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NOTE

Coccoquinones A and B, new anthraquinone derivatives produced by *Staphylotrichum coccosporum* PF1460

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Activity of p53, a known tumor suppressor gene, is regulated by Mdm2, an E3 ubiquitin ligase.[1] Mdm2 binds to the N-terminal transactivation domain of p53, and then Mdm2 mediates the ubiquitination of p53. Finally, ubiquitinated p53 is degraded by the proteasome. Inhibitors of the p53–Mdm2 interaction suppress p53 degradation and potentiate the transcription of p53. Furthermore, these inhibitors induce p53-dependent cell death and suppress osteosarcoma tumor growth in a xenograft model.[2–6] Therefore, inducers of p53-dependent cell death may provide a promising strategy for anticancer therapies.

To discover new compounds that induce p53-dependent cell death, we screened microbial metabolites using glioblastoma LNZTA3 cells in which the expression levels of wild-type p53 can be regulated by a tetracycline-inducible system.[7] In our previous report, we isolated five novel compounds that induce p53-dependent cell death, quinofuracins A–E, from fungus *Staphylotrichum boninense* PF1444.[8] Further investigation of microbial metabolites led to the discovery of new anthraquinones, named coccoquinones A (**1**) and B (**2**) from another fungus *Staphylotrichum coccosporum* PF1460 (Figures 1a and b). Here we describe the isolation, structural elucidation and biological properties of **1** and **2**, as well as their ability to suppress p53-dependent growth in LNZTA3 cells.

The fungal strain PF1460 was isolated from a soil sample collected in Ishigaki Island, Okinawa prefecture, Japan. This strain was deposited as NITE P-02037 at the NITE Patent Microorganisms Depository, Japan. The following media were used to identify strain PF1460: potato dextrose agar, 2% malt agar, oatmeal agar and corn meal agar. The formed colonies were observed during the 1- to 2-week incubation period at 25 °C. The 28S rRNA-D1/D2 and ITS-5.8S rRNA sequences of strain PF1460 were identical to those of *Staphylotrichum coccosporum* NBRC33272 (100%). Therefore, this strain was identified as *Staphylotrichum coccosporum* PF1460. The sequence data of *S. coccosporum* PF1460 were deposited in GenBank as LC061579 and LC061580.

The strain *S. coccosporum* PF1460 was grown in 100-ml Erlenmeyer flasks, each containing 20 ml of a seed medium comprising 2% soluble starch, 1% glucose, 0.5% polypeptone, 0.6% wheat germ, 0.3% yeast extract, 0.2% soybean meal and 0.2% $CaCO_3$ in deionized water adjusted to pH 7.0 with NaOH solution before sterilization. The flasks were incubated at 25 °C for 4 days on a rotary shaker at 220 r.p.m. Aliquots of 3 ml of this seed culture were inoculated by spot inoculation into 500-ml Erlenmeyer flasks each containing a solid production medium, which consisted of 2 g of oatmeal and 80 g of water-absorbed brown rice. The flasks were gently shaken and incubated statically at 25 °C for 14 days.

Four-hundred-gram grown solid medium of *S. coccosporum* PF1460 was extracted with 800 ml of 67% aqueous acetone. The filtrate of the extracts was concentrated under reduced pressure, and the remaining residue was partitioned between EtOAc (400 ml) and H_2O (400 ml). The organic layer was concentrated under reduced pressure, and the remaining residue was chromatographed using a silica gel column (Merck, Darmstadt, Germany) with a $CHCl_3$–MeOH solvent system. The active fraction 1 (eluent $CHCl_3$–MeOH = 25:1) was purified by reverse-phase HPLC (CapcellPak UG120, ϕ 20 mm × 250 mm, Shiseido, Tokyo, Japan; 40% aqueous CH_3CN containing 0.1% AcOH) to give **1** (6.3 mg). The active fraction 2 (eluent $CHCl_3$–MeOH = 10:1) was purified using same method as **1** to give **2** (1.5 mg).

