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Peptides from the Soft Coral-associated Fungus Simplicillium sp. SCSIO41209

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ABSTRACT

Five new peptides, Sinulariapeptides A−E together with seven known peptides (**6–12**) were isolated from the soft coral associated fungus *Simplicillium* sp. SCSIO 41209. The structures of the new compounds and their absolute configurations were established on the basis of spectroscopic analysis including NMR, MS and ECD. All the Compounds (except sinulariapeptides B−D) were tested for the inhibitory activities of *Mycobacterium tuberculosis* protein tyrosine phosphatase B (MptpB), and antifungal activities against five plant pathogenic fungi. Simplicilliumtides B and cyclo(L-Val-L-Pro) showed inhibitory activity with the IC_{50} values of 35.0 and 25.9 μM, sinulariapeptides A, simplicilliumtides J, verlamelins A and B exhibited potent inhibition against *Colletotrichum asianum* with the MIC values of range from 4.9 to 9.8 μg/mL and simplicilliumtides J, verlamelins A and B displayed inhibition against *Pyricularia oryza* Cav with the MIC values in the range of 19.5–78.1 μg/ml, respectively.

1. Introduction

Coral represent was a vast and large resource for biodiversity that has the potentially to yield structurally unique and biologically active novel metabolites (Parvatkar et al., 2009; Zhukova and Titlyanov, 2003). However, the supply has become a serious obstacle to the ultimate development of these bioactive substances. Coral-derived fungi, which were regarded as the true producers of their host-derived compounds, have proven to be a promising source of new bioactive specialized metabolites that have become interesting and significant resources for drug discovery (Liu et al., 2016; YL et al., 2016; Zhuang et al., 2011). In previous study, the specialized metabolites from the soft corals of the South China Sea were investigated, and a variety of new and bioactive compounds were obtained (Yang et al., 2012, 2013, 2014). To search for a resource for bioactive compounds, we have focused our efforts on fungi derived from corals in the South China Sea, and about 80 fungi have been isolated and purified from corals in recent years. A fungal strain SCSIO41209 authenticated as *Simplicillium* sp. was isolated from the soft coral *Sinularia* sp., collected near the Yongxing Island.

The genus *Simplicillium* has been known to be a major contributor to peptides. Natural peptides exhibited a wide range of biological activities, and some are in clinical use or have entered human clinical trials as antibiotic or anticancer agents (Hancock and Diamond, 2000; Lehrer et al., 1993).

The fungal strain *Simplicillium* sp. SCSIO 41209 resulted in the isolation and identification of an undescribed cyclodepsipeptide, three undescribed linear peptides, and an undescribed cyclic dipeptide, named sinulariapeptides A−E (**1–5**), together with seven known peptides (**6–12**) simplicilliumtides A, B and J(Liang et al., 2017), verlamelins A and B (Ishidoh et al., 2014), Hirsutellic acid A(Thongtan et al., 2006), cyclo(L-Val-L-Pro) (Fig. 1). In the present study, the isolation and structure elucidation, enzyme-inhibitory and antifungal activities were reported.

2. Results and discussion

Compound **1** was obtained as a pale yellow oil. The molecular formula was determined as $C_{45}H_{69}N_7O_{11}$ by high-resolution electrospray ionization mass spectrometry (HRESIMS) (*m/z* 906.4964 $[M + Na]^+$).

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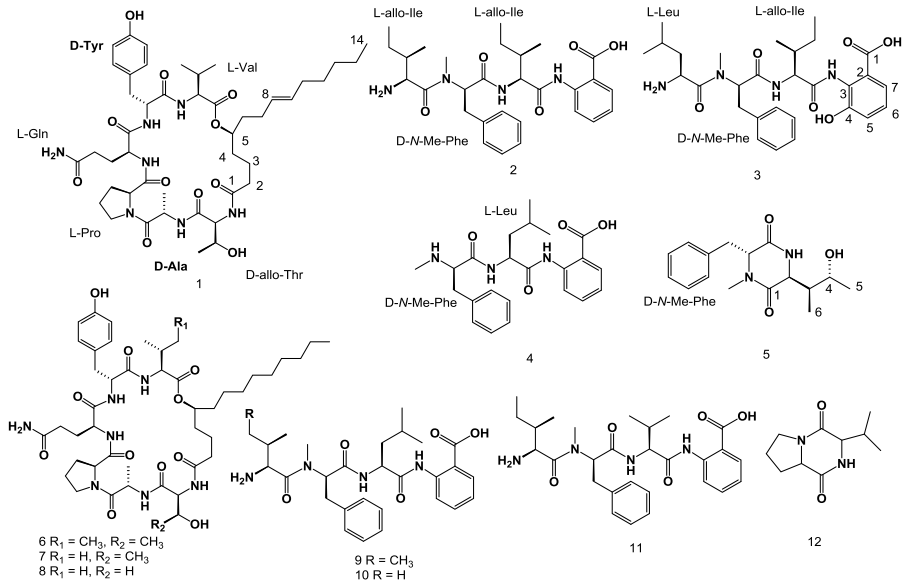

Fig. 1. Structures of compounds **1–12**.

Table 1
^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data for **1** in CD_3OD (δ_H and δ_C in ppm).

