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NOTE

JBIR-120: a new growth inhibitor of hormone-refractory prostate cancer cells

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Prostate cancer is a common nondermatological cancer in older adult men. Androgen receptor (AR) signaling has a central role in prostate cancer cell growth and survival,[1] and therefore, androgen ablation therapy is recognized as a standard regimen for the treatment of advanced and metastatic prostate cancers.[2] However, most patients who undergo androgen ablation progress from being androgen-dependent to developing hormone-refractory prostate cancer within 2 years after initiating therapy. Although the recurrent tumors are often resistant to standard AR-targeting agents, which cause deprivation of androgens or block androgen–AR interaction, AR-mediated signaling still has a key role in the development and maintenance of hormone-refractory prostate cancers.[3–5] Thus, identifying new therapeutic agents targeting the AR signaling pathway may possibly control the occurrence of hormone-refractory prostate cancers. Mashima *et al.*[6] earlier reported that nigericin can block AR-mediated signaling in hormone-refractory prostate cancer cells. In the current study, we discovered a novel compound—JBIR-120 (**1**)—extracted from the culture broth of a new *Streptomyces* strain, RI104-LiC104. This paper describes the isolation, structure elucidation and biological activity of **1**.

Streptomyces strain RI104-LiC104 was isolated from a lichen obtained from the Rishiri Island, Hokkaido Prefecture, Japan, by using the moist incubation and desiccation method.[7] To identify the strain, the 16S rRNA gene sequence of RI104-LiC106 was determined (DDBJ accession number, AB693145) and compared with sequences in the Eztaxon-type strain database.[8] Sequence analysis showed that the strain suspected as a new species of the genus *Streptomyces*.

The strain was cultivated in 50-ml test tubes containing 15 ml of a seed medium consisting of 1.0% starch (Kosokagaku, Tokyo, Japan), 1.0% polypeptone (Nihon Pharmaceutical, Tokyo, Japan), 1.0% molasses (Dai-Nippon Meiji Sugar, Tokyo, Japan) and 1.0% meat extract (Extract Ehlrich; Wako Pure Chemical Industry, Osaka, Japan) at pH 7.2 (adjusted before sterilization). The test tubes were maintained on a reciprocal shaker (320 r.p.m.) at 27 °C for 2 days. Aliquots (2.5 ml each) of the broth were transferred to 500-ml baffled Erlenmeyer flasks containing 100 ml of a production medium consisting of 2.0% glycerol (Nacalai Tesque, Kyoto, Japan), 1.0% molasses (Dai-Nippon Meiji Sugar), 0.5% casein (Kanto Chemical, Tokyo, Japan), 0.1% polypeptone (Nihon Pharmaceutical) and 0.4% $CaCO_3$ (Kozaki Pharmaceutical, Tokyo, Japan) at pH 7.2 (adjusted before sterilization), and cultured on a rotary shaker (180 r.p.m.) at 27 °C for 5 days.

The fermentation broth (2 l) was separated by centrifugation. The supernatant was extracted using EtOAc (1.8 l × 3). The mycelial cake was extracted using acetone (600 ml) and filtered, and the filtrate was concentrated *in vacuo*. The residual aqueous concentrate (80 ml) was partitioned between EtOAc and H_2O (equal volume × 3). After drying with Na_2SO_4, both EtOAc layers were combined and evaporated *in vacuo*. The residue (808 mg) was subjected to normal-phase medium-pressure liquid chromatography (Purif-Pack SI-30; Shoko Scientific, Yokohama, Japan) and successively developed using a gradient system of *n*-hexane–EtOAc (0–15% EtOAc), followed by the stepwise solvent system of $CHCl_3$–MeOH (0, 2, 5, 10, 20, 30 and 100% MeOH). The 5% MeOH-eluted fraction (27 mg) was applied to reversed-phase medium-pressure liquid chromatography (Purif-Pack ODS-30; Shoko Scientific) by using an H_2O–MeOH stepwise solvent system (30, 50, 60, 70, 80 and 90% MeOH). The 70% MeOH fraction was evaporated *in vacuo* to obtain **1** (2.8 mg) as a colorless amorphous product.

