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Lanostane Triterpenoids and Ergostane Steroids from *Ganoderma luteomarginatum* and Their Cytotoxicity

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Abstract: Macrot fungus *Ganoderma luteomarginatum* is one of the main species of *Ganoderma* fungi distributed in Hainan province of China, the fruiting bodies of which have been widely used in folk as a healthy food to prevent tumors. To explore the potential cytotoxic constituents from *G. luteomarginatum*, the phytochemical investigation on the ethyl acetate soluble fraction of 95% ethanolic extract from the fruiting bodies of this fungus led to the isolation of twenty-six lanostane triterpenoids (1–26), including three undescribed ones (1–3), together with eight ergostane steroids (27–34). The structures of three new lanostane triterpenoids were elucidated as lanosta-7,9(11)-dien-3-β-acetyloxy-24,25-diol (1), lanosta-7,9(11)-dien-3-oxo-24,26-diol-25-methoxy (2), and lanosta-8(22)-dien-3,11,23-triioxo-7β,15β-diol-26-oic acid methyl ester (3) by the analysis of 1D, 2D NMR, and HRESIMS spectroscopic data. All isolates were assayed for their cytotoxic activities using three human cancer cell lines (K562, BEL-7402, and SGC-7901) and seven lanostane triterpenoids (1, 2, 7, 13, 18, 22, and 24), and one ergostane steroid (34) showed definite cytotoxicity with IC50 values that ranged from 6.64 to 47.63 µg/mL. Among these cytotoxic lanostane triterpenoids, compounds 2 and 13 showed general cytotoxicity against three human cancer cell lines, while compounds 1 and 18 exhibited significant selective cytotoxicity against K562 cells with IC50 values of 8.59 and 8.82 µg/mL, respectively. Furthermore, the preliminary structure–cytotoxicity relationships was proposed.

Keywords: *Ganoderma luteomarginatum*; lanostane triterpenoids; ergostane steroids; cytotoxic activity

1. Introduction

The genus *Ganoderma*, belonging to the family Ganodermataceae and known as “Lingzhi” in Chinese, has been widely used as traditional Chinese medicine and functional foods for health in China and Southeast Asia for thousands of years [1]. There are more than 100 species in this family growing on cut or rotten trees in China, and 78 wild ones were found in Hainan Province [2]. As the major genus in this family, *Ganoderma* is a prolific producer of novel natural products responsible for its health benefits, mainly containing polysaccharides with an immunostimulative effect and triterpenes with a cytotoxic action [3]. Two main species, *G. lucidum* and *G. sinensis*, are recorded in Pharmacopoeia of the People’s Republic of China and used as an addition to conventional therapy in a clinical treatment of chronic bronchitis, bronchial asthma, leukopenia, coronary heart disease, arrhythmia, and acute infectious hepatitis. Recent research on chemical constituents of *Ganoderma* species showed that lanostane-type triterpenoids are the main characteristic natural products [4], and more than 400 lanostanoids have been isolated from the fungi of *Ganoderma*. These small molecule compounds have attracted considerable attention due to their
extensive biological and pharmacological activities \cite{5,6}, including cytotoxic \cite{7–9}, hep- atoprotective \cite{10,11}, anti-inflammatory \cite{12–14}, antidiabetic \cite{15,16}, neuroprotective \cite{17}, antiviral \cite{18}, antiaging \cite{19}, and antioxidant \cite{20–22} effects. The genus \textit{Ganoderma} is used as a healthy food and has been traditionally used for the prevention of numerous diseases or various pathological conditions, including complementary cancer therapy, especially a broad-spectrum application for the treatment of cancer.

Cancer has been considered as a huge threat to human health, and most governments are committed to diminishing this threat. The prevention and treatment of cancer becomes a key health goal. Finding antitumor drugs with high efficiency and low toxicity has become the urgent task, and countless researchers are dedicated to discovering bioactive ingredients from nature resources. \textit{Ganoderma} is a promising anticancer immunotherapy agent owing to its low toxicology and efficacy as a combination therapy through the regulation of the immune system \cite{23}. Polysaccharides and triterpenes from \textit{Ganoderma} have been known to possess chemopreventive and antitumor activity. Many studies indicate that lanostane-type triterpenoids act as an inhibitor on different cancer cell lines, including the lung, liver, colon, pancreas, breast, skin, and prostate \cite{6}. Among the reported active lanostanoids, the ganoderic acids are the main types of triterpene that play key roles in the biological activity. Lanostane-type triterpenoids can cause cell cycle arrest by the downregulation of cyclin D1 in the G1 phase of cell growth and inhibition of PKC activity in the G2 growth phase. Moreover, lanostane-type triterpenoids also prevent tumor metastasis by modulating MMPs and IL-8 and inhibit the excretion of inflammatory cytokines \cite{24}.

