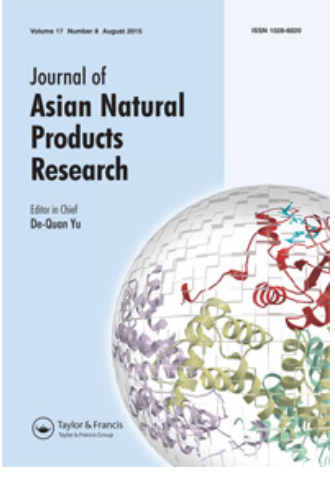

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Four lanostane-type triterpenes from the fruiting bodies of mushroom Laetiporus sulphureus var. miniatus

Xia Yin[a], Zheng-Hui Li[b], Yan Li[b], Tao Feng[b] & Ji-Kai Liu[b]
[a] College of Science, Northwest A&F University, Yangling 712100, China
[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China
Published online: 28 Apr 2015.

To cite this article: Xia Yin, Zheng-Hui Li, Yan Li, Tao Feng & Ji-Kai Liu (2015) Four lanostane-type triterpenes from the fruiting bodies of mushroom Laetiporus sulphureus var. miniatus, Journal of Asian Natural Products Research, 17:8, 793-799, DOI: 10.1080/10286020.2015.1027694

To link to this article: http://dx.doi.org/10.1080/10286020.2015.1027694

Journal of Asian Natural Products Research, 2015
Vol. 17, No. 8, 793–799, http://dx.doi.org/10.1080/10286020.2015.1027694

Four lanostane-type triterpenes from the fruiting bodies of mushroom *Laetiporus sulphureus* var. miniatus

Xia Yin[a], Zheng-Hui Li[b], Yan Li[b], Tao Feng[b]* and Ji-Kai Liu[b]*

[a]*College of Science, Northwest A&F University, Yangling 712100, China;* [b]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China*

(*Received 15 December 2014; final version received 6 March 2015*)

Two new 3,4-*seco*-lanostane-type triterpenes, named as 15α-hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid (**1**), 5α-hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid 3-methyl ester (**2**), and one new lanostane triterpene 15α-acetoxylhydroxytrametenolic acid (**3**) together with a known one versisponic acid D (**4**) were isolated from the fruiting bodies of *Laetiporus sulphureus* var. *miniatus*. Their structures were determined on the basis of extensive spectroscopic methods and comparison with reported data. All four compounds were evaluated for their cytotoxicities against five human cancer cell lines; however, none exhibited inhibitory effects.

Keywords: basidiomycetes; *Laetiporus sulphureus*; lanostane-type triterpenes; cytotoxicities

1. Introduction

Laetiporus sulphureus, belonging to the class Basidiomycetes, is an edible mushroom, also known as Chicken of the Woods and Chicken Mushroom. This kind of mushroom always occurs in the root, butt, or heartwood of living, characterized by sulfur-yellow pore surface [1]. Secondary metabolites of this mushroom represent a wide diversity of chemical species and bioactivities. Polysaccharides were isolated from the fruiting bodies of *L. sulphureus* [2], and then obtained from submerged mycelia culture [3]. Two figments, laetiporic acid A and 2-dehydro-3-deoxylaetiporic acid A were isolated, and the former was considered to be the major orange pigment [4]. Besides, volatile compounds [5], lectins [6], and plant hormones [7] were also isolated from the fruiting bodies. Moreover, lanostane triterpenes, a class of characteristic metabolites were isolated from both the fresh fruiting bodies, and these triterpenes suppressed survival and proliferation of HL-60 cell lines by triggering morphological changes and internucleosomal DNA fragmentation characteristic of apoptotic cell death [8]. Because of its potential for producing high value compounds, the investigation of its secondary metabolites arouse great interest. Our research led to the isolation of four lanostane-type triterpenes including three new ones, namely 15α-hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid (**1**), 5α-hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid 3-methyl ester (**2**), 15α-acetoxyltrametenolic acid (**3**), and a known one versisponic acid D (**4**) [9] (Figure 1). All the compounds were tested for their cytotoxicities against five human cancer cell lines; however, none exhibited inhibitory effects.

