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Antifungal New Oxepine-Containing Alkaloids and Xanthones from the Deep-Sea-Derived Fungus *Aspergillus versicolor* SCSIO 05879

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Supporting Information

ABSTRACT: Phytopathogenic fungi remain a continuous and huge threat in the agricultural fields. The agrochemical industry has made great development of the use of microbial natural products, which has been regarded as an effective strategy against phytopathogenic fungi. Antifungal bioassay-directed fractionation was used to isolate two new oxepine-containing alkaloids (**1** and **2**), two new 4-aryl-quinolin-2-one alkaloids (**3** and **4**), and four new prenylated xanthones (**5**−**8**) from the deep-sea-derived fungus *Aspergillus versicolor* SCSIO 05879. Extensive NMR spectroscopic analysis, quantum mechanical calculations, and X-ray single-crystal diffraction were used to elucidate their structures, including their absolute configurations. Versicoloids A and B, versicone A, and cottoquinazoline A showed antifungal activities against three phytopathogenic fungi. The antifungal activities of these bioactive compounds strongly depend on the fungal species. Especially versicoloids A and B showed strong fungicidal effect (MIC of 1.6 μg/mL) against *Colletotrichum acutatum*, compared with that of the positive control cycloheximide (MIC of 6.4 μg/mL). The results of antifungal experiments indicated that versicoloids A and B may be regarded as candidate agents of antifungal agrochemicals.

KEYWORDS: *deep-sea-derived, Aspergillus versicolor, ECD calculations, oxepine, phytopathogenic fungi, antifungal activities*

INTRODUCTION

Effective and sustained control of fungal pathogens is an important project in the agricultural fields. The global losses caused by phytopathogenic fungi are estimated to be ∼20% reductions in the major food and cash crops in spite of the continued release of new resistant cultivars and antifungal agrochemicals.[1,2] Agrochemicals from microbial natural products have been regarded as an effective strategy against phytopathogenic fungi. Many natural antifungal agents such as kasugamycin, polyoxins, validamycin, and blasticidin-*S* and antibacterial agents such as oxytetracyline and streptomycin have been isolated from microbial resources.[3,4]

Over the past 50 years, approximately 20 000 natural products have been reported from marine flora and fauna, but it was rarely reported from deep-water marine organisms, about <2%.[5] Methods for sample collection, identification, and culturing technologies of microorganisms have developed rapidly in recent years. At the same time, chemical investigations on deep-sea microorganisms have shown a sharp increase.[6] As part of our ongoing efforts to discover structurally novel bioactive natural compounds from marine-derived microorganism-inhabiting unique environments,[7−10] a fungal strain SCSIO 05879, identified as *Aspergillus versicolor*, was isolated from a deep-sea sediment (depth = −3927 m), collected from the Indian Ocean (6°00′00″ N, 87°30′50″ E). It was found that *A. versicolor* could produce a number of secondary metabolites with various chemical structures, such as anthraquinones, chromones, lactones, and alkaloids, and some of them exhibited intriguing biological activities.[11−16] The ethyl acetate extract of the fermentation broth of *A. versicolor* SCSIO 05879 displayed significant in vitro antifungal activity against phytopathogenic fungi *Colletotrichum acutatum* and contained a variety of secondary metabolites with similar UV absorptions as shown by high-performance liquid chromatography (HPLC) analysis with photodiode array. Two new oxepine-containing diketopiperazine-type alkaloids, versicoloids A and B (**1** and **2**), two new 4-aryl-quinolin-2-one alkaloids, 3,6-*O*-dimethylviridicatin (**3**) and 3-*O*-methylviridicatol (**4**), and four new prenylated xanthones, versicones A−D (**5**−**8**), together with four known compounds cottoquinazoline A (**9**),[16] (−)-cyclopenol (**10**),[16,17] variecoxanthone A (**11**),[18] and diorcinol (**12**),[19] were isolated from the culture extract of *A. versicolor* SCSIO 05879 (Figure 1). Their structures, including absolute configurations, were elucidated on the basis of extensive NMR spectroscopic data analysis, time-dependent density functional theory ECD calculations, and X-ray single-crystal diffraction. All the isolated compounds (**1**−**12**) were tested for their antifungal activities against three phytopathogenic fungi (*Colletotrichum acutatum*, *Magnaporthe oryzae*, and *Fusarium oxysporum*).

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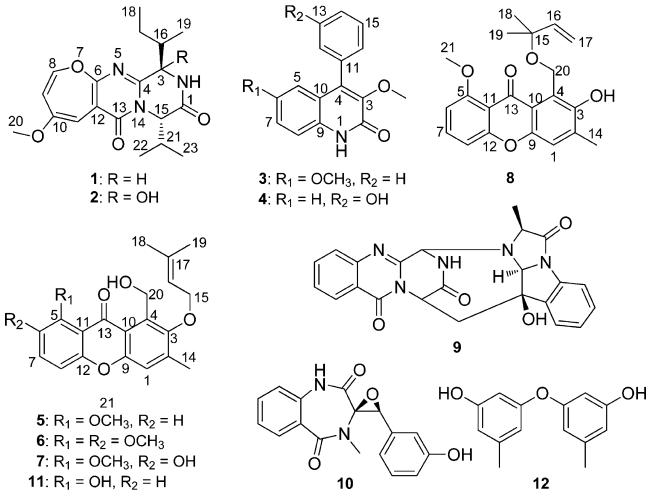

Figure 1. Structures of compounds **1**−**12** from *A. versicolor* SCSIO 05879.

