compounds (**3**–**18**) were isolated from the fruiting bodies of the Vietnamese mushroom *Ganoderma lucidum*. Their chemical structures were determined by extensive spectroscopic (IR, HR-EI-MS, 1D and 2D NMR) analyses. Potential cytotoxic activities of these compounds were evaluated against human non-small cell lung adenocarcinoma (A549), breast adenocarcinoma (MCF-7), and prostatic small cell carcinoma (PC-3). Among the compounds, 3α,12β,15α-triacetoxy-5α-lanosta-7,9(11),24-trien-26-oic acid (**1**) showed significant cytotoxic activity against PC-3 cells with an IC_{50} of 11.5 μM. In studies of anti-angiogenesis activity, ganoderic acid F (**17**) was found to have the most potent inhibitory effect on the formation of capillary-like structures of human umbilical vein endothelial cells.

1. Introduction

Q2 Angiogenesis is the formation of new vessels from an existing vascular network. It is related to cancer, obesity, psoriasis, diabetic retinopathy, and arthritis. Angiogenesis plays an important part in the growth and metastasis of tumor by supplying oxygen and nutrients necessary for the growth of tumor cells (Folkman, 2006). In addition, angiogenesis is required for solid tumors to grow beyond a size of approximately 1–2 mm^3, which is sufficiently small to be treated with conventional chemotherapeutic agents (Folkman, 2006). Therefore, inhibitors of tumor angiogenesis are considered to be an effective strategy for the treatment of cancer.

Within the framework of our research project on Vietnamese traditional medicinal plants, the mushroom *Ganoderma lucidum* (Fr.) P. Karst (Polyporaceae), known locally as "Nam Lim Xanh", was selected. The fruiting bodies of *G. lucidum* are widely used in China, Japan, and Korea as a valuable crude drug, particularly in the treatment of chronic hepatitis, nephritis, hepatopathy, neurasthenia, arthritis, bronchitis, asthma, gastric ulcer, and insomnia (Namba, 1994). Triterpenoids are the main chemical constituents of *G. lucidum*, and these compounds have been shown to produce inhibitory effects on HIV-1 protease (Min et al., 1998), anti-tumor effects (Stanley et al., 2005; Sliva, 2006; Müller et al., 2006), inhibitory effects on histamine release (Kohda et al., 1985), as well as antimicrobial (Wang and Ng, 2006), anti-inflammatory (Tung et al., 2013; Dudhgaonkar et al., 2009), and antioxidant activities (Zhu et al., 1999). In addition, polysaccharides from *G. lucidum* have been shown to possess hypoglycemic (Hikino and Mizuno, 1989), immunostimulant (Kino et al., 1989; Soccol et al., 2010), anti-tumor, and anti-inflammatory activities (Joseph et al., 2011).

In continuing studies toward the discovery of anti-angiogenic agents from natural plants, further fractionation of the chloroform-soluble fraction prepared from the fruiting bodies of Vietnamese *G. lucidum* resulted in the isolation of two new triterpenes (**1** and **2**) along with sixteen known compounds (**3**–**18**). Here, we report on the isolation and structural elucidation of these compounds, as well as the evaluation of their anti-angiogenic effects and cytotoxic properties against some human cancer cell lines.

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2. Results and discussion

The MeOH extract of the fruiting bodies of *G. lucidum* was partitioned into *n*-hexane-, $CHCl_3$-, EtOAc-, and *n*-BuOH-soluble fractions, as well as an H_2O layer. Chromatographic purification of the $CHCl_3$-soluble fraction led to the isolation of two new (**1** and **2**) and sixteen known compounds (**3**–**18**). Known compounds were identified as ganoderic acid DM (**3**) (Wang et al., 1997), ergosta-7,22-dien-2β,3α,9α-triol (**4**) (Lin and Tome, 1991), ganodermanontriol (**5**) (Fujita et al., 1986), ganodermanondiol (**6**) (Fujita et al., 1986), ganoderitriol M (**7**) (Chen et al., 2009), lucidenic acid A (**8**) (Nishitoba et al., 1985), lucidenic acid C (**9**) (Kikuchi et al., 1986), ganoderic acid S_1 (**10**) (Morigiwa et al., 1986), methyl lucidenate Q (**11**) (Kenji et al., 2003), methyl lucidenate L (**12**) (Nishitoba et al., 1987), methyl lucidenate C (**13**) (Kikuchi et al., 1986), lucidadiol (**14**) (González et al., 2002), ganoderiol F (**15**) (Nishitoba et al., 1988), ganoderic acid A (**16**) (Kubota et al., 1982), ganoderic acid F (**17**) (Kikuchi et al., 1986), and methyl lucidenate A (**18**) (Nishitoba et al., 1985) (Fig. 1). The structures of these known compounds were identified by comparison of their spectroscopic data with that reported in the literature.