*Corresponding author. Email: fengtao@mail.kib.ac.cn; jkliu@mail.kib.ac.cn

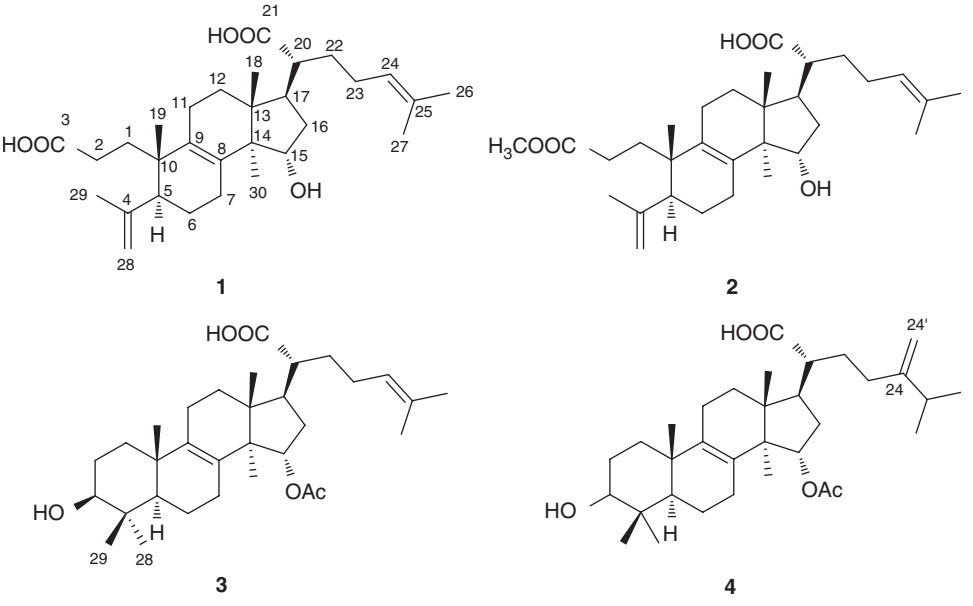

Figure 1. Structures of compounds **1**–**4**.

2. Result and discussion

Compound **1** was isolated as a white powder with the molecular formula of $C_{30}H_{46}O_5$ on the base of HR-ESI-MS at *m/z* 509.3235 $[M + Na]^+$ (calcd for $C_{30}H_{46}NaO_5$, 509.3243), corresponding to eight degrees of unsaturation. ^1H and ^{13}C NMR, as well as the HSQC spectrum revealed 30 carbon resonances, which were ascribed to six methyls, ten methenes including one belonging to a terminal double bound (δ_C 114.2, δ_H 4.97, br s; 4.87, br s), five methines including one oxygenated (δ_C 72.4, δ_H 4.60, dd, $J = 9.0$ and 6.1 Hz) and one sp^2 (δ_C 124.9, δ_H 5.27, t, $J = 6.7$ Hz), and nine quaternary carbons including two carboxyls (δ_C 176.5, 178.8) and four olefinic ones. Thus, except for the five degrees of unsaturation occupied by three double bonds and two carboxyls, the triterpenoid should have a three ring system. These data suggested that compound **1** was a tricyclic 3,4-*seco*-lanostane-type triterpene with a fully substituted double bond and a secondary hydroxyl group in the ring system just like poricoic acid G [10]. However, the difference between compound **1** and poricoic acid G was that the hydroxyl group was located at C-15 instead of C-16, which was supported by the HMBC correlations from H-15 (δ_H 4.60, dd, $J = 9.0$ and 6.1 Hz) to C-14 and C-8. Thus, the planar structure of compound **1** was determined as 15-hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid supported by the $^1H-^1H$ COSY and HMQC spectra (Figure 2). Biogenetically, the configurations of Me-18, 19 and 20 were β oriented, and H-5, Me-30 were α oriented, respectively, which was consistent with the ROESY correlations of H-5/H-1 and H-30/H-17. The configuration of the hydroxyl group was determined to be α according to the ROESY correlations of H-15/H-18/H-20. Finally, compound **1** was elucidated as 15α-hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid.

