

# Penicilazaphilone C, a new antineoplastic and antibacterial azaphilone from the Marine Fungus *Penicillium sclerotiorum*

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**Abstract** Two azaphilonidal derivatives [penicilazaphilones B (**1**) and C (**2**)], have been isolated from the fermented products of marine fungus strain *Penicillium sclerotiorum* M-22, penicilazaphilones C was a new compound. The compound's structures were identified by the analysis of spectroscopic data including 1D and 2D NMR techniques (<sup>1</sup>H-NMR, <sup>13</sup>C-NMR, COSY, HMQC, and HMBC). Biological evaluation revealed that penicilazaphilones B and C showed selective cytotoxicity against melanoma cells B-16 and human gastric cancer cells SGC-7901 with IC<sub>50</sub> values of 0.291, 0.449 and 0.065, 0.720 mM, respectively, while exhibiting no significant toxicity to normal mammary epithelial cells M10 at the same concentration. Moreover, penicilazaphilones C also exhibited strong antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Escherichia coli* with MIC values 0.037–0.150 mM, while penicilazaphilones B's bacteriostatic action was weaker.

**Keywords** Marine fungus · *Penicillium sclerotiorum* · Penicilazaphilones · Antibacterial activity · Cytotoxicity

## Introduction

Marine fungi are potentially prolific sources of highly bioactive secondary metabolites that might serve as useful leads in the development of new pharmaceutical agents (Blunt et al. 2012, 2014). More and more attention has been paid to those obtained from marine fungi in recent years (Li et al. 2013; Bladt et al. 2013; An et al. 2014). Over the course of our search for new antineoplastic and antibacterial lead compounds from marine fungi, we screened various marine-derived fungi strains and found that a strain isolated from rotted leaves at the coast of Haikou, Hainan province in China produces two azaphilone-derived compounds, penicilazaphilones B (**1**) and C (**2**) (Fig. 1) as its major biologically active chemical constituents. There were also reports of four azaphilone derivative compounds (including penicilazaphilones A and B, dechloroisochromophilone III and (–)-sclerotiorin) isolated from the entophytic fungus *P. sclerotiorum* by Arunpanichlert et al. (2010). The authors of the study reported that penicilazaphilones A and B had weak biological activity as antifungal and anti-HIV virus activity, Dechloroisochromophilone III and (–)-sclerotiorin showed strong antimicrobial activities and anti-HIV virus activity. However, azaphilonidal derivatives exhibited broad activity in many biological systems, such as cholesteryl ester transfer protein (Tomoda et al. 1999), endothelin receptor binding (Toki et al. 1999), monoamine oxidase inhibition (Somoza et al. 2012), telomerase inhibition (Tomoda et al. 1999), the induction of Epstein-Barr virus (Akihisa et al. 2005), the inhibition of gp120-CD4 binding (Matsuzaki

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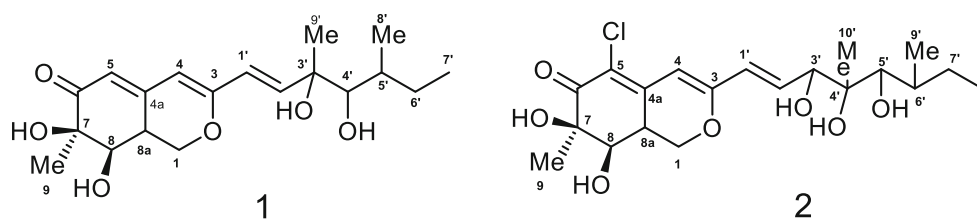
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**Fig. 1** Structures of compounds **1** and **2**

et al. 1995), the inhibition of nitric oxide (NO) production (Quang et al. 2006), cytotoxicity (Yamada et al. 2009), and antimicrobial activities (Li et al. 2010). So we evaluated the two azaphilonic derivatives (**1** and **2**) in terms of antibacterial activity and cytotoxicity.

## Materials and methods

### General experimental procedures

Ultraviolet spectra (UV) were measured with a Hitachi U-2900 spectrophotometer. Principle bands ( $\lambda_{\max}$ ) were recorded as wavelengths (nm) and  $\log \epsilon$  in MeOH solution. Infrared spectra (IR) were determined on a Bruker Tensor 27 spectrometer and recorded on wavenumber ( $\text{cm}^{-1}$ ). ElectroSpray ionization-mass spectrometry (ESI-MS) was recorded on an AB Sciex API4000 mass spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on Bruker AV 400 MHz spectrometers with tetramethylsilane (TMS) as an internal standard. Optical rotations were measured in methanol solution with sodium D line (589.44 nm) on a Schmidt HHW 5 automatic polarimeter. Solvents for extraction and chromatography were distilled at their boiling point ranges prior to use except for ethyl acetate which was an analytical grade reagent. Thin-layer chromatography (TLC) and precoated TLC plating were performed on  $\text{SiO}_2$  GF254 (10–40 mm, Qingdao Marine Chemical Inc., China). Column chromatography was performed on silica gel ( $\text{SiO}_2$ : 200–300 mesh, Qingdao Marine Chemical Inc., China), reverse phase HPLC: RP-18 [LaChrom  $\text{C}^{18}$  (4.6 mm  $\times$  150 mmL) L-2000, Hitachi Co., Japan], preparation HPLC [InertSustain  $\text{C}^{18}$  (20  $\times$  250 mm), LC-8A Shimadzu. Co., Japan].

