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海绵共附生真菌 *Penicillium crustosum* SCSIO 41442 活性代谢产物研究

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摘要: 从海绵共附生真菌 *Penicillium crustosum* SCSIO 41442 中分离得到 1 个新生物碱 diacedolinate(**1**)和 14 个已知化合物(**2~15**)。通过光谱学分析和电子圆二色谱(ECD)测定了所有化合物的结构, 并对这些化合物进行了抗氧化和抗菌活性测试。结果表明, 化合物 **1** 具有弱抗氧化活性, 半抑制浓度(half maximal inhibitory concentration, IC_{50})为(71.00±0.14)μg·mL^{-1}。与阳性对照维生素 C 相比, 化合物 **2** 表现出强抗氧化活性, IC_{50} 为(1.25±0.10)μg·mL^{-1}。化合物 **9**、**10**、**11** 和 **15** 对耐甲氧西林葡萄球菌(methicillin-resistant *Staphylococcus aureus*, MRSA)、*Colletotrichum asianum* HNM 408、*Colletotrichum acutatum* HNM RC178 和 *Alternaria alternate* 多种病原菌表现出广谱的抗菌活性, 最小抑菌浓度(minimum inhibitory concentration, MIC)为 2.5~160μg·mL^{-1}。这些化合物的活性均为首次报道。

关键词: 海绵共附生真菌; *Penicillium crustosum*; 次级代谢产物; 抗氧化; 抗菌

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Bioactive metabolites from the sponge-derived fungus *Penicillium crustosum* SCSIO 41442

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Abstract: A new alkaloid, diacedolinate (**1**), along with fourteen known compounds (**2-15**) was isolated from the sponge associated fungus *Penicillium crustosum* SCSIO 41442. The structures of these compounds were determined by spectrum analysis and ECD. All compounds were evaluated for their antioxidant and antimicrobial activities. The results showed that compound **1** exhibited weak antioxidant activity with an IC_{50} value of (71.00±0.14) μg·mL^{-1}, while compound **2**, in contrast, displayed broad antioxidant activity with an IC_{50} value of (1.25±0.10) μg·mL^{-1}, compared with the positive control, vitamin C. In addition, compounds **9**, **10**, **11**, and **15** demonstrated broad-spectrum antimicrobial activity against a variety of pathogens, including MRSA, *Colletotrichum asianum* HNM

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408, *Colletotrichum acutatum* HNM RC178, and *Alternaria alternate*, with MIC values ranging from 2.5 to 160 μg·mL^{-1}. The bioactivities of these compounds are reported here for the first time.

Key words: sponge-derived fungi; *Penicillium crustosum*; secondary metabolites; antioxidant; antimicrobial

In recent years, antibiotic resistance has emerged as a significant global threat, reducing the effectiveness of curing viral, bacterial, parasitic and fungal infections (Indraningrat et al, 2016). Consequently, the discovery of new bioactive compounds has become very urgent. Marine organisms exhibit substantial differences from their terrestrial counterparts, resulting in distinct metabolic profiles (Carroll et al, 2020). Due to their unique living environments, sponge associated microorganisms are rich in silent genes, which can form a unique physiological metabolism mode and produce secondary metabolites with novel structure and significant activity (Slaby et al, 2017). Furthermore, *Penicillium crustosum* is an important resource that has yielded diverse classes of bioactive compounds (Kozlovskii et al, 2013), such as alkaloids, diketopiperazines, and polyketides.

In our search for additional bioactive natural products from marine sponge associated fungi, the strain *Penicillium crustosum* SCSIO 41442 was isolated from a *Callyspongia* sp. sponge sample collected near Weizhou Island in the Beibu Gulf of the South China Sea. Chemical investigation of this strain led to a new alkaloid (**1**), with fourteen other known metabolites (**2-15**) obtained (Fig. 1). In this paper, we address the isolation, structural elucidation, and biological evaluation of all obtained compounds.

