Antiviral Butyrolactones from the Endophytic Fungus *Aspergillus versicolor*

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Abstract

Versicolactones A–D (**1**–**4**), four new butyrolactones, along with four known butyrolactones (**5**–**8**) were isolated from the fermentation products of the endophytic fungus *Aspergillus versicolor*. The structures of compounds **1**–**4**, including absolute configuration, were elucidated by interpretation of the NMR and CD data. Compound **2** was further confirmed by single-crystal X-ray diffraction analysis. In particular, compound **1** is the first naturally occurring butyrolactone possessing an unusual 2-oxopropyl group. More importantly, compounds **1** and **8** displayed significant antitobacco mosaic virus activities with inhibition rates of 46.4% and 35.4%, even more potent than the positive control ningnanmycin (30.8%). Compound **1** also showed moderate cytotoxicity against A549 and MCF7 cells with IC_{50} values of 3.2 and 2.5 µM, respectively.

Supporting information available online at http://www.thieme-connect.de/products

Introduction

Aspergillus species (Trichocomaceae) from diverse origins (e.g., soil, plants, marine organisms, and even indoor air environments) are often regarded as a prolific source of structurally interesting secondary metabolites, such as alkaloids, polyketides, terpenes, peptides, and lignans, with intriguing biological properties [1–6].

Among them, butyrolactones, a subclass of lignans with a characteristic four-carbon heterocyclic ring, mainly produced by fungi and high plants [7–9], have attracted much attention for their pronounced pharmacological effects including antibacterial [10, 11], cytotoxic [12, 13], anti-inflammatory [14, 15], and antiviral [11, 16] activities.

Herein, our efforts on the endophytic fungus *Aspergillus versicolor* (Vuillemin) Tiraboschi, isolated from the rhizome of *Paris polyphylla* var. *yunnanensis*, collected in Dali, Yunnan, P. R. China, led to the isolation and identification of four new (**1**–**4**) and four known (**5**–**8**) butyrolactones. In this paper, the structure elucidation of compounds **1**–**4**, the antitobacco mosaic virus (anti-TMV) activities of the isolated compounds, and the cytotoxic properties of compounds **1**–**4** against five human tumor cell lines (NB4, A549, SHSY5Y, PC3, and MCF7) are reported.

Results and Discussion

The fermented substrate was extracted with 70% aqueous acetone. The extract was subjected repeatedly to column chromatography on silica gel, MCI, RP-18, and preparative HPLC to afford compounds **1**–**8**, including four new butyrolactones, versicolactones A–D (**1**–**4**), together with four known butyrolactones, butyrolactone I (**5**) [11, 14], butylrolactone VI (**6**) [11, 17], butyrolactone V (**7**) [11, 18], and butylrolactone IV (**8**) [14]. The structures of the compounds **1**–**8** are shown in **Fig. 1**, and the ^1H and ^{13}C NMR data of **1**–**4** are listed in **Table 1**.

Compound **1** was obtained as a white powder. Its molecular formula $C_{23}H_{22}O_8$ was determined by positive HRESIMS at *m/z* 449.1208 $[M + Na]^+$, requiring 13 degrees of unsaturation. The IR spectrum showed broad and intense absorption bands for hydroxy (3447 cm^{-1}), ester/lactone carbonyl (1735, 1727 cm^{-1}) and aromatic rings (1607, 1542, 1468 cm^{-1}). In the ^1H NMR spectrum, the signals of a 1,4-disubstituted benzene moiety (δ_H 7.63, 2H, d, J = 8.6 Hz; 6.85, 2H, d, J = 8.6 Hz), a

