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Cyclopeptides and polyketides from coral-associated fungus, *Aspergillus versicolor* LCJ-5-4

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ABSTRACT

Three new cyclopentapeptides, versicoloritides A–C (**1**–**3**), a new orcinol tetramer, tetraorcinol A (**4**), and two new lactones, versicolactones A and B (**5** and **6**) together with three known metabolites, diorcinol, glyantrypine, and cordyol C were isolated from the fermentation broth of the coral-associated fungus *Aspergillus versicolor* LCJ-5-4. Their structures were elucidated by spectroscopic and chemical methods. The new compounds **1**–**4** were evaluated for their radical-scavenging activity and antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter* aerogenes, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans* and cytotoxicity against P388 and Hela cell lines. Compound **4** showed weak radical-scavenging activity against the DPPH radical with an IC_{50} value of 67 μM.

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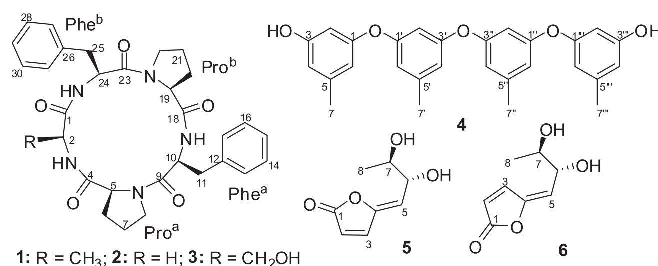
1. Introduction

Coral is an important source of novel, biologically active compounds.[1] For example, pseudopterosin A, a diterpene glycoside derived from the marine octocoral *Pseudopterogorgia elisabethae*, is in Phase II trials as a wound healing agent.[2] Moreover, marine surface-associated microorganisms have proven to be a rich source for novel bioactive because of the necessity to evolve allelochemicals capable of protecting the producer from the fierce competition.[3] However, researches on secondary metabolites of coral-associated microorganisms are rarely reported. During our ongoing pursuit for novel and bioactive natural products from marine surface-associated microorganisms, a fungal strain LCJ-5-4 authenticated as *Aspergillus versicolor*, widely found in human and animal foodstuffs, was firstly isolated from the soft coral *Cladiella* sp. collected from the South China Sea. Previous study had been identified three new quinazolinone alkaloids from *A. versicolor* LCJ-5-4.[4] Continuous chemical study on this fungus resulted in the isolation and identification of three new cyclopentapeptides, versicoloritides A–C (**1**–**3**), a new orcinol tetramer, tetraorcinol A (**4**), and two new lactones, versicolactones A and B (**5** and **6**), together with three known metabolites, diorcinol,[5] cordyol C,[6] and glyantrypine.[7] This paper reports the isolation, structure elucidation, and bioactivity of these new compounds.



2. Results and discussion

Versicoloritide A (**1**) was obtained as a white amorphous powder. Its molecular formula was determined as $C_{31}H_{37}N_5O_5$ according to the HRESIMS at m/z 560.2869 $[M+H]^+$, requiring 16 degrees of unsaturation. Analysis of the NMR data (Table 1) revealed the presence of five carbonyl signals (δ_C 170.6, 169.5, 169.5, 170.6, and 170.2), five normal α-amino acid methine carbon resonances (δ_C 49.7, 60.8, 52.4, 60.9, and 51.7), and three amide protons (δ_H 7.50, 6.38, and 8.31), indicating its peptide nature. $^1H-^1H$ COSY experiment (Fig. 1) constructed the five amino acid residues as an alanine, two phenylalanines, and two prolines. These residues accounted for 15 of the 16 degrees of unsaturation, indicating that **1** is

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Table 1
^{1}H and ^{13}C NMR data for **1**–**3** (600, 150 MHz, DMSO-d_6, TMS, δ ppm)