\textit{Ganoderma luteomarginatum}, used as folk medicinal \textit{Ganoderma} species, is a rare species mainly distributed in Hainan Province in China \cite{25}, where a pharmacodynamic molecular basis has been brought into focus in recent years \cite{26,27}. In our ongoing endeavor to explore bioactive natural products, several species of \textit{Ganoderma} have been studied, and a series of active compounds have been found \cite{7,15,28–31}. The fruiting bodies of \textit{G. luteomarginatum} have been widely used as a healthy food to prevent tumors. To explore the potential cytotoxic constituents from \textit{G. luteomarginatum}, we performed a phytochemical investigation on this fungus, which resulted in the isolation of twenty-six lanostane triterpenoids (1–26) (Figure 1), including three undescribed ones: lanosta-7,9(11)-dien-3β-acetyloxy-24,25-diol (1), lanosta-7,9(11)-dien-3-oxo-24,26-diol-25-methoxy (2), and lanosta-8,20(22)-dien-3,11,23-trioxo-7β,15β-diol-26-oic acid methyl ester (3), together with eight ergostane steroids (27–34) (Figure 1). All these isolates were evaluated for their cytotoxic activity against three human cancer cell lines. Herein, we reported the isolation, structural elucidation, and cytotoxicity of the compounds isolated from the fruiting bodies of \textit{G. luteomarginatum}. 

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Structures of the isolated compounds from \textit{G. luteomarginatum}.}
\end{figure}
Figure 1. The structures of compounds 1–34.

2. Results and Discussion

2.1. Structural Elucidation of Compounds

Compound 1 was obtained as white amorphous powder, and its molecular formula was determined to be C$_{32}$H$_{52}$O$_4$ on the basis of HRESIMS ion at m/z [M + Na]$^+$ 523.3754 (calcld. 523.3763 for C$_{32}$H$_{52}$NaO$_4^+$), indicating seven degrees of unsaturation. The IR spectrum revealed the presence of hydroxyl groups (3475 cm$^{-1}$), double bonds (1641 cm$^{-1}$), and ester carbonyl (1696 cm$^{-1}$). The $^1$H NMR spectral data (Table 1) of 1 revealed the signals for nine methyls ($\delta$H 0.58; 0.89; 0.90; 0.93, d, $J$ = 6.3 Hz; 0.97; 1.02; 1.18; 1.23; 2.07), one proton related to oxygenated carbon ($\delta$H 4.53, dd, $J$ = 11.4, 4.6 Hz), and two olefinic protons ($\delta$H 5.47, t, $J$ = 4.5 Hz; 5.34, d, $J$ = 5.9 Hz). The $^{13}$C NMR and DEPT (Table 1) spectra presented 32 carbon signals for nine methyls; eight methylenes; seven methines (two oxygenated and two olefinic); and eight non-protonated carbons (two olefinic, one oxygenated, and one ester carbonyl at $\delta$C 171.2). The above-mentioned NMR data were closely similar to those of lanosta-7,9(11)-dien-3b-acetyloxy-24,25,26-trihydroxy [29], suggesting that 1 had a lanostane skeleton and structurally similar to this compound. The only difference between them was that the methylol group ($\delta$C 67.8) at C-26 in lanosta-7,9(11)-dien-3b-acetyloxy-24,25,26-trihydroxy was replaced by one methyl ($\delta$C 25.7) in 1, which was confirmed by HMBC correlations (Figure 2) from H$_3$-27 ($\delta$H 1.18) to C-26, C-24 ($\delta$C 79.7) and C-25 ($\delta$C 73.4). The attachment of acetate group to C-3 was proposed by the key HMBC correlation of H-3 ($\delta$H 4.53) with acetal carbonyl ($\delta$C 171.2). The other obvious HMBC correlations
(Figure 2) of 1 from H$_3$-28 (δ$_H$ 0.90) and H$_3$-29 (δ$_H$ 0.97) to C-3, C-4 (δ$_C$ 37.9), and C-5 (δ$_C$ 49.3); from H$_3$-18 (δ$_H$ 0.55) to C-12 (δ$_C$ 37.7), C-14 (δ$_C$ 50.4), and C-17 (δ$_C$ 51.1); from H$_3$-19 (δ$_H$ 1.02) to C-1 (δ$_C$ 35.5), C-5, and C-9 (δ$_C$ 145.7); and from H$_3$-30 (δ$_H$ 0.89) to C-8 (δ$_C$ 142.8), C-13 (δ$_C$ 43.8) and C-15 (δ$_C$ 28.0) further assigned its planar structure of lanostanoid. The relative configuration of the tetracyclic core structure of 1 was determined to be the same as that of lanosta-7,9(11)-dien-3β-acetyloxy-24,25,26-trihydroxy by comparison of their NMR and ROESY spectroscopic data (Figure 1), revealing ROESY correlations of H-18 with H-19 and H-20 (δ$_H$ 1.39). The key ROESY correlation of H-3 (δ$_H$ 4.53) with H$_3$-28 (δ$_H$ 0.90) and H-5 (δ$_H$ 1.18) suggested the assignment of their same orientation. Based on comprehensive analysis of 1D, 2D NMR, and HRESIMS spectrums (see Supplementary Materials), the structure of compound 1 was elucidated to be lanosta-7,9(11)-dien-3β-acetyloxy-24,25-diol.