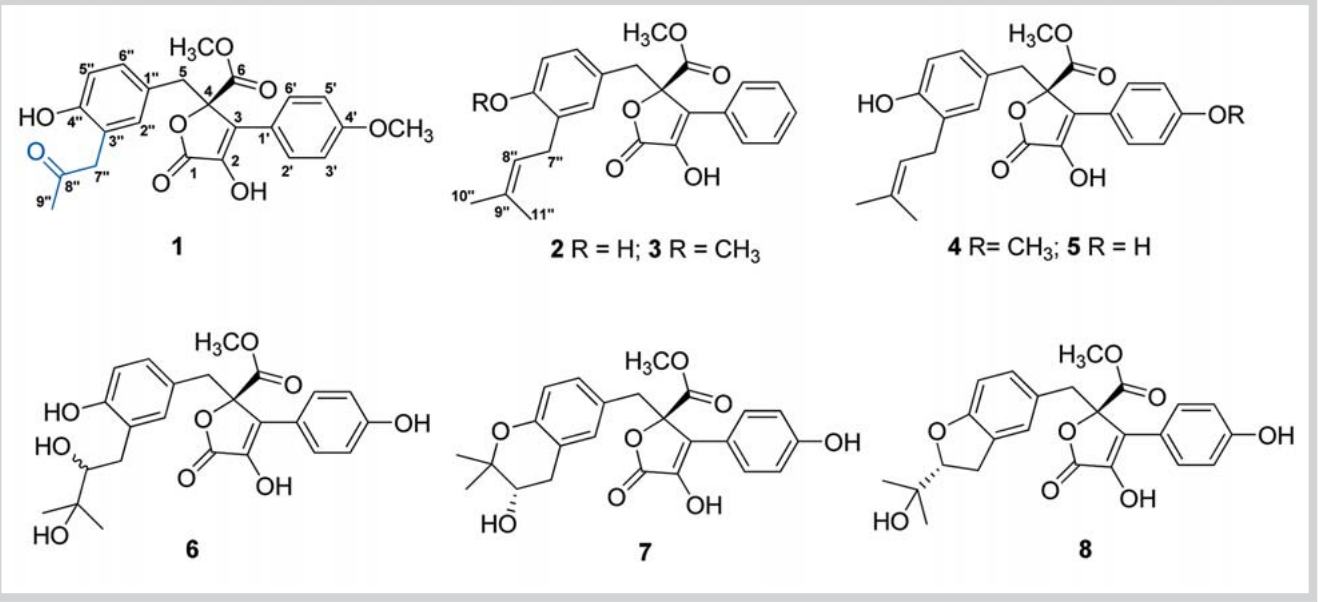

Fig. 1 Chemical structures of compounds **1**–**8**. The absolute stereochemistry of compound **2** was determined by X-ray analysis. The configurations of compounds **1, 3**, and **4** were based on a similarity of Cotton effects compared to compound **2**. The configurations of compounds **5**–**8** are concluded based on previous studies [11, 14, 17, 19]. The wavy bond in the structure of compound **6** indicates an unclear configuration. (Color figure available online only.)

Table 1 ^{1}H- and ^{13}C-NMR data for versicolactones A–D (**1**–**4**) (δ in ppm and J in Hz).

No.	Compound 1[a]		Compound 2[b]		Compound 3[c]		Compound 4[d]	
	δ_C (m)	δ_H (m, J, Hz)	δ_C (m)	δ_H (m, J, Hz)	δ_C (m)	δ_H (m, J, Hz)	δ_C (m)	δ_H (m, J, Hz)
1	168.9 s		169.1 s		168.9 s		169.6 s	
2	140.8 s		140.1 s		140.0 s		139.0 s	
3	127.4 s		127.4 s		127.2 s		127.9 s	
4	85.9 s		85.9 s		85.7 s		86.3 s	
5	38.9 t	3.38, 3.43 d (14.6)	38.4 t	3.43, 3.47 d (14.6)	38.2 t	3.32, 3.36 d (14.6)	38.8 t	3.49, 3.55 d (14.6)
6	170.3 s		170.1 s		169.9 s		170.0 s	
1′	122.8 s		130.1 s		130.0 s		122.4 s	
2′,6′	130.1 d	7.63 d (8.6)	127.7 d	7.63 d (7.6)	127.3 d	7.52 d (7.6)	129.8 d	7.62 d (8.7)
3′,5′	116.5 d	6.85 d (8.6)	129.0 d	7.38 m	129.0 d	7.28 m	116.2 d	6.91 d (8.7)
4′	160.9 s		128.7 d	7.31 d (7.4)	128.7 d	7.18 d (7.4)	160.1 s	
1″	124.5 s		123.8 s		123.6 s		124.8 s	
2″	132.3 d	6.33 d (1.8)	131.5 d	6.37 d (1.6)	130.2 d	6.26 s	131.1 d	6.48 d (1.9)
3″	125.0 s		127.2 s		126.5 s		126.7 s	
4″	152.5 s		153.4 s		156.4 s		153.3 s	
5″	114.9 d	6.51 d (8.2)	114.4 d	6.49 d (8.2)	114.2 d	6.41 d (8.2)	115.3 d	6.53 d (8.2)
6″	128.9 d	6.61 d (1.8, 8.2)	128.9 d	6.53 dd (1.6, 8.2)	128.1 d	6.43 d (8.2)	129.4 d	6.60 d (1.9, 8.2)
7″	48.4 t	4.11 s	28.0 t	3.05 d (7.2)	27.7 t	2.94 d (6.8)	29.2 t	3.14 d (7.2)
8″	206.4 s		121.9 d	5.00 m	121.8 d	4.88 m	121.6 d	5.10 m
9″	29.8 q	1.68 s	132.7 s		132.5 s		132.0 s	
10″			17.5 q	1.51 s	17.3 q	1.40 s	17.9 q	1.63 s
11″			25.6 q	1.59 s	25.4 q	1.48 s	25.8 q	1.68 s
-OMe-6	53.3 q	3.71 s	53.4 q	3.71 s	53.2 q	3.62 s	53.5 q	3.78 s
-OMe-4′	56.0 q	3.78 s					56.1 q	3.80 s
-OMe-4″					56.0 q	3.64 s		