Position	**1**		**2**		**3**	
	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C	δ_H (*J* in Hz)	δ_C
1		170.6, qC		170.7, qC		168.8, qC
2	4.22 (dq, 10.1, 7.3)	49.7, CH	3.91 (dd, 13.7, 10.3); 3.28 (d, 12.4)	42.5, CH_2	4.22 (m)	56.4, CH
3	0.85 (d, 7.3)	19.4, CH_3			3.17 (m), 3.24 (m)	62.0, CH_2
3-OH					4.94 (t, 6.1)	
N–H	7.50 (d, 10.1)		7.85 (d, 9.6)		7.51 (d, 10.1)	
Pro^a 4		169.5, qC		169.4, qC		170.2, qC
5	3.25 (t, 6.4)	60.8, CH	3.01 (m)	60.1, CH	3.21 (m)	60.8, CH
6	1.67 (m), 1.65 (m)	31.1, CH_2	1.70 (m), 1.37 (m)	30.9, CH_2	1.72 (m), 1.62 (m)	31.2, CH_2
7	1.54 (m), 1.49 (m)	22.5, CH_2	1.53 (m), 1.48 (m)	22.1, CH_2	1.64 (m), 1.48 (m)	22.4, CH_2
8	3.30 (m)	46.5, CH_2	3.23 (m)	46.6, CH_2	3.42 (m)	46.5, CH_2
Phe^a 9		169.5, qC		169.4, qC		169.4, qC
10	4.30, m	52.4, CH	4.06 (m)	53.6, CH	4.26 (m)	52.7, CH
11	2.87 (dd, 12.4, 5.0); 2.78 (dd, 12.4, 5.0)	37.9, CH_2	2.97 (dd, 12.8, 5.0); 2.79 (dd, 12.5, 6.8)	38.0, CH_2	2.91 (dd, 12.8, 5.0); 2.79 (dd, 12.3, 4.0)	38.8, CH_2
12		136.1, qC		135.9, qC		136.1, qC
13	7.11 (d, 7.3)	129.6, CH	7.15 (m)	129.3, CH	7.12 (d, 7.3)	129.1, CH
14	7.30 (t, 7.3)	128.5, CH	7.32 (t, 7.3)	128.6, CH	7.31 (d, 7.8)	128.5, CH
15	7.24 (m)	127.0, CH	7.28 (m)	127.2, CH	7.24 (m)	127.0, CH
16	7.30 (t, 7.3)	128.5, CH	7.32 (t, 7.3)	128.6, CH	7.31 (d, 7.8)	128.5, CH
17	7.11 (d, 7.3)	129.6, CH	7.15 (m)	129.2, CH	7.12 (d, 7.3)	129.1, CH
NH	6.38 (d, 7.3)		7.71 (br s)		6.61 (d, 6.4)	
Pro^b 18		170.6, qC		170.8, qC	7.12 (d, 7.3)	170.5, qC
19	4.14 (dd, 7.8, 1.5)	60.9, CH	4.23, dd (7.8, 2.8)	60.3, CH	4.11 (m)	60.8, CH
20	2.01 (m), 1.81 (m)	31.3, CH_2	2.09 (m), 1.92 (m)	32.0, CH_2	2.01 (m), 1.83 (m)	31.4, CH_2
21	1.77 (m), 1.68 (m)	21.5, CH_2	1.68 (m), 1.62 (m)	22.1, CH_2	1.74 (m), 1.68 (m)	21.6, CH_2
22	3.48 (m)	46.9, CH_2	3.44 (m)	46.7, CH_2	3.45 (m)	46.8, CH_2
Phe^b 23		170.2, qC		168.2, qC		169.9, qC
24	4.82 (dt, 15.5, 7.8)	51.7, CH	4.62 (dt, 15.6, 7.3)	51.8, CH	4.72 (dt, 13.9, 6.2)	52.2, CH
25	3.09 (dd, 13.7, 6.9); 2.76 (dd, 13.7, 7.7)	39.4, CH_2	3.01 (m); 2.76 (dd, 13.2, 6.8)	38.2, CH_2	3.05 (dd, 13.7, 7.7); 2.72 (dd, 13.3, 4.8)	38.1, CH_2
26		137.6, qC		137.8, qC		137.8, qC
27	7.18 (d, 7.3)	129.1, CH	7.18 (d, 7.3)	129.2, CH	7.19 (d, 8.2)	129.1, CH
28	7.20 (t, 7.3)	128.0, CH	7.23 (t, 7.3)	128.2, CH	7.23 (d, 7.3)	128.0, CH
29	7.15 (m)	126.3, CH	7.14 (m)	126.3, CH	7.16 (m)	126.2, CH
30	7.20 (t, 7.3)	128.0, CH	7.23 (t, 7.3)	128.2, CH	7.23 (t, 7.3)	128.0, CH
31	7.18 (d, 7.3)	129.1, CH	7.18 (d, 7.3)	129.2, CH	7.19 (d, 8.2)	129.1, CH
NH	8.31 (d, 8.7)		8.34 (d, 8.7)		8.32 (d, 8.3)	

