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Chemical constituents from the fungus *Ganoderma tropicum* (Jungh.) Bres. and their cytotoxic activities

Qing-yun Ma[1#], Li-li Hu[1#], Ling-mei Kong[2], Sheng-zhuo Huang[1], Hao-fu Dai[1], Yan LI[2]* and You-xing Zhao[1]*

[1]Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China.
[2]State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, China.

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The chemical investigation on the fruiting bodies of white rot fungus *Ganoderma tropicum* (Jungh.) Bres. led to the isolation of ten natural compounds by column chromatography on silica gel and sephadex LH-20. Their structures were elucidated by nuclear magnetic resonance (NMR) analysis as well as comparison with those reported in the literatures. Compounds 1-10 were isolated for the first time from this fungus. The bioassay of cytotoxic activities of seven compounds was evaluated against five human tumor cell lines HL-60, SMMC-7721, A-549, MCF-7 and SW-480. Compound jacareubin (1) displayed obvious cytotoxic activities on HL-60, SMMC-7721, A-549, MCF-7 and SW-480 with IC_{50} values of 7.49, 9.04, 4.44, 21.61 and 14.02 μmol.L^{-1}, respectively. Compound 22*E*-7*α*-methoxy-5*α*,6*α*-epoxyergosta-8(14),22-dien-3*β*-ol (9) also exhibited definite cytotoxic activities on HL-60, MCF-7 and SW-480. The discovery of cytotoxic compounds exhibited potential effect on anti-tumor of *G. tropicum*.

Key words: *Ganoderma tropicum*, chemical constituents, cytotoxic activities.

INTRODUCTION

The family Ganodermataceae comprising more than 200 fungi species is mainly distributed in the tropical and subtropical areas of Asia, Australia, Africa and America, among which more than 100 species grow in China and 78 wild species were found in Hainan Province (Wu et al., 1998). *Ganoderma* was the main genus of Ganodermataceae. “Lingzhi” is the Chinese name given to *Ganoderma* as isolated in that vast and diverse country. *Ganoderma* is a basidiomycete white rot fungus which has been used for medicinal purposes for centuries particularly in China, Japan and Korea (Paterson, 2006). A great deal of work has been carried out on *Ganoderma lucidum* and *Ganoderma sinense* which were recorded in Chinese Pharmacopoeia. Recent researches on chemical constituents of *Ganoderma* showed the presence of natural products including triterpenes, steroids, alkaloids, flavonoids, polysaccharides, and fatty acids (Aryantha et al., 2002). Polysaccharides and triterpenes have been most thoroughly investigated from *G. lucidum* and related species, many of which were found to be beneficial for the prevention and treatment of hypertension, diabetes, hepatitis, cancers and AIDS (Liu et al., 2008; Huang and Xiao, 2008). *Ganoderma tropicum* (Jungh.) Bres. is the main wild *Ganoderma* resource distributed in tropical area of China. *G. tropicum* is used widely as a health supplement and ‘‘herbal’’ medicine. It can treat liver disease (Liu et al., 2009). In Fujian province, *G. tropicum* has been also used to treat coronary heart disease (Liu et al., 2009). However, small-molecule natural products of wild *G. tropicum* and their biological activities are still not

*Corresponding author. E-mail: zhaoyx1011@163.com, liyanb@mail.kib.ac.cn.

[#]These authors contributed equally to this work.

reported. In order to find more bioactive constituents from *Ganoderma* resources and develop the wild species, the chemical investigation of *G. tropicum* was carried out. In this paper, we reported the isolation, structural elucidation and cytotoxicities of natural compounds from the fruiting bodies of *G. tropicum.*

MATERIALS AND METHODS

Fungus

Ganoderma tropicum were collected from Lingshui County, Hainan Province, China, in May 2011, and identified by Professor WU Xing-liang of Hainan University. A voucher specimen (No. 2011LZ01) is deposited in the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Science.