[a]500 and 125 MHz, in $CDCl_3$; [b]500 and 125 MHz, in CD_3OD; [c]400 and 100 MHz, in CD_3OD; [d]400 and 100 MHz, in $CDCl_3$

1,3,4-trisubstituted benzene moiety (δ_H 6.33, 1H, d, J = 1.8 Hz; 6.61, 1H, J = 8.2 and 1.8 Hz; 6.51, 1H, d, J = 8.2 Hz), a pair of methylene protons (δ_H 3.38, 1H, d, J = 14.6 Hz; 3.43, 1H, d, J = 14.6 Hz), and two methoxy protons (δ_H 3.71, 3H, s; 3.78, 3H, s) were clearly apparent. The ^{13}C NMR and DEPT data disclosed the existence of three methyls (including two oxygenated ones), two methylenes, seven methines, and eleven quaternary carbons (including seven sp^2 ones, one oxygenated quaternary carbon, and three carbonyl groups). Among them, three carbonyls and fourteen olefinic carbons occupied twelve degrees of unsaturation. These data sug-

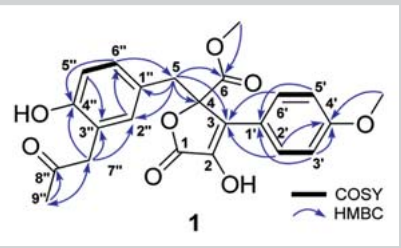

Fig. 2 ^{1}H–^{1}H COSY and selected HMBC correlations of compound **1**. (Color figure available online only.)

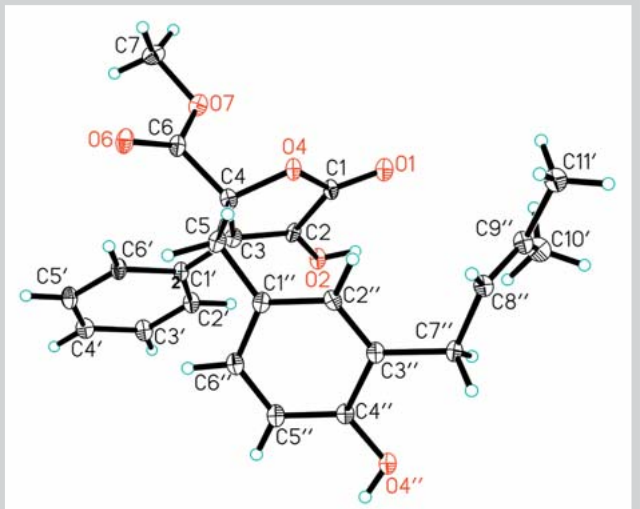

Fig. 3 X-ray structure of compound **2**. (Color figure available online only.)

gested that compound **1** must possess one aliphatic ring in addition to two aromatic rings. The characteristic carbon signals at δ_C 168.9, 140.8, 127.4, 85.9, 38.9, and 170.3 indicated that **1** should be a butyrolactone [14]. A direct comparison of the NMR data of **1** and the known compound butyrolactone I (**5**) suggested that both compounds were similar and had the same basic skeleton. The main difference was that the prenyl group at the C-3″ position in **5** was replaced by an unusual 2-oxopropyl group in **1**. This assignment was supported by the HMBC corrections from H_3-9″ (δ_H 1.68) to C-7″ (δ_C 48.4) and C-8″ (δ_C 206.4), and from H_2-7″ (δ_H 4.11) to C-9″ (δ_C 29.8), C-2″ (δ_C 132.3), and C-4″ (δ_C 152.5) (**Fig. 2**). Furthermore, the HMBC correlation of the methoxy group (δ_H 3.78) with C-4′ (δ_C 160.9) indicated that the methoxy group was located at the C-4′ position. Thus, the structure of **1** was established as shown in **Fig. 1**, and the compound was named versicolactone A. Interestingly, compound **1** is the first described naturally occurring butyrolactone possessing a novel 2-oxopropyl moiety.