Compound **2** was isolated as a white powder. The HR-ESI-MS displayed an $[M + Na]^+$ at *m/z* 523.3402 analyzed for $C_{31}H_{48}NaO_5$, 14 mass units more than that of **1**. The ^1H and ^{13}C NMR data of compound **2** were quite similar to those of

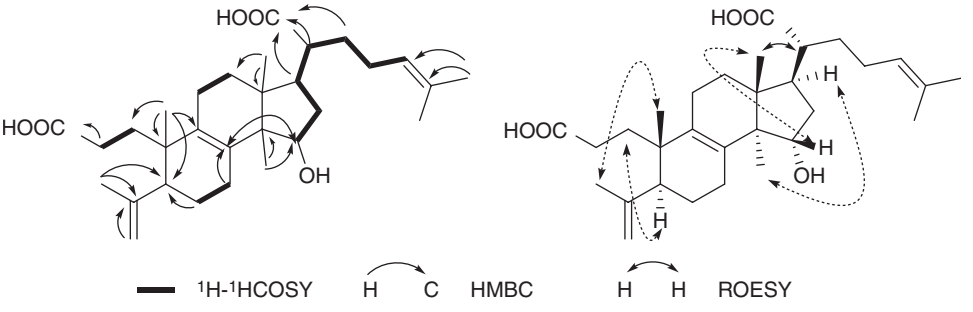

Figure 2. Key 2D correlations of compound **1**.

1, except for an extra methoxy signal (δ_C 51.8, q; δ_H 3.64, s, 3H). According to the HMBC correlations of —OCH_3 (δ_H 3.64, s, 3H) and H-2 (δ_H 2.54–2.55, m, 1H; 2.18–2.19, m, 1H) to the carbonyl (δ_C 174.9, s), the carboxyl group at C-3 was methyl esterified. Detailed analysis of 1D and 2D NMR data (HSQC, $^1H-^1H$ COSY, HMBC) suggested that the remainder of **2** was identical to that of **1** as well as the configurations basing on the ROESY correlations. Hence, compound **2** was established as methyl ester of **1**, named 5α-hydroxy-3,4-*seco*-lanosta-4(28),8,24-triene-3,21-dioic acid 3-methyl ester.

Compound **3**, obtained as a white powder, had the molecular formula of $C_{32}H_{50}O_5$ basing on the HR-EI-MS at *m/z* 514.3667 $[M]^+$, suggesting eight degrees of unsaturation. Of the eight degrees of unsaturation, four were occupied by two double bonds (δ_C 125.2, d; 132.2, s; 133.8, s; 136.6, s) and two carbonyls (δ_C 171.3, s; 179.1, s). The ^1H NMR spectrum exhibited eight methyl singlets (δ_H 1.01, 1.07, 1.13, 1.22, 1.26, 1.61, 1.68 and 2.18), two hydroxymethines at δ_H 3.46 (1H, t, $J = 8.2$ Hz) and 5.43 (1H, dd, $J = 9.1$ and 5.6 Hz) and one olefinic proton at δ_H 5.31 (1H, t, $J = 7.2$ Hz). The ^1H and ^{13}C NMR spectra provided evidences of a lanostane-type triterpene skeleton as 15α-hydroxytrametenolic acid reported in the literatures [11]. The additional acetyl group was attached at OH-15, compared with 15α-hydroxytrametenolic acid, according to the strong HMBC correlations of H-15 (δ_H 5.43, dd, $J = 9.1$ and 5.6 Hz, 1H) to the carbonyl group at δ_C 171.3. Detailed analysis of the 2D spectrum (Figure 3) suggested that the other parts of compound **3** were exactly the same as those of 15α-hydroxytrametenolic acid. In the ROESY spectrum, the

