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Lanostane Triterpenes from the Tibetan Medicinal Mushroom *Ganoderma leucocontextum* and Their Inhibitory Effects on HMG-CoA Reductase and α-Glucosidase

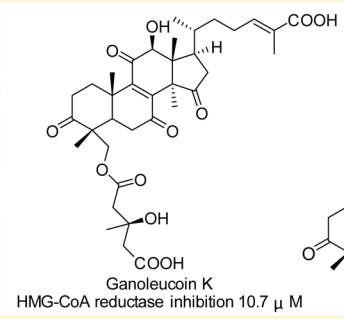
Kai Wang,[†,‡,⊥] Li Bao,[†,⊥] Weiping Xiong,[§,⊥] Ke Ma,[†] Junjie Han,[†] Wenzhao Wang,[†] Wenbing Yin,[†] and Hongwei Liu*[,†]

[†]State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, No. 1 Beichenxi Road, Chaoyang District, Beijing 100101, People's Republic of China

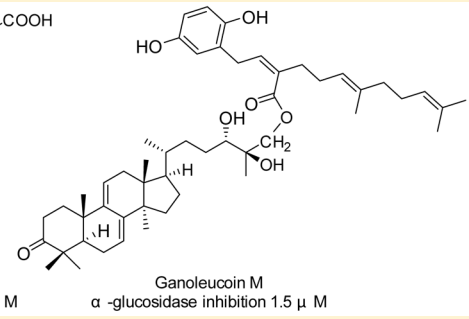
[‡]University of Chinese Academy of Sciences, Beijing 100049, People's Republic of China

[§]Institute of Vegetable Research, Tibet Academy of Agriculture and Animal Science, No. 157 Jinzhuxi Road, Lasa 850000, People's Republic of China

Supporting Information



Ganoleucoin K
HMG-CoA reductase inhibition 10.7 μ M



Ganoleucoin M
α -glucosidase inhibition 1.5 μ M

ABSTRACT: Sixteen new lanostane triterpenes, ganoleucoins A–P (**1**–**16**), together with 10 known tripterpenes (**17**–**26**), were isolated from the cultivated fruiting bodies of *Ganoderma leucocontextum*, a new member of the *Ganoderma lucidum* complex. The structures of the new compounds were elucidated by extensive spectroscopic analysis and chemical transformation. The inhibitory effects of **1**–**26** on HMG-CoA reductase and α-glucosidase were tested *in vitro*. Compounds **1**, **3**, **6**, **10**–**14**, **17**, **18**, **23**, **25**, and **26** showed much stronger inhibitory activity against HMG-CoA reductase than the positive control atorvastatin. Compounds **13**, **14**, and **16** presented potent inhibitory activity against α-glucosidase from yeast with IC_{50} values of 13.6, 2.5, and 5.9 μM, respectively. In addition, the cytotoxicity of **1**–**26** was evaluated against the K562 and PC-3 cell lines by the MTT assay. Compounds **1**, **2**, **6**, **7**, **10**, **12**, **16**, **18**, and **25** exhibited cytotoxicity against K562 cells with IC_{50} values in the range 10–20 μM. Paclitaxel was used as the positive control with an IC_{50} value of 0.9 μM. This is the first report of secondary metabolites from this medicinal mushroom.

Ganoderma species (Ganodermataceae, Polyporales) have a long history of being used as folk medicine for the treatment and prevention of various diseases in China and Japan.[1] They contain lanostane triterpenes, meroterpenes, alkaloids, and polysaccharides as bioactive constituents. The lanostane triterpenes from *Ganoderma* species presented various bioactivities, including cytotoxicity,[2] anti-inflammatory activity,[3] hepatoprotective effect,[4] and inhibitory activities against aldose reductase,[5] 5α-reductase,[6] HMG-CoA reductase,[7] and α-glucosidase.[8]

The Tibet plateau is usually called the "Roof of the World" with an average altitude of 4500 m. The unique ecosystems of the Tibet plateau, such as high-cold bush and meadows, and forests, produce a great biodiversity of edible and medicinal mushrooms.[9] People living in Tibet have a long history of using wild mushrooms as food and folk medicine. Among them, *Ophiocordyceps sinensis* is the most famous medicinal mushroom due to its wide range of pharmacological effects.[10,11] *Ganoderma leucocontextum* was discovered as a new species of *Ganoderma* in Tibet in 2014. *G. leucocontextum* is called Zanglingzhi in the Tibet Autonomous Region and believed to possess beneficial effects on human health.[12] There have been no reports of chemical investigation of *G. leucocontextum* due to its scarcity in nature. We cultivated this new *Ganoderma* species in house for the first time and conducted chemical research on the fruiting bodies obtained. As a result, 16 new lanostane triterpenes, ganoleucoins A–P (**1**–**16**), together with 10 known tripterpenes (**17**–**26**) were isolated. The structures of the new compounds were elucidated by extensive spectroscopic analysis and chemical derivation (Figure 1). The inhibitory activity of compounds **1**–**26** against HMG-CoA reductase and α-glucosidase and their cytotoxicity against K562 and PC-3 cell lines were evaluated *in vitro*.

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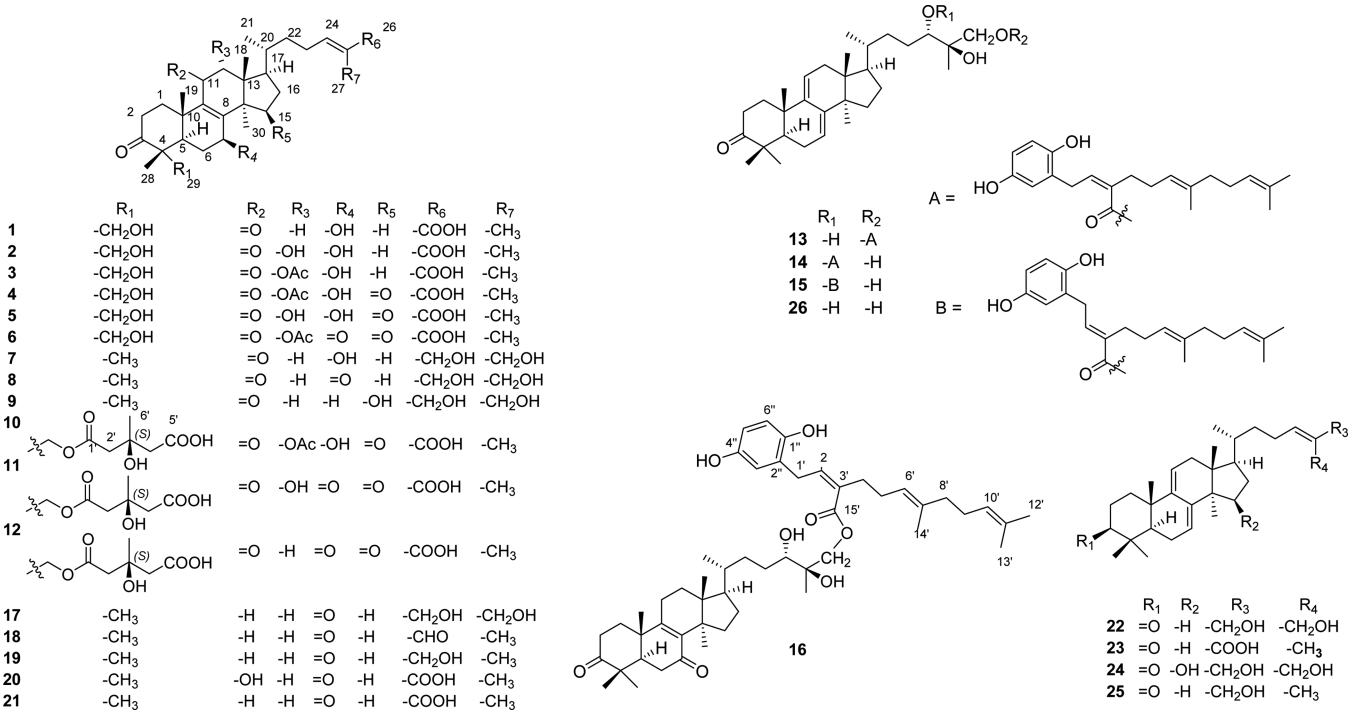

Figure 1. Structures of compounds **1**−**26**.

RESULTS AND DISCUSSION

The ethanol extract of *G. leucocontexum* was partitioned between water and ethyl acetate. The ethyl acetate-soluble fraction was subjected to chromatographic separation using silica gel, ODS, Sephadex LH-20, and preparative HPLC to yield 26 triterpenoids. Ten known compounds (**17**−**26**) were identified as ganoderiol J (**17**),[13] ganoderic acid DM (**18**),[14] ganoderone A (**19**),[15] 11-hydroxy-3,7-dioxo-5-lanosta-8,24(*E*)-dien-26-oic acid (**20**),[16] 3,7-dioxo-8,24(*Z*)-tirucalladien-26-oic acid (**21**),[17] ganoderiol F (**22**),[18] ganoderic acid SZ (**23**),[19] ganoderiol B (**24**),[20] ganoderic acid Y (**25**),[21] and ganodermanontriol (**26**)[22] by comparison of their spectroscopic data with the literature data.

Ganoleucoin A (**1**) was isolated as a colorless powder. The molecular formula of **1** was determined to be $C_{30}H_{44}O_6$ on the basis of HRTOFMS data. The ^{1}H NMR spectrum of **1** showed resonances for six methyl signals [δ_H 0.87 (s), 0.89 (d, J = 6.6 Hz), 1.10 (s), 1.18 (s), 1.34 (s), and 1.85 (s)], one oxygenated methylene [δ_H 4.10 (d, J = 11.3 Hz) and 3.39 (d, J = 11.3 Hz)], one oxygenated methine [δ_H 4.38 (dd, J = 9.2, 5.6 Hz)], and one olefinic proton [δ_H 6.86 (t, J = 7.3 Hz)]. The ^{13}C NMR spectrum of **1** exhibited 30 carbon resonances corresponding to six methyls, nine methylenes (one oxygenated at δ_C 66.0), four methines (one oxygenated carbon at δ_C 73.1), four quaternary carbons, two pairs of olefinic carbons (δ_C 127.1, 138.0, 145.1, and 163.6), and three carbonyl carbons (δ_C 172.5, 198.6, and 222.8, respectively). A lanostane tripterpenoid skeleton was deduced for **1** based on the above NMR data (Table 1 and Table 4). The ^{1}H−^{1}H COSY correlations of H_2-1−H_2-2, H-5−H_2-6−H-7 and H_2-15−H_2-16−H-17−H-20/(H_3-21)−H_2-22−H_2-23−H-24, together with the key HMBC correlations from H_3-18 to C-12, C-13, C-14, and C-17, from H_3-19 to C-1, C-5, C-9, and C-10, from H_2-28 to C-3, C-4, C-5, and C-29, from H_3-29 to C-3, C-4, C-5, and C-28, from H-5 to C-3, C-7, C-28, and C-29, from H-7 to C-5, C-6, C-8, and C-9, from H_2-12 to C-9, C-11, C-13, C-14, C-17, and C-18, from H_3-21 to C-17, C-20, and C-22, from H-24 to C-22, C-25, C-26, and C-27, and from H_3-27 to C-24, C-25, and C-26, confirmed the planar structure of **1** (Figure 2). The relative configuration of **1** was determined by ROESY experiments. The ROESY correlations of H-5 with H-7 and H_2-28, H-17 with H_3-21, H_3-18 with H-12β and H-20, H_3-19 with H-12β and H_3-29, H_3-27 with H_2-23, and H_3-30 with H-7 and H-17 assigned the α-orientation for H-5, H-7, H-17, CH_3-21, $HOCH_2$-28, and CH_3-30, the β-orientation for CH_3-18, CH_3-19, and CH_3-29, and the *E*-geometry for the C-24/C-25 double bond (Figure 3). The absolute configurations of 13*R* and 14*R* were assigned on the basis of the positive and negative Cotton effects at 245 nm ($\Delta\varepsilon$ = +4.2) for a $\pi \rightarrow \pi^*$ transition and 354 nm ($\Delta\varepsilon$ = −0.5) for a $n \rightarrow \pi^*$ transition by the octant rule for the α,β-unsaturated ketone group.[4] Combining the relative configuration established that the absolute configuration of **1** was determined as 4*R*, 5*R*, 7*S*, 10*S*, 13*R*, 14*R*, 17*R*, and 20*R*.