MATERIALS AND METHODS

General. Optical rotations were measured with an MCP 500 automatic polarimeter (Anton Paar) with MeOH as solvent. UV spectra were recorded on a UV-2600 spectrometer (Shimadzu). IR spectra were measured on an IRAffinity-1 Fourier transform infrared spectrophotometer (Shimadzu). Circular dichroism spectra were recorded with a Chirascan circular dichroism spectrometer (Applied Photophysics, Ltd.). ^{1}H, ^{13}C NMR, distortionless enhancement by polarization transfer (DEPT), and 2D-NMR spectra were recorded on the Avance-500 spectrometer (Bruker), using tetramethylsilane (TMS) as an internal standard. High-resolution electrospray ionization mass spectrometry (HRESIMS) and ESIMS spectra data were recorded on a MaXis quadrupole-time-of-flight mass spectrometer and an amaZon SL ion trap mass spectrometer (Bruker), respectively. X-ray diffraction intensity data were collected on a CrysAlis PRO charge-coupled device (CCD) area detector diffractometer with graphite monochromated Cu Kα radiation (λ = 1.54178 Å). Thin-layer chromatography (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, Sweden), respectively. Vacuum-liquid chromatography (VLC) used silica gel H (Qingdao Marine Chemical Factory). All solvents used were of analytical grade (Tianjin Fuyu Chemical and Industry Factory). Semipreparative HPLC was performed using an ODS column (YMC-pack ODS-A, 10 × 250 mm, 5 μm, 4 mL/min).

Fungal Material. The fungus *A. versicolor* SCSIO 05879 was isolated from a marine sediment sample collected in May, 2014, from the Indian Ocean (6°00′00″ N, 87°30′50″ E) at a depth of 3927 m. The fungus was identified using a molecular biological protocol by DNA amplification and sequencing of the ITS region. A BLAST search result showed that the sequence was most similar (100%) to the sequence of *Aspergillus versicolor* (compared to accession no. AY373883). This strain has been deposited in China General Microbiological Culture Collection Center with a deposition number of CGMCC 11123.

Extraction and Isolation. *A. versicolor* SCSIO 05879 was grown under static conditions at 25 °C for 40 days in one hundred 1000 mL conical flasks containing liquid medium (300 mL/flask) composed of starch soluble (10 g/L) and polypeptone (1 g/L), and tap water after adjusting its pH to 7.5. The fermented whole broth (30 L) was extracted three times with EtOAc, and the mycelia were extracted three times by 85% volume aqueous acetone. Both extracts were combined (18.3 g) and then were subjected to vacuum-liquid chromatography (VLC) on a silica gel column using step-gradient elution with MeOH−CH_2Cl_2 (0−100%) to separate into six fractions based on TLC properties. Fraction 1 (2.4 g) was further separated into

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for 1−4 in Dimethyl Sulfoxide (DMSO-d_6) (TMS, δ ppm)

	1		2		3		4	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	166.5, C		166.9, C			12.00, s		12.05, s
2		8.46, s		6.50, s	158.0, C		158.0, C	
3	57.7, CH	4.66, brs	84.8, C		145.6, C		144.9, C	
4	154.0, C		153.6, C		136.9, C		137.6, C	
5					108.3, CH	6.40, d (2.8)	125.8, CH	7.01, d (7.9)
6	159.0, C		159.7, C		154.2, C		121.9, CH	7.07, t (7.5)
7					116.8, CH	7.10, dd (8.5, 2.8)	128.6, CH	7.39, t (7.5)
8	145.1, CH	6.36, d (5.6)	144.5, CH	6.18, d (6.0)	116.4, CH	7.30, d (8.5)	115.1, CH	7.33, d (7.9)
9	115.3, CH	5.65, dd (5.6, 2.0)	115.9, CH	5.53, dd (6.0, 1.8)	130.2, C		135.7, C	
10	156.3, C		157.4, C		120.6, C		119.9, C	
11	95.1, CH	5.72, s	94.9, CH	5.79, s	133.5, C		134.7, C	
12	108.0, C		110.5, C		129.1, CH	7.32, overlap	116.0, CH	6.67, s
13	160.9, C		161.4, C		128.5, CH	7.52, t (7.6)	157.3, C	
14					128.1, CH	7.47, d (7.3)	115.0, CH	6.83, d (7.6)
15	60.5, CH	4.76, d (6.9)	61.2, CH	5.08, d (5.4)	128.5, CH	7.52, t (7.6)	129.5, CH	7.29, t (7.8)
16	35.8, CH	2.40, m	43.9, CH	2.56, m	129.1, CH	7.32, overlap	119.7, CH	6.69, d (7.6)
17	22.9, CH_2	1.25, m	25.2, CH_2	0.97, m				
18	12.3, CH_3	0.81, t (6.9)	11.9, CH_3	0.86, t (7.2)				
19	15.0, CH_3	1.07, d (6.9)	11.0, CH_3	1.12, d (7.2)				
20	55.0, CH_3	3.64, s	55.4, CH_3	3.72, s				
21	30.6, CH	2.19, m	33.1, CH	2.39, m				
22	19.9, CH_3	0.91, d (6.4)	20.2, CH_3	1.14, d (6.9)				
23	18.4, CH_3	0.96, d (6.4)	18.1, CH_3	1.04, d (6.9)				
3-OCH_3					59.4, CH_3	3.68, s	59.5, CH_3	3.67, s
6-OCH_3					55.2, CH_3	3.56, s		
13-OH								9.60, s

