Tetra-, penta-, and hexa-nor-lanostane triterpenes from the medicinal fungus Ganoderma australe

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Abstract: Chemical investigation on the medicinal fungus Ganoderma australe led to the identification of ten new nor-lanostane triterpenes, namely two hexa-nor ones, ganoaustraltetraenones A (1) and B (2), five penta-nor ones, ganoaustraldehydes A–E (3–7), and three tetra-nor ones ganoaustrenoic acids A–C (8–10). The chemical structures along with the absolute configurations were determined by extensive spectroscopic analysis of 1D & 2D NMR and HRESIMS data. The postulated biosynthesis pathways of these compounds were proposed. Ganoaustraldehydes A (3) and B (4) showed moderate inhibition against nitric oxide production in RAW264.7 macrophage cells with the respective IC50 values of 32.5, 34.2 µM (the IC50 of positive control pyrrolidine dithiocarbamate was 20.0 µM).

Keywords: Basidiomycete, Ganoderma australe, Nor-lanostane, Structural determination, Anti-NO production

Graphical Abstract

1 Introduction

The genus Ganoderma contains more than 80 species of wood decaying fungi mostly distributed in tropical and subtropical areas. Different species of Ganoderma have been used in Traditional Chinese Medicine for thousands of years for the treatment of many kinds of diseases, for example, hypertension, respiratory diseases, gastrointestinal disorders, autoimmune diseases [1]. The two species, G. lucidum and G. sinense were recorded in the recent five editions of Chinese Pharmacopoeia with the name “Lingzhi” from the year 2000. Numerous studies have shown that polysaccharides and the main secondary metabolites–triterpenoids, are responsible for the biological activities of Ganoderma, such as immunoregulatory, antiviral, hepatoprotective effects [2–7]. Besides, meroterpenes (farnesyl hydroquinones), the other main constituent in Ganoderma, have attracted much attention in recent years [8, 9]. Ganoderma triterpenes are always characterized by structural poly-oxygenations [6]. Among the reported structures, the popular positions been oxygenated are C-3, C-7, C-11, C-15, C-23,
and C-26. The oxygenations in the side chain sometimes trigger the C-C bond cleavage by retro-aldol reaction or oxidative cleavage to yield nor-lanostane, a minor group of Ganoderma triterpenes, such as C24 (hexa-nor), C27 (tri-nor), and C-25 (penta-nor) lanostanes [5, 6].

Ganoderma australe is regarded as an alternative of the official-recognized species Ganoderma lucidum, and is used as a folk medicine in some ethnic minority areas of Yunnan Province, China. This fungus, however, was chemically under-investigated compared to other easily widely used Ganoderma species, such as Ganoderma lucidum, Ganoderma cochlear, and Ganoderma sinense. Previous studies on the secondary metabolites of Ganoderma australe have led to the isolation of some lanostane triterpenes [10–13], meroterpenoids [14, 15], and alkaloids [14]. In this study, we would like to report ten new nor-lanostane triterpenes isolated from Ganoderma australe, of which the structures are assigned by extensive NMR and HRESIMS spectroscopic analysis. The new structures are classified into tetra-nor-, penta-nor-, and hexa-nor-lanostanes, and are featured by C-6 oxygenation or α,β-unsaturated aldehyde groups, which are unusual modifications in the reported Ganoderma triterpenes, thereby inspiring a biosynthetic proposal of these compounds.

2 Results and discussion

2.1 Structure elucidation of compounds 1–10

Ganoaustratetraenone A (1) (Fig. 1), isolated as pale-yellow needles, has the molecular formula of C_{24}H_{32}O_{4}, which was determined from the HRESIMS analysis (m/z 385.23724 [M+H]^+, calcd. for C_{24}H_{33}O_{4}, 385.23718) (Additional file: 1). The 1D NMR spectroscopic data of 1 (Tables 1 and 2) displayed six singlet methyls, six methylenes, two methines, four quaternary carbons (sp^3 hybridized), a pair of non-protonated olefinic carbons, and four ketone carbonyls. The 1H and 13C NMR data of 1 showed high similarity to those of the previously described compound 4,4,14α-trimethyl-3,7,11,15,20-pentaoxo-5α-pregn-8-en [16], a semisynthetic product of methyl ganoderate O by alkaline treatment. There was only one difference between the structure of ganoaustratetraenone A and 4,4,14α-trimethyl-3,7,11,15,20-pentaoxo-5α-pregn-8-en, which was revealed by analyzing the NMR spectra. The pivotal HMBC correlation from H3-30 (δH 1.27, s) to a methylene at δC 32.3 (Fig. 2) suggested that C-15 of 1 is a methylene instead of being a ketone group in 4,4,14α-trimethyl-3,7,11,15,20-pentaoxo-5α-pregn-8-en. Therefore, compound 1 was elucidated to be a hexa-nor-lanostane derivative.