The molecular formula of ganoleucoin B (**2**) was established as $C_{30}H_{44}O_7$ by HRTOFMS data. Its ^{1}H and ^{13}C NMR spectroscopic data revealed a similar structure to **1** except for an extra oxygenated methine [δ_H 4.29 (s); δ_C 78.8] (Table 1 and Table 4). The location of a hydroxy group at C-12 was confirmed by the HMBC correlations from H_3-18 to C-12, C-13, C-14, and C-17 and from H-12 to C-9 and C-11 (Figure 2). The relative configurations of H-7 and H-12 were assigned as α- and β-orientation, respectively, on the basis of NOE correlations of H-12 with H_3-18 and H_3-19 and of H-7 with H-5 and H_3-30 (Figure 3). The structure of **2** was established by detailed interpretation of its 2D NMR data. As described in **1**, the positive and negative Cotton effects at 237 and 317 nm indicated the 13*R* and 14*R* configurations.

Ganoleucoin C (**3**) was assigned the molecular formula $C_{32}H_{46}O_8$ by HRTOFMS data. A detailed comparison of ^{1}H and ^{13}C NMR spectral data between **3** and **2** indicated that compound **3** was an acetylated derivative of **2** (Table 1 and Table 4). The acetyl moiety was deduced from signals due to one methyl group (δ_H 2.18, s; δ_C 21.2) and one carbonyl carbon (δ_C 170.7). The HMBC correlations from the H-12 to the carbonyl

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Table 1. ^{1}H NMR Spectral Data of Compounds 1−6[a]

no.	1	2	3	4	5	6
1	1.71 m[b]	1.59 m	1.61 m	1.49 m	1.45 m	1.58 m
	2.66 m	2.64 m	2.63 m	2.54 m	2.54 m	2.67 m
2	2.99 dt (14.6, 8.9)	2.84 dt (14.8, 9.0)	2.85 dt (14.6, 8.9)	2.77 dt (14.6, 8.9)	2.77 dt (14.8, 9.0)	2.80 dt (14.6, 8.9)
	2.38 m	2.46 m	2.38 dd (15.8, 8.1)	2.47 dd (15.9, 7.9)	2.54 m	2.45 dd (16.1, 7.9)
5	1.84 m	1.84 m	1.86 m	1.74 m	1.70 m	2.47 m
6	2.50 m	2.52 m	2.51 m	2.19 m	2.32 m	2.71 m
	1.26 m	1.25 m	1.25 m	1.65 m	1.70 m	2.05 m
7	4.38 dd (9.2, 5.6)	4.33 dd (9.2, 5.6)	4.38 t (7.0)	4.84 t (8.4)	4.81 dd (9.0, 5.7)	
12	2.45 m	4.29 s	5.62 s	5.65 s	4.37 s	5.70 s
	2.73 m					
15	1.76 m	1.80 m				
16	1.99 m	2.13 m	2.10 m	2.27 m	2.68 m	2.70 m
	1.77 m	1.80 m	1.78 dd (18.8, 8.1)	2.68 dd (19.0, 8.2)	2.26 m	2.05 dd (19.0, 8.2)
17	1.91 m	2.25 m	2.16 m	2.41 m	2.47 m	2.50 m
18	0.87 s	0.68 s	0.88 s	0.99 s	0.81 s	0.85 s
19	1.10 s	1.21 s	1.34 s	1.30 s	1.38 s	1.33 s
20	1.39 m	1.54 m	1.44 m	1.60m	1.99 m	1.66 m
21	0.89 d (6.6)	1.10 d (6.6)	0.93 d (6.5)	1.02 d (6.5)	1.16 d (6.6)	1.04 d (6.6)
22	2.27 m	2.27 m	2.25 m	2.30 m	2.24 m	2.31 m
	2.14 m	2.15 m	2.13 dt (16.1, 7.9)	2.17 m	2.19 m	2.18 dt (16.1, 8.0)
23	1.17 m	1.16 m	1.19 m	1.17 m	1.15 m	1.22 m
24	6.86 t (7.3)	6.88 t (7.3)	6.86 t (7.0)	6.86 t (7.1)	6.86 t (7.3)	6.88 t (7.1)
27	1.85 s	1.84 s	1.83 s	1.85 s	1.83 s	1.85 s
28	4.10 d (11.3)	4.05 d (11.4)	4.04 d (11.2)	4.05 d(11.3)	4.05 d (11.4)	4.03 d (11.1)
	3.39 d (11.3)	3.45 d (11.4)	3.42 d (11.2)	3.48 d (11.3)	3.52 d (11.3)	3.45 d (11.1)
29	1.34 s	1.32 s	1.32 s	1.30 s	1.29 s	1.31 s
30	1.18 s	1.28 s	1.15 s	1.50 s	1.29 s	1.82 s
2′			2.18 s	2.22 s		2.23 s

[a]Recorded at 500 MHz in $CDCl_3$, δ_H in ppm, J in Hz. [b]"m" means multiplet or overlapped with other signals.

carbon at δ_C 170.7 confirmed the acylation of the hydroxy group at C-12 (Figure 2). The NMR signal assignment was made for **3** by 2D NMR data interpretation. Similar Cotton effects observed at 250 and 347 nm between **3** and **2** confirmed the 13*R* and 14*R* configurations.

Ganoleucoin D (**4**) had a molecular formula of $C_{32}H_{44}O_9$, as determined by HRTOFMS data. The NMR data of **4** were similar to those of **3**, except for the presence of an additional ketone moiety (δ_C 216.7) (Table 1 and Table 4). HMBC correlations from H-12 to C-9, C-11, C-13, C-14, C-17, and C-18, from H_3-18 to C-12, C-13, C-14, and C-17, and from H_3-30 to C-8, C-13, C-14, and C-15 indicated a carbonyl group at C-15 in **4**. The structure of **4** was fully assigned by detailed elucidation of its 2D NMR spectra (Figure 2 and Figure 3). The CD spectrum of **4** showed similar Cotton effects to those of **1**−**3**, indicative of the same 13*R* and 14*R* configurations.

The molecular formula of ganoleucoin E (**5**) was determined as $C_{30}H_{42}O_8$ on the basis of HRTOFMS data. The ^{1}H and ^{13}C NMR spectra of **5** resembled those of **4**, except for the loss of the acetyl group (Table 1 and Table 4). HMBC correlations (Figure 2) from H-5 to C-3, C-7, and C-28, from H-7 to C-5, C-6, C-8, C-9, and C-14, from H-12 to C-9, C-11, C-13, C-14, C-17, and C-18, from H_3-18 to C-12, C-13, C-14, and C-17, from H_3-19 to C-1, C-5, C-9, and C-10, from H_2-28 to C-3, C-4, C-5, and C-29, and from H_3-29 to C-3, C-4, C-5, and C-28 confirmed the substitution of the hydroxy groups at C-7 and C-12 and the carbonyl groups at C-3, C-11, and C-15 in **5**, respectively. NOE correlations of H-12 with H_3-18 and H_3-19 and of H-7 with H-5 and H_{30}-30 determined the α- and β-orientation for H-7 and H-12, respectively.

Ganoleucoin F (**6**) was determined to have a molecular formula of $C_{32}H_{42}O_9$ on the basis of HRTOFMS data. The ^{1}H and ^{13}C NMR data of **6** were similar to those of **4**, except for the presence of an additional ketone group (δ_C 198.2) and the absence of one oxygenated methine (δ_C 65.6) in **4** (Table 1 and Table 4). The HMBC correlations from H-5 to C-3, C-4, C-6, C-7, C-9, C-10, C-19, C-28, and C-29 and from H_2-6 to C-4, C-5, C-7, C-8, and C-10, as well as the ^{1}H−^{1}H COSY correlation of H-5−H_2-6, confirmed the location of a carbonyl group at C-7 (Figure 2). The relative configuration of H-12 was assigned as β-orientation by NOE correlations of H-12 with H_3-18 and H_3-19 (Figure 3). Accordingly, the structure of **6** was identified as described in Figure 1.

Ganoleucoin G (**7**) possessed the molecular formula $C_{30}H_{46}O_5$ on the basis of HRTOFMS data. The NMR data of **7** were similar to those of ganoderiol F (**22**), except for an additional carbonyl moiety (δ_C 200.6) and an oxygenated methine replacing two olefinic methines (Table 2 and Table 5). HMBC correlations (Figure 2) from H-5 to C-3, C-4, C-6, C-7, C-9, C-10, C-19, C-28, and C-29, from H-7 to C-5, C-6, C-8, C-9, and C-14, from H_2-12 to C-9, C-11, C-13, C-14, C-17, and C-18, and from H_3-30 to C-8, C-13, C-14, and C-15, together with the ^{1}H−^{1}H COSY correlations of H-5−H_2-6−H-7, determined the location of a hydroxy group at C-7 and a carbonyl group at C-11, respectively. The relative configuration of **7** was assigned by ROESY experiment (Figure 3). As described in **1**, the absolute configuration at C-13 and C-14 in **7** was determined to be 13*R* and 14*R* by CD data.