Versicolactone B (**2**) showed a similar pattern of ^{13}C NMR signals as butyrolactone I (**5**) [11, 14], but with 16 mass units less as indicated by HR-ESIMS. The main difference was that a 1,4-disubstituted benzene moiety in **5** at the C-3 position was replaced by a monosubstituted benzene moiety in **2**. The structure and absolute configuration of **2** as shown in **Fig. 1** was further confirmed by an X-ray crystallographic study (**Fig. 3**).

Versicolactone C (**3**) was assigned the molecular formula $C_{25}H_{26}O_6$ by HRESIMS at *m/z* 445.1620 $[M + Na]^+$. The ^{1}H and ^{13}C NMR spectra of **3** (**Table 1**) were similar to those of **2**, the major difference being the replacement of an aromatic hydroxy proton signal in **2** by a methoxy signal (δ_C 56.0, δ_H 3.64) in **3**. The HMBC correlation of the methoxy proton (δ_H 3.64) with C-4″ (δ_C 156.4) indicated that the methoxy group was located at C-4″. The structure of **3** was therefore determined.

A direct comparison of the NMR data of **4** and **5** suggested that both compounds were similar and had the same basic skeleton. Just like the difference between compounds **2** and **3**, the only difference between **4** and **5** also arose from the replacement of an aromatic hydroxy proton signal in **5** by a methoxy (δ_C 56.1, δ_H 3.80) signal in **4**. This assignment was supported by the HMBC corrections from the methoxy proton (δ_H 3.80) to C-4′ (δ_C 160.1). Compound **4** was thus defined as shown, and named versicolactone D.

Finally, the absolute configurations of compounds **1**, **3**, and **4** were identical to that of **2**, as determined by their similar Cotton effects in the CD spectra.

Since many butyrolactones have been reported to possess potential antivirus [9, 14] and antitumor properties [12, 13], compounds **1**–**8** (purities > 95%) were tested for anti-TMV, and compounds **1**–**4** (purities > 95%) were screened for cytotoxic activities.

The anti-TMV activities were tested using the half-leaf method [19]. Ningnanmycin (a commercial product for plant disease in China) was used as a positive control. The antiviral inhibition rates at the concentration of 20 µM are listed in **Table 2**. Compounds **1** and **8** showed high anti-TMV activities with inhibition rates of 46.4% and 35.4%, which were even more potent than the positive control ningnanmycin (30.8%). Compounds **2**–**7** showed modest anti-TMV activities with inhibition rates of 18.6–25.4%, respectively.

The cytotoxicities of compounds **1**–**4** were also tested using a previously reported procedure [20]. The cytotoxic abilities against NB4, A549, SHSY5Y, PC3, and MCF7 tumor cell lines by the MTT assay (with taxol as the positive control) are shown in **Table 3**. Previously, the known compounds **5**–**8** have been reported to possess mild cytotoxicity [11, 14, 21]. In this study, the results revealed that compound **1** showed moderate cytotoxicity against A549 and MCF7 cells with IC_{50} values of 3.2 and 2.5 µM. Compounds **2**–**4** also showed moderate cytotoxicity for some tested cell lines with IC_{50} values of less than 10 µM.

Compounds	Inhibition rates at 20 µM (%)	Compounds	Inhibition rates at 20 µM (%)
1	46.4 ± 3.5	**6**	21.5 ± 2.6
2	22.6 ± 3.0	**7**	23.5 ± 2.3
3	25.4 ± 2.8	**8**	35.4 ± 3.3
4	18.6 ± 2.5	ningnamycin	30.8 ± 3.0
5	20.7 ± 2.7		

All results are expressed as mean ± SD; n = 3 for all groups

Table 2 Tobacco mosaic virus infection inhibition activities of compounds **1**–**8**.

Compounds	Cell lines and IC_{50} (μM)				
	NB4	A549	SHSY5Y	PC3	MCF7
1	5.5	3.2	5.3	> 10	2.5
2	> 10	5.8	6.2	> 10	8.5
3	> 10	6.4	8.5	> 10	7.8
4	> 10	> 10	4.8	> 10	8.2
Taxol	0.03	0.02	0.2	0.2	0.1

Table 3 Cytotoxic activity of compounds **1**–**4**.

NB4, human leukemia cell; A549, carcinomic human alveolar basal epithelial cell; SHSY5Y, human neuroblastoma cell; PC3, human prostate cancer cell; MCF7, human breast adenocarcinoma cell

Materials and Methods

General

Optical rotations were measured in a Horiba SEPA-300 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. ECD spectra were measured on a JASCO J-810 spectropolarimeter. IR spectra were obtained in KBr disc on a Bio-Rad Wininfmred spectrophotometer. ESI-MS was measured on a VG Auto Spec-3000 MS spectrometer. ^1H, ^{13}C, and 2D NMR spectra were recorded on Bruker DRX-400 or 500 instruments with tetramethylsilane (TMS) as the internal standard. Column chromatography was performed on silica gel (200–300 mesh) or on silica gel H (10 ~ 40 μm, Qingdao Marine Chemical, Inc.). Final purifications utilized an Agilent 1100 HPLC equipped with a Zorbax-C_{18} (21.2 mm × 250 mm, 7.0 μm) column and DAD detector. Paclitaxel (purity > 95%) was obtained from Sigma-Aldrich Co. Ningnanmycin (purity > 98%) was provided by the Chengdu Institute of Biology, Chinese Academy of Sciences.