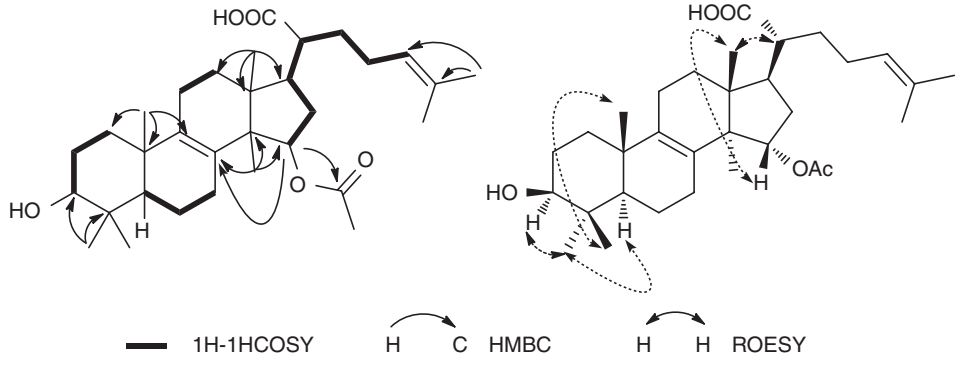

Figure 3.. Key 2D correlations of compound **3**.

correlations of H-3/H-28/H-5 suggested that OH-3 was β-oriented, while the correlations of H-15/H-18/H-20 suggested that the acetoxyl group at C-15 was α-oriented. Finally, compound **3** were assigned to be 15α-acetoxylhydroxytrametenolic acid.

Compounds **1**–**4** were all lanostane-type triterpenes, and among them compounds **1** and **2** were new 3,4-*seco*-lanostane-type triterpenes. All compounds were evaluated for cytotoxicity against five human cancer cell lines using the MTT method as reported previously [12]. Unfortunately, none of the four compounds showed cytotoxicities compared with those of cisplatin.

3. Experimental

3.1. General experimental procedures

Optical rotations were recorded on a JASCO P-1020 digital polarimeter (JASCO, Tokyo, Japan). UV and IR spectra were obtained on a Shimadzu UV2401PC (Shimadzu, Tyoto, Japan) and a Bruker Tensor 27 FT-IR spectrometer (Bruker, Karlsruker, Germany) with KBr pellets. Nuclear Magnetic Resonance (NMR) spectra were obtained on a Bruker Avance III 600 MHz spectrometer (Bruker, Karlsruher, Germany) with tetramethylsilane (TMS) as an internal standard at room temperature. High-resolution (HR) EI-MS were recorded on a Waters AutoSpec Premier P776 (Waters, Milford, USA). High-resolution (HR) ESI-MS were recorded on an Agilent G6230 TOF MS (Agilent Technologies, Santa Clara, USA). Silica gel (200–300 mesh, Qingdao Marine Chemical Ltd, Qingdao, China) and RP-18 gel (20–45 μm, Fuji Silysia Chemical Ltd, Japan) were used for open column chromatography (CC). Preparative HPLC was performed on an Agilent 1100 liquid chromatography system (Agilent Technologies, Santa Clara, USA) equipped with a Zorbax SB-C18 column (9.4 mm × 150 mm, Agilent Technologies, Santa Clara, USA). Fractions were monitored by TLC (GF_{254}, Qingdao Haiyang Chemical Co., Ltd, Qingdao, China). Spots were visualized by heating silica gel plates immersed in Vanillin-H_2SO_4 in ethanol.

3.2. Fungal material

The fruiting bodies of *Laetiporus sulphureus* var. *miniatus* were collected from Ailao Mountain, Yunnan province, in December 2010, and identified by Prof. Zhu-Liang Yang, Kunming Institute of Botany, Chinese Academy of Sciences. A specimen (No. kib201008ls) was deposited at Kunming Institute of Botany, Chinese Academy of Sciences.