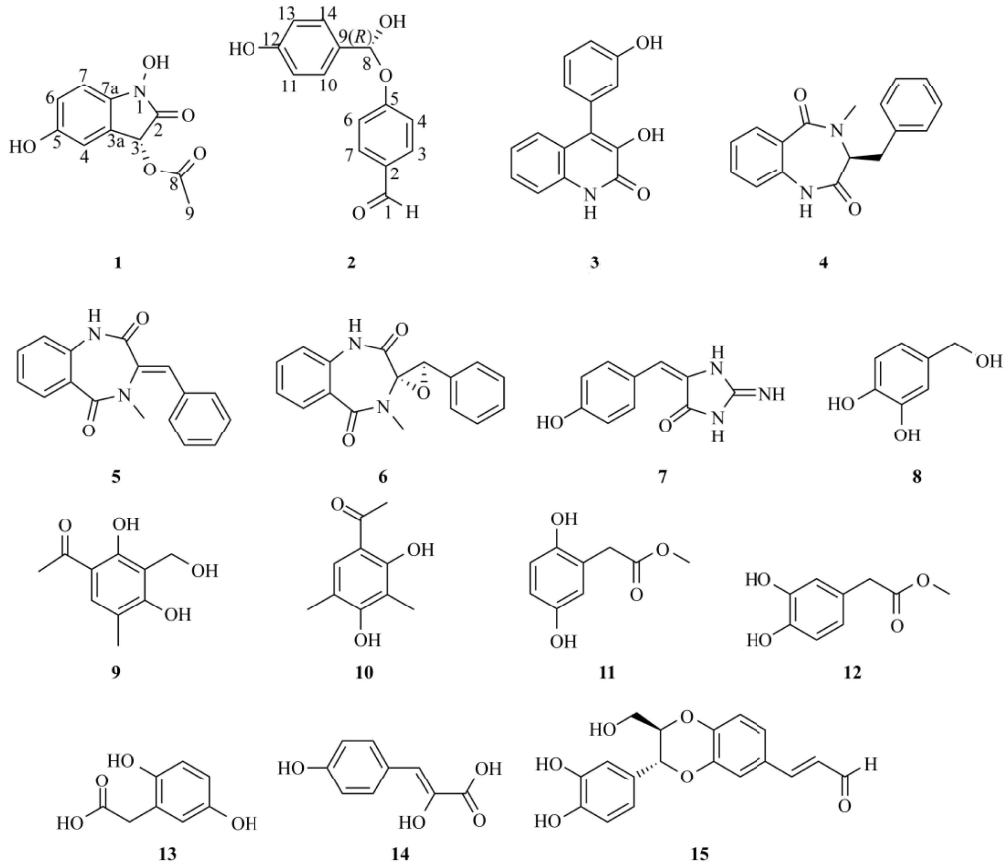

Fig. 1 Chemical structures of compounds **1-15**

1 Materials and Methods

1.1 General experimental procedures

NMR data were acquired using a Bruker Avance spectrometer (Bruker, Billerica, MA, USA) operating at 500 and 700 MHz for ^{1}H NMR and 125 and 175 MHz for ^{13}C NMR. High-resolution mass spectrometry was performed on a Bruker TOF-QII mass spectrometer (Bruker, Bille-Rica, MA, USA). Optical rotations were

measured on a PerkinElmer MPC 500 polarimeter (Waltham, MA, USA). UV and ECD spectra were recorded on a Chirascan circular dichroism spectrometer (Applied Photophysics, Leatherhead Surrey, United Kingdom). TLC and column chromatography (CC) were performed on silica gel GF254 plates (10-40 μm) and silica gel (200–300 mesh; Qingdao Marine Chemical Factory, Qingdao, China), respectively. Semi-preparative HPLC was conducted using an ODS column (YMC-pack ODS-A, 10 mm × 250 mm, 5 μm). All solvents were of analytical grade. Sea salt was obtained from Guangzhou Haili Aquarium Technology Company (Guangzhou, China).

1.2 Fungal material

The fungus *Penicillium crustosum* SCSIO 41442 was isolated from a *Callyspongia* sp. sponge sample collected near Weizhou Island (21.612°N, 108.338°E), Beibu Gulf, South China Sea. The strain was deposited in the Chinese Academy of Sciences Key Laboratory for Tropical Marine Bioresources and Ecology. Based on ITS sequencing, this fungus was identified as *Penicillium crustosum* (GenBank accession number NR_077153.1).

1.3 Fermentation, extraction and isolation

The fermentation of *Penicillium crustosum* SCSIO 41442 was performed on rice for 30 d at 26°C. Then the culture was extracted three times with ethyl acetate by ultrasonication, and the organic solvent was evaporated under reduced pressure to obtain the crude extract. The extract was then dissolved in methanol and partitioned with ethyl acetate (1∶1), yielding 100.5 g of the MeOH-soluble fraction.