### Cell lines and pathogenic bacteria

Melanoma cells B-16, human gastric cancer cells SGC-7901 and normal mammary epithelial cells M-10 were obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae*

ATCC700603, and *Escherichia coli* ATCC 25922 were provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China).

### Fungus identification

The fungus (strain M-22) was isolated from a rotted leaf sample collected on the west coast of Haikou, Hainan province, China (19°59'N, 110°19'E). The chromosomal DNA from M-22 strains was extracted using a fungus genomic DNA extraction kit (Tiangen Co., China), and the isolated DNA was amplified by PCR using universal primers ITS 1 and ITS 4 (Rivera and Seifert 2011), corresponding to a 5.8S rDNA sequence.

For PCR, the reaction elements included 1  $\mu\text{L}$  of the template DNA, 5  $\mu\text{L}$  of 10 $\times$  Taq buffer, 1  $\mu\text{L}$  of 10 mM mixed dNTP, 1  $\mu\text{L}$  of sense-ITS 1 primer (TCCGTAGGTGAACCTGCGG, 10 pmol/ $\mu\text{L}$ ), 1  $\mu\text{L}$  of antisense-ITS 4 primer (TCCTCCGCTTATTGATATGC, 10 pmol/ $\mu\text{L}$ ), and 5 U of Taq DNA polymerase, which were prepared in a final volume of 50  $\mu\text{L}$ . The PCR thermal conditions were set as follows: preheating for denaturation at 94  $^\circ\text{C}$  for 4 min, 30 cycles of denaturation at 94  $^\circ\text{C}$  for 30 s, annealing at 52  $^\circ\text{C}$  for 30 s, extension at 72  $^\circ\text{C}$  for 60 s, and additional extension at 72  $^\circ\text{C}$  for 7 min. The sequencing analysis data obtained from Sinogenomax Co., Ltd. showed that M-22 was most closely related to the partial sequence of *Penicillium sclerotiorum* (99 % identity), identifying this strain as *Penicillium sclerotiorum*.

### Fermentation, extraction and purification of the fungal strain

The fungus was grown under revolving conditions at 28  $^\circ\text{C}$ , 120 rpm for 15 days in 500-ml Erlenmeyer flasks containing liquid medium (200 ml/flask) composed of glucose (10 g/L), yeast extract (1 g/L), tryptone (2 g/L), and aged seawater (600 mL/L). The fermented whole broth (10 L) was filtered through a cheesecloth to separate it into supernatant and mycelia. The former was concentrated to about a quarter of the original volume and then extracted five times with AcOEt to give an AcOEt solution, while the latter was extracted three times with acetone. The acetone solution was concentrated to afford an aqueous solution.

The aqueous solution was extracted three times with AcOEt to give another AcOEt solution. Both AcOEt solutions were combined and concentrated to give a crude extract (7.8 g). The crude extract was applied to CC [SiO<sub>2</sub> (300–400 mesh), step gradient of petroleum ether/CHCl<sub>3</sub> and CHCl<sub>3</sub>/MeOH]: fractions 1–12. Fraction 2 (520 mg) was separated by preparation HPLC (MeOH: H<sub>2</sub>O = 60:40, flow: 15 mL/min, detect wave: 245 nm), which yielded two compounds **1** (10.2 mg) and **2** (18.7 mg).

Penicilazaphilone B (**1**): Yellow gum. <sup>1</sup>HNMR (CDCl<sub>3</sub>, 400 MHz) and <sup>13</sup>CNMR (CDCl<sub>3</sub>, 100 MHz) data see Table 1. IR V<sub>max</sub> (KBr)/cm<sup>-1</sup>: 3405, 1721, 1611. UV λ<sub>max</sub> (MeOH) nm (log ε): 225 (3.96), 244 (3.88), 346 (4.22). HR-EI-MS m/z: 352.1884 (calculated for C<sub>19</sub>H<sub>28</sub>O<sub>6</sub>: 352.1886). EI-MS m/z: 352 (M<sup>+</sup>), 334, 266, 149, 98. [α]<sub>D</sub><sup>25</sup>: -44 (c = 0.30, MeOH).