Table 1 1H NMR spectroscopic data for compounds 1–5

| No. | 1* | 2* | 3* | 4* | 5* |
|-----|----|----|----|----|----|
| 1   | 2.95, overlapped | 2.85, ddd (14.0, 9.6, 7.5) | 2.89, ddd (14.5, 8.6, 6.2) | 2.87, ddd (17.2, 9.9, 7.4) | 2.98, overlapped |
| 2   | 2.60, ddd (15.0, 9.3, 6.0) | 2.74, ddd (14.8, 13.5, 7.5) | 2.71, overlapped | 2.76, overlapped | 2.63, ddd (15.5, 9.5, 5.8) |
| 3   | 2.50, overlapped | 2.34, ddd (14.8, 9.6, 2.6) | 2.45, overlapped | 2.35, overlapped | 2.52, overlapped |
| 4   | 2.24, m | 2.30, d (13.6) | 2.41, overlapped | 2.34, overlapped | 2.31, d (14.7, 2.7) |
| 5   | 2.53, t (14.4) | 4.42, dd (13.6, 3.0) | 2.73, overlapped | 4.47, dd (13.6, 3.2) | 2.58, br. t (14.7) |
| 6   | 2.40, dd (14.4, 2.5) | 2.38, dd (12.7, 2.3) | 2.38, dd (12.7, 2.3) | 2.44, dd (14.7, 2.6) |
| 7   | 2.91, dd (16.4, 1.1) | 2.93, d (16.9) | 3.11, d (16.3) | 3.21, d (16.8) | 3.10 d (16.8) |
| 8   | 2.70, d (16.4) | 2.73, d (16.9) | 3.11, d (16.3) | 3.08, d (16.8) | 3.05 d (16.8) |
| 9   | 1.75, overlapped | 1.68, ddd (19.6, 12.1, 7.2) | 1.93, overlapped | 2.48, dd (12.8, 8.8) | 1.91, td (13.2, 9.8) |
| 10  | 2.26, overlapped | 2.30, overlapped | 2.73, overlapped | 2.70, dd (19.5, 8.8) | 3.12, m |
| 11  | 1.87, overlapped | 1.90, overlapped | 2.62, dd (19.3, 9.3) | 1.80, br. dd (12.8, 10.0) | 2.92, overlapped |
| 12  | 2.98, overlapped | 3.02, dd (9.2, 2.9) | 3.12, s | 3.10, s | 3.07 s |
| 13  | 0.75, s | 0.75, s | 1.21, s | 1.17, s | 1.07, s |
| 14  | 1.28, s | 1.24, s | 1.29, s | 1.25, s | 1.29, s |
| 15  | 2.13, s | 2.13, s | 10.00, s | 10.03, s | 1.80, t (1.6) |
| 16  | 2.17, s | 1.74, s | 1.76, s | 1.76, s | 1.00, s |
| 17  | 2.12, s | 1.14, s | 1.36, s | 1.36, s | 1.15, s |
| 18  | 1.10, s | 1.43, s | 1.12, s | 1.45, s | 1.12, s |
| 19  | 1.27, s | 1.35, s | 1.25, s | 1.30, s | 1.26, s |

* Measured at 600 MHz (CDCl_3); ** Measured at 500 MHz (CD_3OD)
Compound 2 (Fig. 1) was isolated as a white powder. The HRESIMS analysis of 2 gave a protonated ion peak at m/z 401.23224 [M + H]^+*, corresponding to the molecular formula of C_{24}H_{32}O_{5} (calcd. for C_{24}H_{33}O_{5}, 401.23280) (Additional file: 1). The 1D NMR spectroscopic data of 2 (Tables 1 and 2) showed six singlet methyls, five methylenes, three methines including one oxygenated, four quaternary carbons (sp^3 hybridized), a pair of non-protonated olefinic carbons, and four ketone carbonyls. Comparing the NMR data of 1 and 2 suggested that 2 was a structural congener of 1. Compound 2 differed from 1 by the presence of a hydroxy substituent at C-6, which was confirmed by the chemical shift of C-6 (δ_{C} 72.1), by the ^1H–^1H COSY correlation of H-5 (δ_{H} 2.30)/H-6 (δ_{H} 4.42), and by the key HMBC correlations from OH-6 (δ_{H} 3.68) to C-5 (δ_{C} 54.9), C-6, and C-7 (δ_{C} 202.6) (Fig. 2). The ROESY correlation of H_3-19 (δ_{H} 1.25) to C-15 (δ_{C} 31.7), from H_3-18 (δ_{H} 1.74) to C-17 (δ_{C} 170.2), and from H_2-22 (δ_{H} 1.74) to C-17, C-20 (δ_{C} 133.0), and C-21 (δ_{C} 192.1) (Fig. 2) indicated that the C-15 in 3 was a methylene group, C-21 was an aldehyde group, and C-17 and C-20 was a double bond. Therefore, the 2D structure of 3 was elucidated as shown in Fig. 1. The key ROESY correlations between the

