NOTE

Curromycin A as a GRP78 downregulator and a new cyclic dipeptide from *Streptomyces* sp.

Yoichi Hayakawa¹, Minami Akimoto¹, Akari Ishikawa¹, Masumi Izawa¹ and Kazuo Shin-ya²

The Journal of Antibiotics (2016) 69, 187–188; doi:10.1038/ja.2015.115; published online 25 November 2015

Solid cancers are often nutrient-starved because of poor blood circulation and such tumor cells show remarkable tolerance to nutrient deprivation.¹ Thus, antitumor substances targeting resistance to nutrient deprivation may show selective activity against solid cancer cells. In the course of a screening for selective cytotoxic compounds under nutrient-deprived conditions, an actinomycete strain RAI364 was found to produce an active substance. Strain RAI364 was identified as a member of the genus *Streptomyces* based on the 16S rRNA sequence,² which revealed high identity with those of *Streptomyces cuspidosporus* NBRC 12378 (99.3%), *Streptomyces cuspidosporus* NBRC 12379 (99.3%) and *Streptomyces ribosidificus* NBRC 13796 (99.3%).

The producing organism was cultivated in 500-ml Erlenmeyer flasks containing 100 ml of a medium consisting of 2.5% glucose, 1.5% soybean meal (Nisshin Oillio Group, Tokyo, Japan), 0.2% dry yeast (Asahi Food & Healthcare, Tokyo, Japan) and 0.4% calcium carbonate (pH 6.2, before autoclave) on a rotary shaker at 27 °C for 4 days. The culture broth (2 l) was centrifuged and the supernatant was extracted with ethyl acetate. The extract was fractionated on a silica gel column using stepwise elution with chloroform-methanol. The active material eluted with chloroform-methanol (20:1) was subjected to preparative silica gel TLC with chloroform-methanol (10:1). The active fraction was concentrated to dryness to give a colorless powder of **1** (45.4 mg). From the ¹H and ¹³C NMR spectra and the specific rotation ($[\alpha]_D^{22} +23.4$ (c 0.567, MeOH) and $[\alpha]_D^{22} +39.0$ (c 0.115, MeOH) in literature³), **1** was identified as curromycin A^{3,4} (Figure 1). The chloroform-methanol (100:1) fraction on the silica gel column contained an inactive metabolite **2**. A colorless oil of **2** (12.8 mg) was obtained by preparative silica gel TLC with chloroform-methanol (10:1).

The physicochemical properties of **2** are summarized as follows: $[\alpha]_D^{19} -13.9$ (c 0.642, MeOH); high-resolution FAB-MS *m/z* 199.1082 ($[M+H]^+$, calcd. for C₉H₁₅N₂O₃, 199.1083); UV (MeOH) $\lambda_{\max}$ nm (ϵ) 219 (3400); IR (ATR) $\nu_{\max}$ 3207, 1690 cm⁻¹.

The molecular formula of **2** was established as C₉H₁₄N₂O₃ by high-resolution FAB-MS. ¹³C and ¹H NMR data for **2** are summarized in Table 1. The HMQC⁵ spectrum revealed three methyls, including a

methoxy and an *N*-methyl, an olefinic methylene, two methines and three quaternary sp² carbons. Two amide carbonyl groups were identified based on their chemical shifts (δ 165.8, 159.5) and the molecular formula. A COSY experiment displayed a spin network from H-6 to H-10 (Figure 2). This substructure was expanded to include the amide carbonyl (C-5) due to long-range correlations from 6-H and 9-H to C-5 in the HMBC⁶ spectrum (Figure 2). ¹H–¹³C long-range couplings from the methoxy to C-9 and from the *N*-methyl to C-2 and C-6 established their location and an amide bond between C-2 and C-6. The olefinic methylene protons (8-H₂) exhibited long-range correlations to C-2, indicating the presence of an acrylamide moiety. Finally, couplings from an amide proton (4-H) to C-2 and C-6 required a cyclic dipeptide structure consisting of dehydroalanine and *N,O*-dimethylthreonine (or its stereoisomer) residues.

A small coupling constant (4.0 Hz) between 6-H and 9-H arranged them in gauche conformations. The structure of **2**, including the relative stereochemistry, was determined to be cyclo(dehydroalanyl-*N,O*-dimethylthreonyl) by a NOESY experiment, which revealed NOEs between 11-H₃ and 7-H₃, between 7-H₃ and 10-H₃, and between 10-H₃ and 6-H, as shown in Figure 2.