Penicilazaphilone C (**2**): Yellow gum. <sup>1</sup>HNMR (CDCl<sub>3</sub>, 400 MHz) and <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) data see Table 1. IR V<sub>max</sub> (KBr)/cm<sup>-1</sup>: 3412, 1711, 1614. UV λ<sub>max</sub> (MeOH) nm (log ε): 226 (3.98), 245 (3.03), 344 (3.39). HR-EI-MS m/z: 417.1061 (calculated for C<sub>20</sub>H<sub>29</sub>O<sub>7</sub>Cl: 417.1062). EI-MS m/z: 417 (M<sup>+</sup>), 399, 381, 312, 295, 244, 111. [α]<sub>D</sub><sup>25</sup>: -35.1 (c = 0.30, MeOH).

## Cytotoxicity assay

Cytotoxic activity against B-16 (murine melanoma), SGC-7901 (human gastric cancer) and M-10 (normal mammary epithelial cells) cell lines were determined according to previously reported methods (Tavakolinia et al. 2012).

## Antibacterial assay

The two compounds' sensitive bacteria tests were carried out using the disk diffusion method (Niu et al. 2013). Minimal inhibitory concentration (MIC) were determined by double-broth dilution method (Andrews 2001).

## Results and discussion

### Identification of strain

The producing strain, M-22, was isolated from the rotted leaves that were collected from the west coastal of Haikou, Hainan province in China. In general, the mycelium of this strain grow 30–50 mm diameter in 7 days on marine fungus collective medium; the conidial colours are subtle

**Table 1** The NMR Data of Penicilazaphilones B (**1**) and C (**2**)

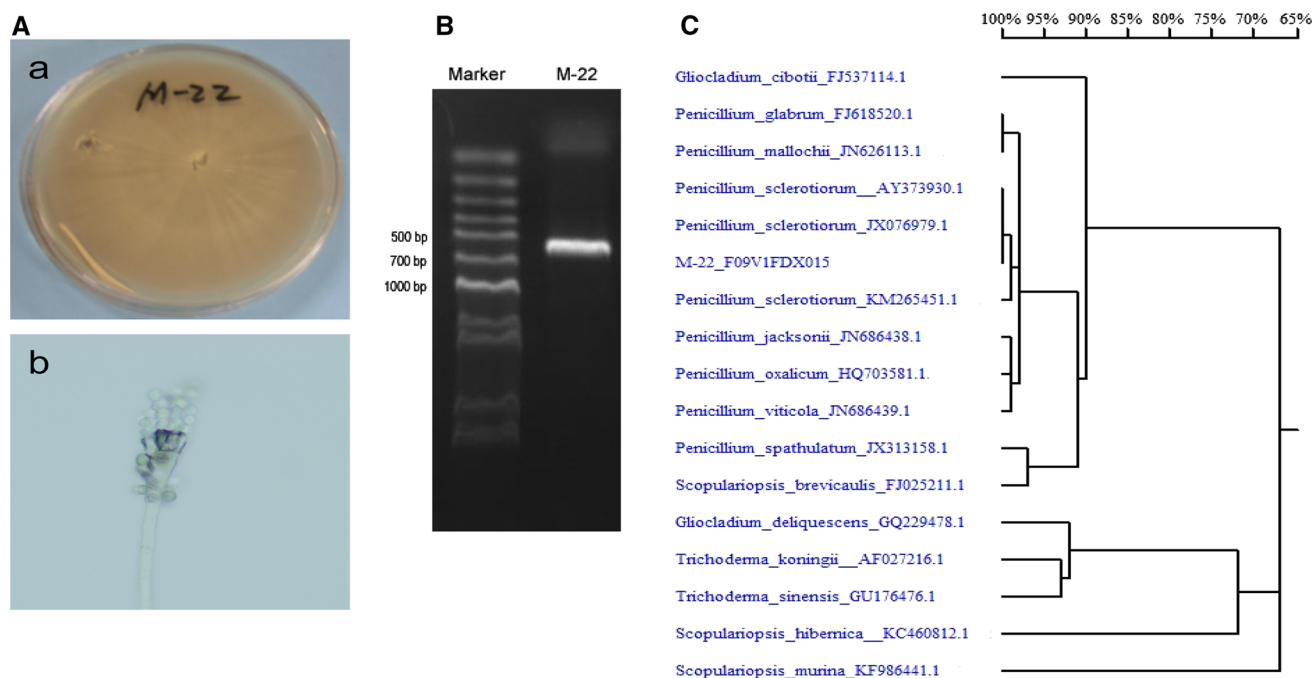
No.	<b>1</b>		<b>2</b>		HMBC correlation
	δH (mult, J, Hz)	δC mult	δH (mult, J, Hz)	δC mult	
1	a: 4.78, dd (10.8, 5.4) b: 3.77, dd (13.5, 10.8)	68.5, CH <sub>2</sub>	a: 4.72, dd (10.1, 4.0) b: 4.15, dd (12.3, 5.4)	68.3, CH <sub>2</sub>	C-3, C-4a, C-8, C-8a
3		161.5, qC		161.8, qC	
4	5.72, s	102.4, CH	6.11, s	101.7, CH	C-3, C-4a, C-5, C-8a, C-1'
4a		152.1, qC		145.0, qC	
5	5.83, s	116.3, CH		111.7, qC	
6		197.3, qC		193.9, qC	
7		74.2, qC		75.4, CH	
8	3.68, d (9.1)	74.5, CH	4.08 d (2.5)	74.4, CH	C-1, C-8a, C-9
8a	3.05, m	36.4, CH	3.19, m	37.3, CH	C-1, C-4a, C-8
9	1.43, s	20.8, CH <sub>3</sub>	1.43, s	22.3 CH <sub>3</sub>	C-6, C-7, C-8
1'	6.25, d (15.2)	122.3, CH	6.32, d (15.6)	121.5, CH	C-3, C-4, C-2', C-4'
2'	6.54, d (15.2)	143.0, CH	6.72, dd (15.6, 15.7)	142.9, CH	C-3, C-1', C-3', C-4',
3'		75.7, qC	3.46, d (15.7)	79.4, CH	C-1', C-2', C-4', C-5', C-10'
4'	3.46, d (2.4)	78.2, CH		77.8, qC	
5'	1.68, m	35.3, CH	3.36, d (16.0)	78.4, CH	C-4', C-10', C-6', C-7', C-9'
6'	a: 1.45, m; b: 1.30, m	28.7, CH <sub>2</sub>	1.74, m	35.1, CH	C-4', C-5', C-7', C-8', C-9'
7'	0.92, m	11.6, CH <sub>3</sub>	a: 1.45, m; b: 1.36, m	28.6, CH <sub>2</sub>	C-5', C-6', C-8', C-9'
8'	0.96, d (7.0)	13.4, CH <sub>3</sub>	0.93, m	11.0, CH <sub>3</sub>	C-6', C-7', C-9'
9'	1.30, s	23.8, CH <sub>3</sub>	0.95, d (7.4)	13.0, CH <sub>3</sub>	C-5', C-6', C-8'
10'			1.32, s	24.9, CH <sub>3</sub>	C-3', C-4', C-5