Table 1. $^1$H (500 MHz) and $^{13}$C NMR (125 MHz) Data of Compounds 1–3 (in CDCl$_3$).

| No. | 1 $^\delta_C$ | 1 $^\delta_H$ (J in Hz) | 2 $^\delta_C$ | 2 $^\delta_H$ (J in Hz) | 3 $^\delta_C$ | 3 $^\delta_H$ (J in Hz) |
|-----|---------------|---------------------------|---------------|---------------------------|---------------|---------------------------|
| 1   | 35.5          | 1.97 m                    | 36.7          | 2.36 m                    | 35.6          | 2.45 m                    |
| 2   | 24.4          | 1.71 m                    | 35.0          | 2.02 m, 2.23 m            | 34.4          | 1.67 m, 1.84 m            |
| 3   | 81.0          | 8.03 m, 11.4 m            | 217.1         | 216.8                     |              |                           |
| 4   | 37.9          | 117.4                     | 46.9          |                           |              |                           |
| 5   | 49.3          | 5.40 d (6.2)              | 199.4         |                           |              |                           |
| 6   | 22.9          | 2.08 m                    | 23.8          | 2.22 m                    | 36.5          | 1.14 m                    |
| 7   | 120.1         | 5.47 t (4.5)              | 120.0         | 5.52 d (6.8)              | 69.5          | 1.48 d (7.1, 9.4)         |
| 8   | 142.8         |                            | 157.0         |                           |              |                           |
| 9   | 145.7         |                            | 143.3         |                           |              |                           |
| 10  | 37.3          | 111.4                     | 38.3          |                           |              |                           |
| 11  | 116.6         | 5.34 d (5.9)              | 117.4         | 5.40 d (6.2)              | 199.4         |                           |
| 12  | 37.7          | 2.23 d (17.6)             | 37.9          | 2.08 m                    | 51.7          | 2.75 d (14.6)             |
| 13  | 43.8          |                            | 48.5          |                           |              |                           |
| 14  | 50.4          |                            | 57.1          |                           |              |                           |
| 15  | 28.0          | 1.29 m                    | 28.0          | 1.27 m                    | 77.8          | 4.33 d (6.7)              |
| 16  | 31.6          | 1.60 m                    | 31.6          | 1.67 m                    | 35.3          | 2.35 m                    |
| 17  | 51.1          | 1.58 m                    | 51.1          | 1.61 m                    | 54.5          | 2.76 m                    |
| 18  | 15.8          | 0.55 s                    | 15.9          | 0.61 s                    | 19.6          | 1.10 s                    |
| 19  | 23.3          | 1.03 s                    | 22.2          | 1.21 s                    | 19.7          | 1.36 s                    |
| 20  | 36.7          | 1.39 m                    | 36.7          | 1.44 m                    | 156.8         |                           |
| 21  | 18.7          | 0.93 d (6.3)              | 18.7          | 0.94 d (6.5)              | 21.3          | 2.16 s                    |
| 22  | 33.6          | 1.76 m                    | 33.8          | 1.86 m                    | 124.7         | 6.16 s                    |
| 23  | 28.8          | 1.56 m                    | 28.4          | 1.63 m                    | 198.4         |                           |
| 24  | 79.7          | 3.31 d (10.2, 2.1)        | 76.8          | 3.59 d (9.4)              | 47.8          | 2.94 m                    |
| 25  | 73.4          |                            | 78.5          |                           | 35.0          | 2.96 m                    |
| 26  | 25.7          | 1.23 s                    | 64.4          | 3.75 d (12.0)             | 176.7         |                           |
| 27  | 22.9          | 1.18 s                    | 16.0          | 1.05 s                    | 17.3          | 1.20 d (6.8)              |
| 28  | 28.2          | 0.90 s                    | 25.4          | 1.10 s                    | 27.0          | 1.13 s                    |
| 29  | 17.1          | 0.97 s                    | 22.6          | 1.14 s                    | 21.0          | 1.12 s                    |
| 30  | 25.7          | 0.89 s                    | 25.6          | 0.89 s                    | 26.0          | 1.24 s                    |
| OAc | 171.2         |                            |               |                           |              |                           |
| OMe | 21.5          | 2.07 s                    |               |                           |              |                           |
Compound 2 had the molecular formula C$_{31}$H$_{50}$O$_4$, as determined by the HRESIMS ion peak at 509.3597 (calcd. 509.3607 for C$_{31}$H$_{50}$NaO$_4^+$). The $^{13}$C NMR and DEPT (Table 1) spectra showed 31 carbon signals for eight methyls (one methoxy at $\delta_C$ 49.5); nine methylenes (one oxygenated at $\delta_C$ 64.4); six methines (one oxygenated at $\delta_C$ 76.8 and two olefinic); and eight non-protonated carbons (two olefinic, one oxygenated, and one ketone carbonyl at $\delta_C$ 217.1). The NMR spectra of 2 resembled those of (24S,25R)-25′-methoxylanosta-7,9(11)-dien-3,24,26-triol [32], except for the presence of a ketone carbonyl of C-3 ($\delta_C$ 217.1) in 2 replacing the hydroxylated methine in (24S,25R)-25′-methoxylanosta-7,9(11)-dien-3,24,26-triol, which was corroborated by the HMBC correlations (Figure 2) from H$_3$-28 ($\delta_H$ 1.10), H$_3$-29 ($\delta_H$ 1.14), and H$_2$-2 ($\delta_H$ 2.38/2.80) to C-3 ($\delta_C$ 217.1). The attachment of methoxy to C-25 was assigned by the key HMBC correlation of the proton signal at $\delta_H$ 3.34 with C-25 ($\delta_C$ 78.5). The other clear HMBC correlations (Figure 2) of 1 from H$_3$-28 ($\delta_H$ 1.10), H$_3$-29 ($\delta_H$ 1.14), and H$_3$-19 ($\delta_H$ 1.21) to C-5 ($\delta_C$ 50.8) and from H$_3$-18 ($\delta_H$ 0.61) and H$_3$-30 ($\delta_H$ 0.89) to C-13 ($\delta_C$ 43.9) and C-14 ($\delta_C$ 50.4) further confirmed its planar structure of lanostanoid. The relative configuration of 2 was established as same as that of (24S,25R)-25′-methoxylanosta-7,9(11)-dien-3,24,26-triol by the ROESY spectrum (Figure 2), revealing key ROESY correlations of H$_3$-30 with H$_3$-17 and H$_2$-18 ($\delta_H$ 6.16) and of H-18 with H-19 and H-20 ($\delta_H$ 1.44), as well as their similar NMR data. On the basis of the above evidence, the structure of 2 was defined as lanosta-7,9(11)-dien-3-oxo-24,26-diol-25-methoxy.