Fungal material

A. versicolor was isolated from the rhizome of *P. polyphylla* var. *yunnanensis*, collected from Dali, Yunnan, People's Republic of China, in 2012. The strain was identified by one of authors (Gang Du) based on the analysis of the ITS sequence (Genbank Accession number KJ801852). It was cultivated at room temperature for seven days on potato dextrose agar at 28 °C. Agar plugs were inoculated into 250-mL Erlenmeyer flasks each containing 100 mL potato dextrose broth and cultured at 28 °C on a rotary shaker at 180 rpm for five days. Large-scale fermentation was carried out in 100 Fernbach flasks (500 mL) each containing 100 g of rice and 120 mL of distilled H_2O. Each flask was inoculated with 5.0 mL of cultured broth and incubated at 25 °C for 45 days.

Extraction and isolation

The fermentation products were extracted four times with 70% acetone (4 × 10 L) at room temperature and filtered. The crude extract (215 g) was applied to silica gel (200–300 mesh, 11 × 120 cm, 1.8 kg) column chromatography, eluting with a $CHCl_3$-$(CH_3)_2CO$ gradient system (20 : 1, 9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5). Six fractions were obtained from the silica gel column and individually decolorized on MCI gel after which they were eluted with 90 : 10 MeOH/H_2O to yield fractions A–F.

The further separation of fraction B (9 : 1, 23.2 g) by silica gel column chromatography (200–300 mesh, 2.5 × 120 cm, 0.2 kg), eluted with petroleum ether-EtOAc (9 : 1, 8 : 2, 7 : 3, 6 : 4, 1 : 1), yielded mixtures B1–B5. Fraction B2 ($CHCl_3$/acetone, 8 : 2; 6.27 g) was subjected to RP-18 column chromatography (8 × 50 cm, MeOH/H_2O 20 : 80 to 70 : 30 gradient) to provide three fractions, B2-1–B2–3. Fraction B2-1 (180 mg) was subjected to preparative HPLC (Zorbax-C_{18}, 7.0 μm, 21.2 mm × 250 mm, flow rate 12 mL/min, UV detection at λ_{max} = 210, 254, and 280 nm, eluted with CH_3OH/H_2O 68 : 32) to give **1** (9.2 mg, t_R = 17 min, purity > 98%), **2** (15.2 mg, t_R = 21 min, purity > 98%), **3** (11.8 mg, t_R = 23 min, purity > 95%), **4** (14.5 mg, t_R = 16 min, purity > 95%), and **5** (14.6 mg, t_R = 14 min, purity > 95%). Fraction B3 ($CHCl_3$/acetone, 7 : 3; 2.86 g) was subjected to RP-18 column chromatography (8 × 50 cm, MeOH/H_2O 20 : 80 to 60 : 40 gradient) to provide five fractions, B3-1–B2–5. Fraction B3-2 (128 mg) was subjected to preparative HPLC (Zorbax-C_{18}, 7.0 μm, 21.2 mm × 250 mm, flow rate 12 mL/min, UV detection at λ_{max} = 210, 254, and 280 nm, eluted with CH_3OH/H_2O 60 : 40) to give **6** (14.5 mg, t_R = 12 min, purity > 95%), **7** (13.6 mg, t_R = 16 min, purity > 95%), and **8** (18.8 mg, t_R = 17 min, purity > 95%).

X-ray crystal structure analysis

The intensity data for versicolactone B (**2**) were collected at 100 K on a Bruker Apex Duo diffractometer equipped with an Apex II CCD using Cu Kα radiation. Cell refinement and data reduction were performed with Bruker SAINT. The structures were solved by direct methods using SHELXS-97, expanded using difference Fourier techniques, and refined by the program and full-matrix least squares calculations. The non-hydrogen atoms were refined anisotropically, and hydrogen atoms were fixed at calculated positions.