Compound **1** was isolated as a red amorphous solid. The UV spectrum of **1** exhibited absorption maxima at 223, 265, 293, 316 and 455 nm in MeOH, closely resembling that of quinofuracins[8] and averantin.[9] The IR spectrum of **1** showed absorption maxima at 3200, 2920, 1710, 1620, 1440, 1400, 1320, 1260, 1170, 1095, 1030 and 770 cm^{-1}. The molecular formula of **1** was determined to be $C_{22}H_{20}O_9$ with high resolution ESI MS (HRESI-MS); (found, *m/z* 429.1182 $(M+H)^+$, calcd for $C_{22}H_{21}O_9$, 429.1180). The ^1H and ^{13}C NMR data for **1** are shown in Table 1. Analysis of ^{13}C NMR and DEPT, along with HMQC spectra, revealed the presence of 22

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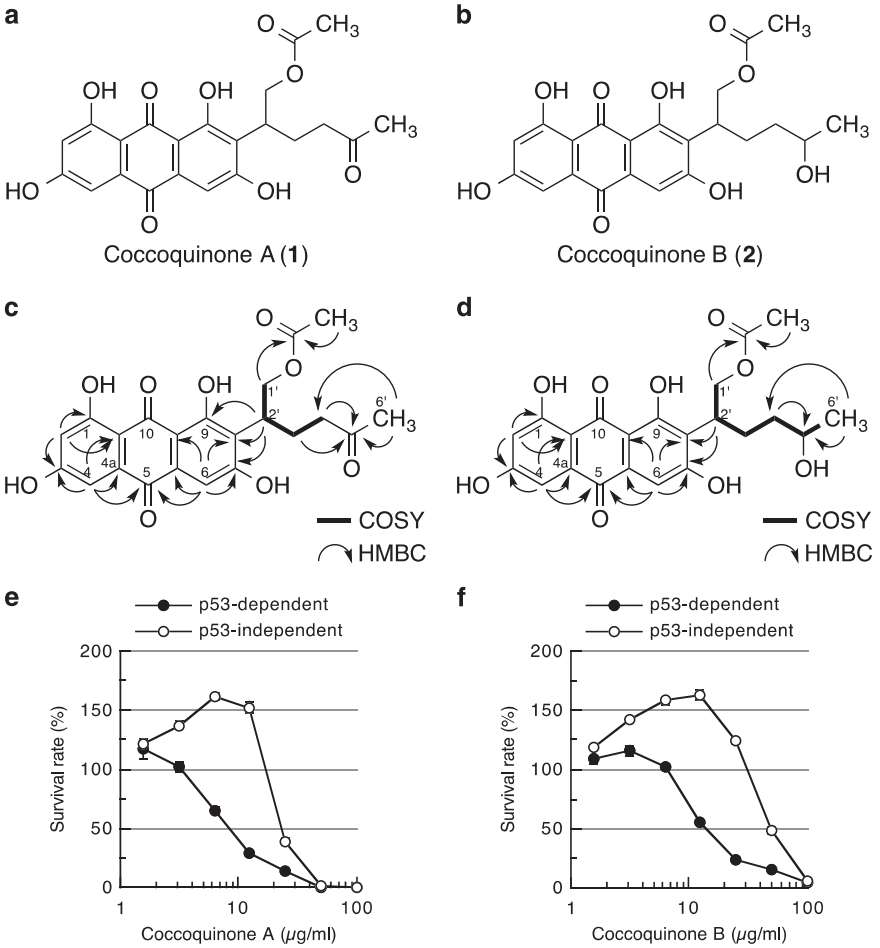

Figure 1 Structures and biological activities of coccoquinones A (**1**) and B (**2**). (**a**) Structure of **1**. (**b**) Structure of **2**. (**c**) Key correlations of **1** obtained by ^{1}H-^{1}H COSY and HMBC spectroscopy. (**d**) Key correlations of **2** obtained by ^{1}H-^{1}H COSY and HMBC spectroscopy. (**e**) p53-dependent growth suppression of **1**. (**f**) p53-dependent growth suppression of **2**.