General experimental procedures

The NMR spectra were recorded on a Bruker AV-400 spectrometer, using TMS as an internal standard. The ESI-MS were measured with an API QSTAR Pulsar mass spectrometer. Column chromatography was carried out with silica gel (Marine Chemical Industry Factory, Qingdao, China) and Sephadex LH-20 (Merck, Darmstadt, Germany). The fractions were monitored by TLC, and spots were visualized by heating Si gel plats sprayed with 5% H_2SO_4 in ethanol. MestReNova version 6.1.0-6224 (Mestrelab Research S.L.) was used for data analysis.

Extraction and isolation

The dry fruiting bodies of *G. tropicum* (6.5 kg) were powdered and extracted with ethanol (95%) at room temperature for three times. The combined extracted ethanol solution was evaporated under reduced pressure, then suspended in water and partitioned with ethyl acetate and *n*- butyl alcohol successively. The ethyl acetate and *n*-butyl alcohol extracts were separately combined and evaporated to dryness under reduced pressure, which obtained and designated as ethyl acetate Fraction (200.0 g) and *n*- butyl alcohol Fraction (32.0 g), respectively. The ethyl acetate Fraction (200.0 g) was separated into 9 fractions (Fr1-Fr9) on silica gel column chromatography (CC) using step gradient elution of petroleum ether- ethyl acetate (20:1, 15:1, 10:1, 8:1, 5:1, 1:1, 1:5 and 0:1). Fraction 5 (5.8 g) was subjected to CC over silica gel eluted with petroleum ether- ethyl acetate (3:1) to give 6 subfractions 5a-5f. Subfraction 5b was chromatographed and using eluent as petroleum ether- ethyl acetate (4:1) to yield compounds **1** (60.0 mg), **2** (8.0 mg) and **4** (34.0 mg). Fraction 6 (15.7 g) was subjected to CC over silica gel eluted with petroleum ether- ethyl acetate (2:1) to give four subfractions 6a-6d. Subfraction 6b (2.8 g) was chromatographed and using eluent as petroleum ether- ethyl acetate (3:1) to yield compounds **3** (6.0 mg), **5** (21.0 mg) and **6** (5.0 mg). Compounds **7** (19.0 mg) and **8** (13.0 mg) were obtained from Subfraction 6c (3.7 g) by repeated silica gel CC with petroleum ether- ethyl acetate (2:1) and then chromatographed over Sephadex LH-20 column, using chloroform-methanol (1:1) as solvent. Subfraction 6d (2.3 g) was subjected to chromatography on silica gel column with petroleum ether-acetone (2:1) as eluent, leading to the isolation of compounds **9** (35.0 mg) and **10** (15.0 mg).

Cytoxicity assay

Each tested compound was dissolved in DMSO to a stock concentration of 10 mM and then diluted to the required concentrations with the medium. Cytotoxicity of compounds against five human tumor cell lines: HL-60 (leukemia), SMMC-7721 (liver carcinoma), A-549 (lung carcinoma), MCF-7 (mammary carcinoma), and SW-480 (colon carcinoma) were measured. Briefly, cells were placed in 96-well plates 12 h before treatment with initial density of 5000 cells/well and continuously exposed to different concentrations (40, 8, 1.6, 0.32, and 0.064 mol.L^{-1}) of compounds for 48 h, with cisplatin (Sigma, USA) as the positive control. Inhibition rates of cell proliferation after compound treatment were determined by MTT assay, as described previously (Niu et al., 2002; Monks et al., 1991), and IC_{50} calculated with Reed and Muench method (Reed and Muench, 1938).

RESULTS

Structural identification

Chemical study on the fruiting bodies of *G. tropicum* resulted in the isolation of ten compounds (Figure 1) by column chromatography on silica gel and sephadex LH-20. These structures were established as jacareubin (**1**), 6-deoxyjacareubin (**2**), 1*H*-indole-3-carboxylic acid (**3**), ganoderic acid Y (**4**), 20(29)-lupen-3-ol (**5**), 3β,5α,9α-trihydroxy-(22*E*,24R)-ergosta-7,22-dien-6-one (**6**), 5α,8α-epidioxyergosta-6,22-dien-3β-ol (**7**), ergosta-8 (14),22*E*-diene-3β,5α,6β,7α-tetraol (**8**), 22*E*-7*α*-methoxy-5*α*,6*α*-epoxyergosta-8(14),22-dien-3*β*-ol (**9**), and 3β,7β-dihydroxy-11,15,23- trioxolanost-8,16-dien-26-oic acid (**10**) by NMR analysis as well as comparison with those reported in the literatures.