The HRESIMS ion peak 529.3160 [M+H]$^+$ of compound 3 gave the molecular formula C$_{31}$H$_{54}$O$_7$, indicating ten degrees of unsaturation. The $^{13}$C NMR and DEPT (Table 1) spectra showed 31 carbon signals for eight methyls (one methoxy at $\delta_C$ 52.0), six methylenes, six methines (two oxygenated, and one olefinic at $\delta_C$ 124.7) and eleven non-protonated carbons (three olefinic; three ketones at $\delta_C$ 216.8, 199.4, and 198.4; and one ester carbonyl $\delta_C$ 176.7). A detailed analysis of the 1D NMR data of 3 suggested that compound 3 possessed the same planar structure as methyl ganoderenate A [33] with a lanostane skeleton. The whole connectivity of compound 3 was also further demonstrated by 2D NMR data, including HMBC spectrum (Figure 2), exhibiting HMBC correlations from H$_3$-28 ($\delta_H$ 1.13), H$_3$-29 ($\delta_H$ 1.12), and H$_3$-19 ($\delta_H$ 1.36) to C-5 ($\delta_C$ 49.3) and from H$_3$-18 ($\delta_H$ 1.10) and H$_3$-30 ($\delta_H$ 1.24) to C-13 ($\delta_C$ 48.5) and C-14 ($\delta_C$ 57.1). The relative configuration of the tetracyclic core structure of 1 was determined to be the same as methyl ganoderenate A, except for chiral C-15 by analysis of its ROESY spectrum (Figure 2). The key ROESY correlations of H-7 ($\delta_H$ 4.80) with H$_3$-30 ($\delta_H$ 1.24) and H-5 ($\delta_H$ 1.67) proposed the $\beta$-orientation of hydroxy at C-7. The $\beta$-orientation of hydroxy at C-15 was assigned by the key ROESY correlation of H$_3$-30 ($\delta_H$ 1.24) with H-15 ($\delta_H$ 4.33). Moreover, the key ROESY correlation of H-22 ($\delta_H$ 6.16) with H-17 ($\delta_H$ 2.76) indicated that the geometry of double bond $\Delta^{20(22)}$ in 3 was $E$ instead of $Z$ in methyl ganoderenate A. Therefore, the structure of compound 3 was established as lanosta-8,20(22)-dien-3,11,23-tri-oxo-7β,15β-diol-26-oic acid methyl ester.