Crystallographic data for versicolactone B (2): $C_{24}H_{24}O_6$, M = 408.43, monoclinic, a = 9.7742(4) Å, b = 8.5413 (4) Å, c = 12.5143 (5) Å, α = 90.00°, β = 98.079 (2)°, γ = 90.00°, V = 1034.38 (8) Å^3, T = 100 (2) K, space group $P21$, Z = 2, μ (CuK-α) = 0.773 mm^{-1}, 8204 reflections measured, 3060 independent reflections (R_{int} = 0.1053). The final R_1 values were 0.1270 ($I > 2\sigma$ (I)). The final wR (F^2) values were 0.3007 ($I > 2\sigma$ (I)). The final R_1 values were 0.1496 (all data). The final wR (F^2) values were 0.3321 (all data). The goodness of fit on F^2 was 1.298; Flack parameter = 0.3 (7).

Antitobacco mosaic virus assays

TMV (U1 strain) was obtained from the Key Laboratory of Tobacco Chemistry of Yunnan Province, Yunnan Academy of Tobacco Science, P.R. China. The virus was multiplied in *Nicotiana tabacum* cv. K326 and purified as described [18]. The concentration of TMV was determined as 20 mg/mL with a UV spectrophotometer [virus concentration = (A_{260} × dilution ratio)/$E^{0.1\%,\ 260nm}_{1cm}$]. The purified virus was kept at − 20 °C and diluted to 32 μg/mL with 0.01 M PBS before use.

Nicotiana glutinosa plants were cultivated in an insect-free greenhouse. *N. glutinosa* was used as a local lesion host. The experiments were conducted when the plants grew to the 5–6 leaf stage. The tested compounds were dissolved in DMSO and diluted with distilled H_2O to the required concentrations. A solu-

tion of equal concentration of DMSO was used as a negative control. The commercial antiviral agent ningnanmycin (purity > 98%) was used as a positive control.

For the half-leaf method [19], the virus was inhibited by mixing it with the solution of the compound. After 30 min, the mixture was inoculated on the left side of the leaves of *N. glutinosa*, whereas the right side of the leaves was inoculated with the mixture of DMSO solution and the virus as the control. The local lesion numbers were recorded 3–4 days after inoculation. Three repetitions were conducted for each compound. The inhibition rates were calculated according to the formula:

$$\text{inhibition rate (\%)} = [(C-T)/C] \times 100\%$$

where C is the average number of local lesions of the control and T is the average number of local lesions of the treatment.

Ningnanmycin, a commercial virucide for plant disease in China, was used as a positive control.

Furthermore, we have designed a simple experiment to assure whether butyrolactone derivatives have a significant effect on *N. glutinosa*'s growth or not. The selected compound **2** (20 µM) was sprayed on one leaf of *N. glutinosa* once a day (seven days), respectively, whereas another leaf was sprayed with DMSO solution as a control. Three repetitions were conducted for each compound. No significant differences between the two conditions were observed after one week. This finding may suggest that pretreatment with the compounds may enhance the resistance of the host plant against TMV infection with low cytotoxicity.

Cytotoxicity assays

The cytotoxicity of compounds was tested using a previously reported procedure [20]. Colorimetric assays were performed to evaluate each compound's activity. NB4 (human acute promyelocytic leukemia), A549 (human lung adenocarcinoma epithelial), SHSY5Y (human neuroblastoma), PC3 (human prostate cancer), and MCF7 (human breast adenocarcinoma) tumor cells were purchased from ATCC. All cells were cultured in RPMI-1640 or DMEM medium (Hyclone) supplemented with 10% FBS (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in the living cells based on the reduction of MTT (Sigma). Briefly, 100 µL of suspended adherent cells were seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition. Furthermore, suspended cells were seeded just before drug addition, with an initial density of 1×10^5 cells/mL in 100 µL of medium. Each tumor cell line was exposed to each test compound at various concentrations in triplicate for 48 h; paclitaxel (purity > 95%) was used as a positive control. After the incubation, MTT (100 µg) was added to each well, and the incubation was continued for 4 h at 37 °C. The cells were lysed with 100 µL of 20% SDS-50% dimethylformamid (DMF) after removal of 100 µL of the medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated by Reed and Muench's method [22].

Versicolactone A (**1**): $C_{23}H_{22}O_8$, obtained as a white amorphous powder; $[\alpha]_D^{24.8}$ +86.4 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 210 (4.48), 252 (3.69), 314 (3.82) nm; ECD (*c* 0.2, MeOH) $\Delta\varepsilon_{205}$ + 15.2, $\Delta\varepsilon_{227}$ − 5.16, $\Delta\varepsilon_{313}$ + 4.29; IR (KBr) ν_{max} 3447, 3032, 2970, 2943, 1735, 1727, 1712, 1607, 1542, 1468, 1425, 1369, 1254, 1163, 1109, 1047, 886, 745 cm^{-1}; ^1H and ^{13}C NMR (500 and 125 MHz, in $CDCl_3$), see **Table 1**; ESIMS (positive ion mode) *m/z* 449 $[M + Na]^+$; HRESIMS (positive ion mode) *m/z* 449.1208 $[M + Na]^+$ (calcd. 449.1212 for $C_{23}H_{22}NaO_8$).