3.3. Extraction and isolation

The dried fruiting bodies of *Laetiporus sulphureus* var. *miniatus* (1.1 kg) were extracted with $CHCl_3$–MeOH (1:1). The extraction was separated by solvent partition between EtOAc and water, and the EtOAc layer was concentrated under reduced pressure to give a crude extract (50 g). The residue was subjected to silica gel CC, eluted with a gradient of $CHCl_3$/MeOH (1:0 → 0:1) to obtain seven fractions (A–D). Fraction A (4.3 g) was separated by silica gel CC (petroleum–acetone, 10:1) to give four subfractions (A1–A4). Fraction A2 (316 mg) was subjected to semipreparative HPLC (60–90%, MeCN–H_2O, 10 ml/min, 20 min) to give two mixtures, A2a and A2b. A2a (117 mg) was further purified by semipreparative HPLC (60%, MeCN–H_2O, 10 ml/min, 20 min) to afford **1** (8.3 mg) and **2** (5.6 mg), while A2b (79 mg) was purified by semipreparative HPLC (70–90%, MeCN–H_2O, 10 ml/min, 20 min) to yield **3** (7.1 mg) and **4** (3.3 mg).

3.3.1. Compound *1*

White powder, $C_{30}H_{46}O_5$, $[\alpha]_D^{21}$ + 78.4 (*c* 0.225, MeOH); UV (MeOH) λ_{max} (log ε)

Table 1. ^{1}H NMR spectral data of compounds **1**–**4** (600 MHz in C_5D_5N).

No.	**1**	**2**	**3**	**4**
1	1.89–1.91, m;	1.93–1.95, m;	1.61–1.62, m;	1.60–1.61, m;
	1.74–1.75, m	1.80–1.81, m	1.17–1.19, m	1.19–1.20, m
2	2.65–2.66, m;	2.54–2.55, m;	1.84–1.84, m	1.86–1.87, m
	2.32–2.33, m	2.18–2.19, m		
3			3.46, t (8.2)	3.47, t (7.9)
5	2.32–2.33, m	2.26–2.26, m	1.19–1.20, m	1.20–1.21, m
6	1.72–1.73, m;	1.58–1.59, m	1.74, dd (12.2, 7.7)	1.72, dd (11.9, 7.5)
	1.59–1.60, m		1.50–1.51, m	1.53–1.54, m
7	2.52–2.53, m	2.52–2.53, m	2.35–2.36, m	2.34–2.35, m
11	2.12–2.13, m	2.08–2.09, m;	2.00–2.01, m;	2.00–2.01, m;
		2.02–2.03, m	1.94–1.95, m	1.94–1.95, m
12	2.18–2.19, m;	1.94–1.95, m;	2.13–2.14, m;	2.14–2.15, m;
	1.95–1.96, m	1.44–1.45, m	1.92–1.93, m	1.74–1.75, m
15	4.60, dd (9.0, 6.1)	4.63, dd (9.2, 5.9)	5.43, dd (9.1, 5.6)	5.44, dd (9.5, 5.5)
16	2.20–2.21, m	2.27–2.28, m	2.38–2.39, m;	2.39–2.40, m;
			1.91–1.92, m	1.95–1.96, m
17	2.66–2.67, m	2.70–2.71, m	2.63–2.64, m	2.64–2.66, m
18	1.18, s	1.21, s	1.13, s	1.15, s
19	0.95, s	0.93, s	1.01, s	1.01, s
20	2.62–2.63, m	2.65–2.66, m	2.60–2.62, m	2.60–2.62, m
22	2.04–2.06, m;	1.93–1.95, m;	1.91–1.93, m;	2.05–2.06, m;
	1.98–1.99, m	1.79–1.80, m	1.66–1.67, m	1.82–1.84, m
23	2.32–2.34, m;	2.35–2.37, m;	2.34–2.35, m;	2.41–2.43, m;
	2.23–2.35, m	2.26–2.28, m	2.10–2.11, m	2.30–2.31, m
24	5.27, t (6.7)	5.30, t (7.19)	5.31, t (7.2)	
25				2.25–2.27, m
26	1.63, s	1.65, s	1.68, s	1.02, d, overlapped
27	1.58, s	1.60, s	1.61, s	1.01, d, overlapped
28	4.97, br s; 4.87 br s	4.97, br s; 4.83, br s	1.26, s	1.27, s
29	1.77, s	1.76, s	1.07, s	1.08, s
30	1.36, s	1.38, s	1.22, s	1.25, s
24′				4.91, brs; 4.89, br s
3–OCH_3		3.64, s		
OAc			2.18, s	2.17, s

235 (1.93), 221 (1.62), 215 (1.57) nm; IR (KBr) ν_{max} 3440, 2940, 1707 and 1636 cm^{-1}; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectral data (C_5D_5N), see Tables 1 and 2; positive ion HR-ESI-MS: *m/z* 509.3235 $[M + Na]^+$ (calcd for $C_{30}H_{46}O_5Na$, 509.3243).