The cytotoxic activity was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method using MKN45 human gastric cancer cells¹ treated with **1** or **2** for 48 h. MTT formazan formed in the cells was dissolved in dimethyl sulfoxide (DMSO) and measured as absorbance at 540 nm. Curromycin A (**1**) showed potent cytotoxicity against MKN45 cells in a nutrient-deprived medium (Earle's salts solution supplemented with 10% fetal bovine serum), with an IC₅₀ of 15 ng ml⁻¹. The growth of MKN45 cells in a normal medium (Dulbecco's modified Eagle's medium with 10% fetal bovine serum) was inhibited ~50% by **1** at the range of 20–20 000 ng ml⁻¹, although no cell death was observed. Anticancer agents such as doxorubicin displayed reverse selectivity in this assay. The IC₅₀ values of doxorubicin were 1.1 µg ml⁻¹ in a normal medium and >20 µg ml⁻¹ in a nutrient-deprived medium. No activity was observed on MKN45 cells treated with **2** at <10 µg ml⁻¹.

¹Department of Medicinal and Life Science, Faculty of Pharmaceutical Sciences, Tokyo University of Science, Chiba, Japan and ²Biological Information Research Center, National Institute of Advanced Industrial Science and Technology, Tokyo, Japan
Correspondence: Professor Y Hayakawa, Department of Medicinal and Life Science, Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan.
E-mail: hykw@rs.noda.tus.ac.jp

Received 11 July 2015; revised 18 September 2015; accepted 9 October 2015; published online 25 November 2015

Figure 1 Structures of metabolites from *Streptomyces* sp. RAI364.

Table 1 ^{13}C and ^1H NMR data for **2** in CDCl_3

No.	δC	δH ($J = \text{Hz}$)
2	159.5 s	
3	133.6 s	
4		8.88 s
5	165.8 s	
6	68.0 d	3.88 d (4.0)
7	36.4 q	3.09 3H s
8	102.4 t	5.57 s, 4.84 s
9	79.0 d	3.69 dq (4.0, 6.5)
10	16.6 q	1.25 3H d (6.5)
11	57.1 q	3.31 3H s

Figure 2 NMR analysis of **2**.

The molecular chaperone GRP78 is involved in the resistance to nutrient-deprivation stress.^{7–9} The effect of **1** on GRP78 gene expression was investigated by the luciferase reporter assay using HT1080 human fibrosarcoma cells transformed with the luciferase gene under the control of the GRP78 promoter (HT1080 G-L).¹⁰ Curromycin A (**1**) dose-dependently inhibited the luciferase expression in the presence of 10 mM of 2-deoxyglucose with an IC_{50} of 4.3 ng ml^{-1} (Figure 3). The antibacterial and cytotoxic activities of **1** have been reported,^{3,4} although the mechanisms of action are unknown. Further biological studies are now under way.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in-Aid for Challenging Exploratory Research (25560407), Japan Society for the Promotion of

Figure 3 Effect of curromycin A (**1**) on the luciferase expression in HT1080 G-L cells. HT1080 G-L cells were treated with **1** in the presence (○) or absence (●) of 2-deoxyglucose (10 mM) for 18 h at 37 °C. The relative luciferase activity compared with non-treated control was measured with a luminometer.

Science (JSPS). We thank F Hasegawa, Tokyo University of Science, for assistance with MS.

- Izuishi, K., Kato, K., Ogura, T., Kinoshita, T. & Esumi, H. Remarkable tolerance of tumor cells to nutrient deprivation: possible new biochemical target for cancer therapy. *Cancer Res.* **60**, 6201–6207 (2000).
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **173**, 697–703 (1991).
- Ogura, M. *et al.* Structure of a new antibiotic curromycin A produced by a genetically modified strain of *Streptomyces hygroscopicus*, a polyether antibiotic producing organism. *J. Antibiot.* **38**, 669–673 (1985).
- Ikeda, Y. *et al.* New triene- β -lactone antibiotics, triedimycins A and B. *J. Antibiot.* **44**, 453–455 (1991).
- Summers, M. F., Marzilli, L. G. & Bax, A. Complete ^1H and ^{13}C assignments of coenzyme B₁₂ through the use of new two-dimensional NMR experiments. *J. Am. Chem. Soc.* **108**, 4285–4294 (1986).
- Bax, A. & Summers, M. F. ^1H and ^{13}C assignments from sensitivity enhanced detection of heteronuclear multiple-bond connectivity by multiple quantum NMR. *J. Am. Chem. Soc.* **108**, 2093–2094 (1986).
- Munro, S. & Pelham, H. R. An Hsp70-like protein in the ER: identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein. *Cell* **46**, 291–300 (1986).
- Fernandez, P. M. *et al.* Overexpression of the glucose-regulated stress gene GRP78 in malignant but not benign human breast lesions. *Breast Cancer Res. Treat.* **59**, 15–26 (2000).
- Yoshida, H., Haze, K., Yanagi, H., Yura, T. & Mori, K. Identification of the cis-acting endoplasmic reticulum stress response element responsible for transcriptional induction of mammalian glucose-regulated proteins. Involvement of basic leucine zipper transcription factors. *J. Biol. Chem.* **273**, 33741–33749 (1998); Erratum in: *J. Biol. Chem.* **274**, 2592 (1999).
- Park, H. R. *et al.* Effect on tumor cells of blocking survival response to glucose deprivation. *J. Natl Cancer Inst.* **96**, 1300–1310 (2004).