The molecular formula of ganoleucoin H (**8**) was determined to be $C_{30}H_{44}O_5$ on the basis of HRTOFMS data. A comparison

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Table 2. ^{1}H NMR Spectral Data of Compounds 7−12[a]

no.	7	8	9	10	11	12
1	1.63 m[b]	1.75 m	1.49 m	1.61 m	1.61 m	1.23 m
	2.51 m	2.61 m	2.48 m	2.71 m	2.68 m	2.61m
2	2.89 dt(14.8, 9.0)	2.96 dt (14.8,9.0)	2.72 dt (15.0, 8.9)	2.88 dt (14.6, 8.9)	2.91 dt (14.6, 8.9)	3.01dt (14.6, 8.9)
	2.36 m	2.54 m	2.31 m	2.43 m	2.58 m	1.69 m
5	1.66 m	2.25 m	2.19 m	1.58 m	2.28 m	2.33 m
6	1.97 m	2.53 m	2.08 m	2.32 m	2.64 m	2.78 m
	1.34 m	2.37 m	1.61 m	1.80 m	2.05 m	1.88 m
7	4.37 dd (9.0, 5.6)		1.99 m	4.89 t (8.2)		
			1.37 m			
12	2.58 m	2.72 m	2.60 t (15.1)	5.65 s	4.53 s	2.79 m
	2.40 m	2.64 m	2.42 m			2.84 m
15	1.62 m	2.00 m	4.52 m			
16	1.83 m	2.15 m	2.52 m	2.70 m	2.64 m	2.63 m
	1.60 m	1.64 m	1.87 m	2.30 m	2.75 dd (18.3, 8.0)	2.71 dd (18.2, 7.9)
17	1.67 m	1.69 m	1.57 m	2.42 m	2.57 m	2.13 m
18	0.81 s	0.82 s	0.68 s	0.98 s	0.64 s	0.86 s
19	0.94 s	1.28 s	1.13 s	1.44 s	1.47 s	1.38 s
20	1.33 m	1.41 m	1.39 m	1.80 m	1.73 m	2.62m
21	0.81 d (6.6)	0.92 d (6.6)	0.94 d (6.6)	1.03 d (6.5)	1.12 d (6.5)	0.99 d (6.6)
22	2.09 m	2.17 m	2.16 m	2.32 m	2.27 m	2.28 m
	1.92 m	2.04 m	1.99 m	2.20 m	2.14 dt (15.1, 7.8)	2.14 m
23	1.05 m	1.14 m	1.12 m	1.19 m	1.16 m	1.23 m
24	5.46 t (6.6)	5.54 t (6.6)	5.54 t (6.6)	6.90 t (7.1)	6.84 t (7.1)	6.84 t (7.1)
26	4.25 s	4.23 s	4.24 s			
27	4.37 s	4.33 s	4.35 s	1.86 s	1.80 s	1.82 s
28	1.05 s	1.12 s	1.11 s	4.65 d (11.2)	4.53 d (11.7)	4.54 d (11.5)
				4.08 d (11.2)	4.11 d (11.7)	4.14 d (11.5)
29	0.98 s	1.11 s	1.10 s	1.21 s	1.20 s	1.22 s
30	1.17 s	1.21 s	1.40 s	1.52 s	1.70 s	1.61 s
2′				2.68 m	2.69 m	2.71 m
4′				2.63 m	2.64 m	2.63 m
6′				1.39 s	1.36 s	1.38 s
2″				2.23 s		

[a]Recorded at 500 MHz in $CDCl_3$, δ_H in ppm, J in Hz. [b]"m" means multiplet or overlapped with other signals.

of NMR data between **8** and **7** indicated the loss of an oxygenated methine and the presence of an additional carbonyl moiety (δ_C 201.4) in **8** (Table 2 and Table 5). Interpretation of its HSQC, HMBC, and ROESY data assigned the structure of **8** (Figure 2). NOE correlations of H_β-12 with H_3-18 and H_3-19, H_3-19 with H_3-29, H-17 with H_3-21 and H_3-30, and H-5 with H_3-28 and H_3-30 determined the β-orientation for CH_3-18, CH_3-19, and CH_3-29 and the α-orientation for H-5, H-17, CH_3-28, and CH_3-30 (Figure 3), respectively.

Ganoleucoin I (**9**) possessed a molecular formula of $C_{30}H_{46}O_5$, as determined by HRTOFMS data. The ^{1}H and ^{13}C NMR data of **9** resembled those of **7**, including five singlet methyls, one doublet methyl, 10 methylenes (two oxygenated), four methines (one oxygenated), four quaternary carbons, two pairs of olefinic carbons, and two carbonyl carbons (Table 2 and Table 5). HMBC correlations from H_3-30 to C-8, C-13, C-14, and C-15 (δ_C 65.9) and from H-15 (δ_H 4.52, m) to C-8, C-13, C-14, C-16, and C-17, as well as the ^{1}H−^{1}H COSY correlations of H-15−H_2-16−H-17, indicated the substitution of a hydroxy group at C-15 in **9** (Figure 2). The planar structure of **9** was established by detailed analysis of its 2D NMR spectra. The relative configuration of H-15 was assigned as α-orientation by the NOE correlations of H-15 with H-17 and H_3-30 (Figure 3). The CD spectrum of **9** was similar to that of **1**, indicating 13*R* and 14*R* configurations.

Compound **10** was isolated as a yellow powder, and its molecular formula was determined to be $C_{38}H_{52}O_{13}$ by HRTOFMS data. The ^{1}H NMR data of **10** exhibited signals due to five tertiary methyls (δ_H 0.98, 1.21, 1.39, 1.44, and 1.52), one acetyl methyl (δ_H 2.23), a vinyl methyl (δ_H 1.86), a secondary methyl [δ_H 1.03 (d, J = 6.5 Hz)], and three oxymethines [δ_H 6.90 (t, J = 7.1 Hz); δ_H 4.89 (t, J = 8.2 Hz); δ_H 5.65 (s)] (Table 2). The ^{13}C NMR and HSQC spectra showed 38 carbon resonances including seven methyls, nine methylenes (one oxygenated at δ_C 66.3), six methines (two oxygenated carbons at δ_C 66.2 and 79.9), four quaternary carbons, one oxygenated tetrasubstituted carbon at δ_C 70.3, four olefinic carbons at δ_C 141.4, 127.5, 144.9, and 156.2, and seven carbonyl carbons (δ_C 170.7, 171.6, 173.0, 174.6, 192.2, 213.1, and 216.8) (Table 5). Furthermore, a lanostane triterpenoid structure identical with that of ganoleucoin D (**4**) was supported by HMBC and ROESY experiments (Figure 2). The remaining signals in the NMR spectra of **10** were elucidated as a 3-hydroxy-3-methylglutaryl (HMG) moiety by HMBC correlations from H-2′ (δ_H 2.68, 2.63) to C-1′ (δ_C 171.6), from H-4′ (δ_H 2.68, 2.63) to C-5′ (δ_C 174.6), and from H-6′ (δ_H 1.39) to C-2′ (δ_C 44.8), C-3′ (δ_C 70.3), and C-4′ (δ_C 44.6). The HMG moiety was attached at C-28 of the triterpenoid skeleton on the basis of HMBC correlations from H_2-28 [δ_H 4.08 (d, J = 11.2 Hz), 4.65 (d, J = 11.2 Hz)] to C-1′ (δ_C 171.6). The absolute configuration of the chiral carbon in the HMG group was

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Table 3. ^{1}H NMR Spectral Data of Compounds 13−16[a]

no.	13	14	15	16
1	2.26 m	2.27 m	2.27 m	2.12 m[b]
	1.78 m	1.75 m	1.76 m	1.79 m
2	2.80 dt (14.5, 9.0)	2.79 dt (14.5, 9.0)	2.77 dt (14.5, 9.0)	2.69 dt (14.6, 8.9)
	2.34 m	2.35 m	2.33 m	2.46 m
5	1.55 m	1.54 m	1.55 m	1.45 m
6	2.05 m	2.07 m	2.06 m	2.33 m
	2.23 m	2.21 m	2.20 m	2.53 m
7	5.39 d (5.8)	5.39 d (5.8)	5.39 d (5.8)	
11	5.50 d (6.5)	5.50 d (6.5)	5.50 d (6.5)	2.11 m
				1.99 m
12	2.21 m	2.21 m	2.22 m	1.79 m
	2.10 m	2.12 m	2.19 m	1.67 m
15	1.93 m	1.95 m	1.95 m	1.99 m
	1.29 m	1.26 m	1.29 m	1.21 m
16	1.65 m	1.63 m	1.63 m	2.32 m
	1.35 m	1.38 m	1.38 m	2.70 m
17	1.54 m	1.54 m	1.54 m	2.15 m
18	0.58 s	0.59 s	0.55 s	0.67 s
19	1.21 s	1.20 s	1.21 s	0.94 s
20	1.48 m	1.45 m	1.49 m	1.80 m
21	0.91 d (6.5)	0.91 d (6.5)	0.91 d (6.5)	0.95 d (6.5)
22	1.63 m	1.63 m	1.63 m	1.68 m
	1.05 m	1.04 m	1.05 m	1.13 m
23	1.62 m	1.62 m	1.58 m	1.60 m
	1.46 m	1.46 m	1.46 m	1.45 m
24	3.44 d (10.2)	4.99 d (9.9)	4.77 d (9.5)	3.43 d (10.2)
26	4.42 d (11.1)	3.49 d (12.1)	3.39 d (11.5)	4.41 d (11.4)
	4.19 d (11.1)	3.32 d (12.1)	3.22 d (11.5)	4.19 d (11.4)
27	1.15 s	1.13 s	1.15 s	1.11 s
28	0.86 s	0.86 s	0.85 s	1.08 s
29	1.13 s	1.13.s	1.13 s	1.12 s
30	1.09 s	1.08 s	1.07 s	1.34 s
1′	3.65 dd (16.1,8.5)	3.65 dd (16.1, 8.6)	3.50 d (7.3)	3.65 dd (16.1, 8.5)
	3.59 dd (16.1,8.5)	3.60 dd (16.1, 8.6)		3.60 dd (16.1, 8.5)
2′	5.97 t (8.5)	6.01 t (8.5)	6.95 t (7.3)	5.96 t (8.5)
4′	2.25 m	2.27 m	2.44 t (7.6)	2.25 m
5′	2.08 m	2.07 m	2.16 m	2.07 m
6′	5.04 m	5.05 m	5.18 t (7.0)	5.04 m
8′	1.97 m	1.98 m	1.96 t (7.0)	1.98 m
9′	2.14 m	2.14 m	2.08 m	2.11 m
10′	5.04 m	5.05 m	5.09 t (6.3)	5.05 m
12′	1.68 s	1.68 s	1.67 s	1.66 s
13′	1.61 s	1.60 s	1.60 s	1.57 s
14′	1.53 s	1.53 s	1.60 s	1.53 s
3″	6.64 d (1.5)	6.63 d (1.5)	6. 89 d (1.5)	6.63 d (1.5)
5″	6.61 dd (8.2, 1.5)	6.60 dd (8.2, 1.5)	6.88 dd (8.2, 1.5)	6.60 dd (8.2, 1.5)
6″	6.73 d (8.2)	6.73 d (8.2)	6.63 d (8.2)	6.71 d (8.2)

[a]Recorded at 500 MHz in $CDCl_3$, δ_H in ppm, J in Hz. [b]"m" means multiplet or overlapped with other signals