Versicolactone B (**2**): $C_{24}H_{24}O_6$, obtained as a white amorphous powder; $[\alpha]_D^{24.5}$ +70.25 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 220 (4.18), 255 (3.52), 300 (2.26) nm; ECD (*c* 0.12 mg/mL, MeOH) $\Delta\varepsilon_{203}$ + 17.8, $\Delta\varepsilon_{230}$ − 4.06, $\Delta\varepsilon_{307}$ + 3.15; IR (KBr) ν_{max} 3462, 3025, 2980, 2925, 1735, 1724, 1606, 1534, 1483, 1432, 1398, 1221, 1168, 1105, 1065, 945, 855, 765 cm^{-1}; ^1H and ^{13}C NMR (500 and 125 MHz, in CD_3OD), see **Table 1**; ESIMS (positive ion mode) *m/z* 431 $[M + Na]^+$; HRESIMS (positive ion mode) *m/z* 431.1475 $[M + Na]^+$ (calcd. 431.1471 for $C_{24}H_{24}NaO_6$).

Versicolactone C (**3**): $C_{25}H_{26}O_6$, obtained as awhite amorphous powder; $[\alpha]_D^{24.6}$ +75.4 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 210 (4.29), 246 (3.52), 300 (3.72) nm; ECD (*c* 0.2, MeOH) $\Delta\varepsilon_{205}$ + 14.8, $\Delta\varepsilon_{225}$ −3.58, $\Delta\varepsilon_{306}$ + 2.92; IR (KBr) ν_{max} 3464, 3023, 2982, 2920, 1737, 1725, 1605, 1527, 1486, 1430, 1386, 1226, 1182, 1118, 1062, 938, 852, 746 cm^{-1}; ^1H and ^{13}C NMR (400 and 100 MHz, in CD_3OD), see **Table 1**; ESIMS (positive ion mode) *m/z* 445 $[M + Na]^+$; HRESIMS (positive ion mode) *m/z* 445.1620 $[M + Na]^+$ (calcd. 445.1627 for $C_{25}H_{26}NaO_6$).

Versicolactone D (**4**): $C_{25}H_{26}O_7$, obtained as a white amorphous powder; $[\alpha]_D^{24.5}$ +82.6 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε): 210 (4.38), 244 (3.56), 305 (3.72) nm; ECD (*c* 0.2, MeOH) $\Delta\varepsilon_{203}$ + 16.2, $\Delta\varepsilon_{228}$ − 5.47, $\Delta\varepsilon_{308}$ + 3.54; IR (KBr) ν_{max} 3460, 3018, 2977, 2934, 1735, 1727, 1605, 1528, 1485, 1434, 1388, 1223, 1178, 1124, 1039, 876, 758 cm^{-1}; ^1H and ^{13}C NMR (400 and 100 MHz, in $CDCl_3$), see **Table 1**; ESIMS (positive ion mode) *m/z* 461 $[M + Na]^+$; HRESIMS (positive ion mode) *m/z* 461.1581 $[M + Na]^+$ (calcd. 461.1576 for $C_{25}H_{26}NaO_7$).

Supporting information

^1H and ^{13}C NMR spectra of compounds **1, 3**, and **4**, ^1H, ^{13}C NMR, DEPT, HSQC, HMBC, COSY, NOESY, and ECD spectra of compound **2**, as well as X-ray data of compound **2** are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

References

1 *Ge HM, Peng H, Guo ZK, Cui JT, Song YC, Tan RX.* Bioactive alkaloids from the plant endophytic fungus *Aspergillus terreus*. Planta Med 2010; 76: 822–824

2 *Zhang HW, Zhang J, Hu S, Zhang ZJ, Zhu CJ, Ng SW, Tan RX.* Ardeemins and cytochalasins from *Aspergillus terreus* residing in *Artemisia annua*. Planta Med 2010; 76: 1616–1621

3 *Ji NY, Liu XH, Miao FP, Qiao MF.* Aspeverin, a new alkaloid from an algicolous strain of *Aspergillus versicolor*. Org Lett 2013; 15: 2327–2329

4 *Gao HQ, Zhou LN, Cai SX, Zhang GJ, Zhu TJ, Gu QQ, Li DH.* Diorcinols B–E, new prenylated diphenyl ethers from the marine-derived fungus *Aspergillus versicolor* ZLN-60. J Antibiot 2013; 66: 539–542