Compound **1**: yellow amorphous powder, $C_{18}H_{14}O_6$; ESI-MS *m/z* 349 $[M+Na]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ 13.51 (1H, s, 1-OH), 7.50 (1H, d, *J* = 8.7 Hz, H-8), 6.93 (1H, d, *J* = 8.7 Hz, H-7), 6.57 (1H, d, *J* =10.1 Hz, H-13), 6.37 (1H, s, H-4), 5.71 (1H, d, *J* = 10.1 Hz, H-14), 1.41 (3H, s, H-15), 1.41 (3H, s, H-16); ^{13}C NMR (125 MHz, DMSO-d_6) δ 159.5 (C-1), 103.7 (C-2), 156.9 (C-3), 94.6 (C-4), 132.5 (C-5), 152.1 (C-6), 113.2 (C-7), 115.9 (C-8), 179.9 (C-9), 78.1 (C-12), 114.5 (C-13), 128.1 (C-14), 27.9 (C-15), 27.9 (C-16), 156.4 (C-4a), 112.9 (C-8a), 102.2 (C-9a), 146.0 (C-10a). Spectral data are consistent with those in the literature of Rukachaisirikul et al. (2003).

Compound **2**: yellow amorphous powder, $C_{18}H_{14}O_5$; ESI-MS *m/z* 333 $[M+Na]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) δ 13.16 (1H, s, OH-1), 7.69 (1H, dd, *J* = 7.8, 1.4 Hz, H-8), 7.24 (1H, dd, *J* = 7.9, 1.4 Hz, H-6), 7.18 (1H, dd, *J* = 7.9, 7.8 Hz, H-7), 6.71 (1H, d, *J* =10.1 Hz, H-13), 6.39 (1H, s, H-4), 5.59 (1H, d, *J* = 10.1 Hz, H-14), 1.46 (3H, s, H-15), 1.46 (3H, s, H-16);^{13}C-NMR (125 MHz, DMSO-d_6) δ 160.9 (C-1), 104.9 (C-2), 156.7 (C-3), 95.1 (C-4), 146.3 (C-5), 120.1 (C-6), 124.2 (C-7), 114.9 (C-8), 180.9 (C-9), 78.1 (C-12), 114.5 (C-13), 128.1 (C-14), 27.9 (C-15), 27.9 (C-16), 156.4 (C-4a), 120.9 (C-8a), 103.2 (C-9a), 146.0 (C-10a). Spectral data are consistent with those in the literature of Goh and Jantan (1991).

Compound **3**: yellow amorphous powder, $C_9H_7NO_2$; ESI-MS *m/z* 162 $[M+H]^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 8.09 (1H, d, *J* = 7.2 Hz, H-4), 7.97 (1H, s, H-2), 7.45 (1H,