green with grey (Fig. 2a). As the colony morphology and conidia or spores of this species is classified as *Penicillium* (Rivera and Seifert 2011; Visagie et al. 2014), this fungus was identified according to analysis of the DNA sequences of the internal transcribed spacer (ITS1-5.8S-ITS4) regions of its ribosomal RNA gene. The ITS sequence (550 bp, shown in Fig. 2b, GenBank Accession Number F09Y7C2T015) matched with three *Penicillium sclerotiorum* sequences from GenBank comprising AY373930.1, JX076979.1 and KM265450.1 with sequence identity of 99.9, 99.9 and 99.8 %, respectively. This marine fungus was then identified as *Penicillium sclerotiorum*. Gene phylogenetic analyses ITS revealed that strains previously identified as *P. sclerotiorum* comprise a complex of phylogenetically distinct species as show in Fig. 2c. *P. sclerotiorum* is now classified as *Penicillium* subgenus *Aspergilloides*, section *Sclerotiora* by Houbraken and Samson (Houbraken and Samson 2011).

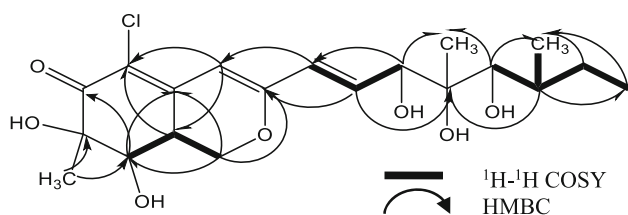
### Structural elucidation of the compounds

Penicilazaphilone C (2) was obtained as a yellow gum. Its IR absorptions at 3412, 1711, and 1614  $\text{cm}^{-1}$  implied that hydroxyl, carbonyl and double bond functional groups existed, respectively. The UV spectrum showed absorption maxima at 226, 245 and 344 nm, indicating the presence of an extended conjugated system. High-resolution mass spectral analysis suggested a molecular formula of