Compound **1** was obtained as colorless oil with an optical rotation of +22.8 (*c* 0.12, $CHCl_3$). Its HR-EI-MS spectrum gave a molecular ion peak at an *m/z* value of 612.3662, which corresponded to the molecular formula $C_{36}H_{52}O_8$. The IR spectrum showed the presence of OH (3424 cm^{-1}), and C=O (1718 cm^{-1}) absorptions. The ^1H NMR spectrum of compound **1** (Table 1) displayed signals for five tertiary methyls at δ_H 0.67 (3H, s, H-18), 1.04 (3H, s, H-19), 0.99 (3H, s, H-28), 0.98 (3H, s, H-29), and 0.89 (3H, s, H-30), a secondary methyl at δ_H 0.98 (d, *J* = 3.6 Hz), an allyl methyl at δ_H 1.87 (3H, s, H-27), three *O*-acetyl methyls at δ_H 2.06 (3H, s), 2.07 (3H, s), and 2.09 (3H, s), three oxymethine protons [δ_H 4.68 (1H, s, H-3), 5.04 (1H, t, *J* = 7.2 Hz, H-12), 5. 09 (1H, dd, *J* = 4.4, 10.0 Hz, H-15)], and three olefinic protons [δ_H 5.49 (1H, brs, H-7), 5.32 (1H, d, *J* = 6.4 Hz, H-11), and 6.78 (1H, t, *J* = 7.2 Hz, H-24)]. The ^{13}C NMR spectrum, combined with the DEPT data, showed that **1** had 36 carbon signals consisting of eleven methyls, six methylenes, eight methines and eleven quaternary carbons. Among them, **1** contained distinctively three oxygenated methines [δ_C 78.3 (C-3), 74.7 (C-12), and 77.4 (C-15)], three acetoxy groups [δ_C 170.8, 21.5 (3-OAc), 171.0, 21.6 (12-OAc), and 171.3, 21.3 (15-OAc)], three olefinic quaternary carbons [δ_C 140.2 (C-8), 146.2 (C-9), and 129.5 (C-25)], three olefinic methine carbons [δ_C 121.6 (C-7), 115.6 (C-11), and 139.2 (C-24)], and one carbonyl carbon [δ_C 171.6 (C-26)] (Table 1).

This evidence clearly indicated that **1** was a triacetoxy-ganoderic acid of the 7,9(11),24-triene type (Hirotani et al., 1986). The full NMR assignments and connectivity of **1** were determined by analysis of HMQC and HMBC spectroscopic data. The position of three acetoxyl groups at C-3, C-12, and C-15 were decided by the key HMBC correlations; from H-3 (δ_H 4.68) to C-2/C-4/C-29, and δ_C 170.8, from H-12 (δ_H 5.04) to C-9/C-11, and δ_C 171.0,

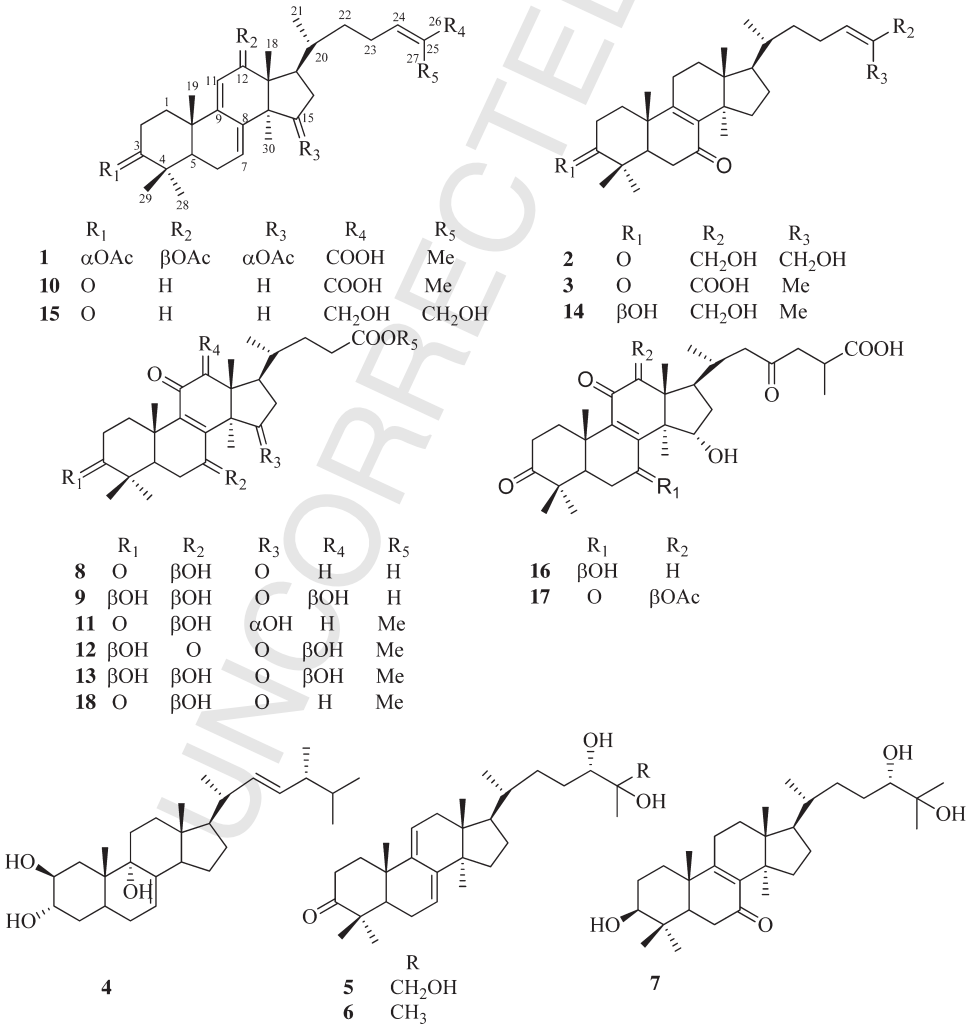

Fig. 1. Chemical structures of isolated compounds (**1**–**18**).

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