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NOTE

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New Naphthoquinones, f13102A and B, from a Fungus Strain F-13102

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Abstract Deficiency of Fas-mediated apoptosis is one of the mechanisms involved in the immune evasion by tumors. Thus, it might be a practical approach for cancer treatment that Fas-mediated apoptosis in tumor cells is modified by drugs. In the course of screening, we have isolated two new naphthoquinones, f13102A and B, from the culture broth of fungus strain F-13102. Coumpound f13102A sensitizes Fas-resistant human lung adenocarcinoma A549 cells to apoptosis.

Keywords chemotherapy, tumor immunity, naphthoquinone, Fas, CD95

Fas (CD95/APO-1), a member of the TNF receptor family, is a death receptor that induces apoptosis upon ligation with its intrinsic ligand, Fas ligand (FasL). Fas-mediated apoptosis plays important roles in the immune system, including an elimination of tumor cells by cytotoxic T lymphocytes [1～4]. Fas expression is in fact shown as a significant prognostic factor in the human malignancies [5～7]. In addition, we have recently demonstrated that the Fas-mediated apoptosis of tumor cells and host immune defense are involved in an antitumor effect of drugs [8]. Thus, it might be a practical approach for cancer treatment that Fas-mediated apoptosis in tumor cells is modified by drugs. In the course of screening, we have found that a culture broth of fungus strain F-13102 sensitizes Fas-resistant human lung adenocarcinoma A549 cells [9] to apoptosis. We isolated two new compounds, f13102A (**1**) and B (**2**), from the culture broth. In this paper, we describe the fermentation, isolation, physico-chemical properties, structure elucidation, and biological activities of **1** and **2**.

Fungus strain F-13102 was isolated from a soil sample collected in Hahajima, Ogasawara Islands, Tokyo Prefecture, Japan. A slant culture of the strain F-13102 was inoculated into 100 ml of a seed culture medium containing glucose 1%, potato starch 2%, soybean meal 2%, KH_2PO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.05%, and five glass beads, and cultured at 25°C for 3 days on a rotary shaker (225 rpm). For production of **1** and **2**, the seed culture (5 ml) was inoculated into a 500-ml flask containing 100 ml of a culture medium containing glycerin 5%, potato extract 25%, malt extract 0.5%, and yeast extract 0.5%, and cultured at 25°C for 4 days on a rotary shaker (225 rpm).

The cultured broth (10.0 liter) was extracted with an equal volume of EtOAc and filtered. The organic layer was concentrated to give a brown oily material (7.04 g). The material was dissolved in a small volume of 90% MeOH and washed with *n*-hexane. The residue was concentrated *in vacuo* to give a brown oily material (1.33 g). The oily material was applied on a silica gel column and the active substances were eluted with $CHCl_3$-MeOH (100 : 1). The active fractions were collected and concentrated to give a yellow oil (212.5 mg). The yellow oil was subjected to reversed phase HPLC (Inertsil ODS-3, GL Science) with 40% CH_3CN. The active fractions containing **1** were

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concentrated to give 23.6 mg of a yellow powder and fractions containing **2** were concentrated to give 86.2 mg of a yellow powder.

The physico-chemical properties of **1**: mp 194～197°C; UV λ_{max} nm (ε) 220 (27,300), 247 (11,000), 294 (11,000), 428 (4,600) in MeOH, 218 (24,400), 247 (10,100), 296 (10,300), 433 (4,600) in 0.01 N HCl - 90% MeOH, 221 (24,400), 250 (10,300), 298 (10,100), 429 (4,300) in 0.01 N NaOH - 90% MeOH, 225 (20,600), 294 (9,000), 430 (2,300), 535 (2,800) in 0.1 N NaOH - 90% MeOH; retention time on HPLC, 5.87 minutes (Inertsil ODS-3, 4.6 i.d.×150 mm, 1 ml/minute, 40% CH_3CN). HRESI-MS; *m/z* found 248.0675 $(M)^+$, calcd for $C_{13}H_{12}O_5$ 248.0685.