3.3.2. *Compound* **2**

White powder, $C_{31}H_{48}O_5$, $[\alpha]_D^{21} + 71.4$ (*c* 0.230, MeOH); UV (MeOH) λ_{max} (log ε) 243 (2.39), 214(2.17), 205 (2.15) nm; IR(KBr) ν_{max} 3437, 2950, 1735, 1438, 1377, 1201 and 1173 cm^{-1}; ^{1}H (600 MHz), and ^{13}C NMR (150 MHz) spectral data (C_5D_5N), see Tables 1 and 2; positive ion HR-ESI-MS: *m/z* 523.34045 $[M + Na]^+$ (calcd for $C_{31}H_{48}O_5Na$, 523.3399).

3.3.3. *Compound* **3**

White powder, $C_{32}H_{50}O_5$, $[\alpha]_D^{21} + 58.5$ (*c* 0.295, MeOH); UV (MeOH) λ_{max} (log ε) 251 (2.52), 222 (2.32), 205 (2.23) nm; IR (KBr): ν_{max} 3439, 2937, 1720 and 1380 cm^{-1}; ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectral data (C_5D_5N), see Tables 1 and 2; positive ion HR-EI-MS: *m/z* 514.3667 $[M]^+$ (calcd for $C_{32}H_{50}O_5$, 514.3658).

Table 2. ^{13}C NMR spectral data of compounds **1**–**4** (150 MHz in C_5D_5N).

No.	1	2	3	4
1	33.3, t	33.7, t	36.5, t	36.4, t
2	30.5, t	30.1, t	29.2, t	29.0, t
3	176.5, s	174.9, s	78.4, d	78.4, d
4	147.9, s	148.1, s	39.9, s	39.9, s
5	47.0, d	47.2, d	51.0, d	51.1, d
6	24.6, t	24.8, t	19.0, t	19.1, t
7	27.2, t	27.7, t	27.2, t	27.2, t
8	139.6, s	140.2, s	133.8, s	133.4, s
9	130.1, s	130.0, s	136.6, s	136.4, s
10	40.8, s	41.2, s	37.8, s	37.9, s
11	21.9, t	22.2, t	21.4, t	21.4, t
12	30.4, t	30.7, t	29.9, t	29.8, t
13	45.2, s	45.6, s	45.6, s	45.6, s
14	53.1, s	53.4, s	51.5, s	52.5, s
15	72.4, d	72.8, d	76.4, d	76.4, d
16	39.1, t	39.5, t	36.4, t	36.4, t
17	46.6, d	47.0, d	46.9, d	46.9, d
18	17.1, q	17.5, q	16.9, q	17.1, q
19	22.6, q	22.8, q	19.7, q	19.7, q
20	48.9, d	49.3, d	49.2, d	49.4, d
21	178.8, s	179.3, s	179.1, s	179.1, s
22	33.5, t	33.5, t	33.7, t	32.4, t
23	26.7, t	27.1, t	27.1, t	33.1, t
24	124.9, d	125.3, d	125.2, d	156.3, s
25	131.7, s	132.2, s	132.2, s	34.6, d
26	25.8, q	26.2, q	26.2, q	19.7, q
27	17.7, q	18.2, q	18.2, q	21.7, q
28	114.2, t	114.7, t	29.1, q	29.1, q
29	23.3, q	23.6, q	16.8, q	16.8, q
30	19.0, q	19.5, q	19.0, q	19.1, q
24′				107.4, t
3-OCH_3		51.8, q		
OAc			171.3, s	171.4, s
			21.7, q	21.7, q

3.3.4. *Versisponic acid D (**4**)*

White powder, $C_{33}H_{52}O_5$, ^1H (600 MHz) and ^{13}C NMR (150 MHz) spectral data (C_5D_5N), see Tables 1 and 2.