| No. | 1^a | 2^a | 3^a | 4^a | 5^a | 6^a | 7^a | 8^a | 9^a | 10^a |
|-----|------|------|------|------|------|------|------|------|------|------|
| 1   | 35.0 | 35.3 | 36.2 | 35.2 | 35.2 | 35.2 | 35.0 | 35.2 | 34.0 | 35.3 | 35.1 | 34.2 |
| 2   | 34.1 | 33.4 | 35.1 | 33.3 | 34.1 | 33.3 | 34.7 | 33.4 | 34.1 | 34.1 | 34.9 | 34.9 |
| 3   | 215.6 | 216.7 | 218.8 | 216.5 | 215.6 | 216.6 | 217.7 | 216.7 | 215.7 | 215.8 | 218.0 | 218.0 |
| 4   | 46.8 | 47.7 | 48.0 | 47.8 | 46.9 | 47.8 | 48.0 | 46.4 | 48.0 | 46.8 | 46.5 | 46.5 |
| 5   | 49.8 | 54.9 | 50.9 | 54.9 | 49.8 | 54.9 | 44.9 | 54.9 | 49.8 | 49.8 | 45.2 | 45.2 |
| 6   | 37.0 | 72.1 | 38.0 | 72.3 | 37.2 | 72.2 | 72.7 | 37.1 | 29.4 | 37.1 | 29.4 | 29.4 |
| 7   | 201.1 | 202.6 | 202.9 | 202.5 | 201.1 | 202.6 | 67.0 | 202.6 | 201.2 | 67.3 | 67.3 | 67.3 |
| 8   | 150.7 | 148.0 | 150.1 | 151.5 | 147.4 | 149.9 | 147.2 | 158.1 | 148.3 | 151.0 | 159.5 | 159.5 |
| 9   | 150.2 | 150.1 | 151.0 | 149.8 | 150.3 | 150.2 | 150.2 | 140.4 | 150.1 | 150.2 | 140.2 | 140.2 |
| 10  | 39.0 | 39.9 | 40.3 | 40.0 | 39.1 | 40.0 | 37.8 | 39.9 | 39.0 | 38.0 | 38.0 | 38.0 |
| 11  | 200.7 | 199.6 | 201.5 | 198.7 | 200.6 | 199.5 | 198.8 | 200.0 | 201.0 | 199.5 | 199.5 | 199.5 |
| 12  | 49.9 | 49.8 | 52.8 | 51.9 | 49.3 | 49.2 | 49.2 | 49.1 | 49.9 | 49.9 | 503.3 | 503.3 |
| 13^d | 47.3 | 47.0 | 49.0 | 47.7 | 46.5 | 46.2 | 48.6 | 47.7 | 48.3 | 48.3 | 486.6 | 486.6 |
| 14^d | 48.8 | 48.5 | 53.7 | 51.9 | 52.9 | 52.6 | 53.0 | 48.0 | 48.4 | 507.7 | 507.7 | 507.7 |
| 15  | 32.2 | 31.7 | 31.7 | 30.2 | 31.2 | 30.8 | 29.2 | 31.8 | 32.4 | 30.4 | 30.4 | 30.4 |
| 16  | 21.8 | 21.8 | 31.5 | 30.4 | 26.3 | 26.3 | 26.3 | 23.3 | 23.3 | 23.2 | 23.2 | 23.2 |
| 17  | 57.2 | 57.2 | 57.2 | 170.2 | 166.4 | 165.5 | 164.8 | 166.4 | 53.3 | 53.3 | 54.3 | 54.3 |
| 18  | 18.6 | 18.7 | 25.1 | 25.0 | 21.5 | 21.6 | 21.4 | 18.6 | 18.5 | 18.7 | 18.7 | 18.7 |
| 19  | 18.1 | 19.5 | 18.8 | 19.8 | 18.3 | 19.8 | 19.8 | 17.9 | 19.5 | 18.1 | 17.7 | 17.7 |
| 20  | 208.7 | 208.3 | 133.0 | 132.5 | 128.9 | 129.9 | 129.2 | 161.9 | 161.6 | 162.2 | 162.2 | 162.2 |
| 21  | 31.4 | 31.3 | 192.1 | 189.8 | 9.6 | 9.7 | 9.6 | 21.1 | 21.2 | 21.1 | 21.1 | 21.1 |
| 22  | 12.2 | 12.3 | 12.3 | 193.0 | 192.8 | 192.8 | 116.9 | 116.5 | 115.8 | 115.8 | 115.8 | 115.8 |
| 23  | 171.2 | 170.3 | 170.2 | 170.2 | 170.2 | 170.2 | 170.2 | 170.2 | 170.2 | 170.2 | 170.2 | 170.2 |
| 28  | 27.5 | 31.2 | 27.9 | 31.2 | 27.6 | 31.2 | 27.6 | 31.2 | 27.6 | 27.8 | 27.8 | 27.8 |
| 29  | 20.5 | 19.4 | 20.8 | 19.3 | 20.4 | 19.3 | 20.5 | 19.4 | 20.5 | 20.6 | 20.6 | 20.6 |
| 30  | 26.0 | 26.1 | 27.2 | 27.2 | 27.4 | 27.4 | 27.4 | 29.2 | 26.4 | 26.3 | 27.8 | 27.8 |

