NOTE

PM070747, a new cytotoxic angucyclinone from the marine-derived *Saccharopolyspora taberi* PEM-06-F23-019B

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The Journal of Antibiotics (2009) 62, 167–169; doi:10.1038/ja.2008.27; published online 23 January 2009

Keywords: angucyclinone; isolation; PM070747; *Saccharopolyspora taberi*; structural elucidation

The benz[α]anthraquinones are a class of compounds that have attracted a great deal of attention owing to their interesting biological properties.

As part of our on-going screening for new antitumor compounds from marine microorganisms, we have isolated a new angucyclinone, PM070747 (**1**) (Figure 1), produced by the actinomycete *Saccharopolyspora taberi* PEM-06-F23-019B, from a marine sponge collected from near the coast of Tanzania. Additionally, traces of the known angucyclinone, PD116740¹ (**2**), were also found.

Compound **1** is structurally closely related to **2** and TAN-1085² (**3**) (Figure 1). Compound **2** was first isolated from the unknown actinomycete, WP4669, and it was described as a compound with antitumor properties against leukemia and adenocarcinoma cell lines.¹ Recently, an epimer of **2** was isolated from a marine fungus, *Penicillium* sp.³ Compound **3** and its aglycone were described as angiogenesis and aromatase inhibitors in the Japanese patent JP02289532.²

The microbial producer of **1**, *Saccharopolyspora taberi* PEM-06-F23-019B, was isolated by spreading the homogenized ectosome of the sponge on Bennett's agar medium⁴ plates supplemented with nalidixic acid (0.02%) and cycloheximide (0.02%). The plates were incubated at 28 °C for 30 days.

The strain was subjected to a phylogenetic analysis based on 16S rRNA sequences analyzed by BLAST (Basic Local Alignment Search Tool) against the NCBI (National Center for Biotechnology Information) database,⁵ and showed a high identity with that of *Saccharopolyspora*, such as *Saccharopolyspora taberi* DSM 43856 (956/968, 98.8%) (sequence AF002819).⁶

The seed culture was developed in two scale-up steps, first in 100-ml Erlenmeyer flasks containing 20 ml of seed medium, and then in 250-ml Erlenmeyer flasks with 50 ml of the same medium. The seed culture was

grown on a medium containing dextrose (0.1%), soluble starch 2.4%, soy peptone 0.3%, yeast extract 0.5%, Tryptone 0.5%, soya flour 0.5%, NaCl 0.54%, KCl 0.02%, MgCl₂ 0.24%, Na₂SO₄ 0.75% and CaCO₃ 0.4% in tap water, and cultured at 28 °C on an orbital shaker for 72 h. For production, 12.5 ml of the seed medium was transferred into 2000-ml Erlenmeyer flasks containing 250 ml of fermentation medium, comprising yeast extract 0.5%, soy peptone 0.1%, dextrose 0.5%, soya flour 0.3%, Glucidex 2.0%, NaCl 0.53%, KCl 0.02%, MgCl₂·6H₂O 0.24%, Na₂SO₄ 0.75%, MnSO₄·4H₂O 0.00076%, CoCl₂·6H₂O 0.0001%, K₂HPO₄ 0.05% and CaCO₃ 0.4%. The culture was grown at 28 °C using an orbital shaker (5-cm eccentricity, 220 r.p.m.) for 5 days.

The bioassay-guided isolation of **1** from the fermentation broth is depicted in Figure 2 and summarized below.

The fermentation broth (6l) was subjected to centrifugation, and the clarified broth was extracted with ethyl acetate 1:1 (v/v). The extract was concentrated to give a broth extract (382 mg). It was subjected to reversed-phase vacuum chromatography by a filter funnel on Polygoprep 100–50 μ m C18, using a H₂O–MeOH–CH₂Cl₂ mixture as the eluting solvent. The cytotoxic activity was detected in fractions eluted with H₂O:MeOH 3:1 (35 mg), 1:1 (100 mg) and 1:3 (50 mg). As these three fractions displayed similar HPLC profiles, they were combined and evaporated under reduced pressure, resulting in a crude extract (185 mg). This extract was then chromatographed on a reversed-phase (C-18) column eluted in a stepwise gradient with increasing ratios of MeOH in water, followed by CH₂Cl₂ in MeOH. Three out of the fourteen fractions eluted with H₂O–MeOH mixtures showed antitumor activity and similar HPLC profiles. Further purification of two of these active fractions by preparative HPLC was carried out at room temperature using a C-18 Atlantis column (19×150 mm, 5 μ m, Waters). A gradient of CH₃CN/H₂O (15–60% in 20 min) was