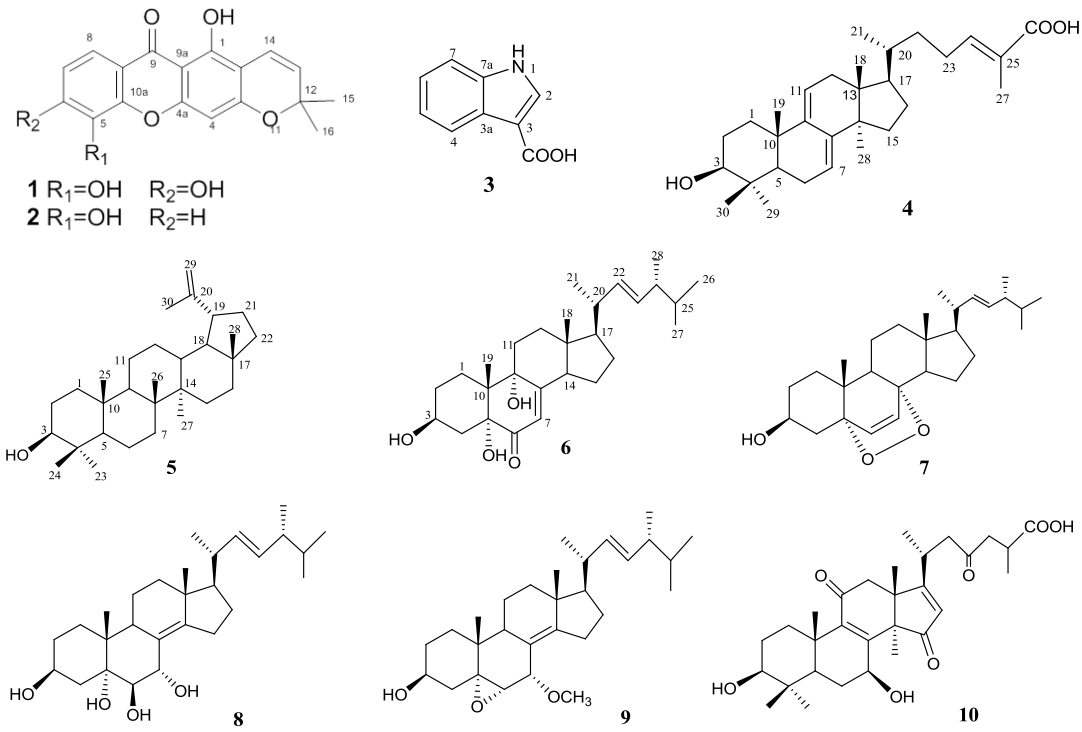

Figure 1. The structures of compounds **1-10**.

d, *J* = 7.2 Hz, H-7), 7.21|(2H, m, H-5 and H-6); ^{13}C NMR (125 MHz, CD_3OD) *δ* 133.4 (C-2), 127.6 (C-3), 122.4 (C-4), 123.6 (C-5), 122.0 (C-6), 112.9 (C-7), 108.7 (C-3a), 138.2 (C-7a), 169.2 (COOH). Spectral data are consistent with those in the literature of Lai et al. (2008).

Compound **4**: needle crystals, $C_{30}H_{46}O_3$; ESI-MS *m/z* 453 $[M-H]^-$; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 6.75 (1H, t, *J* = 7.0 Hz, H-24), 5.42 (1H, s, H-7), 5.26 (1H, s, H-11), 3.18 (1H, dd, *J* = 4.7, 11.2 Hz, H-3), 1.76 (3H, s, H-27), 0.93 (3H, s, H-19), 0.92 (3H, s, H-29), 0.87 (3H, d, *J* = 5.6 Hz, H-21), 0.81 (6H, s, H-18, H-28), 0.50 (3H, s, H-30); ^{13}C NMR (125 MHz, $CDCl_3$) as shown in Table 1. Spectral data were consistent with those in the literature of Toth et al. (1983).

Compound **5**: white amorphous powder, $C_{30}H_{50}O$; ESI-MS *m/z* 449 $[M+Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 4.68 (1H, d, *J* = 2.2 Hz, H-29) and 4.56 (1H, d, *J* = 2.2 Hz, H-29), 3.19 (1H, dd, *J* = 4.9, 11.4 Hz, H-3), 1.68 (3H, s, H-30), 1.03 (3H, s, H-24), 0.96 (3H, s, H-23), 0.94 (3H, s, H-27), 0.83 (3H, s, H-26), 0.79 (3H, s, H-28), 0.76 (3H, s, H-25); ^{13}C NMR (125 MHz, $CDCl_3$) as shown in Table 1. Spectral data are consistent with those in the literature of Reynolds et al. (1986).

Compound **6**: white needle crystals, $C_{28}H_{44}O_4$; ESI-MS *m/z* 467 $[M+Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 5.57 (1H, s, H-7), 5.19 (1H, dd, *J* = 7.4, 15.2 Hz, H-23), 5.12 (1H, dd, *J* = 8.3, 15.2 Hz, H-22), 3.92 (1H, m, H-3), 0.98 (3H, d, *J* = 6.6 Hz, H-21), 0.93 (3H, s, H-19), 0.87 (3H, d, *J* = 6.8 Hz, H-28), 0.78 (6H, d, *J* = 7.1 Hz, H-26 and H-27), 0.57 (3H, s, H-18); ^{13}C NMR (125 MHz, $CDCl_3$) as shown in Table 1. Spectral data are consistent with those in the literature of Yaoita et al. (1998).