Nine fractions (P.Fr.1-9) were obtained by fractionation using silica gel CC (200−300 mesh) with an increasing polarity gradient (CH_2Cl_2/MeOH, from 100∶1 to 0∶1, *V*/*V*). ODS CC (Spherical C18, 20−45 μm, 100 A) and MeOH/H_2O (*V*/*V*, 1∶9−10∶0) were performed on P.Fr.3 (2.4 g) to obtain five subfractions (P.Fr.3.1-3.5). Then, P.Fr.3-2 (216.2 mg), P.Fr.3-3 (154.7 mg) and P.Fr.3-4 (207.5 mg) were purified by semi-preparative HPLC using an ODS column (YMC-pack ODS-A, 10 × 250 mm, 5 μm) to gain **2** (3.0 mg, 45%MeCN/H_2O, 2.0 mL·min^{-1}, t_R= 28.0 min), **4** (3.5 mg, 55%MeOH/H_2O, 2.0 mL·min^{-1}, t_R=18.0 min), **5** (1.6 mg, 55%MeOH/H_2O, 2.0 mL·min^{-1}, t_R=20.0 min), and **6** (3.0 mg, 55%MeOH/H_2O, 2.0 mL·min^{-1}, t_R =12.0 min). ODS CC and MeOH/H_2O (1∶9–10∶0, *V*/*V*) were performed on P.Fr.4 (2.6 g) to obtain four subfractions (P.Fr.4.1-4.4). Then, P.Fr.4-1 (287.3 mg) and P.Fr.4-3 (246.9 mg) were purified by semi-preparative HPLC to gain **3** (4.5 mg, 55%MeOH/H_2O, 3.0 mL·min^{-1}, t_R=15.5 min), **7** (3.8 mg, 15%MeOH/H_2O, 2.5 mL·min^{-1}, t_R=32.0 min), and **14** (1.6 mg, 15%MeOH/H_2O, 2.5 mL·min^{-1}, t_R=16.8 min). P.Fr.2 (7.9 g), 5 (10.8 g), 6 (2.7 g), and 7 (1.8 g) were purified by HPLC to obtain **1** (3.2 mg, 10%MeCN/H_2O, 2.0 mL·min^{-1}, t_R=8.0 min), **8** (5.2 mg, 20%MeOH/H_2O, 2.5 mL·min^{-1}, t_R=9.2 min), **9** (3.1 mg, 45%MeCN/H_2O, 2.0 mL·min^{-1}, t_R=28.0 min), **10** (33.0 mg, 45%MeCN/H_2O, 2.0 mL·min^{-1}, t_R=21.0 min), **11** (8.9 mg, 25%MeOH/H_2O, 2.0 mL·min^{-1}, t_R=19.0 min), **12** (3.5 mg, 25%MeOH/H_2O, 2.0 mL·min^{-1}, t_R=26.0 min), **13** (27.6 mg, 25%MeOH/ H_2O, 2.0 mL·min^{-1}, t_R=12.0 min), and **15** (5.0 mg, 25%MeOH/H_2O, 2.0 mL·min^{-1}, t_R=16.0 min).

Diacedolinate (1) : yellowish oil; $[\alpha]_D^{25}$ -3 (c 0.10, MeOH); UV (MeOH) λmax (log ε) 262 (3.71); 210 (5.13) nm; ECD (0.3mg·mL^{-1}, MeOH) λmax ($\Delta\varepsilon$) 238 (−3.08); 213 (+4.69) nm; HRESIMS: m/z 224.0555 $[M+H]^+$ (calcd for $C_{10}H_{10}NO_5$ 224.0553); for NMR data of ^{1}H and ^{13}C, see Tab. 1.

(*R*)-4-hydroxy (4-hydroxyphenyl) methoxy) benzaldehyde (2) : Yellow oil; $[\alpha]_D^{25}$ -2 (c 0.10,MeOH); ^{1}H NMR (500 MHz, CD_3OD-d_4) δ_H 9.77 (1H, brs, H-1), 6.92 (1H, m, H-3), 7.78 (1H, m, H-4), 7.78 (1H, m, H-6), 6.92 (1H, m, H-7), 4.38 (1H, s, H-8), 7.77 (1H, m, H-10), 6.91 (1H, m, H-11), 6.91 (1H, m, H-13), 7.77 (1H, m, H-14); ^{13}C NMR (125 MHz, CD_3OD-d_4) δ_C 192.82 (CHO-1), 130.37 (C-2), 116.56 (CH-3), 130.29 (CH-4), 165.23 (C-5), 130.29 (CH-6), 116.56 (CH-7), 106.14 (CH-8), 130.37 (C-9), 133.43 (CH-10), 116.88 (CH-11), 162.05 (C-12), 116.88 (CH-13), 133.43 (CH-14).

Viridicatol (3): Pink powder; ^{1}H NMR (500 MHz, CD_3OD-d_4) δ_H 7.34 (1H, m, H-5), 7.12 (1H, td, *J* = 8.2, 6.3, 1.4 Hz, H-6), 7.24 (1H, dd, *J* = 8.4, 1.4 Hz, H-7), 7.35 (1H, m, H-8), 6.81 (1H, m, H-2′), 6.87 (1H, dd, *J* = 8.4, 2.8 Hz, H-4′), 7.32 (1H, d, *J* = 7.7 Hz, H-5′), 6.83 (1H, m, H-6′); ^{13}C NMR (125 MHz, CD_3OD-d_4) δ_C

160.59 (C-2), 143.21 (C-3), 123.82 (C-4), 123.06 (C-4a), 126.32 (CH-5), 127.97 (CH-6), 122.17 (CH-7), 116.20 (CH-8), 134.33 (C-8a), 136.23 (C-1′), 116.46 (CH-2′), 158.67 (C-3′), 115.95 (CH-4′), 130.56 (CH-5′), 117.93 (CH-6′).