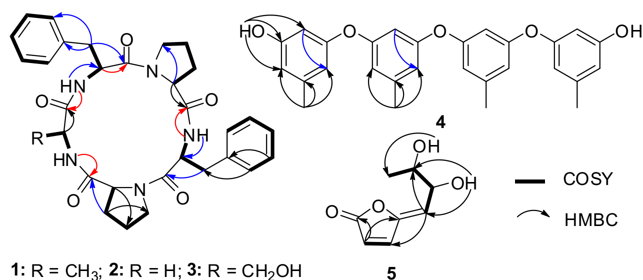

Fig. 1. The key ^{1}H–^{1}H COSY and HMBC correlations of **1**–**5**.

a cyclopeptide. Diagnostic 2D NMR (DMSO-d_6) HMBC correlations (Fig. 1) from 24-NH to C-1, 2-NH to C-4, and 17-NH to C-18 established the sequence Phe^b-Ala-Pro^a and Phe^a-Pro^b that combined together by cyclization between the Pro nitrogen and the Phe carboxyl from the molecular formula. The complete amino acid sequence of **1** was also confirmed on the basis of the results of Q-TOF-MS[2] experiments. Although there was more than one possible ring-opening position for the peptide, the preferred ring-opening of **1** occurred at the Pro–Phe amide bond. One ion series started with loss of 147 amu due to Pre, leaving *m*/*z* 413 (–Ala-Pro-Phe-Pro plus H), which then lost 71 amu (Ala), affording *m*/*z* 342 (–Pro-Phe-Pro plus H). The latter fragment lost 97 amu (Pro), yielding *m*/*z* 245 (–Phe-Pro plus H) (Fig. 2). Another pathway left the major fragment *m*/*z* 463 $[M+H-Pro]^+$ after loss of 97 amu (Pro) from *m*/*z* 560 $[M+H]^+$, which then lost sequentially 147 amu (Phe), leaving *m*/*z* 316 $[M+H-Pro-Phe]^+$ (Fig. S30, Supplementary data). Furthermore, The geometry of the amide bonds of both Pro^a and Pro^b were *cis*, on the basis of the large difference in the ^{13}C NMR chemical shifts of Pro^a ($\Delta\delta C_{6(\beta)}-C_{7(\gamma)}$=8.6 ppm) and Pro^b ($\Delta\delta C_{20(\beta)}-C_{21(\gamma)}$=9.8 ppm).[8,9] The absolute configuration of the hydrolyszaes with authentic samples (co-injection) revealed that the amino acids were L-Ala, L-Pro, and L-Phe (Fig. S26, Supplementary data). The structure of versicoloritide A (**1**) was consequently elucidated as *cyclo*-(L-Ala-*cis*-L-Pro-L-Phe-*cis*-L-Pro-L-Phe).

The molecular formula of versicoloritide B (**2**) was established as $C_{30}H_{35}N_5O_5$ from the pseudomolecular ion peak at *m*/*z* 546.2703 $[M+H]^+$ in the HRESIMS, indicative of a CH_2 homologue of **1**. Contrastive analysis of NMR spectra (Table 1) between **1** and **2** revealed that a methylene signal ($\delta_{H/C}$ 3.91 and3.28/42.5) replaced a methyl signal ($\delta_{H/C}$ 0.85/19.4) and a methine signal ($\delta_{H/C}$ 4.22/49.7) in **1**, indicating that **2** is the Gly-substituted analogue for Ala of **1**. This deduction was further supported by analysis of ^{1}H–^{1}H COSY correlations (Fig. 1) and the Q-TOF-MS[2] experiments (Fig. 2)