carbons in **1**, which were categorized as 2 methyl, 2 methylene, 1 oxymethylene, 1 sp^3 methine, 3 sp^2 methine, 9 sp^2 quaternary, and 4 carbonyl carbons. The structure of **1** was elucidated primarily using 2D NMR experiments (^{1}H-^{1}H COSY and HMBC) as illustrated in Figure 1c. In the HMBC spectrum, correlations between aromatic protons at H-2, H-4, H-6 and a methine proton at H-2′ and relevant carbons in the anthraquinone skeleton suggested that **1** was a penta-substituted anthraquinone moiety. In the ^{1}H-^{1}H COSY spectrum, a spin network was observed from the oxymethylene protons at H-1′ to the methylene protons at H-4′ through one methine proton at H-2′ and one methylene proton at H-3′. In the HMBC spectrum, methyl protons at H-6′ correlated with the methylene carbon at C-4′ and the carbonyl carbon at C-5′, and two methylene protons at H-3′ and H-4′ correlated with the carbonyl carbon at C-5′. Furthermore, acetyl methyl protons (δ_H 1.99) correlated with the acetyl carbonyl carbon (δ_C 173.1), and the oxymethylene protons at H-1′ correlated with the acetyl carbonyl carbon, suggesting that an acetyl group bound to the 1′-oxygen. On the basis of these findings, the structure of **1** was closely related to versicolorone[10] and paeciloquinones,[11] and was determined to be 1′-*O*-acetylversicolorone (Figure 1a).

Compound **2** was isolated as a red amorphous solid. The UV spectrum of **2** exhibited absorption maxima at 224, 263, 294, 314 and 469 nm in MeOH. The IR spectrum of **2** showed absorption maxima at 3200, 2920, 1715, 1625, 1415, 1400, 1310, 1260, 1170, 1125, 1030 and 770 cm^{-1}. The ^{1}H and ^{13}C NMR data for **2** are shown in Table 1. These spectroscopic data of **2** were similar to those of **1**. The molecular formula of **2** was determined to be $C_{22}H_{22}O_9$ by HRESI-MS (found *m/z* 431.1338 $(M+H)^+$, calcd for $C_{22}H_{23}O_9$, 431.1337), indicating that **2** had two additional hydrogen atoms in comparison with **1**. The absence of a carbonyl carbon signal (δ_C 211.7) and the presence of an oxymethine signal (δ_C 68.3, δ_H 3.73) were observed in the NMR spectra of **2**, suggesting reduction of a carbonyl carbon at C-5′. Indeed, a spin network was observed from the oxymethylene protons at H-1′ to the methyl protons at H-6′ in the ^{1}H-^{1}H COSY spectrum (Figure 1d). Furthermore, in the HMBC spectrum, methyl protons at H-6′ correlated with the methylene carbon at C-4′ and oxymethine at C-5′, and the methylene protons at H-4′ correlated with the oxymethine carbon at C-5′. Thus, the structure of **2** was determined to be a dihydro derivative of **1** (Figure 1b).

The p53-dependent growth suppression of **1** and **2** was determined using human glioblastoma LNZTA3 cells. These cells contain a wild-type p53-encoding sequence controlled by tetracycline. Wild-type p53 is produced in the absence of tetracycline, whereas, p53 is not produced in the presence of tetracycline. LNZTA3 cells were treated with **1** or **2** in the presence or absence of tetracycline for 72 h, and then the cell viability was determined by MTT assay (Figures 1e and f). Compounds **1** and **2** showed preferential cytotoxicity to LNZTA3 cells expressing p53 compared with LNZTA3 cells not expressing p53,

Table 1 ^{13}C (150 MHz) and ^{1}H (600 MHz) NMR spectroscopic data for **1** and **2** in CD_3OD