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Table 2. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for 5−7 (TMS, δ ppm)

	5[a]		6[a]		7[b]		8[b]	
position	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	119.0, CH	7.20, s	119.0, CH	7.17, s	118.8, CH	7.40, s	118.0, CH	7.29, s
2	141.0, C		141.2, C		140.4, C		134.3, C	
3	152.9, C		152.4, C		151.9, C		151.4, C	
4	134.3, C		134.1, C		133.8, C		121.3, C	
5	160.5, C		148.7, C		145.0, C		159.8, C	
6	105.4, CH	6.77, d (8.3)	149.1, C		148.8, C		105.8, CH	6.90, d (7.7)
7	134.9, CH	7.56, t (8.3)	120.6, CH	7.30, d (9.2)	123.9, CH	7.35, d (9.0)	134.6, CH	7.64, t (7.8)
8	109.7, CH	6.99, d (8.3)	112.6, CH	7.14, d (9.2)	112.8, CH	7.21, d (9.0)	109.0, CH	7.02, d (7.7)
9	152.7, C		153.0, C		152.3, C		149.0, C	
10	120.7, C		120.0, C		119.2, C		118.8, C	
11	112.6, C		117.1, C		116.7, C		112.3, C	
12	157.3, C		150.5, C		146.6, C		156.3, C	
13	179.7, C		179.7, C		178.1, C		176.7, C	
14	17.6, CH_3	2.40, s	17.6, CH_3	2.39, s	17.0, CH_3	2.37, s	16.9, CH_3	2.27, s
15	72.1, CH_2	4.43, d (7.2)	72.1, CH_2	4.42, d (7.2)	71.3, CH_2	4.38, d (7.1)	76.7, C	
16	119.9, CH	5.59, t (7.2)	119.9, CH	5.59, t (7.2)	120.1, CH	5.56, t (7.1)	143.1, CH	6.00, dd (16.8, 10.6)
17	138.8, C		138.8, C		137.7, C		114.7, CH_2	5.27, d (16.8); 5.20, d (10.6)
18	25.9, CH_3	1.78, s	25.9, CH_3	1.78, s	25.5, CH_3	1.76, s	25.5, CH_3	1.35, s
19	18.2, CH_3	1.70, s	18.2, CH_3	1.70, s	17.9, CH_3	1.67, s	25.5, CH_3	1.35, s
20	57.2, CH_2	5.02, s	57.2, CH_2	5.00, s	55.6, CH_2	4.90, d (6.9)	60.7, CH_2	5.26, s
21	56.6, CH_3	4.01, s	61.7, CH_3	3.97, s	60.9, CH_3	3.82, s	56.1, CH_3	3.87, s
6-OCH_3			57.3, CH_3	3.89, s				
6-OH						9.51, s		
20-OH						4.72, t (6.9)		

[a]Recorded in $CDCl_3$. [b]Recorded in DMSO-d_6.

nine subfractions by reverse-phase silica gel (ODS) using step-gradient elution with MeOH−H_2O (20%−100%). Subfraction 1-6 was directly separated by HPLC (55% MeOH/H_2O) to yield **2** (4.1 mg, t_R = 21.6 min). Fraction 1-7 was directly separated by HPLC (65% MeOH/H_2O) to yield **12** (3.6 mg, t_R = 16.3 min) and **1** (2.6 mg, t_R = 22.6 min). Fraction 1-8 was further purified by HPLC (57% acetonitrile/H_2O) to yield **7** (9.7 mg, t_R = 16.3 min), **5** (28.6 mg, t_R = 23.1 min), and **6** (42.8 mg, t_R = 28.2 min), respectively. Fraction 1-9 was directly separated by HPLC (77% MeOH/H_2O) to yield **11** (44.5 mg, t_R = 28.5 min) and **8** (6.4 mg, t_R = 32.0 min). Fraction 3 was divided into five parts (fractions 3-1−3-5) by Sephadex LH-20 (MeOH). Fraction 3-3 was further purified by HPLC (57% MeOH/H_2O) to yield **3** (5.1 mg, t_R = 18.8 min). Fraction 4 was divided into three parts (fractions 4-1−4-3) by Sephadex LH-20 (MeOH). Fraction 4-3 was further purified by HPLC (41% MeOH/H_2O) to yield **10** (131.7 mg, t_R = 8.0 min) and **4** (3.5 mg, t_R = 22.2 min). Fraction 5 was divided into three parts (fractions 5-1−5-3) by Sephadex LH-20 (MeOH). Fraction 5-3 was further purified by HPLC (50% MeOH/H_2O) to yield **9** (15.9 mg, t_R = 33.0 min).