Table 4. ^{13}C NMR Spectral Data of Compounds 1−6[a]

no.	1	2	3	4	5	6
1	35.0	34.4	34.4	34.9	35.0	34.2
2	34.6	34.0	34.3	34.5	34.8	33.8
3	222.8	221.4	221.9	219.1	218.3	218.8
4	49.9	50.2	50.1	50.3	50.9	50.1
5	52.3	52.1	52.1	49.5	49.9	51.5
6	29.6	29.6	29.5	27.0	27.3	39.0
7	73.1	72.9	72.5	65.6	65.8	198.2
8	163.6	165.4	163.5	157.0	158.3	146.2
9	138.0	136.7	138.0	140.9	140.0	149.7
10	36.7	36.5	36.7	37.5	37.6	36.8
11	198.6	199.7	192.5	192.4	199.9	194.2
12	51.8	78.8	80.4	79.7	78.6	79.3
13	46.8	53.4	51.5	60.5	60.3	58.7
14	53.6	54.4	54.8	49.7	51.7	47.7
15	18.0	18.0	29.8	216.7	217.4	206.0
16	38.8	36.9	37.1	37.7	37.6	37.6
17	49.1	49.1	48.7	46.1	46.7	45.3
18	17.4	11.3	12.6	13.6	12.4	12.3
19	20.2	20.1	18.8	19.1	19.3	19.6
20	36.0	34.1	34.1	31.9	31.9	33.4
21	18.1	20.0	20.1	20.9	21.0	20.4
22	34.5	34.2	34.4	33.1	33.4	34.2
23	25.9	26.7	26.5	26.5	26.9	26.6
24	145.1	145.6	144.8	143.8	144.5	144.4
25	127.1	126.8	127.3	127.6	127.4	127.4
26	172.5	172.3	172.5	172.2	172.6	172.0
27	12.2	12.2	12.2	12.1	12.2	12.3
28	66.0	65.8	65.9	65.5	65.6	65.5
29	22.7	22.6	22.7	22.0	21.9	22.8
30	19.0	18.0	19.9	24.5	23.5	21.0
1′			170.7	170.4		170.3
2′			21.2	20.7		21.0

[a]Recorded at 125 MHz in $CDCl_3$, δ_C in ppm.

determined as *S* by using the reference method (Scheme 1).[23−26] Compound **10a** was obtained by amidation of **10** with (*S*)-1-phenylethylamine. Reduction of **10a** with $LiBH_4$, followed by acetylation, gave 5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide (**10b**), which had the same ^{1}H NMR data as those of (3*R*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide.[24] Compound **10** was designated as ganoleucoin J.

Ganoleucoin K (**11**) had a molecular formula of $C_{36}H_{48}O_{12}$, as determined by HRTOFMS data. The ^{1}H and ^{13}C NMR data of **11** resembled those of **10**, except for the absence of the acetyl group and the presence of an additional carbonyl group (δ_C 197.8) instead of an oxygenated methine (δ_C 66.3) in **10** (Table 2 and Table 5). The HMBC correlations from H-5 (δ_H 2.28, m) and H_2-6 (δ_H 2.64, m) to C-7 (δ_C 197.8), as well as the ^{1}H−^{1}H COSY correlation of H-5−H_2-6, confirmed the location of a carbonyl moiety at C-7. A detailed examination of its 2D NMR spectroscopic data confirmed the structure of **11** (Figure 2). The relative configuration of H-12 was assigned as β-orientation by NOE correlations of H-12 with H_3-18 and H_3-19.

Ganoleucoin L (**12**) was obtained as a yellow powder. The molecular formula of $C_{36}H_{48}O_{11}$ was confirmed on the basis of HRTOFMS data. The ^{1}H and ^{13}C NMR data of **12** were similar to those of **11**, except for the absence of an oxygenated methine (δ_C 77.8) in **11** and the presence of an extra methylene (δ_C 44.1) (Table 2 and Table 5). The HMBC correlations from H_3-18 (0.86, s) to C-12, C-13, C-14, and C-17 and from H_2-12 to C-9, C-11, C-13, C-14, C-17, and C-18 confirmed the structural variation at C-12 between **11** and **12**. Structural determination and full signal assignment of **12** were solved on the basis of 2D NMR spectral analysis (Figure 2). The absolute configuration of the HMG group in compounds **11** and **12** was determined as *S* by using the same method as described for **10**. The HMG group has been found to be conjugated in flavonoids from *Oxytropis falcata*,[23] triterpenoids from *Piptoporus betulinus*[24] and *Ganoderma resinaceum*,[27] and phenylpropanoids from *Edgeworthia chrysantha*.[28]

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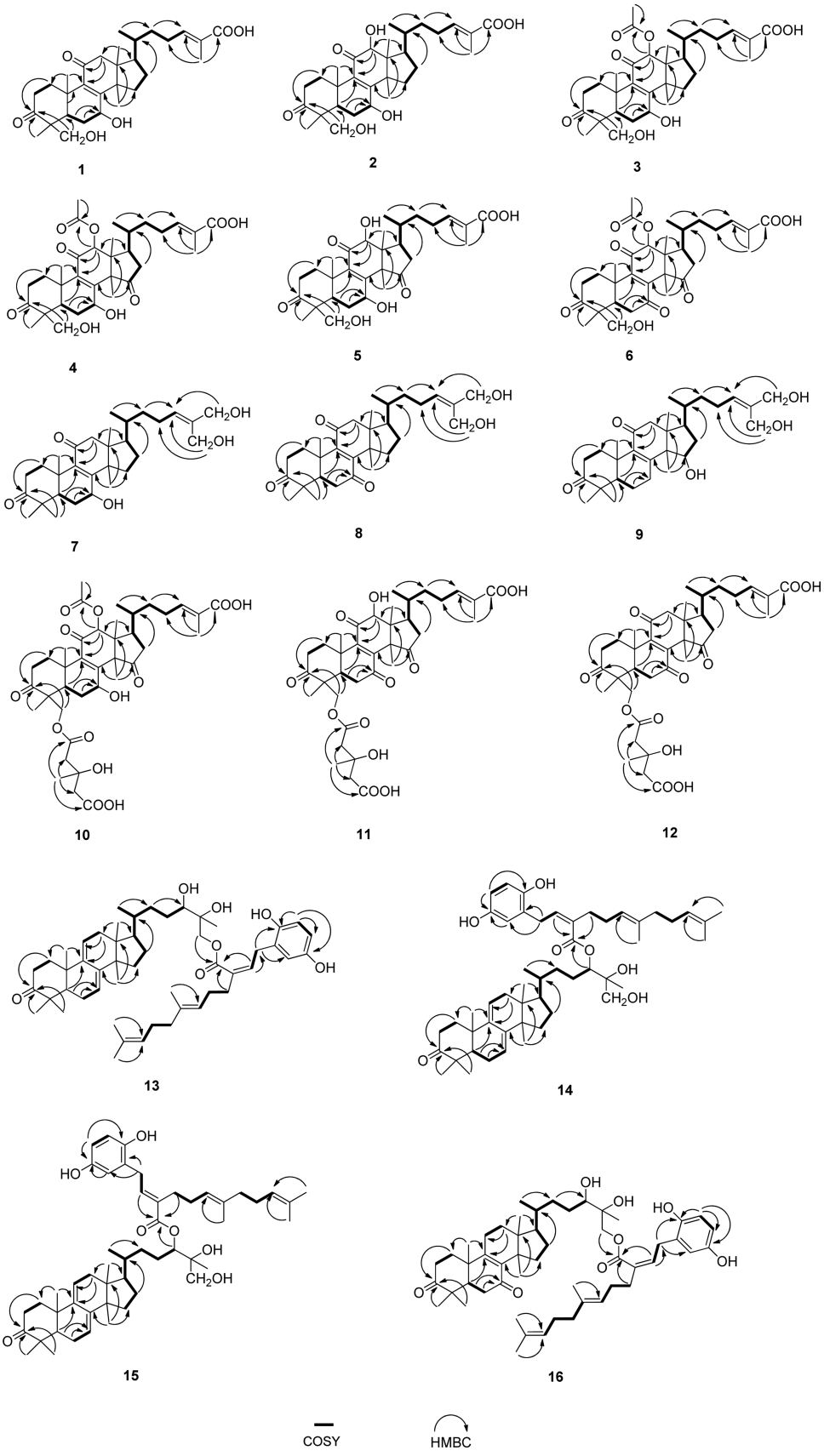

Figure 2. Key $^{1}H-^{1}H$ COSY and HMBC correlations of **1**−**16**.

Ganoleuconin M (**13**) was assigned the molecular formula $C_{51}H_{74}O_7$ on the basis of HRTOFMS data. The ^{1}H NMR and ^{13}C NMR spectra of **13** exhibited signals due to nine singlet methyl groups, two doublet methyl groups, 14 methylenes

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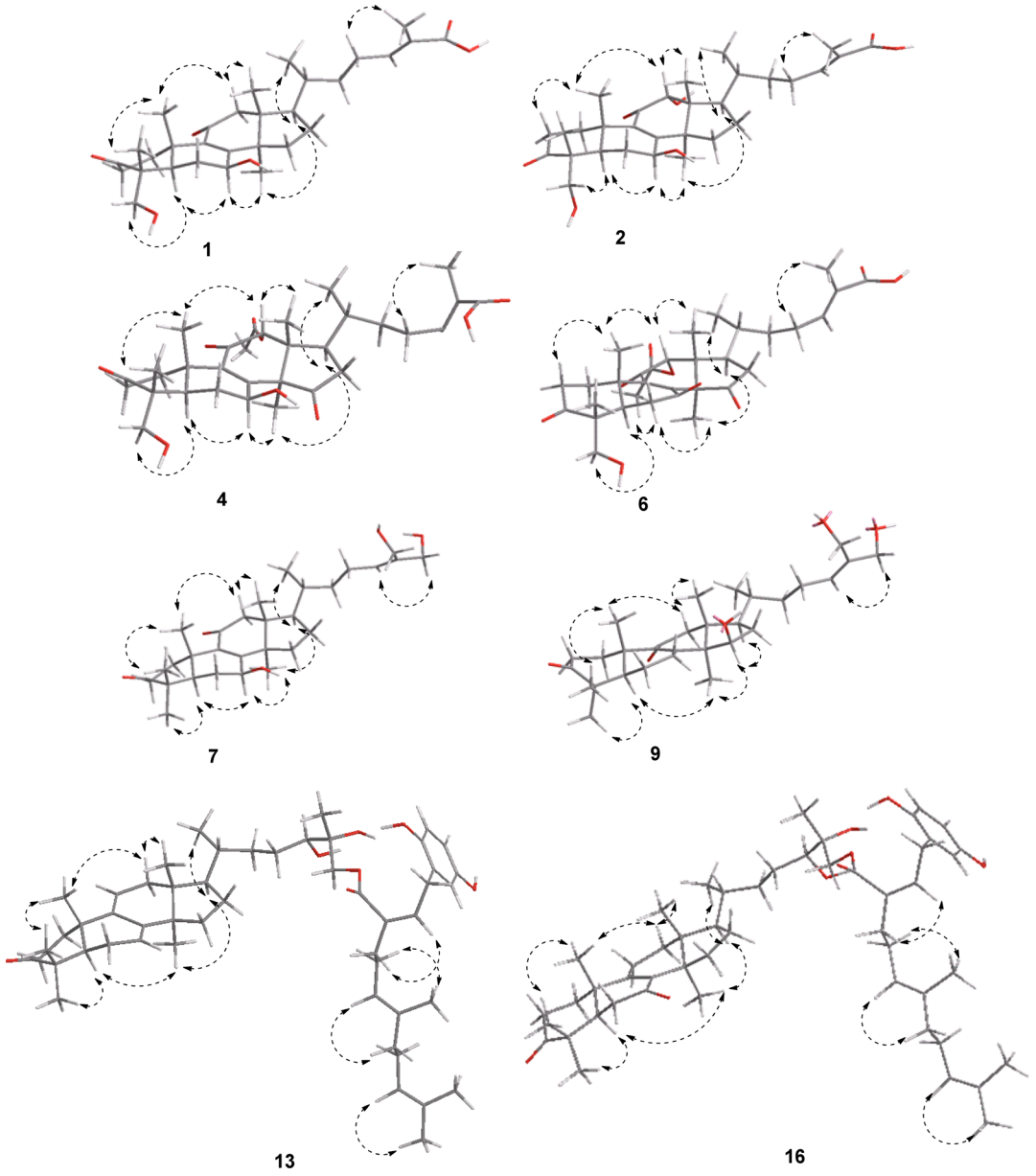