$\text{C}_{20}\text{H}_{29}\text{O}_7\text{Cl}$  ( $m/z$  417.1601  $[\text{M}]^+$ ) with six degrees of unsaturation, which was consistent with the structural information provided by  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra. The  $^1\text{H-NMR}$  spectral data (Table 1) and HMQC spectrum composed of signals for trans-olefinic protons [ $\delta$  6.72 (1H, dd,  $J = 15.6, 15.7$  Hz, H-2') and 6.32 (1H, d,  $J = 15.6$  Hz, H-1')], three oxymethine protons [ $\delta$  4.08 (1H, d,  $J = 2.5$  Hz, H-8), 3.46 (1H, d,  $J = 12.0$  Hz, H-3') and 3.36 (1H, d,  $J = 16.0$  Hz, H-5')], two methine protons [ $\delta$  3.19 (1H, m, H-8a) and  $\delta$  1.74 (1H, m, H-6')], two nonequivalent methylene protons [ $\delta$  1.45 (1H, m, H<sub>a</sub>-7') and  $\delta$  1.36 (1H, m, H<sub>b</sub>-7')] and four methyl groups [ $\delta$  1.43 (3H, s, H-9), 1.32 (3H, s, H-10'), 0.95 (3H, d,  $J = 7.4$  Hz, H-9'), 0.93 (3H, m, H-8')]. The remaining proton signals belonged to one olefinic proton ( $\delta$  6.11, 1H, s, H-4), two non-equivalent oxymethine protons [ $\delta$  4.72 (1H, dd,  $J = 10.1, 4.0$  Hz, H<sub>a</sub>-1) and 4.15 (1H, dd,  $J = 12.3, 5.4$  Hz, H<sub>b</sub>-1)].  $^1\text{H-}^1\text{H}$  COSY analysis of 2 revealed three partial structural units as shown by bold-faced lines in Fig. 3, the cross peaks of H<sub>ab</sub>-1 ( $\delta$  4.72, 4.15)/H-8a ( $\delta$  3.19), H-8a ( $\delta$  3.19)/H-8 ( $\delta$  4.08), H-1' ( $\delta$  6.32)/H-2' ( $\delta$  6.72), H-2' ( $\delta$  6.72)/H-3' ( $\delta$  3.46), H-5' ( $\delta$  3.36)/H-6' ( $\delta$  1.74) and H<sub>ab</sub>-7' ( $\delta$  1.45, 1.32)/H-8' ( $\delta$  0.93). The chemical shifts of C-7 ( $\delta$  75.4) and C-8 ( $\delta$  74.4) suggested that these carbons are oxygenated. C-1' ( $\delta$  121.5) of the octenyl unit was connected with C-3 ( $\delta$  161.8) of the azaphilone unit by the key HMBC correlations from H-2' to C-3 and from H-1' to C-4. The connection of these units and the remaining functional groups

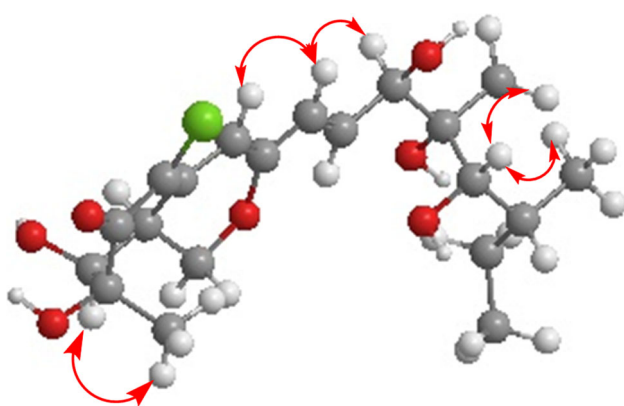


**Fig. 2** **A** Morphology of strain M-22, the characterization of strain (a) and conidiophores of strain (b); **B** PCR product of ITS rDNA sequence of strain; **C** homology tree of strain M-22 isolated to other fungi from GeneBank, deduced from sequence of ITS rDNA phylogenetic tree of the 5.8S rRNAs



**Fig. 3** Selected COSY and HMBC correlations for **2**

was determined on the basis of the key HMBC correlations summarized in Fig. 3. The connection of a chlorine atom to C-5 was reasonable from its chemical shift ( $\delta$  C 111.7). Thus, the planar structure of penicilazaphilone C was established as that depicted in **2**. The relative stereoconfiguration of **2** was established by the analysis of its  $^1\text{H}$  NMR  $J$ -values and NOEDIFF data combined with three-dimensional molecular modelling. The large coupling constant (15.6 Hz) observed for H-1' and H-2' suggested that these protons had a *trans*-diaxial relationship. NOE correlations of H-1' to H-4 ( $\delta$  6.11) and H-3' ( $\delta$  3.46) indicated that they were cofacial and that the absolute configuration for C-3' was *S*. Irradiation of H-5' in the NOEDIFF experiment enhanced signal intensity of H<sub>3</sub>-9' and H<sub>3</sub>-10' but not H-6' suggesting that these protons were cofacial but have opposite orientation to H-6', thus indicating that the absolute configuration at C-4', C-5' and C-6' was then proposed to be *S*, *R* and *R*, respectively. In addition, irradiation of H-8 in the NOEDIFF experiment enhanced signal intensity of H<sub>3</sub>-9, but not H-8a (3.19), indicating that H-8 was *trans* to H-8a but *cis* to H<sub>3</sub>-9. These data revealed that the absolute configuration of this moiety in **2** was proposed to be 7*R*, 8*R*, and 8*aR*. The relative configuration of penicilazaphilone C (**2**) was thus established as that shown in Fig. 4. Consequently, penicilazaphilone C (**2**) was assigned as a new polyketide azaphilone.