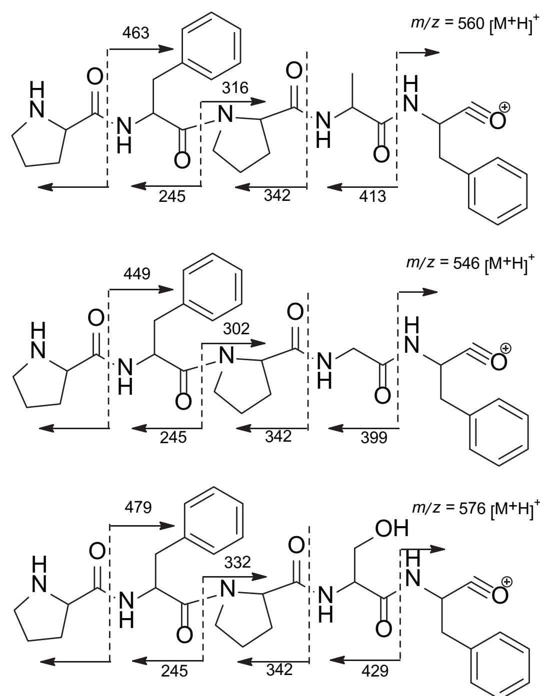

Fig. 2. Q-TOF-MS[2] sequence ions (m/z) for protonated molecular $[M+H]^+$ ions of **1**–**3**.

and the acidic hydrolysis experiment (Fig. S27, Supplementary data).[10] Versicoloritide B (**2**) was thus identified as *cyclo*-(Gly-*cis*-L-Pro-L-Phe-*cis*-L-Pro-L-Phe).

Versicoloritide C (**3**) showed a pseudomolecular ion peak at m/z 576.2797 $[M+H]^+$, indicative of a molecular formula of $C_{31}H_{37}N_5O_6$, with one oxygen more than **1**. Compared the NMR data with **1**, compound **3** showed an additional oxygenated methylene signal ($\delta_{H/C}$ 3.17 and 3.24/62.0), while the methyl signal ($\delta_{H/C}$ 0.85/19.4) in **1** disappeared. These data indicated that the Ser residue in **3** replaced the Ala residue in **1**, which was confirmed by HMBC correlations between NH protons and neighboring residue carbonyls and $^1H-^1H$ COSY correlations (Fig. 1) and further by the Q-TOF-MS[2] experiments (Fig. 2). The absolute configurations of both Pro and Phe were determined by Marfey's analysis as L- (Fig. S28, Supplementary data).[10] The absolute configuration of Ser was determined as L- by amino acids analysis on a chiral Crownpak CR(+) HPLC column (Fig. S28, Supplementary data).[11,12] The structure of versicoloritide C (**3**) was accordingly determined to be *cyclo*-(L-Ser-*cis*-L-Pro-L-Phe-*cis*-L-Pro-L-Phe).

Tetraorcinol A (**4**) was assigned a molecular formula of $C_{28}H_{26}O_5$, according to HREIMS at m/z 442.1785 $[M+H]^+$, requiring 16 sites of unsaturation. NMR spectra only showed 14 carbon and 13 proton signals including six aromatic methines, six aromatic quaternary carbons (four oxygenated), two methyl groups ($\delta_{H/C}$ 2.18/21.1 and 2.24/21.2), and one exchangeable proton (δ_H 9.47), indicating a symmetrical structure. The ^1H NMR data also showed two sets of coupled ^1H NMR signals at δ_H 6.16, 6.25 (br s), 6.34 (br s) and 6.50 (br s), 6.62 (br s), 6.75 (br s) assigned by $^1H-^1H$ COSY, revealing the presence of two 1,3,5-trisubstituted benzene nucleus moieties similar to those of diorcinol.[5] These data revealed **4** as a diorcinol dimer via C–O–C bridge that was further confirmed by HMBC correlations from H_3C-7/7′ to C-4/4′, C-5/5′, and C-6/6′, from HO-3 to C-2/2′, C-3/3′, and C-4/4′, and from H-2/2′ to C-6/6′. Therefore, the structure of **4** was elucidated as 3,3′-{[oxybis(5-methyl-1,3-phenylene)]bis (oxy)} bis(5-methylphenol).