*X-ray Crystal Data for **4**, **6**, and **8**.* Yellow crystals of **4**, **6**, and **8** were obtained in the solvent of methanol. Crystal data of **4** (Cu Kα radiation) and **6** (Cu Kα radiation) were obtained on a CrysAlis PRO CCD area detector diffractometer with graphite monochromated Cu Kα radiation (λ = 1.54178 Å). Crystal data of **8** (Mo Kα radiation) were obtained on a Bruker Smart CCD area detector diffractometer with graphite monochromated Mo Kα radiation (λ = 0.71073 Å). Crystallographic data for **4**, **6**, and **8** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 1416778, 1416787, and 1417166, respectively. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB21EZ, U.K. [Fax: + 44 (0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

*X-ray Crystallographic Data of **4**.* Monoclinic, space group P2(1)/c with a = 12.0134(10) Å, b = 7.8855(8) Å, c = 13.9221(11) Å, V = 1304.1(2) Å^3, Z = 4, D_{calc} = 1.361 g/cm^3, R = 0.0999, wR_2 = 0.1045, T = 293(2) K. Crystal size, 0.20 × 0.08 × 0.06 mm^3.

*X-ray Crystallographic Data of **6**.* Monoclinic, space group P2(1)/c with a = 4.1217(2) Å, b = 39.0750(2) Å, c = 14.1681(8) Å, V = 2275.6(2) Å^3, Z = 4, D_{calc} = 1.122 g/cm^3, R = 0.1004, wR_2 = 0.2142, T = 293(2) K. Crystal size, 0.30 × 0.14 × 0.10 mm^3.

*X-ray Crystallographic Data of **8**.* Triclinic, space group P-1 with a = 7.2746(7) Å, b = 12.0963(12) Å, c = 12.4067(14) Å, V = 979.61(17) Å^3, Z = 2, D_{calc} = 1.310 g/cm^3, R = 0.0888, wR_2 = 0.1737, T = 298(2) K. Crystal size, 0.43 × 0.38 × 0.21 mm^3.

*Versicoloid A (**1**).* Yellow oil; $[\alpha]_D^{25}$: −230.4 (c 0.3, MeOH); UV (MeOH) λ_{max} (log ε): 344 (3.65) nm; IR (KBr) ν_{max}: 1661, 1653, 1582, 1537, 1404, 1377, 1290, 1219, 1192, 1173, 1103, 1015 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRESIMS m/z 382.1726 [M + Na]$^+$ (calcd for $C_{19}H_{25}N_3NaO_4$, 382.1737).

*Versicoloid B (**2**).* Yellow oil; $[\alpha]_D^{25}$: −114.9 (c 0.4, MeOH); UV (MeOH) λ_{max} (log ε): 253 (3.78), 350 (3.66) nm; IR (KBr) ν_{max}: 3246, 1657, 1616, 1584, 1537, 1406, 1379, 1290, 1217, 1192, 1173, 1098, 1057, 1013 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRESIMS m/z 398.1689 [M + Na]$^+$ (calcd for $C_{19}H_{25}N_3NaO_5$, 398.1686).

*3,6-O-Dimethylviridicatin (**3**).* Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 205 (4.14), 238 (4.13), 347 (3.42) nm; IR (KBr) ν_{max}: 1653, 1647, 1558, 1541, 1506, 1489, 1456, 1423, 1339, 1277, 1217, 1152, 1020 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRESIMS m/z 304.0938 [M + Na]$^+$ (calcd for $C_{17}H_{15}NNaO_3$, 304.0944).

*3-O-Methylviridicatol (**4**).* Faint yellow crystal; UV (MeOH) λ_{max} (log ε): 203 (4.02), 267 (3.47), 322 (3.15) nm; IR (KBr) ν_{max}: 1684, 1647, 1636, 1558, 1541, 1506, 1489, 1456, 1423, 1339, 1298, 1271, 1209 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 1; HRESIMS m/z 268.0960 [M + H]$^+$ (calcd for $C_{16}H_{14}NO_3$, 268.0968).

*Versicone A (**5**).* Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 204 (4.20), 237 (4.21), 243 (4.21), 251 (4.20), 286 (3.87), 357 (3.68) nm; IR (KBr) ν_{max}: 1616, 1601, 1474, 1420, 1379, 1265, 1211, 1190, 1098, 1084, 1007, 957 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; HRESIMS m/z 377.1355 [M + Na]$^+$ (calcd for $C_{21}H_{22}NaO_5$, 377.1359).

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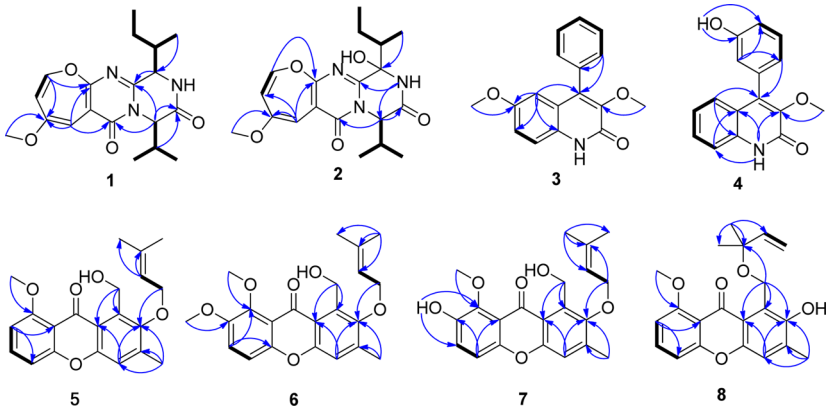

Figure 2. Key $^{1}H-^{1}H$ COSY (bold) and HMBC (arrows) correlations of **1**−**8**.