*Measured at 150 MHz (CDCl_3); ^b Measured at 125 MHz (CD_2OD); ^c Measured at 200 MHz (CDCl_3); ^d The data were assigned with the aid of software estimation.
The white amorphous powder ganoaulaldehyde B (4) (Fig. 1) returned a protonated ion peak at \( m/z \) 413.23221 [M + H]\(^+\) in the HRESIMS analysis, suggesting the molecular formula of \( \text{C}_{25}\text{H}_{32}\text{O}_5 \) (calcd. for \( \text{C}_{25}\text{H}_{33}\text{O}_5 \), 413.23225) (Additional file: 1). The 1D NMR spectroscopic data of 4 (Tables 1 and 2) highly resembled to those of compound 3, indicating the analogous structures.
of 3 and 4. The signal of an oxymethine at $\delta_C 72.3$ (C-6) in the $^{13}$C NMR spectra of 4 compared to those of 3, along with the 2D NMR correlations of 4, including the $^1$H-$^1$H COSY correlations between H-5 ($\delta_H$ 2.34) and H-6 ($\delta_H$ 4.47), and HMBC correlations from 6-OH ($\delta_H$ 3.69) to C-6 ($\delta_C$ 72.3) and C-7 ($\delta_C$ 202.5) (Fig. 2) suggested that there was a hydroxy group substituted at C-6 in 4.

The stereochemistry of the C-17-C-21 double bond was assigned as Z configuration according to the ROESY cross peaks between H-21 ($\delta_H$ 10.03) and H-12 ($\delta_H$ 3.08) (Fig. 3). The OH-6 was determined to be $\alpha$ configuration by the diagnostic ROESY signals of H-6/H-3-19 ($\delta_H$ 1.25) (Fig. 3). Therefore, compound 4 was elucidated as shown in Fig. 1.

The molecular formula of compounds 5 and 6 (Fig. 1) were same with those of 3 and 4, respectively. The 1D NMR data of 5 and 6 (Tables 1, 3 and 2) also exhibited high similarity to those of 3 and 4, respectively, thus indicating that 5 and 6 were the respective structure congeners of 3 and 4. Analysis of the 2D NMR spectra of 5 and 6 enabled us to identify the only difference between these two pairs of compounds, which was the configuration of C-17-C-21 double bond (Fig. 2). The diagnostic ROESY correlations of H$_3$-21($\delta_H$ 1.80)/H-12 ($\delta_H$ 3.05, 3.10), and H-22 ($\delta_H$ 10.00)/H-16 ($\delta_H$ 3.12 in 5; 3.19 in 6) (Fig. 3) suggested the E configuration of C-17-C-21 double bonds of 5 and 6 (Fig. 3). The configuration of OH-6 of 6 was determined as $\alpha$ orientation by the ROESY correlation of H-6/H$_3$-19 (Fig. 3). Therefore, compounds 5 and 6 were identified as ganoausraldehydes C and D, respectively.

