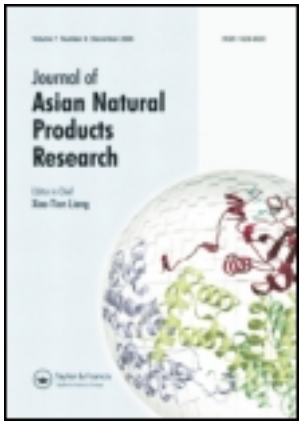

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Three new compounds from the cultures of basidiomycete *Boreostereum vibrans*

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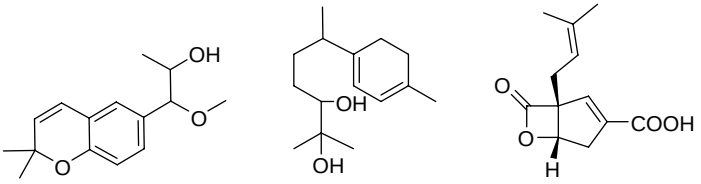
Three new compounds, vibranether (**1**), myrrhlalkyldiol (**2**), vibralactone H (**3**), together with three known compounds, 2-methyl-6-*p*-tolylheptane-2,3-diol (**4**), 2-hydroxy-2-methyl-6-*p*-tolylheptan-3-one (**5**), and vibralactone (**6**), were isolated from the cultures of the basidiomycete *Boreostereum vibrans*. Their structures were determined on the basis of spectroscopic evidences (1D and 2D NMR, HRMS, UV, and IR data), chemical methods, and the literature data. The new compounds displayed no significant cytotoxicities against five human cancer cell lines ($IC_{50} > 40\ \mu M$).



Keywords: *Boreostereum vibrans*; basidiomycete; vibralactone derivatives

1. Introduction

Our previous study on the secondary metabolites of the basidiomycete *Boreostereum vibrans* resulted in the isolation of a series of vibralactone analogs [1,2]. Of them, vibralactone showed strong inhibitory activity against pancreatic lipase with an IC_{50} value of 0.4 μg/ml [3], vibralactones D–F showed inhibitory activities against human and mouse 11β-HSD1 and 11β-HSD2, respectively [4]. These natural products have attracted great interests of synthetic chemists. In 2008, vibralactone and vibralactone C have been totally synthesized [5,6]. Recently, vibralactone has been reported as a tool to study the activity and structure of the ClpP1P2 complex from *Listeria monocytogenes* [7], while the biosynthetic pathway of vibralactone has also been reported to be found in 2013 [8]. Due to these reasons, we continued to search for new natural products from *B. vibrans*. In this paper, we report three new compounds, vibranether (**1**), myrrhlalkyldiol (**2**), and vibralactone H (**3**), together with three known compounds from the cultures of *B. vibrans*. These new structures were established by means of spectroscopic

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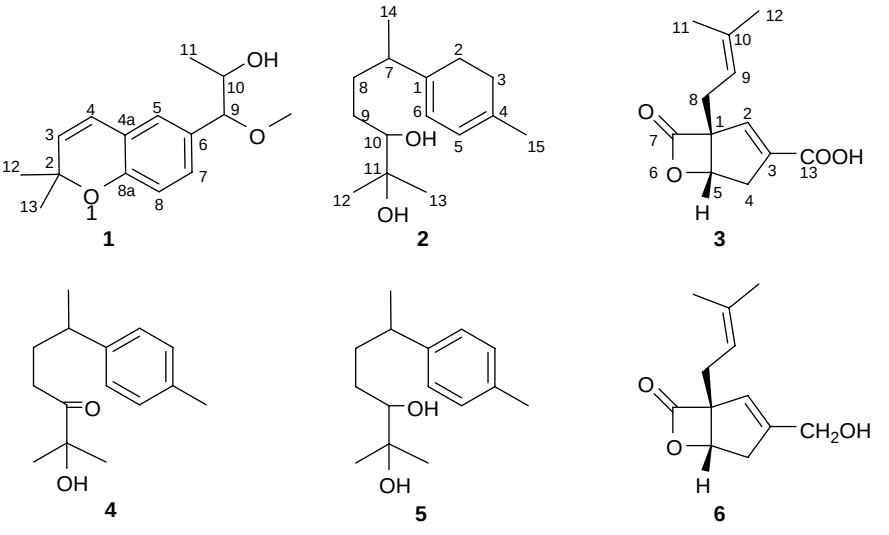