The molecular formula of **1** was determined as $C_{18}H_{18}N_2O_3$ through high-resolution ESI MS (*m/z* 309.1237 $[M–H]^-$; calcd.

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309.1239). The optical rotation was $[\alpha]_D^{25}-10.5$ (*c* 0.12, in MeOH), and the UV spectrum showed maximal absorptions at 249 (ε, 6400), 304 (ε, 3500) and 342 (ε, 3700) nm in MeOH. The IR absorptions (attenuated total reflectance) at 3470, 1714 and 1670 cm^{-1} showed the presence of hydroxy, carbonyl and imino groups, respectively. The planar structure was clarified by a series of 2D NMR analyses, including double quantum-filtered COSY, heteronuclear single-quantum coherence and constant-time HMBC[9] spectra. The ^{13}C and ^{1}H NMR data for **1** are listed in Table 1.

The sequence from an aromatic methine proton 3′-H (δ_H 6.31) to an *ortho*- and *meta*-coupled aromatic methine proton 6′-H (δ_H 7.93; $J = 1.8$, 8.4 Hz) through aromatic methine protons 4′-H (δ_H 7.14) and 5′-H (δ_H 6.54) observed in the double quantum-filtered COSY spectrum showed the presence of a 1,2-disubstituted benzene ring. Strong couplings from 4′-H and 6′-H to an aromatic quaternary carbon C-2′ (δ_C 150.5), and from 3′-H and 5′-H to another aromatic quaternary carbon C-1′(δ_C 111.0) established the assignments of the benzene ring moiety. Additional HMBC from 6′-H to a carbonyl carbon C-7′(δ_C 171.3) and the molecular formula of **1**(*vide infra*) revealed that a carboxylic acid functional group is substituted at position C-1′.

The sequence from terminal methyl protons 10-H_3 (δ_H 1.07) to a low-field-shifted sp^3 methine proton 8-H (δ_H 5.48) through the aliphatic methylene protons 9-H_2 (δ_H 1.91; 1.84) established a propyl unit. ^{1}H-^{13}C long-range couplings from 8-H to C-2′, together with the ^{13}C chemical shift value at C-8 (δ_C 51.7), proved that the propyl and the benzene ring moieties were joined by a nitrogen atom. Furthermore, HMBC correlations from 8-H to aromatic quaternary carbons C-7a (δ_C 140.3), C-7 (δ_C 139.0), and an aromatic methine carbon C-6 (δ_C 146.1), established the relationships of these carbons. Strong couplings from the aromatic methine protons 6-H (δ_H 8.58) and 4-H (δ_H 8.72) to the aromatic carbon C-7a elucidated their positions and assignments, as shown in Figure 1b.

A spin coupling between methylene protons 2-H_2 (δ_H 2.83, 2H) and 3-H_2 (δ_H 3.26, 2H) were also observed. On the basis of the HMBC correlations from 2-H and 3-H to an α,β-unsaturated ketone carbonyl carbon C-1(δ_C 208.5), aromatic quaternary carbons C-3a (δ_C 149.3) and C-7a were shown to consist of a 5-membered ring system. According to the molecular formula and unsaturated number, the sequence from C-3a to C-7a forms a pyridine moiety, whereas C-7′ consists of a carboxylic-acid functional group. These results indicated the presence of a 6,7-dihydro-5*H*-cyclopenta[*c*]pyridin-5-one moiety, with C-8 attached to C-7. By taking into consideration these results, the gross structure of **1** is shown in Figure 1. Compound **1** is the first example of a louisianin- and ulupyrinone-type compound containing 2-aminobenzoic acid.[10,11]

We evaluated the inhibitory effect of **1** on the growth of the AR-positive human prostate cancer cell line, 22Rv1,[12] which is known to be involved with AR signaling. Cell growth with or without androgen (dihydrotestosterone) was examined. The cells were treated with 0 and 80 μM of **1** in the presence or absence of 10 nM dihydrotestosterone for 5 days, and the cell growth was measured by a WST-8 colorimetric assay (Cell Counting Kit; Dojindo, Kumamoto, Japan). Compound **1** showed weak cytotoxicity against 22Rv1 cells ($IC_{50} = 150$ μM). Rather, **1** effectively suppressed the cell growth activated by dihydrotestosterone, which shows 2.7-fold rise compared with dihydrotestosterone-depleted condition (Figure 2).

