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New bioactive pyrrospirones C–I from a marine-derived fungus *Penicillium* sp. ZZ380

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ABSTRACT

Seven rare pyrrospirones C–I (**1**–**7**) as well as 18 known compounds were isolated from a marine-derived fungus *Penicillium* sp. ZZ380. Structures of the new pyrrospirones were elucidated by extensive NMR spectroscopic analyses, HRESIMS data, and Mosher's method. Pyrrospirone D (**2**) was also confirmed by X-ray diffraction analysis. Pyrrospirone G (**5**) showed potent activity in inhibiting the proliferation of different glioma cells with IC_{50} values of 1.06–8.52 μM and pyrrospirones C (**1**), F (**4**), and I (**7**) had antimicrobial activity against the growth of both methicillin-resistant *Staphylococcus aureus* and *Escherichia coli* with MIC values of 2.0–5.0 μg/mL.

1. Introduction

Glioma is the most common type of primary malignant brain tumor[1] and surgery following by adjuvant radiotherapy and chemotherapy are always considered in all patients.[2] Because glioma usually locates at important brain function areas, which makes surgical resection extremely difficult, so chemotherapy has played a more important role in the treatment and prevention of glioma. So far, very few drugs, including temozolomide, carmustine, and lomustine, have been approved for treating glioma. Moreover, most of the current anti-glioma drugs are alkylating agents with limited efficacy and serious side effects.[3,4] Therefore, there is an urgent need to discover lead compounds for the development of novel anti-glioma drugs. Marine-derived natural products are important sources for the discovery of new anticancer agents.[5–7]

As a part of our ongoing project for the discovery of novel anti-glioma agents from marine microorganisms,[8–15] a marine fungus strain ZZ380 was isolated from a sample of wild crab. This marine fungus was assigned as *Penicillium* sp. ZZ380 (Fig. S_1 and Table S_1) based on its ITS rDNA sequence result (Fig. S_2). A crude extract prepared from the culture of this isolated fungus in BMPM medium showed activity in inhibiting the proliferation of human glioma U87MG cells with an inhibition rate of 55.1%. Chemical investigation of this active extract resulted in the isolation of seven new pyrrospirones C–I (**1**–**7**) and 18 known compounds (**8**–**25**) (Fig. 1 and Supplementary Data Fig. S_3). Herein, we described the isolation and culture of strain ZZ380, the isolation and structural elucidation of new pyrrospirones, and their activities in inhibiting the proliferation of glioma cells and the growth of methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Candia albicans*.

2. Results and discussion

A large culture of *Penicillium* sp. ZZ380 was conducted in BMPM liquid medium and a crude extract prepared from the culture was separated by column chromatography, followed by HPLC purification, to afford compounds **1**–**25**. Based on the NMR and HRESIMS data and the comparison to the reported data, 18 known compounds were identified as GKK1032B (**8**),[16] chrysophanol (**9**), emodin (**10**),[17] citreorosein-3-*O*-sulphate (**11**), emodin-3-*O*-

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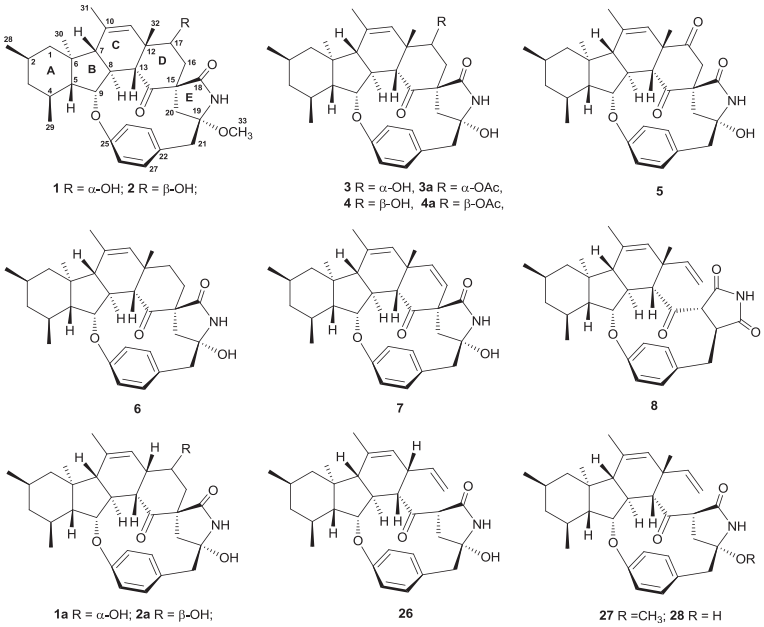