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Received 8 August 2008; accepted 18 December 2008; published online 23 January 2009

Figure 1 Structures of PM070747 (**1**), PD 116740 (**2**) and TAN-1085 (**3**).

employed as a mobile phase, with a flow rate of $13.65 \text{ ml min}^{-1}$ with UV detection at 215 nm. Under these conditions, a total amount of 5 mg of pure **1** was isolated, along with traces of **2**.¹

The structure of **1** was established as shown in Figure 1 on the basis of analysis of the physico-chemical properties (Table 1) and NMR data (Table 2), and by comparison with data reported for the related angucyclinone **2**.¹

The physico-chemical properties of **1** are summarized in Table 1. Compound **1** was obtained as a reddish powder. Its molecular formula was determined as $\text{C}_{21}\text{H}_{18}\text{O}_6$, on the basis of high resolution electrospray ionization mass spectrum (HR-ESI-MS) and the NMR data (Table 2), indicating 13 degrees of unsaturation.

The ESI-MS experiments showed ions at m/z 367 ($M+H$) and 389 ($M+Na$), which confirmed the molecular mass. The UV spectrum of **1** in MeOH exhibited absorption maxima at 203 and 408 nm, indicating the presence of a substituted anthraquinone moiety. The IR indicated the presence of hydroxyl groups (3353 cm^{-1}). In addition, a strong absorption peak at 1639 cm^{-1} and the absence of a peak near 1670 cm^{-1} indicated that both carbonyls were involved in hydrogen bonding to neighbouring hydroxyls.^{7,8} As described for **2**, the absence of an IR band at around $1700\text{--}1725 \text{ cm}^{-1}$, assigned to the C-1 carbonyl in related antibiotics, suggested the presence of a hydroxyl group, hydrogen bonded to the C-12 quinone carbonyl, attached to the C-1 position (see Table 1).

The ^1H NMR spectrum of **1** exhibited a signal at δ 4.00 for an aromatic methoxy group (Table 2). Moreover, a pair of coupled signals at δ 4.59 (H-5) and 5.12 (H-6) revealed the presence of a 5,6-diol moiety in **1**. Two more signals were observed as doublets with a small coupling constant at δ 6.76 (H-2) and 6.86 (H-4), each integrating for one proton, representing meta-coupled aromatic protons. In addition, an aromatic ABX system with a pattern very similar to that observed for **2**¹ was present in the ^1H NMR spectrum of **1**. However, no signals for a benzylic hydroxymethyl group were observed compared with **2**. Instead, coupled signals at δ 1.26 ($\text{H}_3\text{-14}$) and 2.64 ($\text{H}_2\text{-13}$) clearly indicated the presence of an ethyl group attached to the C-3 position. Assignment of the rest of the benz[α]anthraquinone signals was confirmed by 2-D NMR experiments. In the $^1\text{H}\text{--}^1\text{H}$ COSY, correlations of H-10 with H-9 and H-11, H-5 with H-6 and H-13 with H-14 revealed the linkages in these substructures. A correlation between the H-5 and H-6 protons in the COSY, together with the observed coupling constant for both protons (3 Hz), suggested the same *trans* stereochemistry as that of PD 116740, the X-ray diffraction analysis of

Figure 2 Isolation procedure for PM070747 (**1**) from the culture of *Saccharopolyspora taberi* PEM-06-F23-019B.

which has been described in the literature.¹ The rest of the correlations observed in the ROESY (that is, H-4 with H-13; H-2 with H-13; H-9 with H-15) were consistent with the proposed structure (Figure 3). The relationship between these fragments was established through the heteronuclear multibond correlation (HMBC) NMR spectrum. The $\text{H}_3\text{-15}$ methoxy group at δ 4.00 was coupled to the aromatic carbon C-8, indicating its position. Moreover, long-range couplings observed between H-13 with C-2 and C-4 showed the position of the ethyl group (Figure 3). Long-range couplings observed between H-4 with C-2, C-12b and C-5, and between H-6 with C-4a, C-12a and the carbonyl group C-7 indicated two substructures fused through C-6–C-6a and C-12a–C-12b, which was further supported by cross peaks between H-5 with C-6a and between H-2 with C-12b in HMBC as complementary evidence (Figure 3). On the other hand,