Figure 3. Key ROESY correlations of **1**, **2**, **4**, **6**, 7, **9**, **13**, and **16**.

including one oxygenated methylene [δ_H/δ_C 4.42 (d, J = 11.1), 4.19 (d, J = 11.1 Hz)/69.2], four methines including one oxymethine (δ_C 77.2), four quaternary carbons, one oxygenated tetrasubstituted carbon (δ_C 74.1), five pairs of double bonds including five protonated olefinic carbons (δ_H/δ_C 5.96 (t, J = 8.5 Hz)/140.3, 5.50 (d, J = 6.5)/117.3, 5.39 (d, J = 5.8 Hz)/120.1, 5.04 (m)/124.3, and 5.05 (m)/122.5), a 1,2,4-trisubstituted aromatic ring [δ_H 6.61 (dd, J = 8.2, 1.5 Hz), 6.64 (d, J = 1.5 Hz), 6.73 (d, J = 8.2 Hz); δ_C 115.1, 117.1, 117.4, 124.3, 149.0, and 149.4], and two carbonyl carbons (δ_C 170.4 and 217.2) (Table 3 and Table 6). A detailed analysis of its 2D NMR spectra revealed the presence of ganodermanontriol or its side-chain triol diastereomers and a 12-deoxylganomycin A unit in **13**. The HMBC correlations of H_2-26 (δ_H 4.42 and 4.19) with C-15′ (δ_C 170.4) indicated that the ganodermanontriol moiety was linked with a 12-deoxylganomycin A unit by an ester linkage (C-26 to C-15′). In its ROESY spectrum, the NOE correlations of H-2′ with H-4′, H-5′ with H_3-14′, and H-6′ with H_2-8′ assigned the Z and *E* configurations for the C-2′−C-3′ and C-6′−C-7′ double bonds, respectively. Ganodermanontriol (**26**) and its three stereoisomeric triols have been synthesized in an early report and have been reported to have different chemical shifts of C-24, C-25, and C-26 in the side chain.[29] To confirm the absolute configuration in the side chain of the tripterpenoid moiety in **13**, compound **13** was hydrolyzed in 2% sodium methoxide−methanol to afford ganodermanontriol (**26**). Thus, the absolute configuration of C-24 and C-25 in **13** was assigned as 24*S* and 25*R*.

The molecular formula of ganoleuconin N (**14**) was determined as $C_{51}H_{74}O_7$ by HRTOFMS data. The NMR data of **14** were similar to those of **13**, including the NMR signals due to a ganodermanontriol moeity and a farnesyl hydroquinone unit (Table 3 and Table 6). The structure of **14** was confirmed and fully assigned by detailed analysis of its 2D NMR data. In the HMBC spectrum, the correlation of H-24 at δ_H 4.99 (d, J = 9.9 Hz) with the carbonyl group at δ_C 171.3 confirmed the linkage of a 12-deoxylganomycin A unit (A) with ganodermanontriol through an ester bond between C-24 and C-15′. The absolute

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Table 5. ^{13}C NMR Spectral Data of Compounds 7−12[a]

no.	7	8	9	10	11	12
1	35.0	35.0	35.0	34.9	34.4	34.3
2	34.2	34.0	34.8	33.2	33.8	34.2
3	218.4	215.8	214.8	213.1	211.2	211.8
4	45.1	46.8	47.6	50.9	50.6	51.6
5	51.0	49.7	50.9	50.6	51.7	50.5
6	27.4	37.1	32.8	27.2	39.3	39.5
7	67.3	201.4	28.1	66.3	197.8	198.7
8	160.3	151.6	159.1	156.2	147.5	147.0
9	140.0	149.7	142.2	141.4	148.8	149.9
10	37.9	38.8	40.2	37.4	37.4	40.2
11	200.6	202.1	199.7	192.2	201.7	199.6
12	51.6	51.2	37.7	79.9	77.8	44.1
13	47.2	46.7	48.3	60.8	57.9	57.3
14	46.5	48.7	47.7	49.9	49.5	49.1
15	29.3	27.4	65.9	216.8	207.0	208.1
16	30.2	31.9	44.7	38.1	37.3	37.1
17	50.2	49.0	49.8	46.1	45.7	45.3
18	17.8	16.9	17.1	13.6	11.0	16.2
19	17.2	18.0	25.2	18.6	18.5	18.6
20	36.0	35.9	36.1	32.0	32.9	35.7
21	18.4	18.4	18.6	21.0	20.4	18.5
22	36.2	35.8	36.0	34.9	33.7	34.5
23	24.5	24.3	24.4	26.7	26.7	25.8
24	131.9	131.4	132.1	144.9	144.8	144.5
25	136.7	136.8	136.4	127.5	127.3	127.5
26	68.0	67.8	67.9	173.0	173.2	173.0
27	60.4	60.5	60.5	12.2	12.1	12.2
28	27.8	27.5	25.4	66.2	65.7	65.8
29	20.6	20.3	21.7	21.7	22.3	22.5
30	27.6	25.9	19.4	24.4	20.2	21.2
1′				171.6	171.5	171.6
2′				44.8	44.8	44.8
3′				70.3	69.8	69.9
4′				44.6	44.7	44.7
5′				174.6	175.7	175.2
6′				27.4	27.3	27.3
1″				170.7		
2″				20.9		

[a]Recorded at 125 MHz in $CDCl_3$, δ_C in ppm.

configuration of the triterpene moiety in **14** was determined by the same method as described in **13**.

Ganoleuconin O (**15**) was obtained as a yellow powder. It had the same molecular formula of $C_{51}H_{74}O_7$, as determined by HRTOFMS data. The NMR data of **15** were similar to those of **13** (Table 3 and Table 6). A significant difference observed in the ^{1}H and ^{13}C spectra of **13** and **15** lies in the farnesyl hydroquinone unit, especially for signals due to the olefins and 1,2,4-trisubstituted benzene. A detailed examination of its 2D NMR spectral data (^{1}H−^{1}H COSY, HSQC, HMBC, and ROESY) confirmed the presence of ganodermanontriol and a farnesyl hydroquinone unit (B). The HMBC correlation of H-24 at δ_H 4.77 (d, J = 9.5 Hz) with the carbonyl group at δ_C 169.6 concluded the attachment of the moiety B at C-24. The alkaline hydrolysis of **15** also afforded ganodermanontriol (**26**). The NOE correlations of H_2-1′ with H_2-4′, H_2-5′ with H_3-14′, and H-6′ with H_2-8′ suggested the *E* and *E* configurations for the C-2′−C-3′ and C-6′−C-7′ double bonds, respectively. Thus, the structure of **15** was assigned as shown in Figure 1.

Ganoleuconin P (**16**) was determined to have a molecular formula of $C_{51}H_{74}O_8$ on the basis of HRTOFMS data. The IR spectrum showed the presence of carbonyl (1719 and 1700 cm^{-1}) groups. A comparison of NMR data between **13** and **16** indicated that **16** was a triterpene-farnesyl hydroquinone conjugate. The ^{1}H and ^{13}C NMR spectra of **16** exhibited signals corresponding to dihydroxy-3,7-dioxo-8-ene-lanosta-26-ol[30] and 12-deoxylganomycin A units (Table 3 and Table 6). Detailed analysis of the 2D NMR spectra of **13** established its structure. The HMBC correlations of H_2-26 (δ_H 4.19 and 4.41) with C-15′ (δ_C 170.3) indicated that the dihydroxy-3,7-dioxo-8-ene-lanosta-26-ol moiety was conjugated with the 12-deoxylganomycin A unit by an ester linkage (C-26 to C-15′). In its ROESY spectrum, the NOE correlations of H-2′ with H_2-4′, H_2-5′ with H_3-14′, and H-6′ with H_2-8′ assigned the Z and *E* configurations for the C-2′−C-3′ and C-6′−C-7′ double bonds, respectively. Due to the structural similarity in the side chain between **13** and **16**, the absolute configurations of C-24 and C-25 in **16** were deduced by NMR data comparison. The ^{13}C NMR data of C-22, C-23, C-24, C-25, and C-26 in **16** were almost the same as those of **13**, indicating the same absolute configuration of 24*S* and 25*R*. To date, only three triterpene-farnesyl hydroquinone conjugates (ganosinensins A−C) have been reported from the fruiting body of *G. sinense*.[31]

The medicinal mushroom *G. lucidum* was reported to have hypolipidemic and antidiabetic effects. The intake of powdered mycelium of *G. lucidum* was able to decrease total plasma cholesterol, total liver triglyceride, and total liver cholesterol levels in animals.[32] The water extract of *G. lucidum* was confirmed to have beneficial effects in treating type 2 diabetes mellitus through the suppression of hepatic PEPCK gene expression.[33] 7-Oxo-ganoderic acid Z and 15-hydroxy-ganoderic acid S from *G. lucidum* exhibited inhibitory activities against HMG Co-A reductase with IC_{50} values of 22.3 and 21.7 μM, respectively.[7] Ganoderol B was found to possess α-glucosidase inhibition with an IC_{50} of 119.8 μM.[8] Glucosidase and HMG Co-A reductase inhibitors have been considered to be valuable compounds for the development of hypolipidemic and antidiabetic drugs.[34−36]

