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Burkholone, A New Cytotoxic Antibiotic against IGF-I Dependent Cells from *Burkholderia* sp.

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Abstract In the course of our screening program for new inhibitors of IGF-I signaling, we isolated a new cytotoxic antibiotic, burkholone, from the culture broth of *Burkholderia* sp. QN15488. The structure of burkholone was determined to be (*E*)-3-methyl-2-(2-octenyl)-4-quinolone by a series of NMR analyses. Burkholone induced cell death 32D/GR15 cells with an IC_{50} value of 160 nM in IGF-I containing medium, while no cell death was observed in IL-3 containing medium even at the concentration of 37 μM.

Keywords burkholone, insulin-like growth factor, quinolone, cytotoxic antibiotic

Insulin-like growth factors (IGFs) play in part a key role in human cancer progression. IGF signals through IGF-I receptor are known to be significant for tumor cell growth and survival [1]. Thus, inhibitors of IGF signal transduction are expected to be promising drugs for cancer chemotherapy. To evaluate the anti-IGF activity, we employed IGF-I receptor harboring 32D/GR15 cells which were derived from hematopoietic cell line 32D cells by transformed with the IGF-I receptor [2]. Viability and proliferation of 32D/GR15 cells are strictly dependent on cytokines such as interleukin-3 (IL-3). The IL-3-dependent 32D/GR15 cells undergo rapid apoptosis in the absence of IL-3. However, 32D/GR15 cells completely survive in an IL-3-free medium containing IGF-I [2]. Using 32D/GR15 cells enables to distinguish the survival signaling between IL-3 and IGF-I [2]. In the course of our screening program for new inhibitors of IGF-I signaling, a new quinolone compound, (*E*)-3-methyl-2-(2-octenyl)-4-quinolone designated as burkholone (**1**, Fig. 1) was isolated from the culture broth of *Burkholderia* sp. QN15488.

In this paper, we report the production, isolation, physico-chemical properties, structure elucidation and brief biological properties of **1**.

Strain QN15488 was isolated from a soil sample

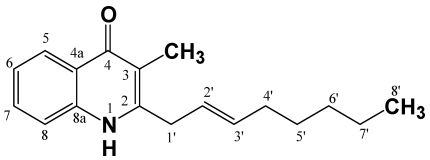

Fig. 1 Structure of burkholone (**1**).

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Table 1 Physico-chemical properties of burkholone (**1**)

Appearance	White powder
MP	218～220°C
Molecular formula	$C_{18}H_{23}NO$
HRFAB-MS (*m/z*)	
Found:	270.1866 $(M+H)^+$
Calcd.:	270.1858 $(M+H)^+$
UV λ_{max} nm (ε) in MeOH	335 (7,400), 322 (7,100), 240 (20,000), 212 (17,000)
IR ν_{max} (KBr) cm^{-1}	1730, 1630, 1610

collected on Ishigaki island, Okinawa Prefecture, Japan. The partial 16S rDNA gene sequence of strain QN15488 showed high levels of identity with strains from the genus *Burkholderia*. The producing organism was inoculated into a seed medium consisting of glucose 1.0%, potato starch 2.0%, Polypepton 0.5%, yeast extract 0.5% and $CaCO_3$ 0.4% (pH 7.0), and cultured on a rotary shaker at 28°C for 3 days. The seed culture was transferred to 500-ml Erlenmeyer flasks each containing 100 ml of a production medium composed of glucose 0.5%, casamino acid 0.5%, Polypepton 3.0%, yeast extract 0.5%, Na_2HPO_4 0.5%, KH_2PO_4 0.1%, NH_4Cl 0.1% and $MgSO_4 \cdot 7H_2O$ 0.05% (pH 7.0). The fermentation was carried out at 28°C for 4 days on a rotary shaker.

The mycelial cake of the broth (10 liters) was extracted with acetone. The acetone extract was evaporated to an aqueous concentrate and then partitioned between ethyl acetate and water. The organic layer was evaporated *in vacuo* to dryness. The crude material (900 mg) was subjected to silica gel column chromatography and developed with $CHCl_3$ - MeOH (90 : 1). The active fraction was purified by HPLC using a Senshu-Pak PEGASIL ODS column (particle size: 7 μm, 20 i.d.×250 mm) with 70% MeOH to give a colorless powder of **1** (1.54 mg).

The physico-chemical properties of **1** are summarized in Table 1. The molecular formula of burkholone was established to be $C_{18}H_{23}NO$ by high-resolution FAB-MS. The direct connectivity of protons and carbons was established by the HMQC spectrum and the tabulated ^{13}C- and ^1H-NMR spectral data for burkholone are shown in Table 2. The sequence from the methylene proton H-1′ (δ_H 3.47) to a methyl proton H-8′ (δ_H 0.85) through H-2′ (δ_H 5.50), H-3′ (δ_H 5.62), H-4′ (δ_H 2.00), H-5′ (δ_H 1.32), H-6′ (δ_H 1.25) and H-7′ (δ_H 1.27) observed in the DQF spectrum revealed the presence of a 2-octene moiety (Fig. 2). In the same manner, a 1,2-disubstituted benzene substructure was also established by the correlations among aromatic protons H-5 (δ_H 8.35), H-6 (δ_H 7.25), H-7 (δ_H 7.50) and H-8 (δ_H 7.45) (Fig. 2). The heteronuclear multiple-bond correlation (HMBC) spectrum displayed the long-range ^1H-^{13}C couplings from a methyl proton (δ_H 2.15, 3-CH_3) to C-2 (δ_C 147.5), C-3 (δ_C 115.7) and a carbonyl carbon C-4 (δ_C 178.1), which was in turn long-range coupled to H-5 (δ_H 8.35). A methylene proton H-1′ (δ_H 3.47) was long-range coupled to C-2 and C-3. Thus, the 2-octene and methyl residues were revealed to be substituted at the C-2 and C-3 position, respectively. Long-range ^1H-^{13}C couplings on the benzene ring moiety established the assignment of the remaining aromatic carbons C-4a (δ_C 123.6) and C-8a (δ_C 139.0) as shown in Fig. 2. By taking into consideration the molecular formula of **1**, C-2 and C-8a should be connected through a nitrogen atom. The geometrical configuration of C-2′ was proved to