*Versicone B (**6**).* Yellow crystal; UV (MeOH) λ_{max} (log ε): 203 (4.15), 243 (4.22), 260 (4.11), 374 (3.46) nm; IR (KBr) ν_{max}: 1636, 1609, 1489, 1456, 1420, 1317, 1277, 1231, 1200, 1128, 1069, 1007, 959, 939 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 407.1469 $[M + Na]^+$ (calcd for $C_{22}H_{24}NaO_6$, 407.1465).

*Versicone C (**7**).* Yellow amorphous solid; UV (MeOH) λ_{max} (log ε): 203 (4.23), 241 (4.21), 266 (4.13), 312 (3.39), 375 (3.43) nm; IR (KBr) ν_{max}: 3308, 1732, 1616, 1481, 1458, 1425, 1383, 1296, 1207, 1196, 1055, 1036, 978, 951 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 393.1314 $[M + Na]^+$ (calcd for $C_{21}H_{22}NaO_6$, 393.1309).

*Versicone D (**8**).* Yellow crystal; UV (MeOH) λ_{max} (log ε): 203 (3.99), 245 (4.03), 286 (3.58), 366 (3.39) nm; IR (KBr) ν_{max}: 1645, 1603, 1574, 1464, 1420, 1379, 1335, 1271, 1256, 1140, 1098, 1084, 1063, 1040, 1007, 939 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Table 2; HRESIMS *m/z* 377.1364 $[M + Na]^+$ (calcd for $C_{21}H_{22}NaO_5$, 377.1359).

Computational Methods. The electron-capture dissociation (ECD) spectra of versicoloids A and B (**1** and **2**) and models I and II were calculated as described previously.[7,8]

Antifungal Activity Assay. The antifungal activities against three phytopathogenic fungi (*C. acutatum*, *M. oryzae*, and *F. oxysporum*) were evaluated in 96-well microtiter plates using a modification of the broth microdilution method.[3] Arrayed stock solutions of the tested compounds dissolved in methanol were diluted 100-fold with the proper culture medium for each pathogenic fungi and tested at final concentrations between 0.1 and 200 μg/mL. Under the sterile environment, fungal suspensions (50 μL) of each pathogenic fungi were poured into the wells containing 50 μL of 2-fold serially diluted single compounds in the corresponding culture medium for a final volume of 100 μL. The negative controls were treated with 1% MeOH, which corresponds to the highest concentration. Under the same concentrations, the blank wells were prepared with corresponding culture medium containing the tested compounds. The inoculated plates were incubated at 28 °C. After an incubation of 48 h, the optical density (OD) of each well was measured using a microplate reader at 600 nm. The growth inhibition of each dilution was calculated using the following formula:

$$\%\text{inhibition} = 100 \times [1 - \text{OD of treated well} / \text{OD of negative control well}]$$

The minimum inhibitory concentration (MIC) values were derived from Probit analysis of the concentration−response data, with serially diluted concentrations of the tested compounds. The dilutions of the tested compounds were independently performed at least three times. Cycloheximide was used as the positive control against three phytopathogenic fungi (*C. acutatum*, *M. oryzae*, and *F. oxysporum*) with MIC values of 6.4, 12.8, and 12.8 μg/mL, respectively.

RESULTS AND DISCUSSION

The ethyl acetate extract of the fermentation broth of *A. versicolor* SCSIO 05879 was subjected to silica gel column chromatography and further purified by HPLC to obtain eight new (**1**−**8**) and four known (**9**−**12**) compounds (Figure 1). The known compounds were identified by comparison of spectroscopic data with those reported in the literature as cottoquinazoline A (**9**),[16] (−)-cyclopenol (**10**),[16,17] variecoxanthone A (**11**),[18] and diorcinol (**12**).[19]