(3*S*)-1, 4-benzodiazepine-2, 5-diones (4): Yellow oil; $[\alpha]_D^{25}$-7 (c 0.10, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 10.51 (1H, s, H-1), 4.33 (1H, s, H-3), 7.07 (1H, d, *J* = 8.0 Hz, H-6), 7.19 (1H, t, *J* = 7.8 Hz, H-7), 7.85 (1H, t, *J* = 8.1 Hz, H-8), 7.57 (1H, d, *J* = 7.6 Hz, H-9), 2.87 (2H, s, H_2-10), 7.02 (1H, d, *J* = 7.2 Hz, H-14), 7.50 (1H, t, *J* = 7.4 Hz, H-15), 7.72 (1H, t, *J* = 7.6 Hz, H-16), 7.50 (1H, t, *J* = 7.4 Hz, H-17), 7.02 (1H, d, *J* = 7.2 Hz, H-18), 2.95 (3H, s, H_3-19); ^{13}C NMR (175 MHz, DMSO-d_6) δ_C 169.22 (C-2), 55.99 (CH-3), 167.67 (C-5), 120.69 (CH-6), 124.10 (CH-7), 136.64 (CH-8), 132.04 (CH-9), 31.49 (CH_2-10), 126.99 (C-11), 137.44 (C-12), 130.71 (CH-13), 126.49 (CH-14), 128.41 (CH-15), 129.02 (CH-16), 128.41 (CH-17), 126.49 (CH-18), 28.78 (CH_3-19).

7-Hydroxy-3, 10-dehydrocyclopeptine (5): Yellow oil; ^{1}H NMR (700 MHz, CD_3OD-d_4) δ_H 7.43 (1H, dd, *J* = 8.3, 1.3 Hz, H-6), 7.90 (1H, dd, *J* = 7.9, 1.6 Hz, H-7), 7.36 (1H, dd, *J* = 7.9, 1.6 Hz, H-8), 7.35 (1H, dd, *J* = 8.3, 1.3 Hz, H-9), 6.93 (1H, s, H-10), 7.29 (1H, dd, *J* = 7.9, 1.1 Hz, H-2), 7.41 (1H, t, *J* = 6.8 Hz, H-3′), 7.54 (1H, ddd, *J* = 8.1, 7.3, 1.6 Hz, H-4′), 7.38 (1H, t, *J* = 7.3 Hz, H-5′), 7.13 (1H, dd, *J* = 8.1, 1.1 Hz, H-6′), 3.12 (3H, s, H_3-4-NCH_3); ^{13}C NMR (175 MHz, CD_3OD-d_4) δ_C 172.40 (C-2), 130.96 (C-3), 36.12 (CH_3-4-NCH_3), 169.03 (C-5), 133.63 (C-5a), 131.84 (CH-6), 125.90 (CH-7), 134.11 (CH-8), 122.02 (CH-9), 137.95 (C-9a), 131.83 (CH-10), 135.35 (C-1′), 130.18 (CH-2′), 130.24 (CH-3′), 126.75 (CH-4′), 130.24 (CH-5′), 130.18 (CH-6′), 128.41 (CH-17), 126.49 (CH-18), 28.78 (CH3-19).

Cyclopenin (6): Yellow oil; $[\alpha]_D^{25}$-14 (c 0.10, MeOH); ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 7.23 (1H, dd, *J* = 6.2, 1.4 Hz, H-4), 7.54 (1H, td, *J* = 7.9, 7.6, 1.7 Hz, H-5), 7.09 (1H, td, *J* = 7.6, 7.5, 1.1 Hz, H-6), 6.92 (1H, dd, *J* = 7.9, 1.6 Hz, H-7), 3.07 (3H, s, H_3-10), 6.62 (1H, d, *J* = 1.6 Hz, H-12), 7.16 (1H, t, *J* = 7.0 Hz, H-13), 7.30 (1H, t, *J* = 7.4 Hz, H-14), 7.21 (1H, t, *J* = 7.2 Hz, H-15), 6.64 (1H, d, *J* = 2.0 Hz, H-16), 4.36 (1H, s, H-17); ^{13}C NMR (175 MHz, DMSO-d_6) δ_C 165.92 (C-1), 70.15 (C-2), 165.32 (C-3), 131.03 (CH-4), 132.40 (CH-5), 124.22 (CH-6), 121.16 (CH-7), 135.17 (C-8), 127.90 (C-9), 30.84 (CH_3-10), 130.90 (C-11), 126.10 (CH-12), 128.72 (CH-13), 130.47 (CH-14), 128.81 (CH-15), 126.42 (CH-16), 63.72 (CH-17).

Hemimycalin D (7): Yellow oil; ^{1}H NMR (500 MHz, DMSO-d_6) δ_H 6.23 (1H, s, H-4), 6.72 (2H, d, *J* = 9.0 Hz, H_2-7, 9), 7.43 (2H, d, *J* = 9.0 Hz, H_2-6, 10); ^{13}C NMR (175 MHz, DMSO-d_6) δ_C 155.40 (C-1), 163.20 (C-2), 126.50 (C-3), 116.50 (CH-4), 123.50 (C-5), 131.60 (CH-6, 10), 114.80 (CH-7, 9), 157.90 (C-8).