The thirty-one known compounds, including twenty-one lanostane triterpenoids (4–26) and eight ergostane steroid (27–34), were identified as lanosta-8,24E-dien-7-oxo-3β-acycloxy-26-ol (4) [26], lanosta-8,24E-dien-7-oxo-3β-acycloxy-26-al (5) [26], lanosta-7,9(11),24-tri-3β-acycloxy-26-ol (6) [26], (24R,25S)-lanosta-7,9(11)-dien-3β,24,26-triol-25-methoxy (7) [16], lanosta-7,9(11)-dien-3β-acycloxy-24,25,26-triol (8) [29], lanosta-7,9(11)-dien-3β-acycloxy-24,26-dihydroxy-25-methoxy (9) [29], ganodermanondiol (10) [34], lu-
cidumol B (11) [34], 26-hydroxy-ganodermanondiol (12) [35], ganoderiol A (13) [36], ganoderone A (14) [37], lucidadiol (15) [38], ganoderadiol (16) [39], lanosta-7,9(11),24E-trien-3β-acetoxy-26,27-diol (17) [29], ganoderic F (18) [40], lanosta-8-en-7,11-dioxo-3β-acetoxy-24,25,26-triol (19) [29], ganoderol D (20) [40], lanosta-8-en-7-oxo-3β-acetoxy-24,25,26-trihydroxy (21) [34], lucidumol A (22) [41], lanosta-7,9(11),24E-trien-3-oxo-26-al (23) [42], lanosta-7,9(11),24-triene-3β-ol-26-al (24) [42], lucidone H (25) [29], lucidalone H (26) [43], ergosta-7,9(11),22E-triene-3β,5α-diol-6β-methoxy (27) [29], ergosta-7,22E-dien-3-one (28) [44], ergosta-7,22E-dien-3β-ol (29) [45], ergosta-4,6,8(14),22E-tetraen-3-one (30) [44], ergosterol peroxide (31) [46], ergosta-3β,5α-diol-7,22E-dien-6β-methoxy (32) [47], ergosta-7,22E-dien-3β,5α,6β-triol (33) [48], and calvasterol B (34) [49] by comparing their NMR data with those reported in the literature.

*Ganoderma* has been used as a healthy food and medicinal purposes for centuries particularly in China, Japan, and Korea. A great deal of work has been carried out on over thirty species of *Ganoderma*. Two types of natural products, lanostane triterpenoids (1–26) and ergostane steroids (27–34), were discovered from the fruiting bodies of *G. luteomarginatum* collected in Hainan Province, China. These two types of compounds are widely found in genus *Ganoderma* [1], and lanostaneoids (*Ganoderma* triterpenoids) were the characteristic active metabolites in species of *Ganoderma*, which are a class of compounds with various chemical structures. Here, the isolated lanostane triterpenoids were divided into two groups according to the conjugated system at C-7, C-8, C-9, and C-11. The first group possessed the conjugated double bond $\Delta^7$ as shown in 1, 2, 6–18, 23, and 24. The second group had (A$^7$)-β-unsaturated ketone at C-7 or C-9. The C-26 in lanostanoid structures is often oxidized to alcohols, aldehydes, and acids [5]. Among the structures of identified lanostanoids (1–26), ganoderol derivative was the main type. In addition, three ganoderic aldehydes (5, 23, 24) and one ganoderic acid (3) were also found. The β-configuration of OH-15 in new ganoderic acid (3) from *G. luteomarginatum* was consistent with the previously discovered ganoderic acid derivatives from this fungus [27], which was opposite of that shown in the corresponding compounds from other *Ganoderma* species [7,12,50]. Moreover, norlanostanoids with 24 carbon atoms often occur in *Ganoderma*. In present study, two hexanorlanostanoids (25 and 26) were isolated. Some lanostane triterpenoids besides the three new ones in our study are structurally different from those previously reported lanostanoids [26,27] from *G. luteomarginatum* collected in Guangxi Province, China. This may be related to the different growth environment of this fungus or different growth period for collection, which needs further comparative analysis in our subsequent studies.

