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# Triterpenes and meroterpenes from *Ganoderma lucidum* with inhibitory activity against HMGs reductase, aldose reductase and α-glucosidase

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ABSTRACT

Seven new compounds including four lanostane triterpenoids, lucidenic acids Q-S (**1–3**) and methyl ganoderate P (**4**), and three triterpene-farnesyl hydroquinone conjugates, ganolucinins A-C (**5–7**), one new natural product ganomycin J (**8**), and 73 known compounds (**9–81**) were isolated from fruiting bodies of *Ganoderma lucidum*. The structures of the compounds **1–8** were determined by spectroscopic methods. Bioactivities of compounds isolated were assayed against HMG-CoA reductase, aldose reductase, α-glucosidase, and PTP1B. Ganolucidic acid η (**39**), ganoderenic acid K (**44**), ganomycin J (**8**), and ganomycin B (**61**) showed strong inhibitory activity against HMG-CoA reductase with $IC_{50}$ of 29.8, 16.5, 30.3 and 14.3 μM, respectively. Lucidumol A (**67**) had relatively good effect against aldose reductase with $IC_{50}$ of 19.1 μM. Farnesyl hydroquinones ganomycin J (**8**), ganomycin B (**61**), ganomycin I (**62**), and triterpene-farnesyl hydroquinone conjugates ganoleuconin M (**76**) and ganoleuconin O (**79**) possessed good inhibitory activity against α-glucosidase with $IC_{50}$ in the range of 7.8 to 21.5 μM. This work provides chemical and biological evidence for the usage of extracts of *G. lucidum* as herbal medicine and food supplements for the control of hyperglycemic and hyperlipidemic symptoms.

## 1. Introduction

The traditional Chinese medicine (TCM), *Ganoderma* (G.) *lucidum* Karst is a well-known mushroom which has been used clinically in some Asian countries. It is termed “Lingzhi” in China, “Reishi” in Japan and “Yeongji” in Korea. It has long been used in TCM for the promotion of longevity and maintenance of vitality [1]. Modern chemical researches on *G. lucidum* have revealed a wide array of bioactive metabolites, such as polysaccharides, triterpenoids, meroterpenoids and sterols, alkaloids [2]. Bioactivity studies has shown that *G. lucidum* possess various biological properties, such as antihypertensive, anticancer, antiviral and immunomodulatory activities [3–6].

Metabolic syndrome characterized by insulin resistance, central obesity, elevated blood pressure and dyslipidemia have become a worldwide public health issue [7,8]. The current clinical drugs for the treatment of diabetes and metabolic syndrome (e.g. insulin, statins, fibrates and angiotensin-converting enzyme inhibitors) are still facing some problems due to the limitation of the therapeutic efficacy and the accompanying side effects. Considerable efforts have been made to develop a new drug that can ameliorate the metabolism of both glucose and lipids without side effects in the pharmaceutical industry. HMG-CoA reductase, aldose reductase and α-glucosidase are targets of various drugs to treat metabolic syndrome. In our continuous researches on bioactive components from *Ganoderma* species, some meroterpenoids and triterpenoids showed strong inhibitory activities against HMG-CoA reductase, aldose reductase or α-glucosidase activity [5,9].

In an early report, an in silico screening method was used to predict the interaction of *Ganoderma* constituents with the targets involved in the metabolic syndrome [10]. A number of triptepenes from *G. lucidum* were disclosed as putative bioactive agents for the treatment of metabolic syndrome. To confirm the hypoglycemic and hypolipidemic bioactivities of *Ganoderma* tripterpenes in vitro, we conducted chemically investigated on the fruiting bodies of *G. lucidum* collected from Sichuan and Fujian Province of China. As a result, 73 known compounds (Fig. 2 & Table 1) and 7 new compounds (Fig. 1) including four lanostane triterpenes (**1–4**) and three triterpene-farnesyl hydroquinone conjugates (**5–7**) were obtained. And one farnesyl hydroqui-

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**Table 1**
Name and formula of known compounds **9–81**.