3, 4-Dihydroxybenzyl alcohol (8): Black powder; ^{1}H NMR (500 MHz, CD_3OD-d_4) δ_H 6.46 (1H, s, H-2), 6.42 (1H, d, *J* = 8.2 Hz, H-5), 6.53 (1H, d, *J* = 8.4 Hz, H-6), 3.30 (2H, s, H_2-7); ^{13}C NMR (125 MHz, CD_3OD-d_4) δ_C 123.62 (C-1), 113.67 (CH-2), 148.67 (C-3), 149.37 (C-4), 116.01 (CH-5), 117.24 (CH-6), 63.09 (CH_2-7).

Communal G (9): Yellow powder; ^{1}H NMR (700 MHz, CD_3OD-d_4) δ_H 7.60 (1H, s, H-6), 4.68 (2H, s, H_2-7), 2.17 (3H, s, H_3-8), 2.54 (3H, s, H_3-10); ^{13}C NMR (175 MHz, CD_3OD-d_4) δ_C 110.92 (C-1), 162.36 (C-2), 117.81 (C-3), 163.52 (C-4), 113.68 (C-5), 133.84 (CH-6), 65.62 (CH_2-7), 15.82 (CH_3-8), 204.57 (C-9), 26.26 (CH_3-10).

Clavatol (10): Yellow powder; ^{1}H NMR (500 MHz, CD_3OD-d_4) δ_H 2.53 (3H, s, H_3-1), 7.46 (1H, s, H-6′), 2.07 (3H, s, H_3-7′), 2.18 (3H, s, H_3-8′); ^{13}C NMR (125 MHz, CD_3OD-d_4) δ_C 26.19 (CH_3-1), 204.4 (C-2), 113.76 (C-1′), 162.16 (C-2′), 111.83 (C-3′),162.33 (C-4′), 117.25 (C-5′), 131.17 (CH-6′), 7.93 (CH_3-7′), 16.28 (CH_3-8′).

2, 5-Dihydroxy-phenylacetic acid methyl ester (11): Brown powder; ^{1}H NMR (500 MHz, CD_3OD-d_4) δ_H 6.62 (1H, d, *J* = 8.6 Hz, H-3), 6.54 (1H, dd, *J* = 8.6, 2.9 Hz, H-4), 6.59 (1H, d, *J* = 2.9 Hz, H-6), 3.55 (2H, s, H_2-7), 3.67 (3H, s, H_3-9); ^{13}C NMR (125 MHz, CD_3OD-d_4) δ_C 123.22 (C-1), 149.56 (C-2), 116.67 (CH-3), 115.67 (CH-4), 151.04 (C-5), 118.56 (CH-6), 36.47 (CH_2-7), 174.56 (C-8), 52.33 (CH_3-9).

3, 4-Dihydroxyphenylacetic acid methyl ester (12): Brown powder; ^{1}H NMR (500 MHz, CD_3OD-d_4) δ_H 6.70 (1H, d, *J* = 2.1 Hz, H-2), 6.68 (1H, d, *J* = 8.1 Hz, H-

5), 6.56 (1H, dd, *J* = 8.1, 2.1 Hz, H-6), 3.31 (2H, s, H_2-7), 3.66 (3H, s, H_3-9); ^{13}C NMR (125 MHz, CD_3OD-d_4) δ_C 126.94 (C-1), 116.30 (CH-2), 146.32 (C-3), 145.57 (C-4), 117.34 (CH-5), 121.61 (CH-6), 41.18 (CH_2-7), 174.57 (C-8), 52.37 (CH_3-9).

Homogentisic acid (13): Brown powder; ^{1}H NMR (500 MHz, CD_3OD-d_4) δ_H 6.63 (1H, d, *J* = 8.6 Hz, H-3), 6.55 (1H, dd, *J* = 8.6, 2.9 Hz, H-4), 6.62 (1H, d, *J* = 2.9 Hz, H-6), 3.53 (2H, s, H_2-7); ^{13}C NMR (125 MHz, CD_3OD-d_4) δC 123.58 (C-1), 149.56 (C-2), 116.78 (CH-3), 115.59 (CH-4), 150.98 (C-5), 118.57 (CH-6), 36.75 (CH_2-7), 176.21 (C-8).

***p*-Hydroxyphenylpyruvic acid (14):** White powder; ^{1}H NMR (700 MHz, DMSO-d_6) δ_H 6.79 (1H, s, H-3), 7.27 (2H, d, *J* = 8.6 Hz, H_2-5, 9), 6.77 (2H, d, *J* = 8.6 Hz, H_2-6, 8); ^{13}C NMR (175 MHz, DMSO-d_6) δ_C 168.0 (C-1), 138.7 (C-2), 127.2 (CH-3), 124.8 (C-4), 131.0 (CH-5/9), 115.1 (CH-6/8), 156.2 (C, C-7).

