The Journal of Antibiotics (2017) 70, 219–221
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www.nature.com/ja

NOTE

A new cyano-substituted anthracycline metabolite from *Streptomyces* sp. HS-NF-1006

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The Journal of Antibiotics (2017) **70,** 219–221; doi:10.1038/ja.2016.112; published online 7 September 2016

Anthracyclines, produced by various *Streptomyces* species, have been proved to be important components in the treatment of cancer chemotherapy.[1–3] The first clinically important anthracycline (daunorubicin) was isolated from the pigment-producing *Streptomyces peucetius* early in the 1960s. In the concerted effort to yield new and improved anthracyclines, doxorubicin subsequently appeared. Although this agent differed by only one hydroxyl group from daunorubicin, it was soon realized that this minor difference was sufficient to endow the drug with a greatly superior spectrum of activity.[4] So a great effort has been made to develop various analogs and derivatives of anthracycline compounds expecting for more improved antitumor drugs.[5–8] As part of our continuous screening for more secondary metabolites, we investigated the bioactive constituents of the strain *Streptomyces* sp. HS-NF-1006 and this led to the isolation of an interesting anthracycline analog, designated 6''-cyano-6''-deoxy-TAN-1120 (**1**, Figure 1), from the fermentation broth of this strain. Here, the details of fermentation, isolation, structure characterization and bioactivity of compound **1** are described.

Strain *Streptomyces* sp. HS-NF-1006 was isolated from a soil sample collected from a pasture, located in Hailaer, Inner Mongolia Autonomous Region, China. The strain was identified as the genus *Streptomyces* because its 16S rRNA sequence (accession no: KU848003) exhibited a high sequence similarity of 99.45% with that of *Streptomyces rishiriensis* strain NBRC13407 (accession no: AB184383) and it was deposited in the Pharmaceutical Research Culture Collection, Zhejiang Hisun Group Co., Ltd., with accession no: HS-NF-1006.

This strain was incubated for 6–7 days at 28 °C on the medium containing glucose (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) 4 g, malt extract (Bei Jing Ao Bo Xing, Beijing, China) 10 g, yeast extract (Oxoid Ltd, Basingstoke, UK) 4 g, $CoCl_2 \cdot 6H_2O$ 0.005 g and agar (Becton, Dickinson and company, Franklin Lake, NJ, USA) 18 g in 1.0 l of water, pH 7.0–7.2. The seed medium consisted of glucose 4 g, malt extract 10 g, yeast extract 4 g and $CaCO_3$ 2 g in 1.0 l water, pH 7.2–7.4. All the media were sterilized at 121 °C for 20 min. Fermentation was carried out in a 50 l fermentor (containing 30 l of production medium; Shanghai Guoqiang Bioengineering Equipment, Shanghai, China). The producing medium was composed of glucose 1%, soluble starch (Haiyan Liuhe Starch Chemical Co, Ltd., Haiyan, China) 4%, yeast extract 0.4%, malt extract 1%, $CaCO_3$ 0.2%, $FeSO_4 \cdot 7H_2O$ 0.1%, $ZnSO_4 \cdot 7H_2O$ 0.1%, and $MnCl_2 \cdot 4H_2O$ 0.1% at pH 7.2–7.4 before sterilization. The fermentation was conducted at 28 °C for 7 days by stirring at 200 r.p.m. with an aeration rate of 1000 l of air per hour.