Compound **7**: needle crystal, $C_{28}H_{44}O_3$; ESI-MS *m/z* 451 $[M+Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 6.49 (1H, d, *J* = 8.5 Hz, H-7), 6.23 (1H, d, *J* = 8.5 Hz, H-6), 5.23 (1H, dd, *J* = 7.6, 15.2 Hz, H-23), 5.14 (1H, dd, *J* = 8.2, 15.2 Hz, H-22), 3.96 (1H, m, H-3), 0.99 (3H, d, *J* = 6.6 Hz, H-21), 0.91 (3H, d, *J* = 3.5 Hz, H-28), 0.88 (3H, s, H-19), 0.82 (3H, s, H-18), 0.81 (6H, d, *J* = 3.0 Hz, H-26 and H-27); ^{13}C NMR (125 MHz, $CDCl_3$) as shown in Table 1. Spectral data are consistent with those in the literature of Wan et al. (1999).

Compound **8**: needle crystals, $C_{28}H_{46}O_4$; ESI-MS *m/z* 469 $[M+Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 5.23 (1H, m, H-23), 5.19 (1H, m, H-22), 4.41 (1H, d, *J* = 3.6 Hz, H-6), 3.91 (1H, m, H-3), 3.14 (1H, d, *J* = 3.6 Hz, H-7), 1.01 (3H, d, *J* = 6.6 Hz, H-21), 0.91 (3H, d, *J* = 6.9 Hz, H-26), 0.86 (6H, s, H-18 and H-19), 0.83 (3H, d, *J* = 6.9 Hz, H-28), 0.82 (3H, d, *J* = 6.9 Hz, H-27); ^{13}C NMR (125 MHz, $CDCl_3$) as shown in Table 1. Spectral data are consistent with those in the literature of Sun et al. (2006).

Compound **9**: colorless oil, $C_{29}H_{46}O_3$; ESI-MS *m/z* 465 $[M+Na]^+$; ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 5.23 (1H, m, H-23), 5.18 (1H, m, H-22), 4.16 (1H, d, *J* = 3.2 Hz, H-7), 3.92 (1H, m, H-3), 3.41 (3H, s, 7-OCH_3), 3.19 (1H, d, *J* = 3.2 Hz, H-6), 1.01 (3H, d, *J* = 6.7 Hz, H-21), 0.91 (3H, d,

Table 1. ^{13}C NMR chemical shifts of compounds **4 - 10** (125 MHz).

No.	4	5	6	7	8	9	10
1	35.8	39.0	25.6	37.1	32.4	32.3	**34.8**
2	27.9	27.7	29.8	34.8	31.2	31.3	**27.8**
3	78.8	79.3	66.9	66.6	68.8	68.9	**78.4**
4	38.7	39.2	36.2	28.8	39.4	39.8	**38.8**
5	48.7	55.6	79.1	82.3	67.9	65.3	**49.5**
6	23.0	18.6	199.2	135.3	65.2	58.7	**26.2**
7	120.3	34.6	119.7	130.9	61.5	72.8	**67.3**
8	142.6	40.3	164.9	79.6	125.3	122.6	**158.4**
9	146.0	50.7	74.6	51.2	38.9	40.4	**142.1**
10	37.4	37.5	41.7	37.1	36.0	36.1	**39.3**
11	116.2	21.2	28.5	20.8	19.1	19.4	**198.5**
12	37.8	25.4	34.9	39.5	36.7	36.6	**44.5**
13	43.8	38.3	45.3	44.7	43.0	43.3	**51.8**
14	50.3	43.1	51.8	51.8	152.7	153.4	**58.8**
15	27.5	27.7	22.4	23.5	25.1	25.0	**211.2**
16	31.5	35.9	27.9	30.2	27.3	27.4	**123.6**
17	50.9	43.3	56.0	56.3	57.0	56.9	**189.1**
18	15.7	48.6	12.3	13.0	17.8	18.3	**31.5**
19	22.7	48.3	20.2	18.3	18.2	16.7	**18.7**
20	36.2	151.3	40.3	39.8	39.7	39.4	**28.4**
21	18.3	30.1	21.1	21.0	21.4	21.4	**21.0**
22	34.9	41.1	135.2	135.6	135.4	135.5	**49.8**
23	25.8	28.3	132.5	132.4	132.4	132.3	**206.4**
24	144.0	15.7	42.9	42.9	43.1	43.0	**45.6**
25	127.2	16.4	33.1	33.2	33.2	33.2	**35.0**
26	170.9	16.3	19.7	19.8	16.7	20.1	**179.5**
27	12.1	14.8	20.0	20.1	19.8	19.8	**17.2**
28	25.5	18.3	17.6	17.7	20.1	17.7	**33.1**
29	28.1	109.6	-	-	-	-	**28.4**
30	15.8	19.6	-	-	-	-	**15.7**
OMe	**-**	**-**	**-**	**-**	**-**	**54.7**	**-**