Versicolactone A (**5**) owned molecular formula $C_8H_{10}O_4$ from the HRESIMS peak at m/z 193.0486 $[M+Na]^+$, requiring four sites of unsaturation. ^1H and ^{13}C NMR (Table 2) revealed the presence of a carbonyl carbon (δ_C 169.6), a sp^2 quaternary carbon (δ_C 169.6), three sp^2 methine ($\delta_{H/C}$ 6.43/119.3, 7.87/145.5 and 5.47/117.2), together with a methyl ($\delta_{H/C}$ 1.02/19.0), and two oxygenated methines ($\delta_{H/C}$ 3.60/69.4 and 4.26/70.0). Two fragments, CH_3–CH(OH)–CH(OH)–CH and CH=CH, could be deduced from the $^1H-^1H$ COSY correlations of H-8/H-7/H-6/H-5, and H-2/H-3 (Fig. 1). Furthermore, 5-(2,3-dihydroxybutylidene)furan-2(5*H*)-one skeleton was established on the base of the key HMBC correlations from H-2 (δ 6.43) to C-1 (δ 169.6) and C-4 (δ 149.1), from H-3 (δ 7.87) to C-1, and from H-5 (δ 5.47) to C-3 (δ 145.5), C-4 and C-7 (δ 69.4). The double bond in the side chain was assigned as *Z*-configuration based on the NOE difference experiments. When H-3 and H-5 were radiated, the signals of H-5 and H-3 were enhanced by 5.1% and 7.0%, respectively, indicating H-3 and H-5 was in the same side. Versicolactone B (**6**) was the isomer of **5**, with the same molecular formula $C_8H_{10}O_4$ from the HRESIMS peak at m/z 193.0468 $[M+Na]^+$. Its NMR data were almost identical to those of **5**. The differences were that H-3 and H-5 and C-3 undergo large downfield shifts and large upfield shift (Table 2) for de-steric and steric effects

Table 2
^1H and ^{13}C NMR data for **4**–**6** (600, 150 MHz, DMSO-d_6, TMS, δ ppm)

Position	4		5		6	
	δ_H (J in Hz)	δ_C	δ_H (J=Hz)	δ_C	δ_H (J=Hz)	δ_C
1 (1‴)		157.7, qC		169.6, qC		169.6, qC
2 (2‴)	6.16, br s	102.9, CH	6.43, dd (0.6, 5.4)	119.3, CH	6.46, dd (1.7, 6.1)	120.1, CH
3 (3‴)		158.6, qC	7.87,.d (5.4)	145.5, CH	8.16, d (6.1)	142.8, CH
4 (4‴)	6.34, br s	111.3, CH		149.1, qC		150.3, qC
5 (5‴)		140.2, qC	5.47, d (9.2)	117.2, CH	5.78, dd (1.2, 8.6)	117.0, CH
6 (6‴)	6.25, br s	109.9, CH	4.26, ddd (5.1, 5.1, 5.1)	70.0, CH	4.16, ddd (5.3, 5.4, 8.6)	71.2, CH
7 (7‴)	2.18, s	21.1, CH_3	3.60, 'dq' like (5.1, 9.2)	69.4, CH	3.57, 'dq' like (5.4, 6.3)	69.6, CH
8			1.02, d (6.4)	19.0, CH_3	1.06, d (6.3)	19.6, CH_3
1′ (1″)		154.6, qC				
2′ (2″)	6.62, br s	108.3, CH				
3′ (3″)		156.8, qC				
4′ (4″)	6.50, br)	114.1, CH				
5′ (5″)		139.5, qC				
6′ (6″)	6.75, br s	116.1, CH				
7′ (7″)	2.24, br s	21.2, CH_3				
3 (3‴)-OH	9.47, br s					
6-OH			5.16, d (5.1)		5.23, d (5.2)	
7-OH			4.66, d (5.1)		4.66, d (5.3)	

between H-3 and H-5, and between CH(OH)-6 and C-3, respectively, indicating the double bond in the side chain of **6** was *E*-form. The large coupling constants of $^3J_{6,7}$ (5.1, 5.4 Hz) and downfield shifts of δ_{CH3} (19.0, 19.6) of **5** and **6** (Table 2) indicated that both compounds are *threo*-configuration. According to the literatures, the *threo*-1-alkenylpropane-1,2-diol displayed larger coupling constants of $^3J_{1,2}$ (5–7 Hz) and larger δ_{CH3} value (19–20) than *erythro*-1-alkenylpropane-1,2-diol ($^3J_{1,2}$ 3–4 Hz, δ_{CH3} 17–18).[13–21] The steric hindrance among HO–, CH_3– and alkenyl resulted in upfield shift of CH_3– in *erythro*-molecule (Fig. 3). The absolute configurations of compounds **5** and **6** could be deduced by comparison of their specific rotations with those of their analogues, 7-*epi*-goniobutenolides A and B in which the only difference is the replacement of phenyl by the corresponding methyl groups.[22,23] The same sign of specific rotations of **5** (−31.5) and **6** (+19.8) as those of 7-*epi*-goniobutenolide A (−246.5) and B (+95.5) indicated both compounds **5** and **6** are (*R*,*R*)-configurations. Thus, the structures of versicolactones A and B (**5** and **6**) were deduced as (4*Z*,6*R*,7*R*)-5-(2,3-dihydroxybutylidene)furan-2(5*H*)-one and (4*E*,6*R*,7*R*)-5-(2,3-dihydroxybutylidene)furan-2(5*H*)-one, respectively.