**Fig. 4** Selected NOE correlations for **2**

Penicilazaphilone B was obtained as a yellow gum. The HR-EI-MS showed the molecular formula C<sub>19</sub>H<sub>28</sub>O<sub>6</sub> corresponding to six degrees of unsaturation. The general features of its UV, IR, and NMR spectra (Table 1) resembled those of **2** except less one carbon atom, one oxygen atom and helium atom, and the  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR spectral data as shown in the Table 1. According to the  $^1\text{H}$ -NMR,  $^{13}\text{C}$ -NMR spectral data and relevant literature can be identified as penicilazaphilone B by comparison with the data given in the corresponding references (Arunpanichlert et al. 2010).

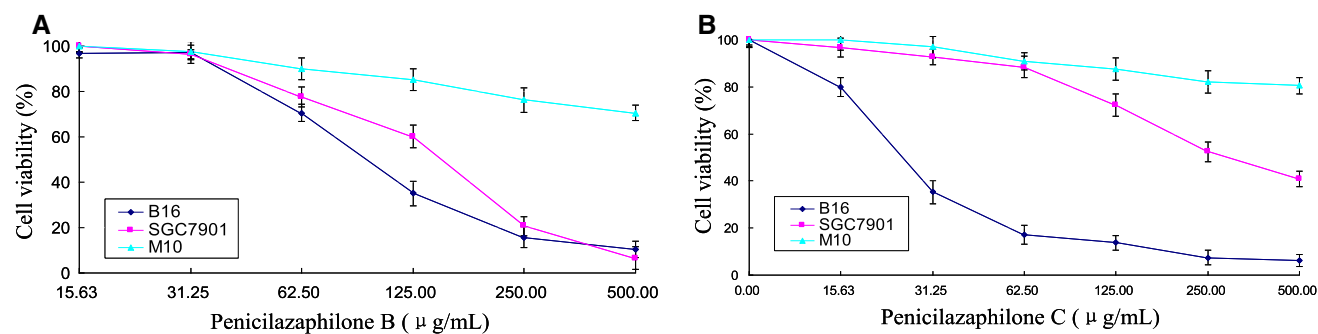
#### Antineoplastic activities of the isolated compounds

The *in vitro* cytotoxicity experiments were performed with the two compounds against melanoma cells B-16, human gastric cancer cells SGC-7901 and normal mammary epithelial cells M-10 to study cell proliferation (Fig. 5). The MTT assay was employed and showed that the two compounds have good antiproliferation against cancer cells but weak inhibition proliferation for normal M-10 cells. The inhibition of cell proliferation was stronger when those two compounds' concentration increased. IC<sub>50</sub> (the inhibition of 50 % cell growth) was determined by interpolation from dose–response curves. As shown in Fig. 5, penicilazaphilone B exhibited potent cytotoxic ability against B-16 and SGC-7901 cell lines, and its IC<sub>50</sub> values were 0.291 and 0.449 mM respectively. Thus penicilazaphilone C against the two cancer cell lines showed antiproliferation and IC<sub>50</sub> values of 0.065 and 0.720 mM, respectively. The results suggested that penicilazaphilone B and C were both selectively cytotoxic to cancer cell lines.

#### Antibacterial activities of the isolated compounds

According to the determination standard for the qualitative result of bacteriostasis *in vitro*, the two compounds were all sensitive to four pathogenic bacteria (*S. aureus*, *P. aeruginosa*, *K. pneumonia* and *E. coli*). The penicilazaphilone C antibacterial force to *K. pneumoniae* was the strongest. The results of the drug-sensitive test of two compounds to the four pathogenic bacteria are shown in Table 2 and Fig. 6.