Compound **1** was initially obtained as a yellow oil. Its molecular formula, $C_{19}H_{25}N_3O_4$, was established by HRESIMS at *m/z* 382.1726 $[M + Na]^+$ (calcd 382.1737), implying the presence of 9 degrees of unsaturation. The ^{1}H NMR and heteronuclear multiple quantum correlation (HMQC) spectra (measured in DMSO-d_6) indicated the presence of 25 protons, including one exchangeable proton signal at δ_H 8.46 (2-NH), three unsaturated proton signals [δ_H 6.36 (d, *J* = 5.6 Hz, H-8), 5.72 (s, H-11), and 5.65 (dd, *J* = 5.6, 2.0 Hz, H-9)], two proton signals attached heteroatoms at δ_H 4.76 (d, *J* = 6.9 Hz, H-15) and δ_H 4.66 (brs, H-3), one methoxyl group at δ_H 3.64 (H_3-20), two methine signals at δ_H 2.40 (H-16) and δ_H 2.19 (H-21), one methylene at δ_H 1.25 (H_2-17), and four methyl groups [δ_H 1.07 (d, *J* = 6.9, H_3-19), 0.96 (d, *J* = 6.4, H_3-23), 0.91 (d, *J* = 6.4, H_3-22), and 0.81 (t, *J* = 6.9, H_3-22)]. The ^{13}C NMR (Table 1) and DEPT spectra revealed the presence of 19 carbons, namely, 5 methyls (one oxygenated), 1 methylene, 4 saturated methines, 3 olefinic methines, and 6 quaternary carbons (including 4 olefinic ones and 2 carbonyls), which accounted for the 9 degrees of unsaturation. Four olefinic signals ($\delta_{H/C}$ 6.36/145.1, 5.72/95.1, 5.65/115.3, and δ_C 156.3) were typical for an oxepine ring,[20,21] which was confirmed by the correlation spectroscopy (COSY) correlations of H-8/H-9 and by the HMBC correlations of H-8 with C-6, C-9 and C-10 and H-11 with C-6, C-9, and C-10. Detailed analysis of the 1D- and 2D-NMR spectra of **1** revealed that they were very similar to those of oxepinamides B,[20] indicating that they shared the same skeleton. The signals for valine were observed in **1**, instead of the corresponding unit of alanine in oxepinamides B; meanwhile, one methine signal ($\delta_{H/C}$ 4.66/57.7) replaced the oxygenated quaternary carbon (C-3) in oxepinamides B. This deduction was further supported by the COSY correlations of H-15/H-21/H_3-22(H_3-23) and H-3/H-16/H_2-17(H_3-19)/H_3-18, as well as by the heteronuclear multiple bond correlation (HMBC) of H-15 with C-1, C-4, and C-13 and H-3 with C-16, C-17, and C-19 (Figure 2). The observed nuclear Overhauser enhancement spectroscopy (NOESY) correlation between H-3

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and H-21 in the NOESY spectrum indicated the noncofacial orientation of H-3 and H-15 (Figure 3). Because the chiral

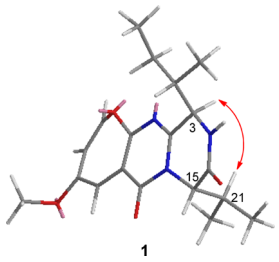

Figure 3. Key NOESY correlation of compound **1**.

center C-16 in **1** was far from the chromophore, the simplified structure of **1** (Model-I) was used for conformational analysis.[22,23] Therefore, the absolute configurations of C-3 and C-15 in **1** were determined as 3*R* and 15*S*, respectively, by comparing the calculated ECD spectrum with its experimental values (Figure 4).

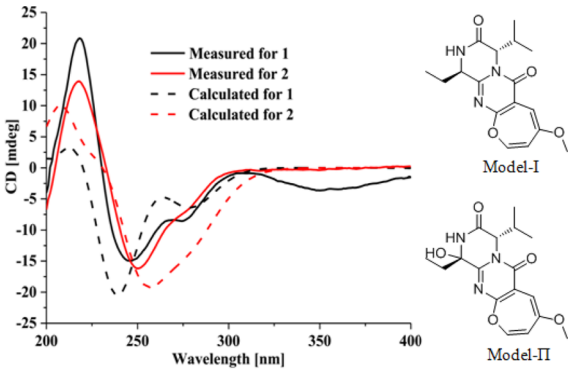

Figure 4. CD and calculated ECD spectra of **1** and **2**.

Compound **2**, yellow oil, was assigned the molecular formula $C_{19}H_{25}N_3O_5$ on the basis of HRESIMS data, with one oxygen atom more than **1**, and showed similar spectral features to those of **1** (Table 1). However, significant differences in chemical shift between **1** and **2** were observed, particularly for H-3 and C-3. The chemical shifts of the methine (CH-3, $\delta_{H/C}$ 4.66/57.7) in **1** were changed dramatically to the oxygenated quaternary carbon (C-3, δ_C 84.8) in **2**, which was supported by HMBC correlations from H_2-17 (δ_H 0.97, m) and H_3-19 (δ_H 1.12, d, J = 7.2 Hz) to C-3 (Figure 2). In addition, the similarity of the CD curve [CD (c 0.02, MeOH) ($\Delta\varepsilon_{max}$) 217 (+13.5), 252 (−16.4) and specific rotation ($[\alpha]^{25}_D$: −114.9°)] of **2** with those of **1** [CD (c 0.02, MeOH) ($\Delta\varepsilon_{max}$) 218 (+21.1), 247 (−15.0) and specific rotation ($[\alpha]^{25}_D$: −230.4°)] indicated the same absolute configuration between **2** and **1** (Figure 4). Similarly, this deduction was further supported by the ECD calculations of the simplified analogue (Model-Π) with that determined experimentally. Because compounds **1** and **2** exhibited almost identical ECD spectra, the absolute configuration of **2** was also established as 3*R* and 15*S* (Figure 4).