Table 1 Physico-chemical properties of PM070747 (**1**)

Appearance	Reddish powder
Molecular formula	C ₂₁ H ₁₈ O ₆
Molecular weight	366.364
HR-ESI-MS (pos) <i>m/z</i>	(M+Na) ⁺
Calculated	389.0995
Found	389.0988
UV λ _{max} (MeOH) nm	203, 408
IR ν _{max} (neat) cm ⁻¹	3353, 2959, 1730, 1639, 1376, 1340, 860, 839, 801, 738, 663
(α) _D ²⁵ (c=0.32, MeOH)	+220

Figure 3 Summary of ¹H-¹H COSY, HMBC and ROESY correlations in PM070747 (**1**).**Table 2** ¹H and ¹³C NMR (MeOD, 500 and 125 MHz) assignments of PM070747 (**1**)

Position C/H No.	¹ H shifts ^{a,b,c}	¹³ C shifts ^a	HMBC
1	—	157.34	—
2	6.76 d, 1H (<i>J</i> =2)	118.53	C-1, C-13, C-12b
3	—	150.40	—
4	6.86 d, 1H (<i>J</i> =2)	123.21	C-2, C-5, C-12b, C-13
4a	—	143.04	—
5	4.59 d, 1H (<i>J</i> =3)	72.79	C-4, C-12b, C-6a
6	5.12 d, 1H (<i>J</i> =3)	64.69	C-7, C-4a, C-12a
6a	—	141.88	—
7	—	184.32	—
7a	—	120.80	—
8	—	160.70	—
9	7.50 d, 1H (<i>J</i> =8)	119.14	C-11
10	7.77 dd, 1H (<i>J</i> =7.5, 8)	136.22	C-8, C-11a
11	7.69 d, 1H (<i>J</i> =7.5)	120.18	C-9, C-7a
11a	—	137.58	—
12	—	187.49	—
12a	—	140.27	—
12b	—	114.42	—
13	2.64 q, 2H (<i>J</i> =7.5)	29.66	C-2, C-3, C-4
14-Me	1.26 t, 3H (<i>J</i> =7.5)	15.56	C-3
15-Me	4.00 s, 3H	56.83	C-8

^aChemical shifts (δ) are in ppm.^bCoupling constants (*J*) in hertz (Hz) are given in parentheses.^cs: singlet; d: doublet; t: triplet; q: quartet.

C-7a was assigned by its correlation with H-11, and the C-11a position was revealed by a cross peak between H-10 and C-11a in HMBC (Figure 3).

The antitumor activity of **1** was evaluated against a panel of three tumor cell lines: human breast adenocarcinoma MDA-MB-231 cells, human colorectal adenocarcinoma HT-29 cells and human lung carcinoma A-549 cells (concentration of 50% inhibition on cell growth (GI₅₀) 0.71, 1.42 and 3.28 μM, respectively), according to the procedure described in the literature.⁹ Cell survival was estimated using the National Cancer Institute algorithm.¹⁰ Three dose-response parameters were calculated for **1** (Table 3).

Table 3 Cell-survival values (μM) for three tumor cell lines treated with PM070747 (**1**)

	A549	HT29	MDA-MB-231
GI ₅₀	3.28	1.42	0.71
TGI	4.64	1.97	1.06
LC ₅₀	6.82	> 2.73	1.72

GI₅₀, compound concentration that produces 50% inhibition on cell growth as compared with control cells; TGI, compound concentration that produces total growth inhibition as compared with control cells; and LC₅₀, compound concentration that produces 50% cell death as compared with control cells.

ACKNOWLEDGEMENTS

We gratefully acknowledge the assistance of our PharmaMar colleagues, Dr Luis Francisco Garcia for the cytotoxicity assay, Dr Susana González for performing the NMR experiments, Jesus Garcia for the microbial cultures, and Dr Simon Munt and Dr Fernando Reyes for the revision of this paper.

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