Figure 1. The structures of compounds **1**–**6**.

methods, while the known compounds were identified as 2-methyl-6-*p*-tolylheptane-2,3-diol (**4**) [9], 2-hydroxy-2-methyl-6-*p*-tolylheptan-3-one (**5**) [10], and vibralactone (**6**) (Figure 1) [3] by comparison with data in the literature. The new compounds were screened for their cytotoxicity against five human cancer cell lines.

2. Results and discussion

Compound **1** was obtained as a colorless oil. Its molecular formula was established as $C_{15}H_{20}O_3$ by the HR-EI-MS (*m/z* 248.1413 $[M]^+$, calcd for $C_{15}H_{20}O_3$, 248.1412). The IR spectrum exhibited absorption bands for hydroxy (3453 cm^{-1}) and an aromatic moiety (1683, 1640, and 1489 cm^{-1}). The latter was also indicated by the ^1H NMR and ^{13}C NMR data (Table 1). Three aromatic protons of H-7 (dd, $J = 8.2$ and 2.0 Hz), H-8 (d, $J = 8.2$ Hz), and H-5 (d, $J = 1.9$ Hz) indicated a 1,2,4-trisubstituted benzene ring. Besides the six carbon signals occupied by the benzene ring, the ^{13}C NMR spectrum displayed nine carbons ascribable for two olefinic carbons, one oxygenated sp^3 quaternary carbon (δ_C 76.4), two oxygenated methines (δ_C 89.1, 71.4), and four methyls (including one OMe at δ_C 56.6). The ^1H–^1H COSY spectrum revealed three fragments as shown in Figure 2. In the HMBC spectrum (Figure 2), the correlation from the olefinic proton at δ_H 6.31 (1H, d, $J = 9.8$ Hz, H-3) to the sp^3 quaternary carbon at δ_C 76.4 (s, C-2) and the correlation from two methyls at δ_H 1.43 (6H, s, Me-12 and

Table 1. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **1** (δ in ppm, J in Hz).

No.	δ_C	δ_H
2	76.4, s	
3	122.1, d	6.31, d (9.8)
4	130.9, d	5.62, d (9.8)
4a	121.1, s	
5	125.4, d	6.87, d (2.0)
6	130.4, s	
7	128.4, d	6.99, dd (8.2, 2.0)
8	116.2, d	6.74, d (8.2)
8a	152.9, s	
9	89.1, d	3.72, d (8.3)
10	71.4, d	3.77, m
11	18.0, q	0.96, d (6.1)
12	28.0, q	1.43, s
13	28.0, q	1.43, s
OMe	56.6, q	3.21, s

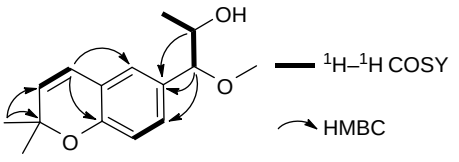
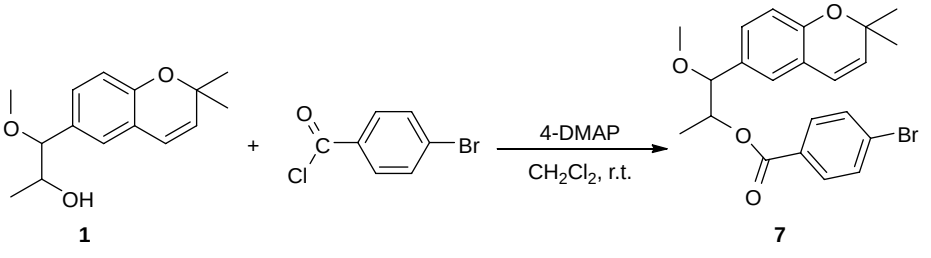


Figure 2. ^{1}H–^{1}H COSY and HMBC correlations for compound **1**.