Isoamericanin A (15): Brown powder; $[\alpha]_D^{25}$ +2 (c 0.10, MeOH); ^{1}H NMR (500 MHz, CD_3OD-d_4) δ_H 6.77 (1H, d, *J* = 2.1 Hz, H-2), 6.74 (1H, d, *J* = 7.9 Hz, H-5), 6.72 (1H, dd, *J* = 8.1, 1.9 Hz, H-6), 4.59 (1H, d, *J* = 7.6 Hz, H-7), 4.30 (1H, ddd, *J* = 7.8, 4.7, 2.8 Hz, H-8), 4.23 (1H, dd, *J* = 12.5, 2.6 Hz, H-9a), 3.38 (1H, dd, *J* = 12.4, 4.5 Hz, H-9b), 6.87 (1H, d, *J* = 2.1 Hz, H-2′), 6.80 (1H, d, *J* = 8.2 Hz, H-5′), 6.92 (1H, dd, *J* = 8.2, 2.1 Hz, H-6′), 7.31 (1H, d, *J* = 15.8 Hz, H-7′), 6.66 (1H, dt, *J* = 15.8, 7.9 Hz, H-8′); ^{13}C NMR (175 MHz, CD_3OD-d_4) δ_C 126.41 (C-1), 115.33 (CH-2), 146.29 (C-3), 147.21 (C-4), 116.55 (CH-5), 121.05 (CH-6), 75.58 (CH-7), 75.69 (CH-8), 61.04 (CH_2-9), 130.73 (C-1′), 118.88 (CH-2′), 146.21 (C-3′), 147.33 (C-4′), 118.88 (CH-5′), 123.64 (CH-6′), 153.76 (CH-7′), 126.41 (CH-8′), 193.06 (C-9′).

1.4 ECD calculation

The Molecular Merck force field was used to perform the conformational search of compound **1** using Spartan' 14. Conformers with a Boltzmann population of more than 1% were optimized with Gaussian 09 at the B3LYP/6-31G (d) level in methanol (Cammi et al, 1995). Stable conformers were then chosen for ECD calculations at the B3LYP/6-311G (d, p) level in methanol. The overall ECD data were weighted by Boltzmann distribution, and the ECD curves and enantiomeric ECD curves were produced with a half-band width of 0.33 eV by Guassian view 6.0 software. The UV-corrected Boltzmann calculated contributions for each conformation were used for this purpose.

At the same time, compound **1** conformers whose Boltzmann population is more than 1% are selected for NMR chemical shift calculations. DFT was used to optimize these conformers again at the B3LYP/6-31 G (d) level using GAUSSIAN 09 program. The GIAO method was used to calculate the chemical shift of the NMR data at the PCM/mPW1PW91/6-31 G (d, p) level in methanol. In order to get the final spectrum, we used the Boltzmann distribution theory to average the spectra of the conformers. Unscaled shifts were used to analyze the probability of DP4$^+$. The DP4$^+$ calculations were performed with an Excel spread sheet (sarotti-NMR.weebly.com).

1.5 Antioxidant assay

DPPH radical scavenging activity was tested with minor adjustments to a literature protocol (Sharma et al, 2009). Test compounds and the positive control (vitamin C) were dissolved in methanol to make 2 mg·mL^{-1} solution and 0.25 mg·mL^{-1} solution, respectively. Samples with different concentrations (10, 50, 100, 500, 1000 μg·mL^{-1}) were added to 100 μL DPPH (0.2 mmol·L^{-1}). The mixture was stirred vigorously and left in darkness for 30 minutes at room temperature. The absorbance of the resulting solution was measured at 517 nm. Lower absorbance indicates higher free radical scavenging activity, and vice versa for higher absorbance. Vitamin C was used as reference compound and three parallel experiments were designed for each compound. The DPPH radical scavenging activity (%) was calculated as $[(A_{control}-A_{sample})/A_{control}]\times100\%$. Based on the inhibition to the terminal concentration, the inhibitory concentration (IC_{50}), which results in a 50% clearance of the DPPH radical, was estimated.

1.6 Antimicrobial assay

Antimicrobial activities were measured against twelve strains, including *Staphylococcus aureus*, MRSA, *Enterococcus faecalis*, *Micrococcus luteus*, *Colletotrichum asianum* HNM 408, *Colletotrichum acutatum* HNM RC178, *Colletotrichum gloeosporioides*

HNM 1003, *Pyricularia oryaza* HNM 1003, *Fusarium oxysporum* HNM 1003, *Curvularia australiensis*, *Alternaria alternate* and *Rhizoctonia solani* by the broth microdilution method. Pathogenic and phytopathogenic bacteria were cultivated at 28 °C for 24 h and 48 h respectively on a rotating shaker (180 r·min^{-1}). The cultures were diluted with sterile water to achieve an optical absorbance of 0.4~0.6 at 600 nm, before they were transferred into 96-well microtiter plates. Three replicates of each compound were tested in a dilution sequence between 640 and 0.625 μg·mL^{-1}. After 24 hours of culture, the optical absorbance was measured at 600 nm. The lowest concentration which completely inhibited visible growth was recorded in three separate experiments.