| No. | Name | Chemical formula | Reference |
|---|---|---|---|
| 9 | Lucidenic acid N | $C_{27}H_{40}O_6$ | [32]; |
| 10 | Ganoderenic acid C | $C_{30}H_{44}O_7$ | [33] |
| 11 | Lucidenic acid P | $C_{29}H_{42}O_8$ | [34] |
| 12 | Ganoderic acid ζ | $C_{30}H_{42}O_7$ | [32]; |
| 13 | Ganoderic acid N | $C_{30}H_{42}O_8$ | [35] |
| 14 | Ganoderenic acid H | $C_{30}H_{40}O_7$ | [36] |
| 15 | Methyl lucidenate E | $C_{30}H_{42}O_8$ | [37] |
| 16 | Ganoderic acid C | $C_{30}H_{46}O_7$ | [33] |
| 17 | Ganoderic acid GS-2 | $C_{30}H_{44}O_7$ | [38] |
| 18 | Methyl ganoderate K | $C_{33}H_{48}O_9$ | [35] |
| 19 | 7β-Hydroxy-3,11,15-trioxolanosta-8,24(*E*)-dien-26-oic acid methyl ester | $C_{31}H_{42}O_7$ | [39] |
| 20 | 12β-Acetoxy-3β-hydroxy-7,11,15,23-tetraoxo-lanost-8,20E–diene-26-oic acid | $C_{32}H_{42}O_9$ | [40] |
| 21 | Lucidenic acid H | $C_{32}H_{44}O_9$ | [35] |
| 22 | Lucidenic acid C | $C_{27}H_{40}O_7$ | [41] |
| 23 | Ganoderic acid C6 | $C_{30}H_{42}O_8$ | [42] |
| 24 | Methyl lucidenate N | $C_{28}H_{40}O_7$ | [43] |
| 25 | Methyl lucidenate P | $C_{30}H_{44}O_8$ | [34] |
| 26 | Methyl ganoderenate A | $C_{31}H_{44}O_7$ | [44] |
| 27 | Methyl ganoderate A | $C_{31}H_{46}O_7$ | [45]; |
| 28 | 23-Dihydroganoderic acid N | $C_{31}H_{46}O_7$ | [39] |
| 29 | Methyl ganoderenate D | $C_{31}H_{42}O_7$ | [39] |
| 30 | Methyl lucidenate Q | $C_{28}H_{42}O_6$ | [34] |
| 31 | Methyl lucidenate A | $C_{28}H_{40}O_6$ | [34] |
| 32 | Methyl lucidenate F | $C_{28}H_{38}O_6$ | [46] |
| 33 | Ganolucidic acid γa | $C_{30}H_{46}O_7$ | [47] |
| 34 | Methyl ganoderate F | $C_{33}H_{44}O_9$ | [34] |
| 35 | Ganoderic acid θ | $C_{30}H_{42}O_8$ | [48] |
| 36 | Ganoderic acid ε | $C_{30}H_{44}O_7$ | [48] |
| 37 | Ganolucidic acid D | $C_{33}H_{44}O_6$ | [48] |
| 38 | Ganoderic acid J | $C_{30}H_{42}O_7$ | [41] |
| 39 | Ganoderic acid η | $C_{30}H_{44}O_7$ | [48] |
| 40 | Ganoderic acid G | $C_{30}H_{44}O_8$ | [33] |
| 41 | Ganoderenic acid B | $C_{30}H_{42}O_7$ | [33] |
| 42 | Ganoderic acid B | $C_{30}H_{44}O_7$ | [49] |
| 43 | Lucidenic acid E | $C_{29}H_{40}O_8$ | [41] |
| 44 | Ganoderenic acid K | $C_{32}H_{44}O_9$ | [50] |
| 45 | Ganoderic acid K | $C_{32}H_{46}O_9$ | [51] |
| 46 | Methyl ganoderate H | $C_{33}H_{46}O_9$ | [43] |
| 47 | Methyl ganoderate D | $C_{31}H_{44}O_7$ | [43] |
| 48 | Methyl ganoderate E | $C_{31}H_{42}O_7$ | [43] |
| 49 | Ganoderic acid F | $C_{32}H_{42}O_9$ | [33] |
| 50 | Ganoderic acid D | $C_{30}H_{42}O_7$ | [33] |
| 51 | Ganoderic acid A | $C_{30}H_{44}O_7$ | [49] |
| 52 | Ganolucidic acid A | $C_{30}H_{44}O_8$ | [52] |
| 53 | Methyl lucidenate D | $C_{30}H_{40}O_8$ | [37] |
| 54 | Ganoderic acid I | $C_{30}H_{44}O_8$ | [52] |
| 55 | 20-Hydroxyganoderic acid AM1 | $C_{30}H_{42}O_8$ | [53] |
| 56 | Methyl ganoderate A | $C_{31}H_{46}O_7$ | [54] |
| 57 | Ganoderic acid E | $C_{30}H_{40}O_7$ | [37] |
| 58 | Methyl ganoderate J | $C_{31}H_{44}O_7$ | [55] |
| 59 | Ganolucidic acid E | $C_{30}H_{44}O_5$ | [56] |
| 60 | Ganoderiol J | $C_{30}H_{46}O_4$ | [57] |
| 61 | Ganomycin B | $C_{21}H_{28}O_4$ | [58] |
| 62 | Ganomycin I | $C_{21}H_{26}O_4$ | [59] |
| 63 | 3,7,11-trioxo-5a-lanosta-8,24(E)-dien-26-oic acid | $C_{30}H_{42}O_5$ | [60] |
| 64 | Ganoderone A | $C_{30}H_{46}O_3$ | [61] |
| 65 | 15-Acetoxyganolucidic acid E | $C_{32}H_{46}O_6$ | [62] |
| 66 | Lucidadiol | $C_{30}H_{48}O_3$ | [63] |
| 67 | Lucidumol A | $C_{30}H_{48}O_4$ | [64] |
| 68 | Ganoderic acid DM | $C_{30}H_{44}O_4$ | [65] |
| 69 | Ganoderic acid TR | $C_{30}H_{44}O_4$ | [66] |
| 70 | Ganoderic acid T-Q | $C_{32}H_{46}O_5$ | [67] |
| 71 | Ganoderic acid S | $C_{30}H_{44}O_3$ | [68] |
| 72 | Ganoderic acid Sz | $C_{30}H_{44}O_3$ | [69] |
| 73 | Ganoderol A | $C_{30}H_{46}O_2$ | [61] |
| 74 | *Ganoderma* nondiol | $C_{30}H_{48}O_3$ | [70] |
| 75 | Ganoderic acid X | $C_{32}H_{48}O_5$ | [71] |
| 76 | Ganoleuconin M | $C_{51}H_{74}O_7$ | [5] |
| 77 | Ganoleuconin P | $C_{51}H_{74}O_8$ | [5] |
| 78 | Ganoleuconin N | $C_{51}H_{74}O_7$ | [5] |
| 79 | Ganoleuconin O | $C_{51}H_{74}O_7$ | [5] |
| 80 | Lucialdehyde A | $C_{30}H_{46}O_2$ | [72] |
| 81 | Ganoderal A | $C_{30}H_{44}O_2$ | [51] |