The physico-chemical properties of **2**: mp 141～144°C; UV λ_{max} nm (ε) 220 (31,200), 248 (11,100), 292 (11,600), 428 (4,800) in MeOH, 220 (28,200), 250 (10,600), 293 (10,800), 428 (4,800) in 0.01 N HCl - 90% MeOH, 220 (28,000), 250 (10,600), 294 (10,900), 428 (4,500) in 0.01 N NaOH - 90% MeOH, 225 (27,100), 290 (10,000), 524 (3,800) in 0.1 N NaOH - 90% MeOH; retention time on HPLC, 2.61 minutes (Inertsil ODS-3, 4.6 i.d.×150 mm, 1 ml/minute, 40% CH_3CN). HRESI-MS; *m/z* found 287.0528 $(M+Na)^+$, calcd. for $C_{13}H_{12}Na_1O_6$ 287.0532.

The UV absorpotion maxima of **1** and **2** are closely resemble to that of misakimycin [10] indicating the presence of naphthoquinone moieties in **1** and **2**. The HRESI-MS spectrum of **1** led to the molecular formula $C_{13}H_{12}O_5$.

The ^{13}C NMR, DEPT, and HMQC spectra of **1** revealed the presence of thirteen carbon signals consisting of two methyls, one methylene, two methines, and eight quaternary carbons. The ^1H NMR spectrum of **1** indicated a chelated OH group at δ_H 12.6. The aromatic region of the spectrum indicated two singlets at δ_H 6.1 and 7.8 suggesting the presence of a tetra-substituted naphthoquinone. The aliphatic region of the spectrum indicated singlets at δ_H 2.2, 3.9 and 4.8 due to naphthoquinone-bound methyl, methoxy and hydroxymethyl protons, respectively. The conectivity of these functional groups and naphthoquinone ring was elucidated by HMBC experiments as shown in Fig. 1. The observed correlations were as follows; from 2-OCH_3 to C-2, from 3-H to C-1, C-2, and C-4a, from 5-OH to C-4a, C-5, and C-6, from 6-CH_3 to C-5, C-6, and C-7, from 8-H to C-1, C-4a, C-5, C-7′, and from 7′-H_2 to C-8. Thus, the structure of **1** was concluded as 5-hydroxy-7-hydroxymethyl-2-methoxy-6-methyl-1,4-naphthoquinone.

The molecular formula of **2** was determined to be $C_{13}H_{12}O_6$ based on the HRESI-MS. The ^1H and ^{13}C NMR spectra of **1** and **2** were similar to each other. In DEPT spectra, one methyl carbon in **1** was replaced to one methylene carbon in **2**. Further analysis confirmed that 6-methyl in **1** was replaced to hydroxymethyl in **2** as shown in Fig. 1. The observed correlations in **2** were as follows; from 2-OCH_3 to C-2, from 3-H to C-1, C-2, C-4, and C-4a, from 8-H to C-1, C-4a, C-6, and C-7′, from 7′-H_2 to C-6, C-7, and C-8, and from 6′-H_2 to C-5, C-6, and C-7. Thus, the structure of **2** was concluded as 6,7-dihydroxymethyl-5-hydroxy-2-methoxy-1,4-naphthoquinone. The ^1H and ^{13}C NMR assignments of **1** and **2** are summarized in Table 1.

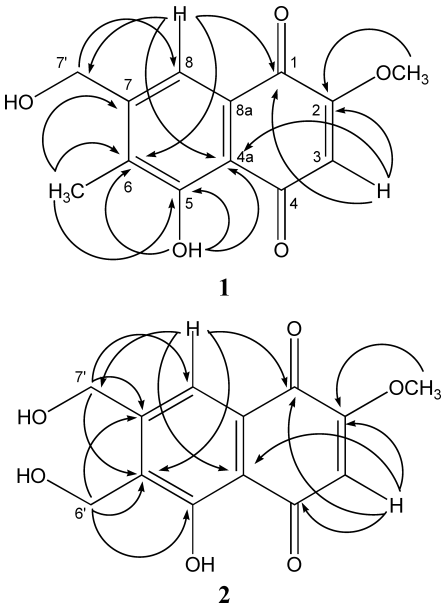

Fig. 1 Structures and ^{13}C-^1H-long range couplings of f13102A(**1**) and B(**2**).