Table 2 ^1H- and ^{13}C-NMR data of **1** in $CDCl_3$

No.	δ_C	δ_H (multiplicity, *J*=Hz)
2	147.5	
3	115.7	
4	178.1	
4a	123.6	
5	126.0	8.35 (dd, *J*=8.0, 1.0)
6	123.1	7.25 (dt, *J*=8.0, 1.0)
7	131.1	7.50 (dt, *J*=8.0, 1.0)
8	117.4	7.45 (d, *J*=8.0)
8a	139.0	
1′	35.5	3.47 (2H, d, *J*=7.0)
2′	123.1	5.50 (ddt, *J*=15.0, 7.0, 1.0)
3′	136.0	5.62 (ddt, *J*=15.0, 7.0, 1.0)
4′	32.5	2.00 (2H, dd, *J*=15.0, 7.0)
5′	28.8	1.32 (2H, m)
6′	31.4	1.25 (2H, m)
7′	22.5	1.27 (2H, m)
8′	14.0	0.85 (3H, t, *J*=7.0)
3-Me	10.5	2.15 (3H, s)
1-NH		10.12 (br s)

^{13}C- and ^1H-NMR spectra were recorded at 125 MHz and 500 MHz, respectively.

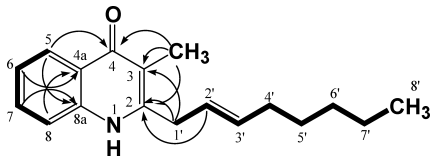

Fig. 2 COSY and HMBC analyses for **1**. Bold lines show proton spin networks and arrows indicate ^1H-^{13}C long-range correlations.

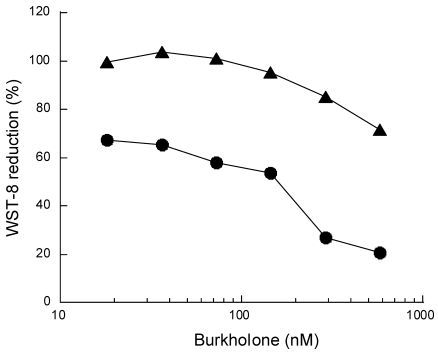

Fig. 3 Effects of **1** on the viability and growth of 32D/GR15 cells.

▲, IL-3; ●, IGF-I. 32D/GR15 cells were cultured for 36 hours with various concentrations of **1** and then the survival was measured by the WST-8 assay.

be *E* by a large coupling constant ($J_{2'H\text{-}3'H}$=15.0 Hz). The correspondence of ^{13}C chemical shifts assigned to the chromophore moiety between **1** and quinolone compounds such as PSC-D, YM-30059 and CJ-13,136 strongly supported the structure of **1** [3～5]. Thus, the structure of **1** was established to be (*E*)-3-methyl-2-(2-octenyl)-4-quinolone as shown in Fig. 2.

32D/GR15 cells were cultured for 36 hours with various concentrations of **1** and then the survival was measured by WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium monosodium salt] assay. **1** induced cell death in 32D/GR15 cells in the IL-3-free medium containing IGF-I (50 ng/ml) with an IC_{50} value of 160 nM. Contrary to the potent cytotoxicity in IL-3 free medium, burkholone did not exhibit cytotoxic activity in IL-3 (2 ng/ml) containing medium (IC_{50} >37 μM, Fig 3). Several human cancer cells were cultured for 72 hours with various concentrations of **1** and then the survival was measured by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. **1** also induced cell death to IGF-I high dependent MCF-7 [6] human breast cancer cells (IC_{50} 240 nM) and IGF-I low dependent HeLa [6] human cervical cancer cells (IC_{50} 140 nM). Such activity of **1** was not observed in IGF-I low dependent HT1080 human fibrosarcoma cells (IC_{50} 15 μM) and IGF-I low dependent SiHa [7] human cervical cancer cells (IC_{50} >37 μM). Expression of antisense RNA directed against the IGF-IR mRNA lowers the expression of the IGF-IR in MCF-7cells, HeLa cells and SiHa cells and slightly inhibits their growth [6, 7]. These findings suggested that **1** induced cell death not only by direct action on IGF-I-dependent pathway but also involves other action mechanism. Although, some kinase inhibitors of IGF-IR were reported [8, 9], there is only one report that synthetic quinolones possessed inhibitory effects against EGFR tyrosine kinase [10]. Quinolones are reported to show antitumor effects targeting DNA related factors such as topoisomerases I and II [11～15]. A variety of 3-methyl-2-alkenyl-4-quinolones shows antibacterial activities against Gram-positive bacteria [16], including multiple-drug resistant *Staphylococcus aureus* and *S. epidermidis* [4], plant growth promoting activities and antifungal activities [3]. But **1** did not induce antibacterial activity against *Bacillus subtilis* at 370 μM. Further biological studies on **1** are now under way.

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