Me-13) to C-3 indicated the presence of an isoprenyl moiety. Meanwhile, the HMBC correlation from H-9 at δ_H 3.72 (1H, d, $J = 8.3$ Hz) to C-6 at δ_C 130.4 (s) and from the methoxy protons at δ_H 3.21 (3H, s, OMe) to C-9 indicated that another side chain was connected to the benzene ring at C-6 (Figure 2). The ^{1}H–^{1}H COSY correlation between H-10 at δ_H 3.77 (1H, m) and Me-11 at δ_H 0.96 (3H, d, $J = 6.1$ Hz), as well as the HMBC correlation from H-11 to C-10 at δ_C 71.4 (d), suggested that C-10 was an oxygenated methine (Figure 2). These data suggested that compound **1** possessed a similar structure to that of 6-(2-hydroxyethyl)-2,2-dimethyl-2*H*-1-benzopyran [11], in particular with the same bicyclic core due to the closely related NMR data. To determine the absolute configuration of **1**, a reagent of 4-bromobenzoyl chloride was used for producing a derivative for a single crystal (Scheme 1). Unfortunately, a single crystal could not be obtained after many attempts. It was worth mentioning that this derivative (**7**) further supported that C-10 possessed a hydroxy group due to the significant downfield shifts of CH-10 (δ_H 5.26; δ_C 85.6) in the 1D NMR spectra. Therefore, compound **1** was established and named as vibranether.

Compound **2**, a colorless oil, possessed a molecular formula $C_{15}H_{26}O_2$, as established by the HR-EI-MS at *m/z* 238.1943 $[M]^+$. The IR absorption band at 3423 cm^{-1} revealed the presence of hydroxy groups. The ^{13}C NMR data (Table 1) revealed 15 carbon signals assigned to two double bonds, one oxygenated quaternary carbon, two sp^3 methines (one oxygenated), four sp^3 methylenes, and four methyls. These NMR data were closely similar to those of compound **5**, and the main difference was that the benzoyl group in **5** was hydrogenated to a cyclohexadiene group H-5/H-6 (δ_H 5.43) and H-2/3 (δ_H 2.56) in **2**. Detailed analysis of 2D NMR data indicated that the other parts were the same as those of **5**. Therefore, compound **2** was determined as shown in Figure 1, and named as myrrhlalkyldiol.

Compound **3** was isolated as a colorless oil. Its molecular formula was established as $C_{12}H_{14}O_4$ by HR-EI-MS (*m/z* 222.0902 $[M]^+$, calcd for $C_{12}H_{14}O_4$ at *m/z* 222.0892). The IR spectrum showed absorption bands for hydroxyl (3442 cm^{-1}), carboxyls (1823 and 1699 cm^{-1}), and double bands (1627 and 833 cm^{-1}). The ^{1}H and ^{13}C NMR data of **3** were very similar to those of **6** except that C-13 was oxygenated into a carboxyl carbon at δ_C 168.4 in **3**, as supported by the HMBC correlation from H-2 at δ_H 6.72 (1H, s) and H-4 at δ_H 2.99 (2H, m) to C-13 at δ_C 168.4 (s). Detailed analysis of other 2D NMR data (HSQC, HMBC, ^{1}H–^{1}H



Scheme 1. Acylation of **1**.

COSY, and ROESY) suggested that the other parts were the same as those of **6**. Therefore, compound **3** was established as depicted, and named as vibralactone H.

Three known compounds were identified as 2-methyl-6-*p*-tolylheptane-2,3-diol (**4**) [9], 2-hydroxy-2-methyl-6-*p*-tolylheptan-3-one (**5**) [10], and vibralactone (**6**) [3], respectively, by comparing their physical and spectroscopic data with those reported in the literature.

The new compounds were evaluated for their cytotoxicities against five human cancer cell lines, SK-BR-3 breast, SMMC-7721 hepatocellular carcinoma, HL-60 myeloid leukemia, PANC-1 pancreatic cancer, and A-549 lung cancer, using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [12]. Unfortunately, no significant activity was detected ($IC_{50} > 40\ \mu M$).