### 2.2. Cytotoxic Activities of Compounds

The cytotoxic activities of all the isolates were evaluated by MTT method toward three human cancer cell lines (K562, BEL-7402, and SGC-7901). The results were presented in Table 2. Of the compounds tested, seven lanostane triterpenoids (1, 2, 7, 13, 18, 22, and 24) showed definite cytotoxicity against K562 with IC$_{50}$ values range from 6.64 to 17.38 µg/mL, among which compounds 1, 13 and 18 showed the IC$_{50}$ values of 8.59, 6.64, and 8.82 µg/mL, respectively. Compounds 2 and 13 also showed moderate cytotoxicity against two human cancer cell lines (BEL-7402 and SGC-7901). Moreover, compound 7 showed moderate cytotoxicity against human cancer cell lines BEL-7402 with IC$_{50}$ value of 20.05 µg/mL. Compounds 1, 18, 22, and 24 had no obvious cytotoxicity on BEL-7402 and SGC-7901 cell lines (IC$_{50}$ > 50 µg/mL). Among these cytotoxic lanostanoids, compounds 2 and 13 showed general cytotoxicity against three human cancer cell lines, while compounds 1 and 18 exhibited significant selective cytotoxicity against K562 cell lines. One ergostane steroid (34) showed general cytotoxicity against three human cancer cell lines.
| Compounds | K562  | BEL-7402 | SGC-7901 |
|-----------|-------|----------|----------|
| 1         | 8.59  | >50      | >50      |
| 2         | 16.05 | 24.27    | 33.38    |
| 7         | 11.69 | 20.05    | >50      |
| 13        | 6.64  | 13.49    | 15.62    |
| 18        | 8.82  | >50      | >50      |
| 22        | 16.95 | >50      | >50      |
| 24        | 17.38 | >50      | >50      |
| 34        | 22.81 | 47.63    | 26.06    |
| Paclitaxel| 5.62  | 3.26     | 3.41     |

Lanostane-type triterpenoids of *Ganoderma* are considered to be the major pharmacologically active compounds that contribute to its antitumor efficacy. The lanostane-type triterpenoids were extensively evaluated for cytotoxic activities against a series of tumor cell lines [5] related to lung, liver, colon, pancreas, breast, skin, and prostate [6]. The lanostanoids with structural complexity and functional group variety may be specific to different cell lines and the structure-cytotoxicity relationships could be raised. Compounds 1, 18, 22, and 24 exhibited selective cytotoxicity against K562 cell lines may be due to their unique structures. From the results of isolated lanostanoids against three human cancer cell lines (K562, BEL-7402, and SGC-7901), the conjugated double-bond \( \Delta^7,9(11) \) system in tetracyclic skeleton (1, 2, 7, 13, 18, and 24) seemed to be more important than (\( \Delta^8 \))\( \alpha,\beta \)-unsaturated ketone system for potent cytotoxic activity. Comparing the cytotoxicity between 7/9, 18/17, and 24/5 with only difference at C-3, it suggested that acetylation may be the negative factor for cytotoxic activity. In addition, compound 13 exhibited significant cytotoxicity, while their keto-3 analog 12 was inactive, assumed that reduction of the keto-3 group to OH-3 in lanostane triterpenoids would improve the cytotoxicity against K562, BEL-7402, and SGC-7901 significantly. The above preliminary structure-cytotoxicity relationships provide an approach to understanding the structural requirements of lanostane-type triterpenoids.

3. Materials and Methods

3.1. General Experimental Procedures

The NMR spectra were recorded with a Bruker AV-500 spectrometer (Bruker, Bremen, Germany) with TMS as an internal standard. HRESIMS data were determined on a mass spectrometer API QSTAR Pulsar (Bruker, Bremen, Germany). Optical rotations were measured on a Rudolph Autopol III polarimeter (USA). UV spectra were obtained on a Shimadzu UV-2550 spectrometer. IR spectra were obtained on a Nicolet 380 FT-IR spectrometer with KBr pellets. Silica gel (60–80 and 200–300 mesh, Marine Chemical Industry Factory, Qingdao, China), Rp-C18 (20–45 mL; Fuji Silysis Chemical Ltd., Aichi, Japan), and Sephadex LH-20 (Merck, Germany) were used for column chromatography. Fractions were monitored by TLC and spots were visualized by heating after spraying with 5% \( \text{H}_2\text{SO}_4 \) in ethanol.

