

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

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Received: June 6, 2007 / Accepted: October 22, 2007

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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 IC₅₀ 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

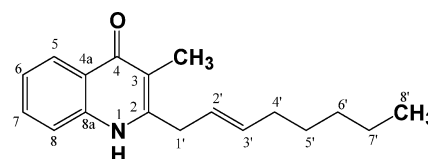


Fig. 1 Structure of burkholone (**1**).

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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 (<i>m/z</i>)	
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 CaCO₃ 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%, Na₂HPO₄ 0.5%, KH₂PO₄ 0.1%, NH₄Cl 0.1% and MgSO₄·7H₂O 0.05% (pH 7.0). The fermentation was carried out at 28°C for 4 days on a rotary shaker.

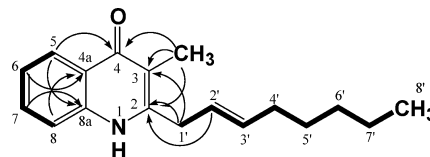
The mycelial cake of the broth (10 liters) was extracted with acetone. The acetone extract was evaporated to an aqueous concentrate and then partitioned between ethyl acetate and water. The organic layer was evaporated *in vacuo* to dryness. The crude material (900 mg) was subjected to silica gel column chromatography and developed with CHCl₃-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 C₁₈H₂₃NO by high-resolution FAB-MS. The direct connectivity of protons and carbons was established by the HMQC spectrum and the tabulated ¹³C- 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

Table 2 ¹H- and ¹³C-NMR data of **1** in CDCl₃

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)

¹³C- 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-¹³C long-range correlations.

(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-¹³C couplings from a methyl proton (δ_H 2.15, 3-CH₃) 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-¹³C 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

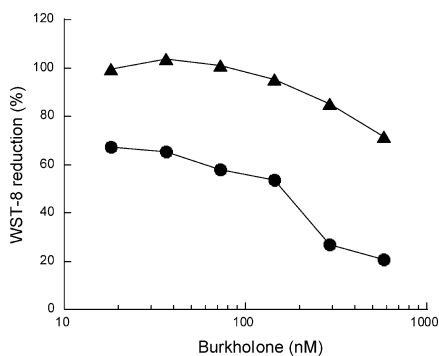


Fig. 3 Effects of **1** on the viability and growth of 32D/GR15 cells.

▲, 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 ($J_{2'H-3'H} = 15.0$ 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 ($\text{IC}_{50} > 37 \mu\text{M}$, 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 ($\text{IC}_{50} > 37 \mu\text{M}$). Expression of antisense RNA directed against the IGF-IR mRNA lowers the expression of the IGF-IR in MCF-7 cells, 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.

Acknowledgements We are grateful to Dr. Renato Baserga (Kimmel Cancer Center, Thomas Jefferson University) for providing 32D/GR15 cells. This work was supported by New Energy and Industrial Technology Development Organization (NEDO) of Japan.

References

1. Baserga R, Hongo A, Rubini M, Prisco M, Valentini B. The IGF-I receptor in cell growth, transformation and apoptosis. *Biochim Biophys Acta* 1332: F105–F126 (1997)
2. Peruzzi F, Prisco M, Dews M, Salomoni P, Grassilli E, Romano G, Calabretta B, Baserga R. Multiple signaling pathways of the insulin-like growth factor I receptor in protection from apoptosis. *Mol Cell Biol* 19: 7203–7215 (1999)
3. Moon S, Kanga PM, Park KS, Kim CH. Plant growth promoting and fungicidal 4-quinolones from *Pseudomonas cepacia*. *Phytochemistry* 42: 365–368 (1996)
4. Kamigiri K, Tokunaga T, Shibasaki M, Setiawan B, Rantiatmodjo RM, Morioka M, Suzuki K. YM-30059, a novel quinolone antibiotic produced by *Arthrobacter* sp. *J Antibiot* 49: 823–825 (1996)
5. Dekker KA, Inagaki T, Gootz TD, Huang LH, Kojima Y, Kohlbrenner WE, Matsunaga Y, McGuirk PR, Nomura E, Sakakibara T, Sakemi S, Suzuki Y, Yamauchi Y, Kojima N. New quinolone compounds from *Pseudonocardia* sp. with selective and potent anti-*Helicobacter pylori* activity: taxonomy of producing strain, fermentation, isolation, structural elucidation and biological activities. *J Antibiot* 51: 145–152 (1998)
6. Neuenschwander S, Roberts CT Jr, LeRoith D. Growth inhibition of MCF-7 breast cancer cells by stable expression of an insulin-like growth factor I receptor antisense ribonucleic acid. *Endocrinology* 136: 4298–4303 (1995)
7. Nakamura K, Hongo A, Kodama J, Miyagi Y, Yoshinouchi M, Kudo T. Down-regulation of the insulin-like growth factor I receptor by antisense RNA can reverse the transformed phenotype of human cervical cancer cell lines. *Cancer Res* 60: 760–765 (2000)
8. Parrizas M, Gazit A, Levitzki A, Wertheimer E, LeRoith D. Specific inhibition of insulin-like growth factor-1 and