3.4. *Cytotoxicity assay*

Five human cancer cell lines, breast cancer MCF-7, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, colon cancer SW480, and lung cancer A-549 cells, were used in the cytotoxic assay. All the cells were cultured in RPMI-1640 (Roswell Park Memorial Institute-1640) or DMEM (Dulbecco's-modified Eagle medium) medium (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% CO_2 at 37°C. The cytotoxicity assay was performed according to the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) method in 96-well microplates [12]. Briefly, 100 μl adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition with initial density of 1×10^5 cells/ml. Each tumor cell line was exposed to the test compound dissolved in DMSO at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μmol in triplicates for 48 h, with cisplatin (Sigma, Saint Louis, MO 63103 USA) and vinorelbine (National Institute for the Control of Pharmaceutical and Biological Products, Beijing, China) as positive controls. After compound treatment, cell viability was detected and a cell growth curve was graphed. IC_{50} values were calculated by Reed and Muench's method [13].

Acknowledgments

The authors are grateful to Kunming Institute of Botany, Chinese Academy of Sciences, for the measurement of all spectroscopic data.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was financially supported by Natural Science Foundation of Yunnan Province [grant number 312011FB099].

References

[1] N. Radic, R. Injac, and B. Strukelj, *Int. J. Med. Mushr.* **11**, 103 (2009). doi:10.1615/IntJMedMushr.v11.i2.10.

[2] G. Alquini, E.R. Carbonero, F.R. Rosado, C. Cosentino, and W. Iacomini, *FEMS*

Microbiol. Lett. **230**, 47 (2004). doi:10.1016/S0378-1097(03)00853-X.

[3] H.S. Hwang, S.H. Lee, Y.M. Baek, S.W. Kim, Y.K. Jeong, and J.W. Yun, *Appl. Microbiol. Biotechnol.* **78**, 419 (2008). doi:10.1007/s00253-007-1329-6.

[4] R.W.S. Weber, A. Mucci, and P. Davoli, *Tetrahedron Lett.* **45**, 1075 (2004). doi:10.1016/j.tetlet.2003.11.073.

[5] S. Rapior, G. Konska, J. Guillot, C. Andary, and J.M. Bessiere, *Cryptog. Mycol.* **21**, 67 (2000). doi:10.1016/S0181-1584(00)00109-3.

[6] G. Konska, J. Guillot, M. Dusser, M. Damez, and B. Botton, *J. Biochem.* **116**, 519 (1994).

[7] B. Ozcan and S.F. Topcuoglu, *Turk. J. Biol.* **25**, 453 (2001).

[8] F. León, J. Quintana, A. Rivera, F. Estévez, and J. Bermejo, *J. Nat. Prod.* **67**, 2008 (2004). doi:10.1021/np049762o.

[9] K. Yoshikawa, K. Matsumoto, C. Mine, S. Bando, and S. Arihara, *Chem. Pharm. Bull.* **48**, 1418 (2000). doi:10.1248/cpb.48.1418.

[10] M. Ukiya, T. Akihisa, H. Tokuda, M. Hirano, M. Oshikubo, Y. Nobukuni, Y. Kimura, T. Tai, S. Kondo, and H. Nishino, *J. Nat. Prod.* **65**, 462 (2002). doi:10.1021/np0103721.

[11] Y.M. Ying, W.G. Shan, L.W. Zhang, Y. Chen, and Z.J. Zhan, *Helv. Chim. Acta.* **96**, 2092 (2013). doi:10.1002/hlca.201300002.

[12] T. Mosmann, *J. Immunol. Method.* **65**, 55 (1983). doi:10.1016/0022-1759(83)90303-4.

[13] L.J. Reed and H. Muench, *Am. J. Hygiene.* **27**, 493 (1938).