Compound 7 (Fig. 1), a pale-yellow oil, had a protonated ion peak at $m/z$ 399.25305 in the HRESIMS analysis (calcd. for C$_{25}$H$_{35}$O$_4$, 399.25353) (Additional file: 1). Analysis of the $^1$H and $^{13}$C NMR spectroscopic data of 7 (Tables 3 and 2) revealed that this compound was a structural analogue of 5. The main difference between the two analogues was C-7. In the HMBC spectrum of compound 7, significant correlations from H-7 ($\delta_H$ 4.52) to C-8 ($\delta_C$ 158.1) and C-9 ($\delta_C$ 140.4) were observed (Fig. 2). This evidence together with the $^1$H-$^1$H COSY correlation of H-5/H-6/H-7 (Fig. 2) suggested that C-7 in 7 was an oxygenated methine, instead of being a ketone group in 5. The exocyclic C-17-C-21 double bond was assigned as E configuration by the key ROESY correlations of H$_3$-21 ($\delta_H$ 1.79)/H-12 ($\delta_H$ 3.04) (Fig. 3). The 7-OH was determined to be $\alpha$ configuration by the ROESY cross peak signals of H-15$/\beta$ ($\delta_H$ 2.14)/H-7 ($\delta_H$ 4.52) and the coupling constant of H-7 (t, 3.3 Hz) (Fig. 3) [18]. Therefore, the structure of 7 was assigned as ganoausraldehyde E.
The 1D NMR data of the compounds 8 and 9 (Tables 3 and 2) each showed 26 carbon resonances with a high resemblance to those of compounds 2 and 1, respectively. Further analysis of the NMR spectroscopic data suggested that 8 and 9 differed from 2 to 1 by the existence of a trisubstituted double bond (C-20, C-22) and a COOH group (C-23), while by the absence of a ketone group (C-20). These changes suggested that 8 and 9 were two lanostane triterpenes with six carbon degradation (C-24, C-25, C-26, and C-27). The C-23 in 8 and 9 were carboxylic groups which were assigned by the key ROESY correlations from the proton at H-22 (δH 5.77/5.78 in 8/9) to C-5 (δC 45.2), C-6 (δC 29.4), C-8 (δC 159.5), C-9 (δC 140.2) (Fig. 2) suggested that C-7 was a hydroxymethine in 10 instead of being a ketone group in 9. The ROESY correlations of H3-18 (δH 0.68)/H-15β (δH 2.04)/H-7 (δH 4.48), and the coupling constant of H-7 (t, 3.0 Hz) facilitated to determine the orientation of 7-OH as α (Fig. 3). Therefore, compound 10 was identified as ganoaustrenolic acid C.

Since these nor-lanostane are characterized by unusual C-6 oxygenation, and α,β-aldehyde groups, a biosynthetic proposal is postulated as shown in Scheme 1. The key intermediate lanosterol is biosynthesized from squalene, the common precursor of triterpenes. Further oxygenation on multiple sites of the lanosterol leads to the key intermediate A [19, 20]. Abstraction of the proton of OH-20 by the retro-aldol reaction (route A) give compound 1, which has been further oxygenated at C-6 position to yield 2. Oxidative cleavage of C23-C24, followed by elimination of the C-20 hydroxy group through
E1cb mechanism to produce 9, which is been further oxygenated or reduced to yield 8 and 10. Alternatively, A undergoes oxygenation at C-22, and oxidative cleavage of C22-C23, and further elimination of C-20 hydroxy group giving compounds 3 and 5. Late-stage oxygenation and reduction of 3 and 5 produce 4, 6, and 7.

2.2 The anti-NO production activity of the isolates
All the isolates were subjected to screening their inhibition against the NO production in murine monocytic RAW 264.7 macrophages. As a result, only compounds 3 and 4 displayed moderate inhibitory activities with IC_{50} values of 32.5, 34.2 µM, respectively.

3 Conclusions
Ten previously undescribed nor-lanostane triterpenes were isolated from the fruiting bodies of *Ganoderma australe*. The chemical structures of the compounds were determined with the aid of extensive NMR and HRESIMS spectroscopic analysis. These compounds are featured by tetra-, penta-, and hexa-nor-lanostane scaffold, and by unusual oxidative modifications at C-6. These findings put the diversity of nor-lanostanes of *Ganoderma* origin one step forward.

4 Experimental section
4.1 General experimental procedures
An Autopol IV-T digital polarimeter (Rudolph, Hacketstown, USA) was used to measure the optical rotations. A Shimadzu UV-2401PC UV-vis spectrophotometer (Shimadzu Corporation, Kyoto, Japan) was used to record the UV spectra. The Bruker Ascend 500 MHz, Avance III 600 MHz, or Ascend 800 MHz spectrometers (Bruker Corporation, Karlsruhe, Germany) were used to record the one- and two-dimensional NMR spectra. HRESIMS spectra were measured on a Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, MA, USA). Column chromatography (CC) were run on Sephadex LH-20 (Amersham Biosciences, Uppsala, Sweden) and silica gel (Qingdao Haiyang Chemical Co., Ltd., Qingdao, China). Medium pressure liquid chromatography (MPLC) was performed on an Interchim PuriFlash 450 chromatography system (Interchim Inc., Montlucon Cedex, France). The diameter and length of the column used for MPLC were 14 and 450 mm, respectively. The column was fill with Chromatorex C-18 silica gel (particle size: 40–75 µm, flow rate 40 mL/min, Fuji Silysia Chemical Ltd., Kasugai, Japan). Preparative high performance liquid chromatography (prep.-HPLC) was performed on an Agilent 1260 liquid chromatography system (Agilent
4.2 Fungal material