Fig. 1. Structures of compounds **1**–**8** isolated from *Penicillium* sp. ZZ380.

sulphate (**12**),[18] methyl 8-hydroxy-6-methyl-9-oxo-9H-xanthene-1-carboxylate (**13**),[19] yicathin C (**14**),[20] coniochaetone B (**15**),[21] sclerotinin C (**16**),[22] dihydrocitrinone (**17**),[23] 1,9-dihydroxy-3-(hydroxymethyl)-10-methoxydibenz[*b*,*e*]oxepin-6,11-dione (**18**),[24] moniliphenone (**19**),[25] phenol A (**20**),[26] neocyclocitrinol A (**21**), neocyclocitrinol C (**22**), neocyclocitrinol D (**23**),[27] citrinin H1 (**24**),[28] and penicitrinone A (**25**).[29] Compounds **1**–**7** were identified as new pyrrospirone alkaloids, named as pyrrospirones C–I, respectively. The detailed structural elucidations of these new compounds are described as the following.

Compounds **1** and **2** have the same molecular formula of $C_{33}H_{43}NO_5$ deduced from their HRESIMS and ^{13}C NMR data. The UV spectra of **1** and **2** showed similar absorption at around 278 nm for a phenyl group. Two exchangeable protons (δ 6.49 or 6.60 for a hydroxyl group and δ 9.72 or 9.73 for an amide group) were observed in the ^1H NMR spectra of **1** and **2**. Detailed ^1H, ^{13}C, and HSQC NMR spectroscopical interpretation indicated that both **1** and **2** have two carbonyls, six aromatic carbons, two olefin carbons, four quaternary carbons, two oxymethines, one methoxyl, six methines, five methylenes, and five methyls (Tables 1 and 2). The ^1H NMR spectra of the benzene rings in **1** and **2** did not display a simple A_2B_2 type coupling pattern but rather complex splitting patterns due to the inequality of H-23/H-27 and H-24/H-26 (Table 2). Further NMR analyses including HMBC correlations (Fig. 2) revealed that **1** and **2** have a spiro ring system and are analogues of pyrrospirones A (**1a**) and B (**2a**)[30] with a methyl at C-12 and a methoxyl at C-19.

The relative stereochemistry of **1** was confirmed by a combination of NOE correlations and coupling constants.[30] The β-orientation of H-5, H-7, H-9, H-13, H-17, and H_3-32 was indicated by NOE correlations: βH-17 (δ 5.26) with H-13 (δ 3.88), H-16b (δ 2.66), and H_3-32 (δ 1.83); H-7 (δ 2.25) with H-5 (δ 1.13), H-9 (δ 4.86), H-13, and H_3-32; H-5 with H-9; H-9 with H-13; H-13 with H_3-32 (Fig. 3). NOE correlations of OH-17 (δ 6.49) with H-16a (δ 1.86), H_3-30 (δ 1.36) with αH-1(δ 1.90), H-2 (δ 1.78), H-4 (δ 2.02), and H-8 (δ 3.53) suggested a α-orientation for these protons. The large coupling constants of $^3J_{4,5}$ (11.2 Hz) and $^3J_{7,8}$ (13.6 Hz), together with above the NOE information, supported the *trans*-juncture for A/B and B/C rings. The NOESY spectrum of **1** also showed NOE correlations (Fig. 3) from H-21b (δ 3.29) to NH (δ 9.72) and H-27 (δ 7.33), from H-23 (δ 7.23) to H-20a (δ 3.13) and H-21a (δ 2.97), from H-24 (δ 7.11) to H-8, and from H-20b (δ 1.90) to H_3-33 (δ 3.36), confirming that the relative configuration of the spiro ring system is as shown.[30] Similarly, the relative stereochemistry of **2** was also established