3. Experimental

3.1 General experimental procedures

Optical rotations were obtained on a Horiba SEPA-300 polarimeter (Horiba, Kyoto, Japan). IR spectra were taken on a Bruker Tensor 27 FT-IR spectrometer (Bruker Optics GmbH, Ettlingen, Germany) with KBr pellets. 1D and 2D spectra were run on Bruker DRX-500 and AM-400 spectrometers (Bruker BioSpin GmbH, Rheinstetten, Germany) with tetramethylsilane as an internal standard. Chemical shifts (δ) were expressed in ppm with reference to the solvent signals. EI-MS, ESI-MS, and HR-ESI-MS were measured on Finnigan-MAT 90 and API QSTAR Pulsar i mass spectrometers (MDS Sciex, Concord, Ontario, Canada), respectively. Silica gel 200–300 mesh (Qingdao Marine Chemical, Inc., Qingdao, China) and Sephadex LH-20 (Amersham Biosciences, Upssala, Sweden) were used for column chromatography. MPLC was performed on a Büchi Sepacore System equipping pump manager C-615, pump modules C-605, and fraction collector C-660 (Büchi Labortechnik AG, Flawil, Switzerland), and columns packed with Chromatorex C-18 (40–75 μm, Fuji Silysia Chemical Ltd, Aichi, Japan). Fractions were monitored by Agilent 1200 reversed-phase HPLC (Eclipse XDB-C18 column, 5 μm, 4.6 mm × 150 mm, 25–100% MeOH in H_2O over 8 min followed by 100% MeOH to 11 min, 1 ml/min, 30°C), in combination with thin layer chromatography (Qingdao Marine Chemical, Inc.).

3.2 Fungal material and cultivation

B. vibrans was provided and fermented by Zheng-Hui Li, Kunming Institute of Botany. A voucher specimen (No. 20120920B) of *B. vibrans* was deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. The culture medium consisted of glucose 5%; peptone 0.15%; yeast 0.5%; KH_2PO_4 0.05%; $MgSO_4$ 0.05% in 1 liter of deionized water (pH 6.5 before autoclaving). The fungus was grown in Erlenmeyer flasks (500 ml with 300 ml of medium). Fermentation was carried out in a rotary shaker at 22°C and 200 rpm for 26 days.

3.3 Extraction and isolation

The culture broth (40 liters) of *B. vibrans* was extracted three times with EtOAc (25 liters) after filtration. The organic layer was concentrated under reduced pressure to give a crude extract (24.0 g). The residue was subjected to column chromatography over silica gel (200–300 mesh, 6 cm × 40 cm), eluted with a petroleum ether:EtOAc (20:1, 15:1, 10:1, 5:1, 1:1, 0:1, v/v) gradient, to afford fractions A–E. Fraction B (1.58 g) eluted with petroleum ether:EtOAc (4:1) was separated repeatedly by reversed-phase RP-18 (MeOH/H_2O) column chromatography, followed by Sephadex LH-20 (acetone) column chromatography to give **2** (20 mg), **4** (3.4 mg), and **5** (2.3 mg). Fraction C (2.6 g) was separated by silica gel eluted

Table 2. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data of **2** and **3** (δ in ppm, J in Hz).

	2		**3**	
No.	δ_C	δ_H	δ_C	δ_H
1	138.9, s		76.5, d	
2	26.6, t	2.56, d (4.5)	140.3, d	6.72, s
3	31.5, t	2.56, d (4.5)	137.0, s	
4	131.3, s		36.4, t	2.99, m
5	118.6, d	5.42, brs	78.1, s	4.85, d (5.2)
6	117.8, d	5.43, s		
7	40.7, d	2.08, m	170.5, s	
8	32.0, t	1.60, m	27.3, t	2.68, dd (15.0, 7.4)
		1.37, m		2.52, dd (15.0, 7.4)
9	29.7, t	1.37, m; 1.23, m	116.3, d	5.10, t (7.3)
10	78.9, d	3.30, d (10.3)	137.0, s	
11	73.3, s		18.0, q	1.64, s
12	26.5, q	1.18, s	25.8, q	1.73, s
13	22.9, q	1.13, s	168.4, s	
14	19.6, q	1.01, d (6.9)		
15	23.0, q	1.66, s		

with petroleum ether:EtOAc (2:1) to give three major fractions 1–3. Fraction 3 (106 mg) was purified by preparative HPLC using CH_3CN/H_2O (from 20% to 40%, 20 min) as mobile phase (flow rate 10 ml/min), then separated by Sephadex LH-20 (acetone) column chromatography to afford **1** (8 mg), **3** (9 mg), and **6** (10 mg).