2 Results and Discussion

2.1 Structure elucidation

Compound **1** was obtained as a yellow oil with $C_{10}H_9NO_5$, which was determined by the HRESIMS peak at m/z 224.0555 $[M+H]^+$ (calcd for $C_{10}H_{10}NO_5$ 224.0553), representing 7 degrees of unsaturation. The ^{1}H NMR spectrum (Tab. 1) showed three aromatic proton signals at δ 6.67 (dd, J = 8.3, 2.2 Hz), 6.71 (d, J = 8.7 Hz), and 6.89 (d, J = 2.2 Hz), a signal of oxymethylene at δ 4.17 (s), and a methyl signal at δ 2.56 (s). The ^{13}C NMR (Tab. 1) and HSQC spectra of compound **1** showed 10 carbon signals, including six olefinic carbons at δ 154.52 (C-5), 135.39 (C-7a), 133.42 (C-3a), 116.67 (C-6), 113.05 (C-4), 111.76 (C-7), two carbonyl groups at δ 175.73 (C-8), 174.75 (C-2), an oxygen methylene carbon signal at δ 75.35 (C-3), and a methyl signal at δ 30.07 (9-Me). Based on the unsaturation, compound **1** should contain an additional ring structure apart from the benzene ring. Furthermore, the HMBC correlations of H-4, H-6, H-7/C-5 assigned that the hydroxyl was located at C-5 (Fig. 2). Moreover, in the ECD spectrum, **1** exhibited a positive cotton effect at 213 nm (Δε +4.69), a negative cotton effect at 238 nm (Δε -3.08), and had a shape of curves similar to those of the calculated ECD spectrum of the *R*-isomer (Fig. 3). These results established the absolute configuration of **1** to be 3*R*. Therefore, the structure of compound **1** was elucidated as a novel alkaloid, named diacedolinate.

Tab. 1 ^{13}C- and ^{1}H- NMR data of compound 1 in CD_3OD (500 MHz, 125 MHz)

Position	δ_H, mult (J in Hz)	δ_C, type
2		174.75, C
3	4.17, s	75.35, CH
3a		133.42, C
4	6.89, d (2.2)	113.05, CH
5		154.52, C
6	6.67, dd (8.3, 2.2)	116.67, CH
7	6.71, d (8.7)	111.76, CH
7a		135.39, C
8		175.73, C
9	2.56, s	30.07, CH_3

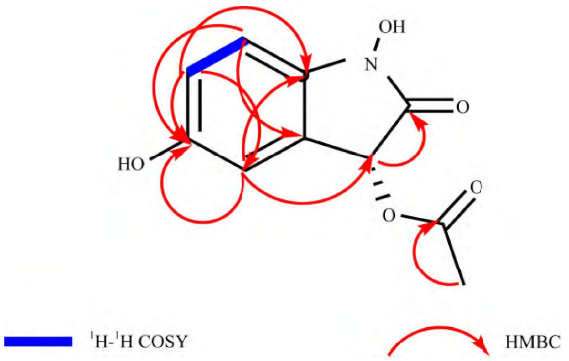

Fig. 2 Key ^{1}H-^{1}H COSY (blue bold lines) and HMBC (red arrows) correlations of compound **1**

The other known compounds were identified as (*R*)-4-hydroxy (4-hydroxyphenyl) methoxy) benzaldehyde (**2**) (Sulistyowaty et al, 2021), viridicatol (**3**) (Liu et al, 2021a), (3*S*)-1, 4-benzodiazepine-2, 5-diones (**4**) (Mao et al, 2021), 7-hydroxy-3, 10-dehydrocyclopeptine (**5**) (Liu et al, 2021b), cyclopenin (**6**) (Liu et al, 2012), hemimycalin D (**7**) (Shaala et al, 2021), 3, 4-dihydroxybenzyl alcohol (**8**) (Du et al, 2011), communal G (**9**) (Newaz et al, 2023), clavatol (**10**) (Yang et al, 2009), 2, 5-dihydroxyphe-nylacetic acid methyl ester (**11**) (Dai et al, 1991), 3, 4-dihydroxyphenylacetic acid methyl ester (**12**) (Li et al, 2019), homogentisic acid (**13**) (Wang et al, 2016), *p*-hydroxyphenylpyruvic acid (**14**) (Bugni et al, 2002), and isoamericanin A (**15**) (Suzuki et al, 2016), by comparing their NMR data in references.

2.2 Antioxidant and antimicrobial activity

All isolated compounds were evaluated for their antioxidant activity. The results showed that compound **1** had weak antioxidant activity with an IC_{50} value of (71.00±0.14) μg·mL^{-1} while compound **2** displayed potent antioxidant activity with an IC_{50} value of (1.25± 0.10) μg·mL^{-1}, compared with the positive control vitamin C (Tab. 2).