Position	δ_C	δ_H, (*J* in Hz)	Position	δ_C	δ_H, (*J* in Hz)
L-Val			4	25.7	2.03 m
1	172.7		5	48.7	3.92 m
2	61.1	3.92 d (6.5)	D-Ala		
3	31.1	2.02 m	1	173.6	
4	19.7	0.87 d (7.0)	2	49.0	4.64 q (6.5)
5	19.1	0.83 d (7.0)	3	16.4	1.36 d (6.5)
D-Tyr			D-allo-Thr		
1	174.2		1	172.9	
2	56.6	4.60 t (8.0)	2	59.4	4.40 d (7.5)
3	39.0	3.04 m	3	68.4	4.24 m
4	129.0		4	20.7	1.27 d (6.0)
5	131.4	7.11 d (8.5)	HTEA		
6	116.3	6.72 d (8.5)	1	176.0	
7	157.4		2	36.5	2.34 m/2.14 m
8	116.3	6.70 d (8.5)	3	21.9	1.54 m
9	131.4	7.09 d (8.5)	4	33.5	1.57 m
L-Gln			5	74.7	4.97 m
1	173.8		6	35.3	1.68 m
2	55.0	4.24 m	7	29.6	2.03 m
3	28.1	2.02 m	8	130.1	5.40 dt (6.0, 15.5)
4	33.2	2.26 m	9	132.5	5.40 dt (6.0, 15.5)
5	177.5		10	30.4	2.03 m
L-Pro			11	34.0	1.31–1.36 m
1	174.3		12	32.5	1.31–1.36 m
2	62.4	4.45 m	13	23.6	1.31–1.36 m
3	30.4	2.04 m	14	14.4	0.90 m

The ^{1}H and ^{13}C NMR data (Table 1) showed the presence of seven amide or ester carbonyl carbon signals at δ_C 177.5, 176.0, 174.3, 174.2, 173.8, 173.6, 172.9, 172.7, and methine carbon signals between δ_C 50–70, which suggested that **1** might be a peptide. Analysis of 2D spectra (Fig. 2) suggested that the six component amino acids were threonine (Thr), glutamine (Gln), proline (Pro), alanine (Ala), tyrosine (Tyr), and valine (Val).

The NMR spectra of **1** were similar to those of verlamelin A (**7**) (Ishidoh et al., 2014; Liang et al., 2017). However, the close comparison of the ^{13}C NMR spectroscopic data of **1** and **7** revealed main difference: a disubstituted double bond was observed in 5-hydroxytetradecanoic acid (HTA) residue. The positions of the hydroxyl group at C-5 and the alkenyl group at C-8 were established by analysis of HMBC and COSY data as shown in Fig. 2. The coupling constant of $J_{H\text{-}8/H\text{-}9}$ (15.5 Hz) revealed *E*-configuration of the double bond C8 = C9. To determine the absolute configuration of C-5, a modified Mosher's method was used (Ishidoh et al., 2014). After the calculation of the $\Delta\delta$ (δ_S-δ_R) values of the Mosher's esters of **1b**, the configuration of the C-5 was identified as *R* (Figs. S1 and S2). Thus, the alkyl chain residue was deduced to be (*R*, *E*)-5-hydroxytetradec-8-enoic acid (HTEA). The six amino acid residues were identified as D-allo-Thr, D-Ala, L-Pro, L-Gln, D-Tyr, and L-Val by Marfey's method and HPLC analysis, respectively (Figs. S47 and S48) (Liang et al., 2017). Furthermore, the HMBC correlations from H-2 of Val to C-1 of Tyr, H-2 of Tyr to C-1 of Gln, H-2 of Gln to C-1 of Pro, H-2 of Pro to C-1 of Ala, H-2 of Ala to C-1 of Thr, H-2 of Thr to C-1 of HTEA, and H-5 of HTEA to C-1 of HTEA, suggested that the sequence of the amino acid residues were cyclo (Val-Tyr-Gln-Pro-Ala-Thr-HTEA).

The HR-ESIMS/MS (Fig. 3, and Fig. S9) showing seven major fragment ions at *m/z* 906.4960 [Val + Tyr + Gln + Pro + Ala + Thr + HTEA + Na]$^+$, 807.4297 [Tyr + Gln + Pro + Ala + Thr + HTEA + Na]$^+$, 644.3632 [Gln + Pro + Ala + Thr + HTEA + Na]$^+$, 516.3049 [Pro + Ala + Thr + HTEA + Na]$^+$, 381.1933 [Ala + Thr + HTEA-O + H]$^+$, 349.2364 [Thr + HTEA + H + Na]$^+$, and 226.1107 [HTEA + 2H]$^+$, further confirmed the sequences. Therefore, the structure of **1** was determined as a sinulariapeptide A.

The molecular formula of sinulariapeptide B (**2**) was determined as $C_{29}H_{40}N_4O_5$ by HRESIMS (*m/z* 525.3081 [M + H] $^+$). Both the ^{1}H, and ^{13}C NMR spectra of **2** showed a close similarity to those of hirsutellic acid A (**9**) (Thongtan et al., 2006). However, the close comparison of the ^{13}C NMR spectra data of **2** and **9** revealed remarkable differences: a Leu residue in **9** was changed to an Ile in **2**.2D NMR data (HSQC and HMBC) correlations (Fig. 2, Figs. S19 and S20) revealed that **2** contained three amino acid residues: two isoleucines (Ile), an *N*-methylphenylalanine (*N*—Me-Phe), and the remaining residue was a 2-aminobenzoic acid (ABA) residue. In the HMBC spectra, the correlations from *N*—CH_3 of *N*—Me-Phe to C-2 of Ile, from the *α*-hydrogen H-2 of *N*—Me-Phe to C-1 of the same Ile, from the other *α*-hydrogen H-2 to C-1 of *N*—Me-Phe, and no correlations from the α-hydrogen H-2 of the first Ile to carbonyl carbon of other residue, no correlations from other *α*-