Herein, we used *in vitro* HMG-CoA reductase and α-glucosidase inhibition assays to test the hypolipidemic and antidiabetic effects of compounds isolated from the fruiting bodies of *G. leucocontextum*. Ganoleuconins A−D (**1**-**4**), F (**6**), H (**8**), and J−N (**10**−**14**), ganoderiol J (**17**), ganoderic acid DM (**18**), 3,7-dioxo-8,24(*Z*)-tirucalladien-26-oic acid (**21**), ganoderic acid S (**23**), ganoderic acid Y (**25**), and ganodermanontriol (**26**) exhibited HMG-CoA reductase inhibition with IC_{50} values in the range 8−100 μM (Table 7). Compounds **1**, **3**, **6**, **10**−**14**, **17**, **18**, **23**, **25**, and **26** showed much stronger inhibitory activity than the positive control atorvastatin. Compound **10** (IC_{50} = 26.4 μM) showed much stronger inhibitory activity than compound **4** (IC_{50} = 97.5 μM), which indicated the significance of the HMG moiety linking at C-28 for the HMG-CoA reductase inhibitory activity. Ganoleuconins E (**5**), M (**13**), N (**14**), and P (**16**) and ganoderone A (**19**) showed strong inhibitory activity against α-glucosidase from yeast with IC_{50} values of 12.7, 13.6, 2.5, 5.9, and 13.7 μM (Table 7). All other compounds isolated presented relatively weak inhibitory activity with IC_{50} values greater than 50 μM. The inhibitory effects of compounds **13**−**16** were further investigated using small intestinal mucosa from rats. Compounds **13**, **14**, and **16** showed much stronger inhibitory activity against α-glucosidase, sucrase, and maltase from rats (Table 7) than the positive control acarbose. Preliminary structure−activity relationship analysis showed that the farnesyl

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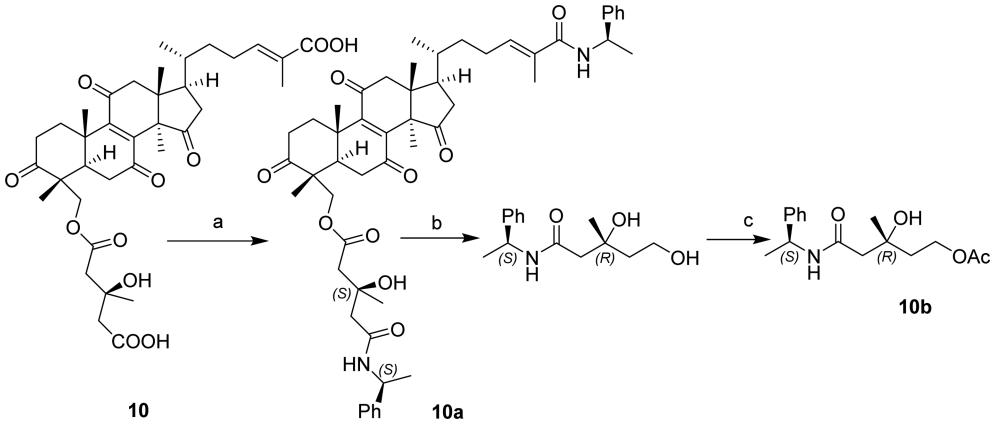
Scheme 1. Determination of the Absolute Configuration in the HMG Moiety of 10[a]



[a]Key: (a) (*S*)-1-phenylethylamine, DMF, Et_3N, PyBOP, HOBt; (b) $LiBH_4$, THF; (c) Ac_2O, pyridine.

Table 6. ^{13}C NMR Spectral Data of Compounds 13−16[a]

no.	13	14	15	16	no.	13	14	15	16
1	36.8	36.8	36.6	35.5	27	22.2	22.2	22.2	21.0
2	35.0	35.0	35.0	34.5	28	25.5	25.5	25.5	25.9
3	217.2	217.2	217.1	215.0	29	25.6	25.6	25.6	21.5
4	47.6	47.6	47.6	47.4	30	21.0	18.3	17.9	18.0
5	51.1	51.0	51.1	50.5	1′	31.9	31.9	29.8	31.9
6	23.8	23.8	23.8	37.3	2′	140.2	141.3	142.0	140.3
7	120.1	120.1	120.0	198.5	3′	131.5	131.6	132.4	131.5
8	143.0	142.9	143.0	163.3	4′	34.5	34.3	27.9	34.5
9	144.6	144.6	144.6	139.7	5′	28.2	25.0	25.3	28.8
10	37.9	37.9	37.9	39.6	6′	124.4	124.3	123.2	124.3
11	117.3	117.3	117.4	25.1	7′	136.8	136.7	136.4	136.7
12	37.3	37.3	37.3	30.2	8′	39.8	39.7	39.8	39.7
13	43.9	43.9	43.9	47.9	9′	27.6	27.9	27.2	27.6
14	50.4	50.4	50.4	45.1	10′	122.5	122.5	124.4	122.5
15	28.0	28.1	28.0	26.6	11′	131.4	131.0	131.7	131.4
16	31.6	31.6	31.6	32.0	12′	25.9	25.9	25.9	25.5
17	50.8	50.8	50.8	49.1	13′	17.9	17.9	17.9	17.9
18	15.9	15.9	15.8	16.0	14′	16.3	16.3	16.2	16.3
19	22.6	22.6	22.6	24.0	15′	170.4	171.3	169.6	170.3
20	36.6	35.8	36.7	36.6	1″	149.4	149.4	149.7	149.3
21	18.8	18.5	18.7	19.0	2″	124.0	124.0	126.5	124.3
22	33.7	32.9	33.4	33.7	3″	117.4	117.2	117.0	117.2
23	26.8	26.8	26.8	26.7	4″	149.0	149.0	147.5	149.1
24	77.2	76.9	76.9	77.4	5″	115.1	115.2	114.3	115.1
25	74.1	73.4	73.5	74.2	6″	117.1	117.2	116.3	117.2
26	69.2	66.9	67.0	69.2					

[a]Recorded at 125 MHz in $CDCl_3$, δ_C in ppm.

hydroquinone moiety in **13**, **14**, and **16** contributed greatly to their inhibitory activity against α-glucosidase.

Triterpenes are considered major pharmacologically active compounds contributing to the antitumor efficacy of *Ganoderma*. Ganoderic acid D induced apoptosis in HeLa cell.[37] Ganoderiol F exhibited cytotoxicity against Lewis lung carcinoma (LLC), sarcoma-180, and T-47D cell lines *in vitro*[38] and induced senescence in hepatoma HepG2 cells.[39] Ganoderic acid T showed significant antitumor effects *in vivo*.[40] Ganoderic acid X inhibited topoisomerases and induced apoptosis of cancer cells.[41] To evaluate the cytotxicity of triterpenes isolated from *G. leucocontexum*, compounds **1**−**26** were tested for their antiproliferative activity against tumor cell lines K562 (human myelogenous leukemia) and PC-3 (human prostate cancer). As shown in Table 8, compounds **1**, **2**, **6**, **7**, **8**, **10**−**14**, **16**−**19**, and **25** exhibited cytotoxicity against K562 cells with IC_{50} values in the range 10 to 50 μM. For the PC-3 cell line, compounds **8**, **14**, **16**, and **19** showed cytotoxicity with IC_{50} values of 24.2, 14.4, 10.8, and 28.5 μM, respectively.

In summary, 26 cytotoxic lanostane triterpenes (**1**−**26**) including 16 new compounds were obtained from the fruiting body of the medicinal mushroom *G. leucocontextum*. The inhibitory activity of compounds **1**−**26** against HMG-CoA reductase and α-glucosidase and cytotoxicity against K562 and PC-3 cell lines were evaluated. This is the first report of bioactive secondary metabolites from this medicinal mushroom, which expanded the chemistry of

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Table 7. HMG-CoA Reductase and α-Glucosidase Inhibitory Activity of 1−26

			small intestinal mucosa from rat		
	HMG-CoA reductase inhibition (IC_{50}, μM)	α-glucosidase inhibition (from baker's yeast) (IC_{50}, μM)	α-glucosidase inhibition (IC_{50}, μM)	sucrase inhibition (IC_{50}, μM)	maltase inhibition (IC_{50}, μM)
1	17.1 ± 3.5	>50	NT[a]	NT	NT
2	66.9 ± 10.3	>50	NT	NT	NT
3	26.6 ± 4.3	>50	NT	NT	NT
4	97.5 ± 9.1	>50	NT	NT	NT
5	>100	12.7 ± 2.1	NT	NT	NT
6	20.5 ± 4.4	>50	NT	NT	NT
7	>100	>50	NT	NT	NT
8	32.9 ± 4.0	>50	NT	NT	NT
9	>100	>50	NT	NT	NT
10	26.4 ± 10.2	>50	NT	NT	NT
11	10.7 ± 2.9	>50	NT	NT	NT
12	13.3 ± 2.7	>50	NT	NT	NT
13	18.9 ± 2.0	13.6 ± 3.1	1.5 ± 0.2	8.4 ± 1.8	8.1 ± 1.7
14	24.1 ± 6.2	2.5 ± 0.7	8.1 ± 2.9	22.5 ± 5.7	10.1 ± 1.9
15	>100	>50	>50	>50	>50
16	>100	5.9 ± 2.2	9.3 ± 1.1	18.5 ± 2.3	16.2 ± 3.0
17	12.6 ± 2.7	>50	NT	NT	NT
18	9.5 ± 1.5	> 50	NT	NT	NT
19	>100	13.7 ± 2.3	NT	NT	NT
20	>100	>50	NT	NT	NT
21	56.1 ± 10.4	>50	NT	NT	NT
22	>100	>50	NT	NT	NT
23	12.5 ± 2.3	>50	NT	NT	NT
24	>100	>50	NT	NT	NT
25	8.6 ± 2.0	>50	NT	NT	NT
26	16.8 ± 5.8	>50	NT	NT	NT
positive control	atorvastatin	acarbose	acarbose	acarbose	acarbose
	32.1 ± 7.7	273.1 ± 30.5	38.1 ± 6.0	20.2 ± 4.5	16.1 ± 4.1

[a]"NT" means "not tested".

Table 8. Cytotoxicity of Compounds 1−26

	K562 (IC_{50}, μM)	PC-3 (IC_{50}, μM)		K562 (IC_{50}, μM)	PC-3 (IC_{50}, μM)
1	17.8 ± 4.6	>200	**14**	48.4 ± 9.7	14.4 ± 4.1
2	19.7 ± 4.6	>200	**15**	103.6 ± 7.9	>200
3	>200	>200	**16**	10.7 ± 1.8	10.8 ± 3.5
4	147.22 ± 13.2	88.3 ± 17.6	**17**	41.6 ± 4.1	>200
5	>200	>200	**18**	18.8 ± 3.5	81.6 ± 13.4
6	18.2 ± 3.3	>200	**19**	27.7 ± 4.5	28.5 ± 3.1
7	11.4 ± 4.3	132.4 ± 25.7	**20**	>200	>200
8	115.4 ± 12.8	24.2 ± 6.6	**21**	55.4 ± 3.7	148.6 ± 19.9
9	>200	>200	**22**	54.9 ± 4.75	79.5 ± 11.3
10	12.3 ± 3.9	110.5 ± 14.1	**23**	65.2 ± 9.9	75.2 ± 17.9
11	22.6 ± 5.2	>200	**24**	76.6 ± 11.9	102.9 ± 25.7
12	14.4 ± 1.8	>200	**25**	17.5 ± 4.5	179.8 ± 22.5
13	48.6 ± 10.3	58.4 ± 11.4	**26**	80.9 ± 8.5	105.8 ± 25.4
positive control	paclitaxel	doxorubicin	positive control	paclitaxel	doxorubicin
	0.9 ± 0.1	2.0 ± 0.4		0.9 ± 0.1	2.0 ± 0.4

the *Ganoderma* species and provided evidence for the usage of *G. leucocontexum* as an herbal medicine and a promising source of new bioactive agents. The discovery of new bioactive lanostane triterpenes from *G. leucocontextum* also supports its phylogenetic taxonomy as a new member of the *G. lucidum* complex.