5 *Hawas UW, El-Beih AA, El-Halawany AM.* Bioactive anthraquinones from endophytic fungus *Aspergillus versicolor* isolated from red sea algae. Arch Pharmacal Res 2012; 35: 1749–1756
6 *Zhou M, Miao MM, Du G, Li XN, Shang SZ, Zhao W, Liu ZH, Yang GY, Che CT, Hu QF, Gao XM.* Aspergillines A–E, highly oxygenated hexacyclic indole-tetrahydrofuran-tetramic acid derivatives from *Aspergillus versicolor*. Org Lett 2014; 16: 5016–5019
7 *Joule JA, Mills K.* Heterocyclic chemistry, 4th ed. Oxford: Blackwell Science Publishing; 2000: 306–307
8 *Mohagheghzadeh A, Schmidt TJ, Bayindir U, Fuss E, Mehregan I, Alfermann AW.* Diarylbutyrolactone lignans from *Linum corymbulosum in vitro* cultures. Planta Med 2006; 72: 1165–1167
9 *Matsumoto T, Hosono-Nishiyama K, Yamada H.* Antiproliferative and apoptotic effects of butyrolactone lignans from *Arctium lappa* on leukemic cells. Planta Med 2006; 72: 276–278
10 *Wang W, Kim H, Nam SJ, Rho BJ, Kang H.* Antibacterial butenolides from the Korean tunicate *Pseudodistoma antinboja*. J Nat Prod 2012; 75: 2049–2054
11 *Haritakun R, Rachtawee P, Chanthaket R, Boonyuen N, Isaka M.* Butyrolactones from the fungus *Aspergillus terreus* BCC 4651. Chem Pharm Bull 2010; 58: 1545–1548
12 *Li DH, Zhu TJ, Liu HB, Fang YC, Gu QQ, Zhu WM.* Four butenolides are novel cytotoxic compounds isolated from the marine-derived bacterium *Strreptoverticillium luteoverticillatum*. Arch Pharm Res 2006; 29: 624–626
13 *Bai ZQ, Lin XP, Wang YZ, Wang JF, Zhou XF, Yang B, Liu J, Yang XW, Wang Y, Liu YH.* New phenyl derivatives from endophytic fungus *Aspergillus flavipes* AIL8 derived of mangrove plant *Acanthus ilicifolius*. Fitoterapia 2014; 95: 194–202
14 *Rao KV, Sadhukhan AK, Veerender M, Ravikumar V, Mohan EV, Dhanvantri SD, Sitaramkumar M, Babu JM, Vyas K, Reddy G.* Butyrolactones from *Aspergillus terreus*. Chem Pharm Bull 2000; 48: 559–562
15 *Qin JJ, Zhu JX, Zeng Q, Cheng XR, Zhu Y, Zhang SD, Shan L, Jin HZ, Zhang WD.* Pseudoguaianolides and guaianolides from *Inula hupehensis* as potential anti-inflammatory agents. J Nat Prod 2011; 74: 1881–1887
16 *Zhang HJ, Van Hung N, Cuong NM, Soejarto DD, Pezzuto JM, Fong HHS, Tan GT.* Sesquiterpenes and butenolides, natural anti-HIV constituents from *Litse verticillata*. Planta Med 2005; 71: 452–457
17 *San-Martín A, Rovirosa J, Vaca I, Vergara K, Acevedo L, Vina D, Orallo F, Chamy MC.* New butyrolactone from a marine-derived fungus *Aspergillus* sp. J Chil Chem Soc 2011; 56: 625–627
18 *Lin T, Lu C, Shen Y.* Secondary metabolites of *Aspergillus* sp. F1, a commensal fungal strain of *Trewia nudiflora*. Nat Prod Res 2009; 23: 77–85
19 *Hu QF, Zhou B, Huang JM, Gao XM, Shu LD, Yang GY, Che CT.* Antiviral phenolic compounds from *Arundina gramnifolia*. J Nat Prod 2013; 76: 292–296
20 *Hu QF, Zhou B, Huang JM, Jiang ZY, Huang XZ, Yang LY, Gao XM, Yang GY, Che CT.* Cytotoxic deoxybenzoins and diphenylethylenes from *Arundina graminifolia*. J Nat Prod 2013; 76: 1854–1859
21 *Parvatkar RR, D'Souza C, Tripathi A, Naik CG.* Aspernolides A and B, butenolides from a marine-derived fungus *Aspergillus terreus*. Phytochemistry 2009; 70: 128–132
22 *Reed LJ, Muench H.* A simple method of estimating fifty percent endpoints. Am J Epidemiol 1938; 27: 493–497