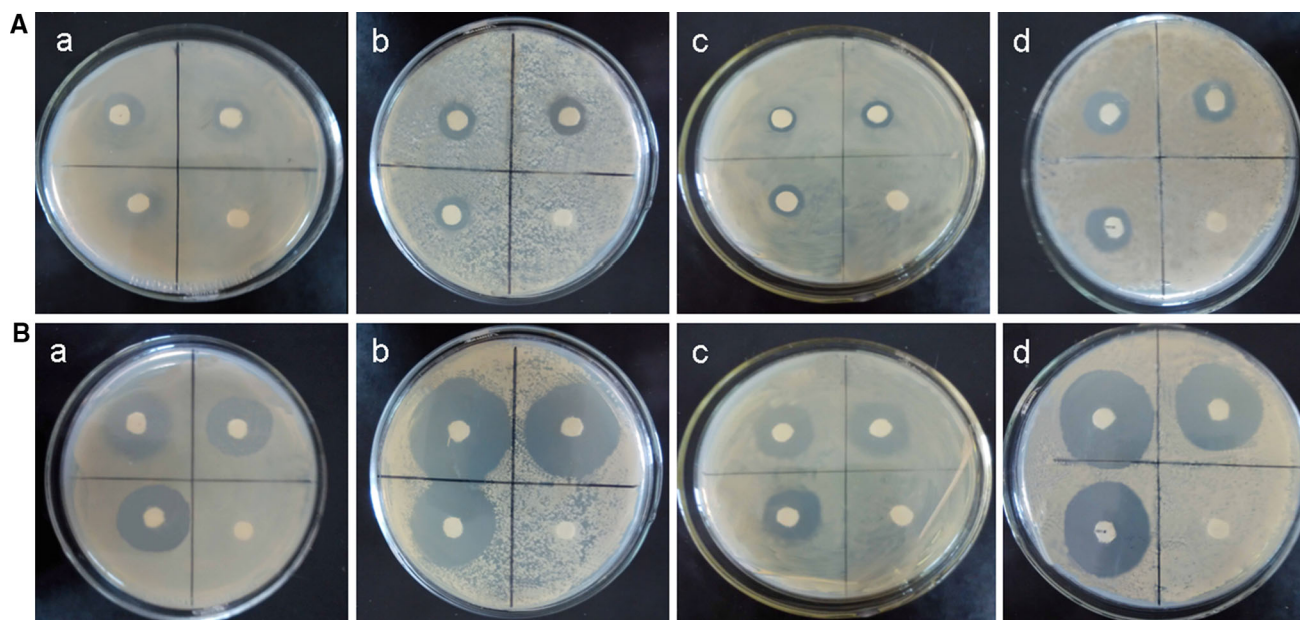
MIC values of the two compounds to four pathogenic bacteria are shown in Table 2. However, penicilazaphilone C has more antibacterial activity than penicilazaphilone B. The latter was weakly inhibited to four pathogenic bacteria, and its lowest MIC value was 250.0  $\mu\text{g}/\text{mL}$ , but penicilazaphilone C inhibited all test pathogenic bacteria, in particular *K. pneumoniae* and *E. coli*, and its MIC values were both 0.037 mM. The results indicated that the chlorine atom might play role in the biological activities.



**Fig. 5** **a** Inhibition growth of penicilazaphilone B on B16, SGC7901 and M10 cell lines; **b** inhibition growth of penicilazaphilone C on B-16, SGC-7901 and M-10 cell lines

**Table 2** Diameter of inhibiting bacteria circle and MIC of drugs

	Penicilazaphilone B		Penicilazaphilone C	
	Diameter of inhibiting bacteria circle (mm)	MIC (μg/mL)	Diameter of inhibiting bacteria circle (mm)	MIC (μg/mL)
<i>S. aureus</i>	11.0 ± 0.50	250.0	23.0 ± 0.87	31.25
<i>P. aeruginosa</i>	10.0 ± 0.50	500.0	14.0 ± 1.32	62.5
<i>K. pneumoniae</i>	10.5 ± 0.53	500.0	25.5 ± 2.02	15.63
<i>E. coli</i>	11.5 ± 0.76	250.0	24.5 ± 1.53	15.63



**Fig. 6** Antibacterial activities of penicilazaphilone B and penicilazaphilone C. **A** Inhibitory effect of penicilazaphilone B on *S. aureus* (a), *P. aeruginosa* (b), *K. pneumoniae* (c), and *E. coli* (d); **B** inhibitory effect of penicilazaphilone C on *S. aureus* (a), *P. aeruginosa* (b), *K. pneumoniae* (c), and *E. coli* (d)

## Conclusion

Two azaphilone derivatives [penicilazaphilones B (**1**) and C (**2**)] were isolated from a marine fungus strain *P. sclerotiorum* M-22 fermented in Erlenmeyer flasks (10 L), and

the penicilazaphilones C (**2**) is a new compound. Two compounds exhibited moderate antineoplastic activity to melanoma cells B16 and human gastric cancer cells SGC-7901 but weak cytotoxic activities to normal mammary epithelial cells M10, and compound **2** showed stronger

antibacterial activity compare to compound **1**. This suggests the two compounds as potential anticancer or antibacterial drugs.