EXPERIMENTAL SECTION

General Experimental Procedures. Solvent used for extraction and chromatographic separation was analytical grade. TLC was carried out on silica gel $HSGF_{254}$, and compounds were visualized by spraying with 10% H_2SO_4 and heating. Silica gel (Qingdao Haiyang Chemical Co., Ltd., People's Republic of China) and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography. HPLC separation was performed on an Agilent 1200 HPLC system using an ODS column (C_{18}, 250 × 9.4 mm, YMC Pak, 5 μm; detector: UV) with a flow rate of 2.0 mL/min. UV and IR spectral data were acquired using a ThermoGenesys-10S UV−vis and Nicolet IS5 FT-IR spectrophotometer, respectively. Optical rotations were measured on an Anton Paar MCP 200 automatic polarimeter. NMR spectral data were obtained with a Bruker Avance-500 spectrometer ($CDCl_3$, δ_H 7.26/δ_C 77.16). The HSQC and HMBC experiments were optimized for 145.0 and 8.0 Hz,

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respectively. HRTOFMS data were measured using an Agilent Accurate-Mass-Q-TOF LC/MS 6520 instrument.

Fungal Material. The strain of *G. leucocontexum* was isolated from its fruiting bodies collected in the Linzhi region of Tibet. *G. leucocontexum* was cultured on slants of potato dextrose agar at 25 °C for 10 days. Agar plugs were inoculated into a bag (18 × 35 cm) with the culture medium composed of 140 g of cotton seed hulls, 36 g of wood powders, 22 g of rice bran, 2 g of $CaSO_4$, and 120 mL of distilled water. Two hundred bags were inoculated and incubated at 25 °C for 90 days in a greenhouse. The fruiting bodies obtained were identified by W.-P.X. (one of the coauthors) by comparing their morphological characteristics with those published for *G. leucocontexum*.[12]

Extraction and Isolation. The air-dried and powdered fruiting bodies of *G. leucocontexum* (3 kg) were extracted three times with ethyl alcohol (3 × 10 L), and organic solvent was evaporated to dryness under vacuum to afford the crude extract (179 g). The ethanol extract (170 g) was partitioned between ethyl acetate and water. The EtOAc extract (43.5 g) was subjected to silica gel column chromatography (CC) using hexane−ethyl acetate in a gradient elution (v/v, 100:0, 100:2, 100:5, 100:8, 100:10, 100:15, 100:20, 100:40, 100:50), followed by dichloromethane−methanol elution (v/v, 100:1, 100:2, 100:3, 100:5, 100:10, 100:15, 100:20, 0:100) to give 23 fractions (GL-1−GL-23).

Fraction 16 (1.42 g) eluted with hexane−ethyl acetate (v/v, 100:50) was subjected to silica gel column chromatography with a $CHCl_3$−MeOH gradient solvent system (100:0−0:100) to yield 10 fractions. Compound **26** (121.1 mg) was obtained from fraction GL-16-3 by recrystallization in MeOH. Compounds **16** (7.2 mg, t_R 27.2 min), **13** (5.1 mg, t_R 28.1 min), **14** (3.5 mg, t_R 25.1 min), and **15** (4.5 mg, t_R 34.2 min) were isolated from fraction GL-16-8 (130.0 mg) by RP-HPLC using 81% methanol in water.

Fraction 17 (3.45 g) eluted with dichloromethane−methanol (v/v, 100:1) was further separated on Sephadex LH-20 CC eluted with 50% methanol in water to give 12 subfractions (GL-17-1−GL-17-12). Fraction GL-17-3 (123 mg) was further purified by Sephadex LH-20 CC eluted with 50% methanol in water to afford compounds **18** (24 mg), **19** (28.1 mg), and **20** (20 mg). Compounds **23** (31.2 mg, t_R 32.2 min), **24** (20.7 mg, t_R 36.5 min), and **25** (50.1 mg, t_R 40 min) were obtained from fraction GL-17-12 (201.2 mg) by RP-HPLC using 75% methanol in water.

Fraction 19 (5.32 g) eluted with dichloromethane−methanol (v/v, 100:2) was further separated on ODS CC using a gradient of methanol−water (30−100%) to give 23 subfractions (GL-19−1−GL-19−23). Compounds **1** (23.2 mg, t_R 32.2 min), **2** (15.2 mg, t_R 34.5 min), and **3** (12.4 mg, t_R 36.2 min) were obtained from fraction GL-19-13 (150.5 mg) by RP-HPLC using 56% acetonitrile in water. Fraction GL-19-12 (352.3 mg) was subjected to Sephadex LH-20 CC eluted with 70% methanol in water to afford five subfractions (GL-19-12-1−GL-19-12-5). Compounds **4** (5.2 mg, t_R 32.3 min) and **5** (7.2 mg, t_R 36.2 min) were purified from subfraction GL-19-12-3 (35.2 mg) by RP-HPLC using 54% acetonitrile in water. Compounds **6** (6.1 mg, t_R 32.1 min), **8** (5.4 mg, t_R 35.2 min), and **21** (7.1 mg, t_R 37.1 min) were isolated from GL-19-12-4 (20.5 mg) by RP-HPLC using 56% acetonitrile in water. Subfraction GL-19-22 (102.5 mg) was separated by RP-HPLC using 60% acetonitrile in water to afford compound **9** (43.1 mg, t_R 39.2 min). Compounds **10** (23.1 mg, t_R 39.8 min), **7** (13.2 mg, t_R 35.1 min), and **22** (3.6 mg, t_R 38.2 min) were purified from fraction GL-19-16 (90.2 mg) by RP-HPLC using 71% methanol in water. Fraction GL-19-15 (123 mg) was further separated by Sephadex LH-20 CC eluted with 50% methanol in water to afford compounds **11** (45 mg), **12** (43.1 mg), and **17** (7.1 mg). The physical properties and spectroscopic data of the new compounds are as follows.

*Ganoleuconin A (**1**):* colorless powder; $[\alpha]^{25}_D$ +72.40 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.10), 258 (2.00) nm; CD (*c* 2.01×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 245 (+4.2), 354 (−0.5) nm; IR (neat) ν_{max} 3315, 2980, 2948, 2840, 2820, 1702, 1696, 1618, 1586, 1429, 1360, 1347, 1236, 856, 612 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 1 and 4; positive HRTOFMS *m*/*z* $[M + H]^+$ 501.3216 (calcd for $C_{30}H_{45}O_6$, 501.3211).

*Ganoleuconin B (**2**):* colorless powder; $[\alpha]^{25}_D$ +53.00 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.04), 260 (1.98) nm; CD (*c* 1.95×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 237 (+3.1), 317 (−0.5) nm; IR (neat) ν_{max} 3319, 2948, 2925, 2840, 2820, 1723, 1702, 1625, 1586, 1425, 1369, 1364, 1226, 866, 754, 612 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 1 and 4; positive HRTOFMS *m*/*z* $[M + H]^+$ 517.3160 (calcd for $C_{30}H_{45}O_7$, 517.3160).

*Ganoleuconin C (**3**):* colorless powder; $[\alpha]^{25}_D$ +90.10 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 220 (4.10), 260 (2.00) nm; CD (*c* 1.85×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 250 (+3.2), 347 (−0.7) nm; IR (neat) ν_{max} 3320, 2979, 2952, 2925, 2835, 2820, 1725, 1702, 1622, 1588, 1441, 1368, 1374, 1236, 856, 612 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 1 and 4; positive HRTOFMS *m*/*z* $[M + H]^+$ 559.3266 (calcd for $C_{32}H_{47}O_8$, 559.3265).

*Ganoleuconin D (**4**):* colorless powder; $[\alpha]^{25}_D$ +83.00 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.10), 262 (2.00) nm; CD (*c* 2.05×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 260 (+3.6), 350 (−0.4) nm; IR (neat) ν_{max} 3320, 2961, 2952, 2925, 2850, 2840, 1714, 1702, 1622, 1588, 1441, 1368, 1374, 1236, 856, 615 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 1 and 4; positive HRTOFMS *m*/*z* $[M + H]^+$ 573.3060 (calcd for $C_{32}H_{45}O_9$, 573.3058).

*Ganoleuconin E (**5**):* colorless powder; $[\alpha]^{25}_D$ +93.50 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 225 (4.10), 260 (2.00) nm; CD (*c* 1.96×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 260 (+4.5), 355 (−0.3) nm; IR (neat) ν_{max} 3325, 2979, 2954, 2910, 2832, 2812, 1725, 1710, 1703, 1622, 1588, 1441, 1368, 1254, 1372, 1236, 836, 612 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 1 and 4; positive HRTOFMS *m*/*z* $[M + H]^+$ 531.2952 (calcd for $C_{30}H_{43}O_8$, 531.2952).

*Ganoleuconin F (**6**):* colorless powder, $[\alpha]^{25}_D$ +106.00 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.10), 262 (2.00) nm; CD (*c* 1.55×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 226 (+3.2), 267 (+2.4), 310 (−0.9) nm; IR (neat) ν_{max} 3315, 3210, 2961, 2952, 2918, 2849, 2828, 1723, 1713, 1700, 1619, 1588, 1425, 1375, 1365, 1230, 856, 736, 615 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 1 and 4; positive HRTOFMS *m*/*z* $[M + H]^+$ 571.2902 (calcd for $C_{32}H_{43}O_9$, 571.2902).

*Ganoleuconin G (**7**):* colorless powder; $[\alpha]^{25}_D$ +50.30 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.50), 254 (2.10) nm; CD (*c* 1.86×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 260 (+3.6), 355 (−0.7) nm; IR (neat) ν_{max} 3310, 2979, 2960, 2920, 2855, 2832, 1713, 1701, 1639, 1579, 1440, 1390, 1360, 870, 725, 620 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 2 and 5; positive HRTOFMS *m*/*z* $[M + H]^+$ 487.3418 (calcd for $C_{30}H_{47}O_5$, 487.3418).

*Ganoleuconin H (**8**):* colorless powder; $[\alpha]^{25}_D$ +36.00 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.50), 254 (2.10) nm; CD (*c* 2.05×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 225 (+3.2), 267 (+2.3), 305 (−0.5) nm; IR (neat) ν_{max} 3310, 2979, 2960, 2920, 2855, 2825, 1723, 1715, 1700, 1639, 1574, 1430, 1380, 1360, 870, 724, 618 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 2 and 5; positive HRTOFMS *m*/*z* $[M + H]^+$ 485.3263 (calcd for $C_{30}H_{45}O_5$, 485.3262).