The fruiting bodies of *Ganoderma australe* were collected in Tongbiuguan Natural Reserve, Dehong, Yunnan Province, China, in 2016, and identified by Prof. Yu-Cheng Dai, who is a mushroom research in Institute of Microbiology, Beijing Forestry University. A voucher specimen of *G. australe* was deposited in the Mushroom Bioactive Natural Products Research Group at South-Central Minzu University.

4.3 Extraction and isolation

10 L of mixed solvent CHCl₃:MeOH (v/v 1:1) was used to extract the constituents from the grounded and dry fruiting bodies of *Ganoderma australe* (3.26 kg) (2.5 L to extract the constituents from the grounded and dry seven fractions (A−D)). The extract was further resuspended in distilled water and partitioned against ethyl acetate (EtOAc) to afford the EtOAc extract (130 g). The EtOAc extract was fractionated on MPLC by using a stepwise gradient of MeOH in H₂O (20-100%) to afford twenty-six subfractions (F1-F26).

Fraction D (2.0 g) was separated by Sephadex LH-20 (CHCl₃:MeOH = 1:1) to afford four subfractions (D1−D4). Subfraction D2 (800 g) was separated by silica gel column chromatography (CC) (petroleum ether-acetone from v/v 15:1 to 1:1) to obtain eleven subfractions (D2a−D2k). Compound 1 (5.2 mg, *t*ᵣ = 12.9 min) was purified from D2k (75 mg) by prep.-HPLC (MeCN−H₂O: 30:70−50:50, 30 min, 4 mL/min) to yield compound 5 (5 mg, *t*ᵣ = 7.80 min, 1.0 mg), 6 (1 mg, *t*ᵣ = 6.50 min, 0.8 mg), and 4 (2 mg, *t*ᵣ = 11.50 min, 1.3 mg). Compound 3 (0.9 mg, *t*ᵣ = 14.4 min) was purified from D2d (11 mg) by prep.-HPLC (MeCN−H₂O: 30:70−50:50, 30 min, 4 mL/min).

Fraction E (1.6 g) was separated by Sephadex LH-20 (MeOH) to afford twelve subfractions (E1-E12). Subfraction E5 was separated by CC on silica gel (petroleum ether-acetone from v/v 15:1 to 1:1) to obtain four subfractions (E5a-E5d). Compound 8 (2.4 mg, *t*ᵣ = 29.4 min) was purified from E5f (23 mg) by prep.-HPLC [MeCN+MeOH (2:1)−H₂O: 50:50, 40 min, 4 mL/min]. Compound 9 (2.1 mg, *t*ᵣ = 20.0 min) was purified from E5c (42 mg) by prep.-HPLC [MeCN+MeOH (2:1)−H₂O: 40:60−60:40, 25 min, 4 mL/min]. Compound 10 (6.5 mg, *t*ᵣ = 13.5 min) was purified from E2e (210 mg) by prep.-HPLC (MeCN-H₂O: 30:70−50:50, 25 min, 5 mL/min). Compound 7 (1.8 mg, *t*ᵣ = 20.0 min) was purified from E2g (13 mg) by prep.-HPLC (MeCN-H₂O: 50:50−70:30, 25 min, 4 mL/min).

4.4 Spectroscopic data of compounds

4.4.1 Ganoaustaternetraenone A (1)

Pale-yellow needles; [α] D₂⁰ +164.0 (c 0.05, MeOH); UV (MeOH) λ max (log ε) 255.0 (3.94); 1H NMR (600 MHz, CDCl₃), see Table 1, 13C NMR (150 MHz, CDCl₃) data, see Table 2; HRESIMS *m/z* 413.23224 [M+H]⁺, calcd. for C25H33O5, 413.23225.

4.4.2 Ganoaustaternetraenone B (2)

White powder; [α] D₂⁰ +259.4 (c 0.09, MeOH); UV (MeOH) λ max (log ε) 260.0 (3.82); 1H NMR (600 MHz, CDCl₃), see Table 1, 13C NMR (150 MHz, CDCl₃) data, see Table 2; HRESIMS *m/z* 397.23724 [M+H]⁺, calcd. for C24H33O4, 397.23718.