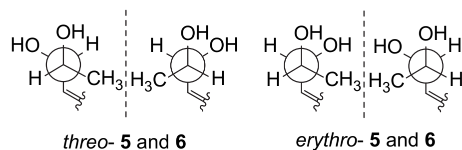

Fig. 3. The major conformations of *threo*- and *erythro*-**5** and **6**.

The new isolates **1**–**4** were evaluated for cytotoxicity against P388 and Hela cells with the MTT method.[24] Their antimicrobial activities against *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Candida albicans* were also evaluated by an agar dilution method.[25] They showed no cytotoxic effect on the tested cancer cell lines (IC_{50} >50 μM) and antimicrobial activities (MIC >150 μM). In the radical-scavenging assay, compound **4** showed weak antioxidative activity against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, with IC_{50} value of 67 μM, ascorbic acid (vitamin C) serving as positive control (IC_{50} 22 μM), as determined by the method of Chen and Ho.[26]

In conclusion, this work describes the discovery of six new compounds from coral-associated fungus, *A. versicolor* LCJ-5-4. Although about 150 compounds of *A. versicolor* were characterized, researches on secondary metabolites of coral-associated *A. versicolor* are rarely reported. The results showed that the coral-associated microorganisms could be valuable organisms to allow construction of diverse chemical space.

3. Experimental section

3.1. General experimental procedures

Optical rotations were obtained on a JASCO P-1020 digital polarimeter. UV spectra were recorded on a Beckman DU® 640 spectrophotometer. IR spectra were recorded on a Nicolet Nexus 470 spectrophotometer as KBr discs. ^1H, ^{13}C NMR, DEPT, and 2D NMR spectra were recorded on a JEOL JNM-ECP 600 spectrometer using TMS as internal standard and chemical shifts were recorded as δ-values. ESI-MS and Q-TOF-MS[2] were measured on a Q-TOF ULTIMA GLOBAL GAA076 LC mass spectrometer. TLC and column chromatography (CC) were performed on plates precoated with silica gel GF_{254} (10–40 μm) and over silica gel (200–300 mesh, Qingdao Marine Chemical Factory), and Sephadex LH-20 (Amersham Biosciences), respectively. Vacuum-liquid chromatography (VLC) was carried out over silica gel H (Qingdao Marine Chemical Factory, Qingdao, China). Semiprepartive HPLC was performed using an ODS column [Shin-pak ODS (H), 20×250 mm, 5 μm, 4 mL/min].

3.2. Fungal material

A. versicolor LCJ-5-4 was isolated from the coral *Cladiella* sp. collected from Lingao, Hainan province of China. It was identified according to its morphological characteristics and 18S rRNA sequences.[4] The voucher specimen is deposited in our laboratory at −80 °C. The producing strain was prepared on Potato Dextrose agar slants at 3.33% salt concentration and stored at 4 °C.

3.3. Fermentation and extraction

A. versicolor LCJ-5-4 was grown under static conditions at 20 °C for 30 days in three hundred 1000 mL conical flasks containing liquid medium (300 mL/flask) composed of sorbitol (20 g/L), maltose (20 g/L), monosodium glutamate (10 g/L), KH_2PO_4 (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.3 g/L), tryptophane (0.5 g/L), yeast extract (3 g/L), and sea salt (33.3 g/L), after adjusting its pH to 6.5. The fermented whole broth (about 90 L) was filtered through cheesecloth to separate into filtrate and mycelia. The filtrate was concentrated under reduced pressure to about a quarter of the original volume and then extracted three times with EtOAc to give an EtOAc solution, while the mycelia was extracted three times with acetone. The acetone solution was concentrated under reduced pressure to afford an aqueous solution. The aqueous solution was extracted three times with EtOAc to give another EtOAc solution. Both EtOAc solutions were combined and concentrated under reduced pressure to give a crude extract (140 g).