	1		*2*	
Position	δ_C *(multiplicity)*	δ_H *(multiplicity, J in Hz)*	δ_C *(multiplicity)*	δ_H *(multiplicity, J in Hz)*
1	166.3 (s)		166.4 (s)	
2	109.3 (d)	6.49 (d, 2.4, 1H)	109.2 (d)	6.50 (d, 2.4, 1H)
3	166.8 (s)		168.1 (s)	
4	110.0 (d)	7.08 (d, 2.4, 1H)	110.7 (d)	7.14 (d, 2.4, 1H)
4a	136.6 (s)		136.6 (s)	
5	183.0 (s)		183.4 (s)	
5a	134.7 (s)		134.5 (s)	
6	109.1 (d)	7.18 (s, 1H)	109.4 (d)	7.21 (s, 1H)
7	164.9 (s)		165.1 (s)	
8	121.5 (s)		122.3 (s)	
9	164.8 (s)		164.8 (s)	
9a	110.2 (s)		110.0 (s)	
10	191.0 (s)		190.7 (s)	
10a	110.3 (s)		109.8 (s)	
1'	67.2 (t)	4.44 (dd. 8.0, 10.6, 1H)	67.4 (t)	4.51 (dd. 7.2, 10.3, 1H)
		4.52 (dd. 7.4, 10.6, 1H)		4.46 (dd. 7.9, 10.3, 1H)
2'	35.7 (d)	3.71 (m, 1H)	36.0 (d)	3.77 (m, 1H)
3'	24.4 (t)	2.05 (m, 1H)	26.4 (t)	2.10 (m, 1H)
		2.18 (m, 1H)		1.77 (m, 1H)
4'	42.3 (t)	2.40 (m, 1H)	38.1 (t)	1.37 (m, 2H)
		2.48 (m, 1H)		
5'	211.7 (s)		68.3 (d)	3.73 (m, 1H)
6'	29.9 (q)	2.08 (s, 3H)	23.4 (q)	1.10 (d, 6.2, 3H)
1'-*CO*CH_3	173.1 (s)		173.1 (s)	
1'-CO*CH_3*	20.9 (q)	1.99 (s, 3H)	20.8 (q)	1.96 (s, 3H)

Chemical shifts are reported in p.p.m. with TMS as an internal standard.

indicating p53-dependent growth suppression in LNZTA3 cells. Further studies of the biological activities of **1** and **2**, and the molecular mechanisms of p53 dependency are currently underway.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Meek, D. W. Tumour suppression by p53: a role for the DNA damage response? *Nat. Rev. Cancer* **9**, 714–723 (2009).
2 Vassilev, L. T. *et al. In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. *Science* **303**, 844–848 (2004).
3 Ding, Q. *et al.* Discovery of RG7388, a potent and selective p53-MDM2 inhibitor in clinical development. *J. Med. Chem.* **56**, 5979–5983 (2013).
4 Tovar, C. *et al.* MDM2 small-molecule antagonist RG7112 activates p53 signaling and regresses human tumors in preclinical cancer models. *Cancer Res.* **73**, 2587–2597 (2013).
5 Rew, Y. *et al.* Structure-based design of novel inhibitors of the MDM2-p53 interaction. *J. Med. Chem.* **55**, 4936–4954 (2012).
6 Yu, S. *et al.* Potent and orally active small-molecule inhibitors of the MDM2-p53 interaction. *J. Med. Chem.* **52**, 7970–7973 (2009).
7 Van Meir, E. G. *et al.* Release of an inhibitor of angiogenesis upon induction of wild type p53 expression in glioblastoma cells. *Nat. Genet.* **8**, 171–176 (1994).
8 Tatsuda, D. *et al.* Quinofuracins A-E, produced by the fungus *Staphylotrichum boninense* PF1444, show p53-dependent growth suppression. *J. Nat. Prod.* **78**, 188–195 (2015).
9 Birkinshaw, J. H., Roberts, J. C. & Roffey, P. Studies in mycological chemistry. Part XIX. 'Product B' (averantin) [1,3,6,8-tetrahydroxy-2-(1-hydroxyhexyl)anthraquinone], a pigment from *Aspergillus versicolor* (vuillemin) tiraboschi. *J. Chem. Soc. Perkin 1* **9**, 855–857 (1966).
10 Yabe, K. *et al.* Enzymatic conversion of averufin to hydroxyversicolorone and elucidation of a novel metabolic grid involved in aflatoxin biosynthesis. *Appl. Environ. Microbiol.* **69**, 66–73 (2003).
11 Fredenhagen, A., Hug, P., Sauter, H. & Peter, H. H. Paeciloquinones A, B, C, D, E and F: new potent inhibitors of protein tyrosine kinase produced by *Paecilomyces carneus*. II. Characterization and structure determination. *J. Antibiot. (Tokyo)* **48**, 199–204 (1995).