4.4.3 Ganoaustaldehyde A (3)

White, amorphous powder; [α] D₃⁰ +10.9 (c 0.05, MeOH); UV (MeOH) λ max (log ε) 250.0 (3.73); 1H NMR (500 MHz, CDCl₃), see Table 1, 13C NMR (125 MHz, CDCl₃) data, see Table 2; HRESIMS *m/z* 397.23721 [M+H]⁺, calcd. for C25H33O₄, 397.23734.

4.4.4 Ganoaustaldehyde B (4)

White, amorphous powder; [α] D₃⁰ +55.8 (c 0.05, MeOH); UV (MeOH) λ max (log ε) 250.0 (3.88); 1H NMR (600 MHz, CDCl₃), see Table 1, 13C NMR (150 MHz, CDCl₃) data, see Table 2; HRESIMS *m/z* 413.23221 [M+H]⁺, calcd. for C25H33O₅, 413.23225.
4.4.5 Ganoaustraldehyde C (5)
White, amorphous powder; [α]D24 +33.5 (c 0.05, MeOH); UV (MeOH) λmax (log ε) 250.0 (4.10); 1H NMR (600 MHz, CDCl3) data, see Table 1; 13C NMR (150 MHz, CDCl3) data, see Table 2; HRESIMS m/z 397.23743[M + H]+, calcd. for C25H33O4, 397.23734.

4.4.6 Ganoaustraldehyde D (6)
White, amorphous powder; [α]D24 +68.4 (c 0.05, MeOH); UV (MeOH) λmax (log ε) 250.0 (3.82); 1H NMR (600 MHz, CDCl3) data, see Table 2; 13C NMR (150 MHz, CDCl3) data, see Table 2; HRESIMS m/z 413.23224[M + H]+, calcd. for C25H33O5, 413.23225.

4.4.7 Ganoaustraldehyde E (7)
Pale-yellow oil. [α]D24 +156.8 (c 0.09, MeOH); UV (MeOH) λmax (log ε) 250.0 (4.62); 1H NMR (600 MHz, CDCl3) data, see Table 2; 13C NMR (150 MHz, CDCl3) data, see Table 2; HRESIMS m/z 399.25305 [M + H]+, calcd. for C26H35O5, 399.25353.

4.4.8 Ganoaustreonoic acid A (8)
White powder. [α]D24 +162.7 (c 0.06, MeOH); UV (MeOH) λmax (log ε) 220.0 (4.08); 1H NMR (600 MHz, CDCl3) data, see Table 2; 13C NMR (150 MHz, CDCl3) data, see Table 2; HRESIMS m/z 443.24299 [M + H]+, calcd. for C26H37O5, 443.24336.

4.4.9 Ganoaustreonoic acid B (9)
White powder. [α]D24 +10.3 (c 0.05, MeOH); UV (MeOH) λmax (log ε) 225.0 (4.23); 1H NMR (600 MHz, CDCl3) data, see Table 2; 13C NMR (150 MHz, CDCl3) data, see Table 2; HRESIMS m/z 427.24808 [M + H]+, calcd. for C26H35O6, 427.24845.

4.4.10 10 ganoaustreonoic acid C (10)
Yellowish oil. [α]D24 +84.3 (c 0.07, MeOH); UV (MeOH) λmax (log ε) 225.0 (3.90); 1H NMR (600 MHz, CDCl3) data, see Table 2; 13C NMR (200 MHz, CDCl3) data, see Table 2; HRESIMS m/z 429.26361 [M + H]+, calcd. for C26H37O5, 429.26410.

4.5 Nitric oxide production in RAW 264.7 macrophages
The murine monocytic RAW 264.7 macrophages were cultured in RPMI 1640 medium 10% Fetal Bovine Serum (FBS). Firstly, the DMSO stocks of the compounds were made, and further been serially diluted in media to make different concentrations of stocks. The cell mixtures were distributed into 96-well plates (2 × 10⁵ cells/well) and preincubated in a humid atmosphere with 5% CO₂ for 24 h at 37°C. Then different concentrations of stocks of the test compounds were added into each well, the maximum concentration of the test compound was 25 μM. Then the lipopolysaccharides (LPS) were added to each cell (final concentration 1 μg/mL), and continued to incubate for another 18 h. After adding 100 μL of Griess reagent to 100 μL of each supernatant from the LPS-treated or LPS- and compound-treated cells in triplicates, and incubated for another 5 min, the nitric oxide (NO) production of each cell was assessed by measuring the absorbance at 570 nm. Pyrrolidine dithiocarbamate (PDTC) was used as the positive control (IC₅₀ 20.0 μM).

Supplementary Information
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Author contributions
LZ and SA carried out the experiments, analyzed the results, and wrote the manuscript; they contributed equally to this work; MXW screened the biological activities; HPC analyzed the results and amended the manuscript; JKL designed and checked the whole manuscript. All authors read and approved the final manuscript.