3.4. Purification

The extract (140 g), was separated into eight fractions on a silica gel VLC column using step gradient elution with $CHCl_3$/petroleum ether (0–100%) and then with MeOH/$CHCl_3$ (0–50%). Fr. 5 (350 mg) was further separated into three subfractions on a silica gel VLC column using step gradient elution with MeOH/$CHCl_3$ (0–50%). Fr. 5–3 (80 mg) was then separated into five subfractions on an RP-18 VLC column using step gradient elution with H_2O/MeOH (0–100%). Fr. 5–3–5 (12 mg) was further purified by semipreparative HPLC (65% MeOH/H_2O) to yield diorcinol (3 mg, t_R 15 min). Fr. 6 (780 mg) from the 50:1 of $CHCl_3$/MeOH eluent was further separated into three subfractions on a silica gel VLC column using step gradient elution with MeOH/$CHCl_3$ (0–50%). Fr. 6–1 (62 mg) was then separated into five subfractions on an RP-18 VLC column using step gradient elution with H_2O/MeOH (0–100%). Fr. 6–1–1 was further purified by Sephadex LH-20, eluting with MeOH/$CHCl_3$ (1:1), and semipreparative HPLC (60% MeOH/H_2O) to yield cordyol C (2.1 mg, t_R 14 min). Fr. 6–1–2 (53 mg) was further purified by Sephadex LH-20 eluting with MeOH, and then by semipreparative HPLC (50% MeOH/H_2O) to yield **4** (15 mg, t_R 10 min). Fr. 6–3 (205 mg) was separated into five subfractions on an RP-18 VLC column using step gradient elution with H_2O/MeOH (0–100%). Fraction 6–3–1 (82 mg) was further purified by Sephadex LH-20 eluting with MeOH, and then by semipreparative HPLC (50% MeOH/H_2O) to yield **1** (30 mg, t_R 25 min), **2** (3 mg, t_R 21 min), and **3** (6 mg, t_R 19 min). Fr. 6–3–3 (38 mg) was further purified by Sephadex LH-20 eluting with MeOH, and then by semipreparative HPLC (55% MeOH/H_2O) to yield glyantrypine (4 mg, t_R 7 min). Fr.6-2 (136 mg) was subjected to silica gel VLC using step gradient elution with acetone/petroleum (0–100%) to afford ten subfractions. Fr. 6–2–8 (36 mg) was subjected to Sephadex LH-20, eluting with MeOH/$CHCl_3$ (1:1), to afford six subfractions. Fr. 6–2–8–6 (12 mg) was further purified

by semipreparative HPLC (10% MeOH/H_2O) to yield **5** (3 mg, t_R 13 min) and **6** (2 mg, t_R 11 min).

3.5. Characteristics of compounds

*3.5.1. Versicoloritide A (**1**).* White amorphous powder; $[\alpha]_D^{25}$ −90.7 (*c* 1.7, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.49), 258 (4.05), 265 (3.97) nm; IR (KBr) ν_{max} 3297, 3064, 3027, 2978, 2963, 1640, 1506, 1449, 1345, 1320, 1189, 1160 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESI-MS *m/z* 560.2869 $[M+H]^+$ (calcd for $C_{31}H_{37}N_5O_5$, 560.2873).

*3.5.2. Versicoloritide B (**2**).* White amorphous powder; $[\alpha]_D^{25}$ −43.6 (*c* 0.1, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.49), 258 (4.05), 265 (3.97) nm; IR (KBr) ν_{max} 3276, 2923, 1680, 1647, 1555, 1543, 1522, 1451, 1344, 748, 702 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESI-MS *m/z* 546.2703 $[M+H]^+$ (calcd for $C_{30}H_{35}N_5O_5$, 546.2716).

*3.5.3. Versicoloritide C (**3**).* White amorphous powder; $[\alpha]_D^{25}$ −118 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.49), 258 (4.02), 265 (3.97) nm; IR (KBr) ν_{max} 3303, 1647, 1555, 1537, 1524, 1450, 1342, 758, 702 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESI-MS m/*z* 576.2797 $[M+H]^+$ (calcd for $C_{31}H_{37}N_5O_6$, 576.2822).