The final 30 l of fermentation broth was centrifuged to separate mycelia and supernatant. The mycelial cake was washed with water (3 l) and subsequently extracted with MeOH (10 l). The supernatant and the wash water were passed through a column of Diaion HP-20 resin (Mitsubushi Chemical Co, Ltd., Tokyo, Japan) equilibrating with water and then eluting with 95% EtOH (5 l). The MeOH extract and EtOH eluent were concentrated under reduced pressure to 1 l at 45 °C, and then extracted three times using an equal volume of EtOAc. The combined organic phase was evaporated under reduced pressure to yield a mixture (26 g). The mixture was subjected to a silica gel (Qingdao Haiyang Chemical Group, Qingdao, Shandong, China; 100–200 mesh) column and eluted stepwise with $CHCl_3$/MeOH (100:0, 98:2, 95:5, 90:10, 85:15 and 80:20, v/v) to give six fractions (Fr.1 to Fr.6) based on the TLC profiles. TLC was performed on silica-gel plates (HSGF254, Yantai Chemical Industry Research Institute, Yantai, China) with solvent system of $CHCl_3$/MeOH (9:1, v/v). The developed TLC plates were observed under a UV lamp at 254 nm, or by heating after spraying with sulfuric acid/ethanol, 5:95 (v/v). After the Fr.3 was concentrated in vacuo, the material (1.2 g) was subjected to Sephadex LH-20 gel (GE Healthcare, Glies, UK) column eluted with $CHCl_3$/MeOH (1:1, v/v) and detected using TLC to afford two fractions (Fr.3-1 and Fr.3-2). The Fr.3-1 was further purified by preparative HPLC (Shimadzu LC-8A, Shimadzu-C18,

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Received 18 May 2016; revised 25 June 2016; accepted 3 August 2016; published online 7 September 2016

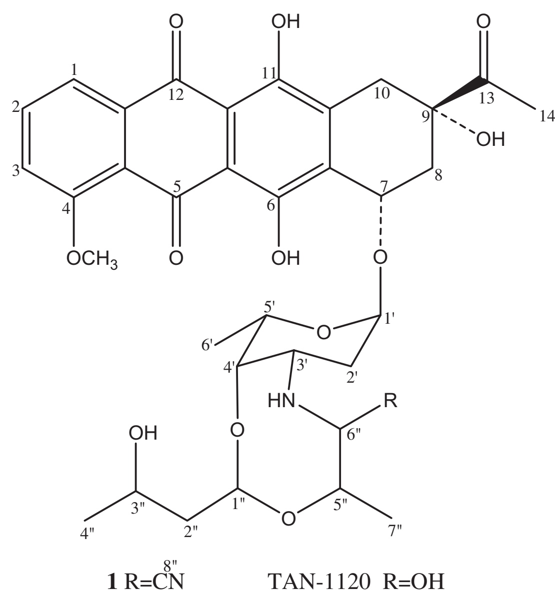

Figure 1 Structures of compound **1** and TAN-1120.

5 μm, 250×20 mm inner diameter; 20 ml min^{-1}; 220 nm/254 nm; Shimadzu, Kyoto, Japan) with a 25 min gradient program of 75–85% MeOH in H_2O to obtain compound **1** (t_R 8.2 min, 16.3 mg). ^1H and ^{13}C NMR spectra were measured with a Bruker DRX-400 (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer (Bruker, Rheinstetten, Germany). The ESIMS and HRESIMS spectra were taken on a Q-TOF Micro LC-MS-MS mass spectrometer (Waters Co, Milford, MA, USA).