Compound **3** was obtained as a yellow amorphous powder with the molecular formula $C_{17}H_{15}NO_3$ as determined by HRESIMS at m/z 304.0938 $[M + Na]^+$ (calcd 304.0944), indicating 11 degrees of unsaturation. In the ^1H NMR spectrum, one exchangeable proton signal was observed at δ_H 12.00 ppm, which indicated that the NH or OH chelated with the carbonyl group. The ^1H, ^{13}C, and DEPT spectra showed the presence of 14 aromatic carbons, including 8 methine ones and 6 aromatic quaternary ones, and 1 amide carbonyl group (δ_c 158.0), which corresponded to 10 degrees of 11 unsaturation equivalents required by the molecular formula and indicated that compound **3** had three rings. The presence of a monosubstituted benzene system was indicated by the ^1H NMR signals at δ_H 7.52 (2H, t, J = 7.6 Hz), 7.47 (1H, d, J = 7.3 Hz), and 7.32 (2H, overlap). Another three aromatic proton signals at δ_H 7.30 (1H, d, J = 8.5 Hz), 7.10 (1H, dd, J = 8.5, 2.8 Hz), and 6.40 (1H, d, J = 2.8 Hz) were assigned to be a typical ABX system. Detailed analysis of the 1D- and 2D-NMR spectra data of **3** revealed that they were very similar to those of 3-*O*-methylviridicatin,[17] indicating that they shared the same skeleton. The main difference in the ^1H NMR spectrum was the presence of a methoxyl group at δ_H 3.56 for 6-OCH_3 in **3**. This deduction was further supported by the HMBC correlation of 6-OCH_3 with C-6 (Figure 2). Therefore, compound **3** was the methylolation derivative of 3-*O*-methylviridicatin and was named 3,6-*O*-dimethylviridicatin.

Compound **4**, a faint yellow crystal, had the molecular formula $C_{16}H_{13}NO_3$ as determined by HRESIMS at m/z 290.0780 $[M + Na]^+$ (calcd 290.0788), indicating 11 degrees of unsaturation. The similarity of ^1H and ^{13}C NMR spectra of **4** with **3** (Table 1) suggested that they were analogues, and the key difference was at the benzene ring. In the ^1H NMR spectrum, the aromatic proton signals and the coupling constants at δ_H 7.39 (1H, t, J = 7.5 Hz), 7.33 (1H, d, J = 7.9 Hz), 7.07 (1H, t, J = 7.5 Hz), and 7.01 (1H, t, J = 7.9 Hz) indicated the presence of an *ortho*-disubstituted benzene system. Another four aromatic proton signals at δ_H 7.29 (1H,

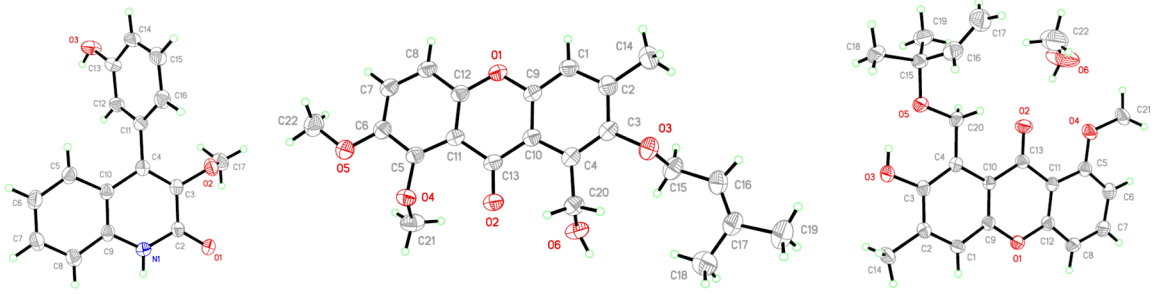

Figure 5. Oak Ridge thermal ellipsoid plot (ORTEP) drawings of compounds **4**, **6**, and **8**.

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t, J = 7.8 Hz), 6.83 (1H, d, J = 7.6 Hz), 6.69 (1H, d, J = 7.6 Hz), and 6.67 (1H, s) were assigned to the 1,3-disubstituted benzene ring. These deductions were further supported by the COSY correlations of H-5/H-6/H-7/H-8 and H-14/H-15/H-16, as well as by the HMBC correlation of H-12 with C-14 and C-16. The exchangeable proton signal at δ_H 9.60 (13-OH) was located at C-13, supported by the HMBC correlation of 13-OH with C-13 and C-14 (Figure 2). To verify the proposed structure, compound **4** was subjected to single-crystal X-ray diffraction analysis (Figure 5). Thus, compound **4** was the methylated derivative of viridicatol[24] and was named 3-*O*-methylviridicatol.

Compound **5** was isolated as a yellow amorphous solid. Its molecular formula was determined as $C_{21}H_{22}O_5$ on the basis of HRESIMS, with 10 degrees of unsaturation. Detailed analysis of the 1D- and 2D-NMR spectra data of **5** revealed that they were very similar to those of **11**,[18] indicating that they shared the same skeleton. The main difference in the ^{1}H NMR spectrum was the presence of a methoxy group at δ_H 4.01 for C-21 in **5**, and in the ^{13}C NMR spectrum, a methoxy group at δ_C 56.6 for 5-OCH_3 was observed in **5**, instead of a hydroxy group located at C-5 in **11**. This deduction was further supported by the HMBC correlation of H_3-21 with C-5 (Figure 2). Compound **5** was thus elucidated as the methylated derivative of **11** and was named versicone A.