none (**8**) (Fig. 1) was first reported as a natural product. Bioactivities of 81 compounds were tested against HMG-CoA reductase, aldose reductase (AR), α-glucosidase, and protein tyrosine phosphatase 1B (PTP1B). Herein, we reported the isolation, structural determination, and bioactivities of compounds isolated from the fruiting bodies of *G. lucidum*.

## 2. Experimental

### 2.1. General experimental procedures

IR spectral data were generated by Nicolet IS5 FT-IR spectrophotometer and UV spectra were measured via Thermo Genesys-10S UV–vis spectrophotometer. Optical rotations were recorded on an Anton Paar MCP 200 Automatic Polarimeter. CD spectra were acquired by the instrument JASCO *J*-815 Spectropolarimeter. NMR spectral data were obtained with Bruker Avance-500 spectrometer ($CDCl_3$, $\delta_H$ 7.26/$\delta_C$77.16) and HSQC and HMBC experiments were optimized for 145.0 and 8.0 Hz, respectively. HR-ESI-MS data were measured using an Agilent Accurate-Mass-Q-TOF LC/MS 6520 instrument. OD absence was tested on Spectra Max 190 microplate reader (Molecular Devices Inc.).

Solvent used for extraction and chromatographic separation was analytical pure, including methanol, dichloromethane, ethyl acetate, etc., while for TLC was carried out on Silica gel $HSGF_{254}$ and the spots were visualized by UV at 254 nm or spraying with 10% $H_2SO_4$ assisted with heating. Silica gel (Qingdao Haiyang Chemical Co., Ltd., People's Republic of China) and Sephadex LH-20 (Amersham Biosciences) were used for column chromatography (CC). 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.

### 2.2. Fungal material

Fruiting bodies of *G. lucidium* from Sichuan province (China) and Fujian province (China) were identified on the basis of morphological characteristics as previously described [11].

### 2.3. Extraction and isolation

The air-dried and powdered fruiting bodies of *G. lucidium* (GF) from Fujian province (2.2 kg) were extracted for three times with ethyl alcohol (3 × 20 L) refluxing 2 hours per time. Then organic solvent was evaporated to dryness under vacuum to afford the crude extract (99.7 g). The ethanol extract (95.4 g) was partitioned between petroleum ether and water, subsequently ethyl acetate and water. The EtOAc extract (45.6 g) was subjected on ODS CC using a gradient of MeOH-$H_2O$ (20% to 100%) to give 13 subfractions (GF-1 to GF-13).

Fraction GF-3 (13.42 g) subjected to silica gel CC was eluted with a $CH_2Cl_2$-MeOH gradient solvent system (100:0 to 0:100, *v/v*) to yield 8 fractions (GF-3-1 to GF-3-8). Fraction GF-3-3-4 was obtained by fraction GF-3-3 (1.82 g) eluted with MeOH-$H_2O$ (35%). Then compound **1** (3.1 mg, $t_R$ 78.2 min) and compounds **9**, **11**, **14** to **16**, **19**, **21** to **24** and **43** to **48** (3.1, 7.2, 3.6, 5.8, 6.5, 5.7, 10.8, 22.6, 13.5, 5.4, 29.0, 30.5, 200.4, 32.8, 5.5 and 2.9 mg, $t_R$ 24.2, 26.3, 29.5, 35.4, 38.2, 40.8, 42.6, 44.4, 48.7, 51.3, 55.6, 60.3, 66.4, 73.3, 81.2 and 91.1 min) were isolated from fraction GF-3-3-4 (942.6 mg) by RP-HPLC (C8) using 28%