3.2. Fungal Material

Fruiting bodies of *G. luteomarginatum* were collected in Qiongzhong County, Hainan Province, China (June, 2012), and identified by Prof. Xing-Liang Wu of Hainan University. A voucher specimen (No. 2012HB01) is deposited at the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences.

3.3. Isolation and Characterization of Compounds

Dried and powdered fruiting bodies of *G. luteomarginatum* (2.5 kg) were extracted with EtOH-H\(_2\)O (10 L, 95:5, \( v/v \)) under reflux conditions three times at a duration of 4 h. The combined extracts were concentrated and suspended in \( \text{H}_2\text{O} \), followed by successive partitioning with EtOAc and n-BuOH, respectively. The EtOAc extract (53.0 g) was sep-
rated by silica gel chromatography (CC) under reduced pressure using a solvent gradient of petroleum ether (PE)-EtOAc (20:1→0:1, v/v) to afford six fractions (Fr1-Fr6). Fr2 (6.2 g) was subjected to silica gel CC under reduced pressure eluted with PE-EtOAc (3:1) to give 3 subfractions: 2a–2c. Subfraction 2a (925 mg) was repeatedly purified by silica gel CC eluted with PE-EtOAc (5:1) to obtain compounds 4 (7.3 mg), 5 (4.2 mg), 6 (5.2 mg), 14 (21.5 mg), 26 (28.7 mg), and 30 (16.4 mg). Subfraction 2b (816 mg) was separated by silica gel column to yield compounds 23 (7.7 mg), 24 (6.0 mg), and 28 (68.3 mg) using an eluent CHCl3-MeOH (15:1), and compounds 25 (2.4 mg) and 29 (81.7 mg) were separated from subfraction 2c (674 mg) using an eluent: CHCl3-MeOH (12:1). Fr3 (9.6 g) was separated by Rp-18 CC with MeOH-H2O (30:70→0:100) to give 3 subfractions: 3a–3c. Subfraction 3a (526 mg) was purified by silica gel CC eluted with PE-EtOAc (3:1) to obtain compounds 1 (5.5 mg), 2 (8.2 mg), 9 (8.6 mg), and 10 (9.0 mg). Subfraction 3b (603 mg) was separated by silica gel CC eluted with PE-EtOAc (2:1) to yield compounds 3 (4.6 mg), 15 (5.5 mg), 16 (5.1 mg), 22 (10.5 mg), and 31 (27.0 mg). Subfraction 3c (839 mg) was subjected to silica gel CC eluted with CHCl3-MeOH (10:1) to yield compounds 17 (8.6 mg), 18 (9.8 mg), 27 (3.9 mg), 32 (3.8 mg), and 34 (5.1 mg). Fr4 (8.5 g) was treated by Rp-18 CC with MeOH-H2O (30:70→0:100) to afford subfractions 4a–4d. Subfraction 4b (406 mg) was subjected to Sephadex LH-20 (CHCl3/Methanol, 1:1), then by silica gel CC with PE-EtOAc (2:1) to yield compounds 7 (8.1 mg), 8 (7.6 mg), and 19 (7.4 mg). Compounds 11 (6.5 mg) and 12 (15.6 mg) was purified from subfraction 4b (365 mg) using an eluent CHCl3-MeOH (8:1). Fr5 (9.0 g) was separated by Rp-18 CC with MeOH-H2O (30:70→0:100) to afford subfractions 5a–5c. Subfraction 5b (382 mg) was subjected to silica gel CC with CHCl3-EtOAc (2:1) to yield compounds 13 (7.8 mg) and 20 (10.2 mg). Subfraction 5c (264 mg) was separated by silica gel CC eluted with CHCl3-MeOH (6:1) to obtain 21 (8.4 mg) and 33 (25.8 mg).

Lanosta-7,9(11)-dien-3β-acetylxylo-24,25-diol (1): White amorphous powder; [α]D27 −2.5° (c 0.02, MeOH); UV (MeOH) λmax (log ε) 252 (3.62), 242 (5.24), 238 (4.50), 210 (1.21); IR (KBr) vmax cm−1 3475, 2938, 1696, 1641, 1302, 1028; 1H and 13C NMR data; see Table 1; HRESIMS m/z [M+Na]+ 523.3754 (calcd. 523.3763 for C32H35NaO4).