- insulin receptor tyrosine kinase activity and biological function by tyrphostins. *Endocrinology* 138: 1427–1433 (1997)
9. Garcia-Echeverria C, Pearson MA, Marti A, Meyer T, Mestan J, Zimmermann J, Gao J, Brueggen J, Capraro HG, Cozens R, Evans DB, Fabbro D, Furet P, Porta DG, Liebetanz J, Martiny-Baron G, Ruetz S, Hofmann F. *In vivo* antitumor activity of NVP-AEW541-A novel, potent, and selective inhibitor of the IGF-IR kinase. *Cancer Cell* 5: 231–239 (2004)
 10. Traxler P, Green J, Mett H, Séquin U, Furet P. Use of a pharmacophore model for the design of EGFR tyrosine kinase inhibitors: isoflavones and 3-phenyl-4(1*H*)-quinolones. *J Med Chem* 42: 1018–1026 (1999)
 11. Robinson MJ, Martin BA, Gootz TD, McGuirk PR, Osheroff N. Effects of novel fluoroquinolones on catalytic activities of eukaryotic topoisomerase II: Influence of the C-8 fluorine group. *Antimicrob Agents Chemother* 36: 751–756 (1992)
 12. Kohlbrenner WE, Wideburg N, Weigl D, Saldivar A, Chu DT. Induction of calf thymus topoisomerase II-mediated DNA breakage by antibacterial isothiazoloquinolones A-65281 and A-65282. *Antimicrob Agents Chemother* 36: 81–86 (1992)
 13. Wentland MP, Lesher GY, Reuman M, Gruett MD, Singh B, Aldous SC, Dorff PH, Rake JB, Coughlin SA. Mammalian topoisomerase II inhibitory activity of 1-cyclopropyl-6,8-difluoro-1,4-dihydro-7-(2,6-dimethyl-4-pyridinyl)-4-oxo-3-quinolinecarb oxylic acid and related derivatives. *J Med Chem* 36: 2801–2809 (1993)
 14. Tomita K, Tsuzuki Y, Shibamori K, Tashima M, Kajikawa F, Sato Y, Kashimoto S, Chiba K, Hino K. Synthesis and structure-activity relationships of novel 7-substituted 1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acids as antitumor agents. Part 1. *J Med Chem* 45: 5564–5575 (2002)
 15. Tsuzuki Y, Tomita K, Shibamori K, Sato Y, Kashimoto S, Chiba K. Synthesis and structure-activity relationships of novel 7-substituted 1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acids as antitumor agents. Part 2. *J Med Chem* 47: 2097–2109 (2004)
 16. Hays EE, Wells IC, Katzman PA, Cain CK, Jacobs FA, Thayer SA, Doisy EA, Gaby WL, Roberts EC, Muir RD, Carroll CJ, Jones LR, Wade NJ. Antibiotic substances produced by *Pseudomonas aeruginosa*. *J Biol Chem* 159: 725–749 (1945)