*Ganoleuconin I (**9**):* colorless powder; $[\alpha]^{25}_D$ +50.20 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.50), 254 (2.10) nm; CD (*c* 1.88×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 255 (+3.9), 345 (−0.8) nm; IR (neat) ν_{max} 3325, 2980, 2948, 2910, 2845, 2835, 1702, 1685, 1619, 1557, 1424, 1378, 1360, 870, 724, 620 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 2 and 5; positive HRTOFMS *m*/*z* $[M + H]^+$ 487.3419 (calcd for $C_{30}H_{47}O_5$, 487.3418).

*Ganoleucoin J (**10**):* yellow powder; $[\alpha]^{25}_D$ +70.50 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.29), 260 (2.12) nm; CD (*c* 1.70×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 250 (+4.1), 352 (−0.7) nm; IR (neat) ν_{max} 3310, 2977, 2969, 2922, 2875, 2860, 1723, 1706, 1685, 1641, 1577, 1457, 1379, 1372, 875, 740, 615 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 2 and 5; positive HRTOFMS *m*/*z* $[M + H]^+$ 717.3480 (calcd for $C_{38}H_{53}O_{13}$, 717.3481).

*Ganoleucoin K (**11**):* yellow powder, $[\alpha]^{25}_D$ +42.40 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.10), 260 (2.12) nm; CD (*c* 1.92×10^{-3} M, MeOH) λ_{max} ($\Delta\varepsilon$) 225 (+4.2), 280 (+2.4), 310 (−0.8) nm; IR (neat) ν_{max} 3308, 2975, 2960, 2912, 2885, 2854, 1715, 1701, 1683, 1625, 1450, 1380, 1372, 826, 739, 615 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 2 and 5; positive HRTOFMS *m*/*z* $[M + H]^+$ 673.3217 (calcd for $C_{36}H_{49}O_{12}$, 673.3219).

*Ganoleucoin L (**12**):* yellow powder; $[\alpha]^{25}_D$ +86.75 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.20), 262 (2.00) nm; CD (*c* 2.01×10^{-3}

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M, MeOH) λ_{max} ($\Delta\varepsilon$) 224 (+3.9), 282 (+2.5), 305 (−0.9) nm; IR (neat) ν_{max} 3325, 2960, 2949, 2910, 2870, 2860, 1716, 1703, 1684, 1625, 1590, 1447, 1380, 1372, 1232, 872, 740, 615 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 2 and 5; positive HRTOFMS m/z $[M + H]^+$ 657.3270 (calcd. for $C_{36}H_{49}O_{11}$, 657.3269).

Ganoleuconin M (13): yellow powder; $[\alpha]^{25}_D$ +23.00 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 248 (4.12), 254 (3.99),377(2.10) nm; IR (neat) ν_{max} 3380, 2930, 2860, 1725, 1704, 1642, 1440, 1403, 1348, 1301, 1239, 1167, 1018, 1000, 745, 649 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 3 and 6; positive HRTOFMS m/z $[M + H]^+$ 799.5508 (calcd for $C_{51}H_{75}O_7$, 799.5507).

Ganoleuconin N (14): yellow powder; $[\alpha]^{25}_D$ +19.20 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 245 (3.60), 254 (3.51), 377(1.99) nm; IR (neat) ν_{max} 3452, 2925, 2820, 1725, 1725, 1630, 1460, 1423, 1345, 1301, 1234, 1190, 1128, 1023, 1000, 654 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 3 and 6; positive HRTOFMS m/z $[M + H]^+$ 799.5510 (calcd for $C_{51}H_{75}O_7$, 799.5507).

Ganoleuconin O (15): yellow powder; $[\alpha]^{25}_D$ +23.20 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 248 (3.52), 254 (3.40), 377 (2.00) nm; IR (neat) ν_{max} 3452, 2939, 2829, 1719, 1680, 1635, 1462, 1425, 1348, 1301, 1234, 1190, 1128, 1023, 980 cm^{-1}; for ^1H and ^{13}C NMR data see Tables 3 and 6; positive HRTOFMS m/z $[M + H]^+$ 799.5510 (calcd for $C_{51}H_{75}O_7$, 799.5507).

Ganoleuconin P (16): yellow powder; $[\alpha]^{25}_D$ +9.00 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 200 (3.50), 254 (3.40), 377 (2.00) nm; IR (neat) ν_{max} 3452, 2925, 2849, 1719, 1700, 1635, 1460, 1423, 1345, 1301, 1234, 1190, 1128, 1023, 1000, 649 cm^{-1}; for ^1H and ^{13}C NMR data see Table 3 and 6; positive HRTOFMS m/z $[M + H]^+$ 815.5456 (calcd for $C_{51}H_{75}O_8$, 815.5456).

Determination of the Absolute Configuration in Compounds 10−12.[23] (*S*)-1-Phenylethylamine (19 μL, 150 μmol), Et_3N (32 μL, 225 μmol), PyBOP (58.5 mg, 115 μmol), and HOBt (20.0 mg, 150 μmol) were added to a solution of compound **10** (50.1 mg, 75 μmol) in 0.5 mL of DMF at 0 °C. The mixture was stirred at room temperature for 5 h. The reaction was quenched with dilute aqueous HCl, and the organic materials were extracted with EtOAc to afford a yellow residue, which was purified by Sephadex LH-20 CC eluting with MeOH to give amide **10a** (54.2 mg). Compound **10a** was further transformed into **10b** (5.4 mg) by the method described in an earlier report.[23] The ^1H NMR spectrum of **10b** was consistent with that of (3*R*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide rather than the 3*S* isomer reported in the literature.[39] The absolute configuration of the HMG moiety in compounds **11** and **12** (each 10.0 mg) was determined using the same method, and all the final products were proved to be (3*R*)-5-*O*-acetyl-1-[(*S*)-phenylethyl]mevalonamide.

(3R)-5-O-Acetyl-1-[(S)-phenylethyl]mevalonamide (10b): colorless oil; $[\alpha]^{25}_D$ −60.10 (c 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 248 (3.52), 254 (3.40) nm; IR (neat) ν_{max} 3452, 2939, 1740, 1640, 1620 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 7.27−7.37 (5H, m, C_6H_5-1′), 6.34 (1H, d, J = 7.3 Hz, NH), 5.14 (1H, quintet, J = 7.2 Hz, H-1′), 4.23 (2H, t, J = 6.8 Hz, H-5), 2.41, 2.28 (each 1H, d, J = 14.4 Hz, H-2), 2.04 (3H, s, CH_3COO), 1.85 (2H, m, H-4), 1.50 (3H, d, J = 6.8 Hz, H-2′), 1.23 (3H, s, H-6); positive HRTOFMS m/z $[M + H]^+$ 277.1623 (calcd for $C_{16}H_{23}NO_3$, 277.1622).

Alkaline Hydrolysis of Compounds 13−15. According to a published procedure,[42] compound **13** (3.0 mg) dissolved in 2% sodium methanolate−methanol (4 mL) was stirred at room temperature for 1 h. The reaction mixture was neutralized with 10% acetic acid. After removal of the solvent from the filtrate under reduced pressure, the residue was dissolved in water (10 mL) and extracted with ethyl acetate (10 mL). The organic solvents were evaporated and purified by preparative TLC on silica gel (dichloromethane−methanol, 20:1) to give compound **26** (1.8 mg). Compounds **14** and **15** were hydrolyzed by the same method to give compound **26**.

Inhibition Assay against α-Glucosidase from Baker's Yeast. As described in our earlier work,[43] the bioassay was conducted using a 96-well plate, and the absorbance was determined at 405 nm using a Spectra Max 190 microplate reader (Molecular Devices Inc.). The control was prepared by adding a phosphate buffer instead of test compounds. The blank was prepared by adding phosphate buffer instead of the α-glucosidase. The inhibition rates (%) = [(OD control − OD control blank) − (OD test − OD test blank)]/(OD control − OD control blank) × 100%. Acarbose was utilized as the positive control with an IC_{50} of 273.1 μM.

Inhibition Assay against α-Glucosidase, Sucrase, and Maltase from Rat's Small Intestinal Mucosa. A slightly modified method of the rat intestinal mucosa assay developed by Kwon et al. was used.[44,45] A total of 1.0 g of rat-intestinal mucosa powder was suspended in 3 mL of 0.9% saline, and the suspension was sonicated at 4 °C 12 times (each 30 s). After centrifugation (10000*g*) at 4 °C for 30 min, the resulting supernatant was used as the enzyme solution for the assay. Each compound dissolved in DMSO (10 μL) was mixed with 20 μL of substrate (maltose, sucrose, *p*-nitrophenyl-α-D-glucopyranoside, respectively), 10 μL of enzyme solution, and 60 μL of 0.1 M phosphate buffer (pH 6.9). After preincubation for 40 min, a commercial kit (GOD assay, Jiancheng Biological Engineering Institue, Najing, China) was used to test the production of glucose. The absorbance was read at 550 nm under a Spectra Max 190 microplate reader (Molecular Devices Inc.). The blank was prepared by adding potassium phosphate buffer instead of enzyme. The control was prepared by adding potassium phosphate buffer instead of test compounds. The inhibition rates (%) = [(OD control − OD control blank) − (OD test − OD test blank)]/(OD control − OD control blank) × 100%.

Inhibition Assay against HMG-CoA Reductase. The HMG-CoA reductase prepared from pig liver microsomes was assayed by the previously described colorimetric method with a slight modification.[46,47] In brief, a mixture containing 10 μL of test compounds (final concentrations of 2.5, 5, 10, 20, 40, 80 μM), 20 μL of prepared enzyme (final concentration of about 4 U/mL), 40 μL of HMG-CoA (final concentration of 200 μM), and 10 μL of 0.1 mol/L potassium phosphate buffer (pH 7.0) (containing 3.5 mmol/L EDTA, 10 mmol/L dithiothreitol, and 0.1 g/L boving serum albumin) was incubated at 37 °C for 5 min. After that, 20 μL of NADPH solution (final concentration of 100 μM) was added to the reaction solution and incubated for 15 min at 37 °C. The assay was conducted in a 96-well plate, and the absorbance was determined at 340 nm using a Spectra Max 190 microplate reader (Molecular Devices Inc.). The blank was prepared by adding potassium phosphate buffer instead of HMG-CoA reductase. The control was prepared by adding potassium phosphate buffer instead of test compounds. The inhibition rates (%) = [(OD test − OD test blank) − (OD control blank − OD control)]/(OD control blank − OD control) × 100%.

Cytotoxicity Assay. The cytotoxicity against K562 and PC-3 cell lines of compounds **1−26** was tested using the MTT method as previously reported.[48]

Statistical Analyses. The bioactivity values were expressed as means of three independent experiments, and each was carried out in triplicate.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00331.

NMR spectra data of compounds **1−26** and **10b** (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +86 10 62566577. E-mail: liuhw@im.ac.cn (H.-W. Liu).

Author Contributions

⊥K. Wang, L. Bao, and W.-P. Xiong contributed equally to this article.

Notes

The authors declare no competing financial interest.

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