Table 1
^{13}C NMR data of pyrrospirones C–I (**1**–**7**, 125 MHz, in pyridine-d_5).

C	1	2	3	4	3a	4a	5	6	7
1	49.3	48.9	49.4	49.0	49.2	48.8	48.7	49.2	49.0
2	28.5	28.5	28.5	28.5	28.5	28.5	28.5	28.6	28.6
3	46.1	46.1	46.1	46.1	46.0	46.1	46.1	46.2	46.2
4	27.9	28.0	27.9	27.9	27.9	28.0	28.0	28.1	28.0
5	61.6	60.8	61.7	60.7	61.5	60.7	61.0	61.3	61.2
6	42.3	42.4	42.3	42.3	42.3	42.4	42.3	42.4	42.5
7	53.4	52.3	53.4	52.4	53.1	52.1	52.0	52.9	52.6
8	46.5	48.5	46.4	48.5	46.0	48.2	45.4	47.0	46.7
9	86.6	89.9	86.7	90.0	86.5	89.8	86.8	88.3	88.3
10	138.8	136.7	138.8	136.7	140.2	138.1	141.6	138.4	138.5
11	126.8	132.3	126.8	132.3	125.6	130.1	126.9	133.1	129.5
12	51.6	48.2	51.5	48.0	49.9	46.5	60.6	46.4	51.4
13	51.7	51.6	51.9	51.7	51.7	51.8	51.9	52.8	51.1
14	201.2	200.1	201.3	200.2	200.1	199.5	202.1	202.6	200.2
15	59.9	59.5	60.4	60.0	59.7	59.7	59.7	60.4	62.2
16	49.9	46.3	50.5	47.0	47.7	42.2	56.5	36.6	132.4
17	71.6	70.8	71.7	70.8	74.9	73.1	208.9	41.6	140.7
18	176.8	177.9	176.2	177.2	175.5	176.5	175.8	176.7	174.0
19	93.2	93.6	88.5	88.7	88.6	88.8	88.9	88.5	88.6
20	36.4	38.8	43.9	46.3	43.5	46.0	42.4	45.1	43.4
21	46.0	46.0	47.7	47.6	45.1	47.7	47.4	47.9	47.8
22	128.8	128.6	129.6	129.4	129.6	129.4	129.6	129.7	129.5
23	133.4	133.4	133.3	133.2	133.3	133.3	133.1	133.3	133.3
24	124.9	124.8	124.9	124.7	124.9	124.8	124.6	124.8	124.8
25	158.9	160.0	158.8	159.8	158.8	159.8	158.8	159.2	159.2
26	120.3	118.6	120.4	118.7	120.4	118.6	120.1	119.8	119.3
27	133.7	135.0	133.7	134.9	133.9	135.1	133.7	134.3	134.3
28	23.4	23.3	23.3	23.2	23.4	23.3	23.3	23.4	23.3
29	20.3	20.3	20.3	20.2	20.3	20.2	20.4	20.3	20.4
30	17.1	16.7	17.1	16.6	17.0	16.6	17.0	16.9	16.9
31	21.5	21.5	21.4	21.3	21.3	21.5	21.0	21.1	21.1
32	27.4	21.0	27.3	20.7	26.6	21.2	23.6	28.0	28.6
33	49.4	49.4	–	–	–	–	–	–	–
OAc-17	–	–	–	–	170.6	170.6	–	–	–
	_	–	–	–	21.3	21.3	–	–	–