3.3.1 *Vibranether (**1**)*

A colorless oil, $[\alpha]_D^{26} + 63.2$ ($c = 0.31$, MeOH). UV (MeOH) λ_{max} nm (log ε): 311 (2.81), 264 (2.88), 223 (3.84), 198 (3.50). IR (KBr) ν_{max} cm^{-1}: 3453, 2976, 2932, 1683, 1640, 1489, 1262, 1139, 1085, 971. For ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data, see Table 1. HR-EI-MS *m/z*: 248.1413 $[M]^+$ (calcd for $C_{15}H_{20}O_3$, 248.1412).

3.3.2 *Myrrhlalkyldiol (**2**)*

A colorless oil, $[\alpha]_D^{25} - 13.0$ ($c = 0.44$, MeOH). UV (MeOH) λ_{max} nm (log ε): 298 (1.99), 274 (1.92), 203 (2.93). IR (KBr) ν_{max} cm^{-1}: 3423, 2962, 2873, 1732, 1382, 1076. For ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data, see Table 2. HR-EI-MS *m/z*: 238.1943 $[M]^+$ (calcd for $C_{15}H_{26}O_2$, 238.1933).

3.3.3 *Vibralactone H (**3**)*

A colorless oil, $[\alpha]_D^{25} - 181.2$ ($c = 0.41$, MeOH). UV (MeOH) λ_{max} nm (log ε): 229 (3.15), 203 (3.36). IR (KBr) ν_{max} cm^{-1}: 3442, 2975, 2919, 1823, 1699, 1277, 1113, 833. For ^{1}H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) spectral data, see Table 2. HR-EI-MS *m/z*: 222.0902 $[M]^+$ (calcd for $C_{12}H_{14}O_4$, 222.0892).

3.4 *Acylation of 1*

A mixture of compound **1** (2 mg, 0.008 mmol), 4-bromobenzoyl chloride (2.6 mg, 0.012 mmol), and 4-dimethylaminopyridine (DMAP) (1.5 mg, 0.012 mmol) in dry CH_2Cl_2 (2 ml) was vigorously stirred at r.t. for 4 h. The reaction mixture was extracted with diethyl ether. The combined extracts were washed with water, brine, dried (Na_2SO_4), and filtered. The solvents were removed, and the residue was purified by flash chromatography (petroleum ether:

diethyl ether, 20:1) to get compound **7** (2.7 mg, 80%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.08 (3H, d, $J = 6.6$ Hz), 1.36 (6H, s), 3.17 (3H, s), 4.08 (1H, d, $J = 6.6$ Hz), 5.26 (1H, m), 5.55 (1H, d, $J = 9.6$ Hz), 6.22 (1H, d, $J = 9.6$ Hz), 6.68 (1H, d, $J = 8.4$ Hz), 6.88 (1H, s), 6.98 (1H, d, $J = 7.8$ Hz), 7.51 (2H, d, $J = 8.4$ Hz), 7.83 (2H, d, $J = 8.4$ Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 16.5, 28.3, 57.3, 73.8, 76.6, 85.6, 116.3, 121.4, 122.3, 125.7, 128.1, 128.7, 129.8, 130.1, 131.3, 131.4, 131.8, 153.1, 165.6; HR-EI-MS: (positive) *m/z* 430.0807 $[M]^+$ (calcd for $C_{22}H_{23}O_4Br$, 430.0780).

3.5 Cell viability

Cell viability was determined by the use of the micro culture tetrazolium technique (MTT). All the cells were cultured in RPMI-1640 or DMEM (Hyclone, Logan, UT, USA), supplemented with 10% fetal bovine serum (Hyclone) in 5% CO_2 at 37°C. The cytotoxicity assay was done according to the MTT method in 96-well microplates [12]. Briefly, 100 μl of adherent cells was 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 an initial density of 1×10^5 cells/ml. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μm in triplicates for 48 h, with cisplatin (Sigma, St Louis, MO, USA) as a positive control. After compound treatment, cell viability was detected and cell growth curve was graphed.

Acknowledgments

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