J = 6.8 Hz, H-28), 0.86 (6H, s, H-18 and H-19), 0.82 (6H, d, *J* = 6.5 Hz, H-26 and H-27); ^{13}C NMR (125 MHz, $CDCl_3$) as shown in Table 1. Spectral data are consistent with those in the literature of Gao et al. (2010).

Compound **10**: yellow powder, $C_{30}H_{42}O_7$; ESI-MS *m/z* 537 $[M+Na]^+$; ^1H NMR (500 MHz, $CDCl_3$): δ 5.73 (1H, s, H-16), 4.79 (1H, dd, *J* = 7.7, 9.6 Hz, H-7), 3.30 (1H, m, H-3), 1.47 (3H, s, H-28), 1.23 (3H, d, *J* = 6.7 Hz, H-21), 1.22 (3H, s, H-18), 1.21 (3H, d, *J* = 3.5 Hz, H-27), 1.18 (3H, s, H-19), 1.04 (3H, s, H-29), 0.85 (3H, s, H-30); ^{13}C NMR (125 MHz, $CDCl_3$) as shown in Table 1. Spectral data are consistent with those in the literature of Guan et al. (2007).

Cytotoxic activity

The cytotoxicity against five tumor cell lines by MTT methods for seven compounds (**1**, **4**, **5**, **7**-**10**) was tested. As a result, compound **1** displayed obvious cytotoxic activities on HL-60, SMMC-7721, A-549, MCF-7 and SW-480 with IC_{50} values of 7.49, 9.04, 4.44, 21.61 and 14.02 $\mu mol.L^{-1}$, respectively. Compound **9** also exhibited definite cytotoxic activities on HL-60, MCF-7, and SW-480 with IC_{50} values of 15.98, 16.28 and 13.26 $\mu mol.L^{-1}$, respectively. While compounds (**4**, **5**, **7**, **8**, **10**) had no cytotoxic activities against five tumor cell lines.

DISCUSSION

Many studies have been carried out on *Ganoderma spp.* especially on *G. lucidum* and *G. sinense* recorded in Chinese Pharmacopoeia (Paterson, 2006). There is little work about *G. tropicum* widely used for medicine by local populace except for its research on cultivation (Liu et al., 2009). The investigation on its chemical components and

pharmacological activities would provide basis for the further development and application of *G. tropicum*. This study on the chemical constituents led to the isolation of four types of 10 known compounds (Figure 1) triterpenoids (**4**, **5**, **10**), steroids (**6**-**9**), xanthones (**1** and **2**), and alkaloids (**3**), from *G. tropicum*. Most types of these natural products were previously found from *Ganoderma* genus. To our knowledge, all these 10 compounds were isolated for the first time from *G. tropicum*. Particularly, xanthones (**1** and **2**) were found for the first time from *Ganoderma* species, which maybe one of the important natural pigment contributed to the yellow color of the fruiting bodies of *G. tropicum*. Simultaneously, the test results of cytotoxicity against five tumor cell lines showed two compounds (**1** and **9**) possessed the cytotoxic activities, suggesting the potential effect on anti-tumor of *G. tropicum*. *Ganoderma* exhibited extensive medicinal value including antitumor effect. From this test, two types of natural products (steroids and xanthones) maybe the main active substance in *G. tropicum* for antitumor, which also could provide the promising molecules for antitumor drug development.

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