Table 1 ^{13}C and ^{1}HNMR spectroscopic data for JBIR-120 (1)

Position	*^{13}C*	*^{1}H (J in Hz)*
1	208.5	
2	36.8	2.83, ddd (5.4, 6.0, 10.5)
3	23.7	3.26, dd (5.4, 6.0)
3a	149.3	
4	148.2	8.72, s
6	146.1	8.58, s
7	139.0	
7a	140.3	
8	51.7	5.48, dd (4.8, 7.2)
9	31.1	1.91, ddq (4.8, 7.8, 15.0); 1.84, ddq (7.2, 7.8, 15.0)
10	10.9	1.07, t (7.8)
1′	111.0	
2′	150.5	
3′	112.2	6.31,d (9.0)
4′	134.8	7.14,ddd (1.8, 9.0, 9.0)
5′	115.4	6.54, dd (8.4, 9.0)
6′	132.7	7.93, dd (1.8, 8.4)
7′	171.3	

^{13}C (150 MHz) and ^{1}H (600 MHz) NMR spectra were taken by 600 NB CL NMR System (Varian, Palo Alto, CA, USA) in $CHCl_3$-*d*/CH_3OH-d_4 (1:1), and the solvent peak was used as an internal standard (δ_C49.0, δ_H3.35 p.p.m.).

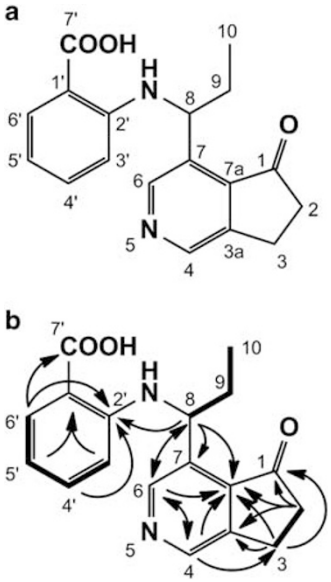

Figure 1 (**a**) Structure of JBIR-120 (**1**). (**b**) Key correlations of double quantum-filtered COSY (bold lines) and CT-HMBC (arrows) spectra of **1**.

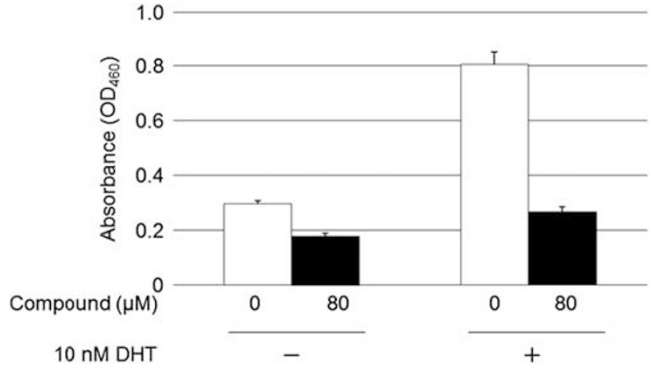

Figure 2 Inhibitory effect of **1** on the growth of AR-positive human prostate cancer cells activated by AR signaling (DHT: dihydrotestosterone).

These results indicate that **1** inhibits androgen-dependent growth of the prostate cancer cells. Louisianin A, which is the analog of **1**, has been reported to show growth inhibition in AR-positive SC 115 cells in the presence of testosterone.[13] The results of our study showed similar outcomes. Further detailed investigations on the biological activities of **1** are currently underway.

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