Declarations

Competing interests
The authors declare that there are no conflicts of interest associated with this work.

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References
1. Kladar NV, Gavarić NS, Božin BN. Ganoderma: insights into anticancer effects. Eur J Cancer Prev. 2016;25(4):462–71.
2. Boh B, Berovic M, Zhang J, Zhi-Bin L. Ganoderma lucidum and its pharmacologically active compounds. Biotechnol Annu Rev. 2007;13:265–301.
3. Amaral AE, Carbonero ER, Rita de Cássia GS, Kadowaki MK, Sassaki GL, Osaku CA, Gorin PAJ, Iacomini M. An unusual water-soluble β-glucan from the basidiocarp of the fungus Ganoderma resinaceum. Carbohydr Polym. 2008;72(3):473–8.
4. Peng XR, Liu JQ, Han ZH, Yuan XX, Luo HR, Qiu MH. Protective effects of triterpenoids from Ganoderma resinaceum on H2O2-induced toxicity in HepG2 cells. Food Chem. 2013;141(2):920–6.
5. Zhao ZZ, Chen HP, Huang Y, Li ZH, Zhang L, Feng T, Liu JK. Lanostane triterpenoids from fruiting bodies of Ganoderma lucidum. Nat Prod Bioprospect. 2016;6:103–9.
6. Babu S, Johnson AJ, Govindan B. Secondary metabolites from Ganoderma. Phytochemistry. 2015;114:66–101.
7. Paterson RRM. Ganoderma—a therapeutic fungal biofactory. Phytochemistry. 2006;67(18):1985–2001.
8. Niedermeyer THJ, Jira T, Lalk M, Lindeque U. In vitro bone inducing effects of Lentilula edodes (shitake) water extract on human osteoblastic cell cultures. Nat Prod Bioprospect. 2013;3:137–40.
9. Peng X, Qiu M. Meroterpenoids from Ganoderma species: a review of last five years. Nat Prod Bioprospect. 2018;8:137–49.
10. Guo JC, Yang L, Ma QY, Ge YZ, Kong FD, Zhou LM, Zhang F, Xie QY, Yu ZF, Dai HF, Zhao YX. Triterpenoids and meroterpenoids with α-glucosidase inhibitory activities from the fruiting bodies of *Ganoderma australe*. Bioorg Chem. 2021;117:105448.
11. León F, Valencia M, Rivera A, Nieto I, Quintana J, Estévez F, Bermejo J. Novel cytostatic lanosteroid triterpenes from *Ganoderma australis*. Helv Chim Acta. 2003;86:3088–95.
12. Isaka M, Chinthanom P, Mayteeworakoon S, Laoteng K, Choowong W, Choeuklin R. Lanostane triterpenoids from cultivated fruiting bodies of the basidiomycete *Ganoderma australe*. Nat Prod Res. 2018;32:1044–9.
13. Jain AC, Gupta SK. The isolation of lanost-7, 9 (11), 24-trien-3β, 21-diol from the fungus *Ganoderma australe*. Phytochemistry. 1984;23:686–7.
14. Zhang JJ, Dong Y, Qin FY, Yan YM, Cheng YX. Meroterpenoids and alka‑loids from *Ganoderma australe*. Nat Prod Res. 2021;35:3226–32.
15. Zhang JJ, Dong Y, Qin FY, Cheng YX. *Australeols A—F*, neuroprotective meroterpenoids from *Ganoderma australis*. Fitoterapia. 2019;134:250–5.
16. Nishitoba T, Sato H, Sakamura S. Triterpenoids from the fungus *Ganoderma lucidum*. Phytochemistry. 1987;26:1777–84.
17. Zhao XR, Huo XK, Dong PP, Wang C, Huang SS, Zhang BJ, Zhang HL, Deng S, Liu KX, Ma XC. Inhibitory effects of highly oxygenated lanostane derivatives from the fungus *Ganoderma lucidum* on P-glycoprotein and α-glucosidase. J Nat Prod. 2015;78:1868–76.
18. Kikuchi T, Kanomi S, Murai Y, Kadota S, Tsubono K, Ogita Z-I. Lanostane triterpenes from the fruiting bodies of Ganoderma lucidum and their inhibitory effects on adipocyte differentiation in 3T3-L1 Cells. Chem Pharm Bull. 1986;34(10):4030–6.
19. Wang WF, Xiao H, Zhong JJ. Biosynthesis of a novel ganoderic acid by expressing CYP genes from *Ganoderma lucidum* in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol. 2022;106(2):523–34.
20. Zhou L, Guo LL, Isaka M, Li ZH, Chen HP. Triterpenes from the fungus *Ganoderma australis*. J Fungi. 2022;8(5):503.

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