The molecular formula of compound **6** was determined to be $C_{22}H_{24}O_6$ by HRESIMS at m/z 407.1469 $[M + Na]^+$ (calcd 407.1465) with 10 degrees of unsaturation. The resemblance of the ^{1}H and ^{13}C NMR data (Table 2) between **6** and **5** indicated that they had the same skeleton. Interpretation of the 2D NMR spectra of **6** revealed that the aromatic proton signal H-6 (δ_H 6.77, d, J = 8.3 Hz) in **5** was replaced by the corresponding methoxy group (δ_C 61.7) in **6**. This deduction was further supported by the HMBC correlation of 6-OCH_3 with C-6 (Figure 2). Ultimately, the structure of **6** was further confirmed by single-crystal X-ray diffraction (Figure 5).

Compound **7**, a yellow amorphous solid, was assigned the molecular formula $C_{21}H_{22}O_6$ on the basis of HRESIMS data, with one CH_2 less than **6**, and showed similar spectral features to those of **6** (Table 2). The distinct differences between them were the disappearance of the methoxy group 6-OCH_3 and the presence of 6-OH in **7**. These deductions were further supported by the HMBC correlation of H-7 with C-5 and C-12, and H-8 with C-6 and C-11. The exchangeable proton signal at δ_H 9.51 (6-OH) was located at C-6, supported by the HMBC correlation of 6-OH with C-5 and C-7 (Figure 2).

The molecular formula of compound **8** was determined to be $C_{21}H_{22}O_5$ by HRESIMS at m/z 377.1364 $[M + Na]^+$ (calcd 377.1359), possessing the same molecular formula with **5**. The resemblance of the ^{1}H and ^{13}C NMR data (Table 2) between **8** and **5** indicated that they had the same backbone, and the key difference between them was at the isopentene group. In the ^{1}H NMR spectrum, the olefinic proton signals and the coupling constants at δ_H 6.00 (1H, dd, J = 16.8, 10.6 Hz), 5.27 (1H, d, J = 16.8 Hz), and 5.20 (1H, d, J = 10.6 Hz) indicated the presence of a terminal double bond. The HMBC correlations from H_3-18/H_3-19 (δ_H 1.35) to C-15 (δ_C 76.7) and C-16 (δ_C 143.1), and H_2-20 (δ_H 5.26) to C-15, revealed the presence of a oxygenated isopentene unit at C-20. To verify the proposed structure, compound **8** was subjected to single-crystal X-ray diffraction analysis (Figure 5). Consequently, the structure of **8** was deduced with the trivial name versicone D.

To discover the structurally novel bioactive natural compounds from the deep-sea-derived microorganism to control fungal pathogens, these compounds (**1**−**12**) produced by the *A. versicolor* SCSIO 05879 isolated from the Indian Ocean deep-sea sediment (depth = −3927 m) were preliminarily investigated for their antifungal activities against three phytopathogenic fungi (*C. acutatum*, *M. oryzae*, and *F. oxysporum*), which commonly infect major food and cash crops. Cycloheximide was used as the corresponding positive control. Among these compounds, versicoloids A and B (**1** and **2**), versicone A (**5**), and cottoquinazoline A (**9**) exhibited antifungal activities against three phytopathogenic fungi (Table 3). The antifungal activities of these bioactive compounds strongly depend on the fungal species. Especially versicoloids A and B (**1** and **2**) showed strong antifungal activities (MIC of 1.6 μg/mL) against *C. acutatum*, compared with that of the positive control cycloheximide (MIC of 6.4 μg/mL). In contrast, other compounds displayed weak antifungal activities with MIC values of >30 μg/mL against the pathogens tested.

Table 3. Inhibitory Effects of Tested Compounds on Phytopathogenic Fungi

	phytopathogenic fungi (MIC, μg/mL)		
compound	*Colletotrichum acutatum*	*Magnaporthe oryzae*	*Fusarium oxysporum*
1	1.6	128	64
2	1.6	128	64
3	>200	>200	>200
4	>200	>200	>200
5	32	>200	128
6	>200	>200	>200
7	>200	>200	>200
8	>200	>200	>200
9	128	128	64
10	>200	>200	>200
11	>200	>200	>200
12	>200	>200	>200
cycloheximide	6.4	12.8	12.8

In conclusion, two new oxepine-containing diketopiperazine-type alkaloids (**1** and **2**), two new 4-aryl-quinolin-2-one alkaloids (**3** and **4**), and four new prenylated xanthones (**5**−**8**) were isolated from the deep-sea-derived fungus *A. versicolor* SCSIO 05879. Their structures were elucidated on the basis of extensive NMR data analysis, time-dependent density functional theory (TDDFT) ECD calculations, and X-ray single-crystal diffraction. To the best of our knowledge, the structures of oxepine-containing diketopiperazine alkaloids are very rarely discovered in nature, featuring both oxepine and diketopiperazine ring systems. The antifungal experiments exhibited that four isolated compounds (**1**, **2**, **5**, and **9**) showed antifungal activities against three phytopathogenic fungi. Moreover, versicoloids A and B (**1** and **2**) were identified as the most active components against *C. acutatum*, and they may be regarded as candidate agents of antifungal agrochemicals in the agricultural field.

■ ASSOCIATED CONTENT

Supporting Information

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ITS gene sequence data of SCSIO 05879 and NMR and HRESIMS spectra of **1**−**8** (PDF)

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Notes

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