Lanosta-7,9(11)-dien-3-oxo-24,26-diol-25-methoxy (2): White amorphous powder; [α]D27 +4.0° (c 0.02, MeOH); UV (MeOH) λmax (log ε) 242 (5.60), 238 (5.03), 212 (2.32); IR (KBr) vmax cm−1 3424, 2952, 1724, 1644, 1386, 1020; 1H and 13C NMR data; see Table 1; HRESIMS m/z [M+Na]+ 509.3997 (calcd. 509.3987 for C31H34NaO4).

3.4. Bioassay of Cytotoxic Activity

All the compounds was assayed for their cytotoxic activity against three human tumor cell lines: K562 (leukemic cell line), BEL7402 (hepatoma cell line), and SGC7901 (gastric cancer cell line) using MTT methods reported previously [7]. Briefly, each tested compound was dissolved with DMSO at concentration of 10 mM and then diluted to the required concentrations with the medium. Cells were cultured in 96-well plates with initial density of 5000 cells/well 12 h before treatment and exposed to different concentrations (40, 8, 1.6, 0.32, and 0.064 µM, respectively) of compounds, with paclitaxel (Sigma, Livonia, MI, USA) as the positive control. After the culturing period, 20 µL of MTT (5 mg/mL) was added per well and incubated for 4 h at 37 °C. Finally, absorbance was measured at 570 nm using a microplate reader. Each assay was replicated three times. The effect of the compounds on cell viability was calculated and expressed as the IC50.

4. Conclusions

Lanostane-type triterpenoids are the main characteristic natural products of the fungi of Ganoderma, which have extensive biological and pharmacological activities, especially possess cytotoxicity. The chemical investigation of the fruiting bodies of G. luteomarginatum
led to the isolation of twenty-six lanostane triterpenoids (1–26), including three undescribed ones (1–3): lanosta-7,9(11)-dien-3β-acetyloxy-24,25-diol (1), lanosta-7,9(11)-dien-3-oxo-24,26-diol-25-methoxy (2), and lanosta-8,20(22)-dien-3,11,23-trioxo-7β,15β-diol-26-oic acid methyl ester (3), together with eight ergostane steroids (27–34). The cytotoxicity assay showed that seven lanostane triterpenoids (1, 2, 7, 13, 18, 22, and 24) revealed definite cytotoxicity against K562, among which compounds 1, 13, and 18 showed the IC_{50} values of 8.59, 6.64, and 8.82 μg/mL, respectively, indicating the prospect of an antitumor. Some preliminary structure–cytotoxicity relationships of these lanostane triterpenoids showed that the conjugated double-bond Δ7,9(11) system in tetracyclic lanostane skeleton seemed to be more important than the (Δ8)x,β-unsaturated ketone system for potential cytotoxic activity. The present study further enriched the understanding of the structural diversity of *G. luteomarginatum*, which also provides theoretical information for its subsequent anticancer drug development.

**Supplementary Materials:** The following supporting information about 1D, 2D NMR, and HRES-IMS data can be downloaded at: https://www.mdpi.com/article/10.3390/molecules27206989/s1, Figure S1-1: ^1^H NMR spectrum of compound 1. Figure S1-2: ^13^C NMR spectrum of compound 1. Figure S1-3: HSQC spectrum of compound 1. Figure S1-4: ^1^H–^1^H COSY spectrum of compound 1. Figure S1-5: HMBC spectrum of compound 1. Figure S1-6: ROESY spectrum of compound 1. Figure S1-7: HRESIMS Data of compound 1. Figure S2-1: ^1^H NMR spectrum of compound 2. Figure S2-2: ^13^C NMR spectrum of compound 2. Figure S2-3: HSQC spectrum of compound 2. Figure S2-4: ^1^H–^1^H COSY spectrum of compound 2. Figure S2-5: ROESY spectrum of compound 2. Figure S2-6: HRESIMS Data of compound 2. Figure S2-7: HRESIMS Data of compound 3. Figure S3-1: ^1^H NMR spectrum of compound 3. Figure S3-2: ^13^C NMR spectrum of compound 3. Figure S3-3 HSQC spectrum of compound 3. Figure S3-4: ^1^H–^1^H COSY spectrum of compound 3. Figure S3-5: HMBC spectrum of compound 3. Figure S3-6: ROESY spectrum of compound 3. Figure S3-7: HRESIMS Data of compound 3.

**Author Contributions:** Q.M.: wrote the manuscript and isolated the compounds. S.Z.: data analysis and isolated the compounds. Q.X.: activity test. L.Y.: structural elucidation. H.D.: method adviser. Z.Y.: revise the manuscript. Y.Z.: theory and orientation adviser. All authors have read and agreed to the published version of the manuscript.

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