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#### Compliance with ethical standards

**Conflicts of Interest** The authors declare no conflict of interest.

## References

- Akihisa T, Tokuda H, Yasukawa K, Ukiya M, Kiyota A, Sakamoto N, Suzuki T, Tanabe N, Nishino H (2005) Azaphilones, furanoisophthalides, and amino acids from the extracts of *Monascus pilosus*-fermented rice (red-mold rice) and their chemopreventive effects. *J Agric Food Chem* 53:562–565
- An CY, Li XM, Li CS, Xu GM, Wang BG (2014) Prenylated indole-diketopiperazine peroxides and related homologues from the marine sediment-derived fungus *Penicillium brefeldianum* SD-273. *Mar Drugs* 12:746–756
- Andrews JM (2001) Determination of minimum inhibitory concentrations. *J Antimicrob Chemother* 49:5–16
- Arunpanichlert J, Rukachaisirikul V, Sukpondma Y, Phongpaichit S, Tewtrakul S, Rungjindamai N, Sakayaroj J (2010) Azaphilone and isocoumarin derivatives from the endophytic fungus *Penicillium sclerotiorum* PSU-A13. *Chem Pharm Bull* 58:1033–1036
- Bladt TT, Frisvad JC, Knudsen PB, Larsen TO (2013) Anticancer and antifungal compounds from *Aspergillus*, *Penicillium* and other filamentous fungi. *Molecules* 18:11338–11376
- Blunt JW, Copp BR, Keyzers RA, Munro MH, Prinsep MR (2012) Marine natural products. *Nat Prod Rep* 29:144–222
- Blunt JW, Copp BR, Keyzers RA, Munro MH, Prinsep MR (2014) Marine natural products. *Nat Prod Rep* 31:160–258
- Houbraken J, Samson RA (2011) Phylogeny of *Penicillium* and the segregation of Trichocomaceae into three families. *Stud Mycol* 70:1–51
- Li LQ, Yang YG, Zeng Y, Zou C, Zhao PJ (2010) A new azaphilone, kasanosin C, from an endophytic *Talaromyces* sp. T1BF. *Molecules* 15:3993–3997
- Li CS, Li XM, Gao SS, Lu YH, Wang BG (2013) Cytotoxic anthranilic acid derivatives from deep sea sediment-derived fungus *Penicillium paneum* SD-44. *Mar Drugs* 11:3068–3076
- Matsuzaki K, Ikeda H, Masuma R, Haruo T, Satoshi O (1995) Isochromophilones I and II, novel inhibitors against gp-120-CD4 Binding Produced by *Penicillium multicolor* FO-2338. *J Antibiot* 48:703–707
- Niu Y, Yi N, Yuan L, Wang R (2013) Study on antibacterial effect of medlar and hawthorn compound extract in vitro. *Afr J Tradit Complement Altern Med* 10:567–573
- Quang DN, Harinantenaina L, Nishizawa T, Hashimoto T, Kohchi C, Soma GI, Asakawa Y (2006) Inhibition of nitric oxide production in RAW 264.7 cells by azaphilones from Xylariaceae fungi. *Biol Pharm Bull* 29:34–37
- Rivera KG, Seifert KA (2011) A taxonomic and phylogenetic revision of the *Penicillium sclerotiorum* complex. *Stud Mycol* 70:139–158
- Somoza AD, Lee KH, Chiang YM, Oakley BR, Wang CC (2012) Reengineering an azaphilone biosynthesis pathway in *Aspergillus nidulans* to create lipoxxygenase inhibitors. *Org Lett* 14:972–975
- Tavakolinia F, Baghipour T, Hossaini Z, Zareyee D, Khalilzadeh MA, Rajabi M (2012) Antiproliferative activity of novel thiopyran analogs on MCF-7 breast and HCT-15 colon cancer cells: synthesis, cytotoxicity, cell cycle analysis, and DNA-binding. *Nucleic Acid Ther* 22:265–270
- Toki S, Tanaka T, Uosaki Y, Yoshida M, Suzuki Y, Kita K, Mihara A, Ando K, Lokkernkkern NA, Matsuda Y (1999) RP-1551s, a family of azaphilones produced by *Penicillium* sp., inhibit the binding of PDGF to the extracellular domain of its receptor. *J Antibiot* 52:235–244
- Tomoda H, Matsushima C, Tabata N, Namatame I, Tanaka H, Bamberger MJ, Arai HFM, Inoue K, Omura S (1999) Structure-specific inhibition of: cholesteryl ester transfer proteins by azaphilones. *J Antibiot* 52:160–170
- Visagie CM, Houbraken J, Frisvad JC, Hong SB, Klaassen CH, Perrone G, Seifert KA, Varga J, Yaguchi T, Samson RA (2014) Identification and nomenclature of the genus *Penicillium*. *Stud Mycol* 78:343–371
- Yamada T, Muroga Y, Tanaka R (2009) New azaphilones, secochaetomugilins A and D, produced by a marine-fish-derived *Chaetomium globosum*. *Mar Drugs* 7:249–257