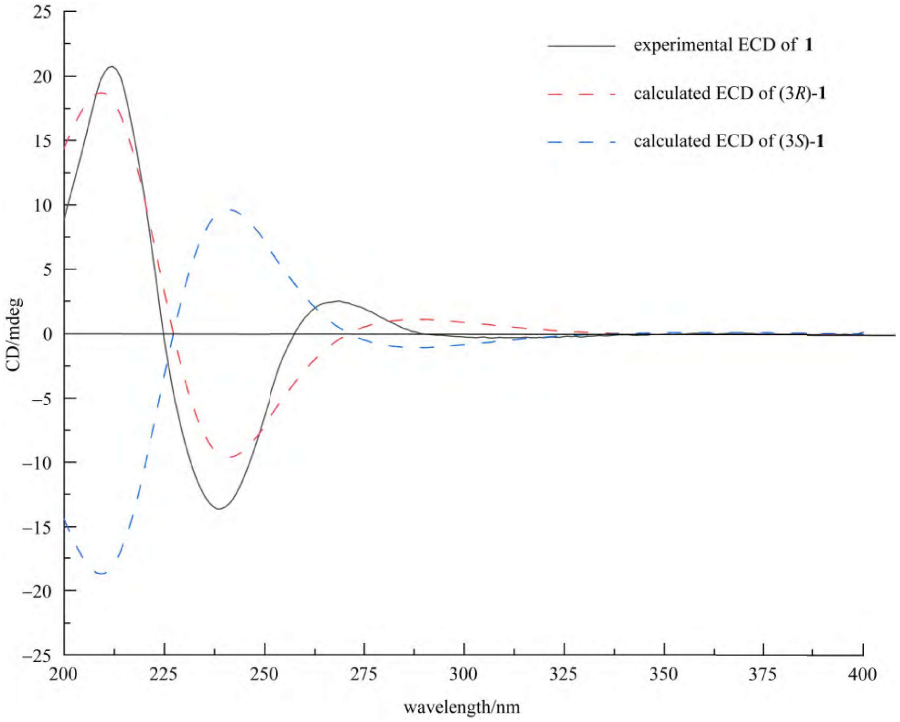

Fig. 3 Experimental and calculated ECD spectra of compound **1**

Tab. 2 Antioxidant activities of compounds 1 and 2

Compound	IC_{50}/(μg·mL^{-1})
1	71.00±0.14
2	1.25±0.10
Vitamin C [a]	5.90±0.05

Note: [a] used as a positive control.

Then, the antimicrobial activities were tested against four pathogenic bacteria, including *Staphylococcus aureus*, MRSA, *Enterococcus faecalis*, and *Micrococcus luteus*, and eight phytopathogenic bacteria, including *Colletotrichum asianum* HNM 408, *Colletotrichum gloeosporioides* HNM 1003, *Colletotrichum acutatum* HNM RC178, *Fusarium oxysporum* HNM 1003, *Pyricularia oryaza* HNM 1003, *Alternaria alternate*, *Curvularia australiensis*, and *Rhizoctonia solani*. Among them, compound **9** inhibited *Colletotrichum acutatum* HNM RC178 with an MIC value of 2.5 μg·mL^{-1}. Clavatol (**10**) showed broad activity against *Colletotrichum asianum* HNM 408 with an MIC value of 5.0 μg·mL^{-1}. Compound **11** inhibited MRSA and *Colletotrichum asianum* HNM 408 with MIC values of 2.5 and 80.0 μg·mL^{-1}, respectively. Compound **15** had weak activity against *Alternaria alternate* with an MIC value of 160.0 μg·mL^{-1} (Tab. 3). The other compounds showed no significant activity.

Tab. 3 Antimicrobial activities of compounds 9, 10, 11, and 15

Compound	MRSA	*C. acutatum* HNM RC178	*C. asianum* HNM 408	*A. alternate*
9	>160	2.5	>160	>160
10	>160	>160	5.0	>160
11	2.5	>160	80.0	>160
15	>160	>160	>160	160.0
Ampicillin [a]	0.625	-	-	-
Nystatin [b]	-	0.625	2.5	5.0

Note: [a] used as a positive control for MRSA; [b] used as a positive control for phytopathogenic strains.

3 Conclusions

In summary, a new alkaloid, diacedolinate (**1**), along with 14 known compounds (**2-15**) were obtained from sponge derived fungus *Penicillium crustosum* SCSIO 41442. Among them, compounds **2**, **4**, **5**, and **15** were isolated for the first time from sponge associated fungi. In addition, compounds **1** and **2** had antioxidant activity with IC_{50} values of (71.00±0.14) μg·mL^{-1}and (1.25±0.10) μg·mL^{-1}, respectively. Compounds **9**, **10**, **11**, and **15** showed broad antimicrobial activity against a

panel of pathogenic strains with MIC values ranging from 2.5 to 160 μg·mL^{-1}. The biological activities of these compounds were reported for the first time. Therefore, this study enriches the chemical diversity of alkaloids and provides a new perspective for natural product drug development.

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