The compound-induced sensitization to Fas-mediated apoptosis was assessed. A549 cells were seeded in 96 well culture plates and CH-11, an anti-Fas agonistic antibody [11], (100 ng/ml), and cycloheximide (1 μg/ml) were added. After 48 hours, the viable cell number was measured by MTT assay. As shown in Fig. 2, compound **1** dose-dependently sensitized cells to Fas-mediated apoptosis (IC_{50} 0.1 μg/ml). In the reference wells (cycloheximide only), antiproliferative effect of **1** was 10 times weaker (IC_{50} 1.0 μg/ml) than that in the presence of CH-11. Compound **1** did not inhibit incorporation of $[^3H]$-uridine and $[^3H]$-leucine at 1 μg/ml (data not shown), suggesting that inhibition of macromolecular synthesis was not involved in the mechanism of the sensitization of A549 cells to Fas-mediated apoptosis. The selective effect was not observed when **2** was used in this assay. Furthermore, other naphthoquinone analogs including 1,4-naphthoquinone, 5,8-dihydroxy-1,4-naphthoquinone, 5-hydroxy-1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone, and 5-hydroxy-2-methyl-1,4-

Table 1 ^{13}C and ^{1}H NMR data of **1** (in chloroform-d_1) and **2** (in DMSO-d_6)

Position	1		2	
	δ_C (ppm)	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)
1	179.7	—	179.0	—
2	161.2	—	161.2	—
2-OCH_3	56.6	3.9	56.9	3.9
3	109.3	6.1	109.5	6.4
4	190.9	—	191.0	—
4a	112.6	—	112.1	—
5	159.5	—	158.2	—
5-OH	—	12.6	—	12.6
6	133.1	—	133.1	—
6′	11.0	2.2	52.4	4.6
7	146.4	—	150.5	—
7′	62.8	4.8	59.8	4.8
8	118.0	7.8	116.6	7.8
8a	128.4	—	129.5	—

Chemical Shifts in ppm from TMS as an internal standard.

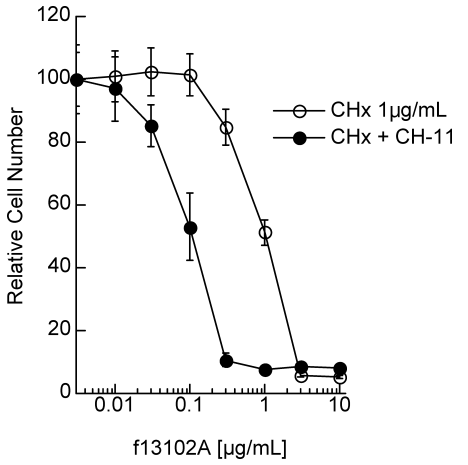

Fig. 2 Effect of f13102A on Fas-mediated apoptosis in A549 cells. Cells were treated with the indicated concentrations of f13102A in the presence of CH-11 plus cycloheximide (closed circle) or the presence of cycloheximide (open circle). After 48 hours, viable cell number was quantified by MTT assay. Values are means for triplicate determinations; bars, S.D.

naphthoquinone neither showed the selective effect. Therefore, compound **1** is considered to have a structurally unique activity.

These compounds showed weak antimicrobial activity. The MIC values were as follows: compound **1**: 4 μg/ml against *Staphylococcus aureus* FDA209P, 16 μg/ml against *S. aureus* MS16526 (MRSA), 4 μg/ml against *Bacillus subtilis* PCI219, 16 μg/ml against *Escherichia coli* NIHJ, 4 μg/ml against *E. coli* BE112i; compound **2**: 4 μg/ml against *S. aureus* FDA209P, 8 μg/ml against *S. aureus* MS16526 (MRSA), 8 μg/ml against *B. subtilis* PCI219, 8 μg/ml against *E. coli* NIHJ, 1 μg/ml against *E. coli* BE1121.

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