*3.5.4. Tetraorcinol A (**4**).* Purple oil; UV (MeOH) λ_{max} (log ε) 223 (4.52), 280 (3.79) nm; IR (KBr) ν_{max} 3417, 2923, 1614, 1585, 1463, 129, 1233, 1159, 1137, 1059, 1036, 971, 840, 782, 677, 662, 634, 596 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESI-MS *m/z* 442.1785 $[M+H]^+$ (calcd for $C_{28}H_{26}O_5$, 442.1780).

*3.5.5. Versicolactone A (**5**).* White oil; $[\alpha]_D^{25}$ −31.5 (*c* 0.02, EtOH); UV (MeOH) λ_{max} (log ε) 272 (3.72) nm; IR (KBr) ν_{max} 1777, 1748, 1683, 1652, 1558, 1537, 1518, 1508, 1125, 1061, 680, 646 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESI-MS m/*z* 193.0486 $[M+Na]^+$ (calcd for $C_8H_{10}O_4Na$, 193.0477).

*3.5.6. Versicolactone B (**6**).* White oil; $[\alpha]_D^{25}$ +19.8 (*c* 0.05, EtOH); UV (MeOH) λ_{max} (log ε) 272 (3.72) nm; IR (KBr) ν_{max} 1754, 1699, 1682, 1650, 1556, 1540, 1204, 1131, 1028, 721, 668 cm^{-1}; ^1H and ^{13}C NMR data, see Table 2; HRESI-MS *m/z* 193.0468 $[M+Na]^+$ (calcd for $C_8H_{10}O_4Na$, 193.0477).

3.6. Determination of absolute configurations of amino acids by Marfey's method[10]

Compounds **1**–**3** (each 1 mg) were hydrolyzed in HCl (6 N; 1 mL) for 20 h at 110 °C. The solution was then evaporated to dryness and redissolved in H_2O (250 μL). A 1% (w/v) solution (100 μL) of L-FDAA in acetone was added to an aliquot (50 μL) of the acid hydrolyzate solution. After addition of $NaHCO_3$ solution (1 N; 20 μL) the mixture was incubated at 45 °C for 1 h. The reaction was then quenched by addition of HCl (2 N, 10 μL). Analyses of the FDAA-derivatized hydrolyzates of compounds **1**–**3** and standard FDAA-derivatized amino acids were carried out by HPLC (Waters 600E; solvents: A. water+0.2% TFA, B. MeCN; linear gradient: 0–5 min, 15% B; 5–50 min, 15–45% B; 50–55 min, 45% B; temperature, 30 °C; flow rate, 1 mL/min; UV detection at λ_{max} 340 nm). Retention times (min) of the amino acids derivatives were as follows: L/D-Ala, t_R 29.6/34.4 min; L/D-Pro, t_R 30.6/32.3 min; L/D-Phe, t_R 40.8/51.2 min. The derivatized hydrolyzates of **1**–**3** showed peaks designated as L-Ala, L-Pro, and L-Phe. All amino acids of these cyclopeptides were established as L-configuration. Due to the bad discrimination between L/D-Ser, they were determined by amino acids analysis on a chiral Crownpak CR(+) HPLC column.[11,12] Compound **3** (1 mg) was dissolved in 1 mL of 6 N HCl and heated in a sealed tube at 110 °C for 12 h. The hydrolyzate was dried and reconstituted in 1 mL of H_2O. The hydrolyzate was then analyzed by chiral HPLC over Crownpak CR(+) column for serine, proline (flow rate 0.5 mL/min; solvent, aqueous $HClO_4$ (pH 1.5); detection, 201 nm; temperature 0 °C), respectively. The retention times of serine in hydrolyzates of **3** and the authentic L/D-Ser and L-Pro were t_R 6.2 min, t_R 6.2/5.2 min, and t_R 5.2 min, respectively. Co-injection of the authentic sample with the hydrolyzate confirmed that the serine residue in compound **3** was L-Ser (Fig. S28).

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Supplementary data

This data include bioassay protocols used, the NMR spectra of compounds **1**–**6**, HPLC profiles of acidic hydrolyzates of **1**–**3**. Supplementary data associated with this article can be found online version, at doi:10.1016/j.tet.2011.07.003.

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