Compound **1** was isolated as a red amorphous powder with $[\alpha]_D^{25}$ +74.2 (*c* 0.03, EtOH) and UV (EtOH) λ_{max} nm (log ε): 201 (4.22), 234 (4.25), 252 (4.12), 290 (3.71), 477 (3.68), 499 (3.70), 533 (3.50), 579 (2.89). Its molecular formula $C_{35}H_{40}N_2O_{12}$ was deduced from the pseudo molecular ion at *m/z* 681.2643 $[M+H]^+$, which indicated 17 degrees of unsaturation. The IR spectrum of **1** showed absorption bands assignable to hydroxy group (3226 cm^{-1}) and carbonyl (1660 cm^{-1}). The ^1H NMR spectrum (Table 1) of **1** displayed two downfield active OH proton signals at δ_H 14.0 (1H, s) and 13.3 (1H, s), a methoxy proton signal at δ_H 4.11 (3H, s), three doublet aliphatic methyls at δ_H 1.26 (3H, d, J=6.2 Hz), 1.30 (3H, d, J=6.2 Hz), 1.31 (3H, d, J=5.2 Hz), a 1, 2, 3-trisubstituted benzene ring system corresponding to three aromatic protons at δ_H 8.06 (1H, d, J=8.1 Hz), 7.80 (1H, t, J=8.1 Hz) and 7.41 (1H, d, J=8.1 Hz), and an acetyl proton corresponding to a singlet methyl proton signal at δ_H 2.44 (3H, s). The ^{13}C NMR spectrum showed signals from 33 carbon atoms, which is incompatible with its molecular formula $C_{35}H_{40}N_2O_{12}$. The ^1H and ^{13}C NMR data assignment were supported by the ^1H-^1H COSY, HMBC and HMQC experiments. In the HMBC spectrum (Figure 2), the correlation from the proton signal of C-2 (δ_H, 7.80, δ_C, 135.6) to C-12a (δ_C 135.6) indicated the carbon signal at δ_C 135.6 containing two resonances. Careful examination of HMBC spectrum revealed a correlation between the acetyl group (δ_H 4.11) and C-9 (δ_C 77.0), which showed that an oxygenated quaternary carbon signal was overlapped with the carbon signal of residual $CHCl_3$. Combined with the DEPT experiment, the 35-carbon resonances can be categorized as 3 carbonyls (δ_C 211.9, 187.1 and 186.8), 2 downfield sp^3 methines (each bonding with two oxygen atoms) (δ_C 107.6, 101.1), 1 oxygenated sp^3 quaternary carbon (δ_C 77.0), 5 oxygenated sp^3 methines (δ_C 79.3, 79.3, 70.2, 65.1, 64.5), 1 methoxy group (δ_C 56.7), 2 sp^3 methines (δ_C 54.4, 48.1), 4 sp^3 methylenes (δ_C 44.0, 35.0, 33.4, 32.1), 4 methyls (δ_C 24.8, 23.6, 21.2, 17.0) and 13 carbon signals in aromatic region. Initial inspection of both the ^1H and ^{13}C NMR spectra (Table 1) indicated compound **1** to be a member of the baumycin-group anthracycline. Comparison of the ^1H and ^{13}C NMR data of **1** with those of TAN-1120[9] revealed significant similarities (Table 1). Taking the molecular formula $C_{35}H_{40}N_2O_{12}$ of **1** into account, compound **1** differs from TAN-1120 by the replacement of a hydroxy group in TAN-1120 with a cyano group. The presence of a nitrile carbon (δ_C 117.5) is evident in the ^{13}C NMR spectrum of **1**. More detailed analysis of the ^{13}C NMR spectrum showed a significant upfield shift of C-6" from δ_C 94.3 to δ_C 54.4 when comparing the NMR spectrum of TAN-1120 with that of **1**. This difference suggests that the cyano-group in **1** is located on C-6". Application of 2D

Table 1 ^1H and ^{13}C NMR data of compounds 1 and TAN-1120

	δ_H (J in Hz)		δ_C	
Position	***1***[a]	*TAN-1120*[b]	***1***[a]	*TAN-1120*[b]
1	8.06 d (8.1)	8.03 br d (7.5)	119.8 (d)	120.0 (d)
2	7.80 t (8.1)	7.83 br t (7.5)	135.6 (d)	136.2 (d)
3	7.41 d (8.1)	7.47 br d (7.5)	118.4 (d)	119.0 (d)
4			161.1 (s)	161.3 (s)
4a			121.1 (s)	120.9 (s)
5			187.1 (s)	187.2 (s)
5a			111.5 (s)	111.7 (s)
6			156.5 (s)	156.4 (s)
6a			134.1 (s)	134.5 (s)
7	5.29 s	5.25 br s	70.2 (d)	70.4 (d)
8	2.35 d (4.8)	2.36 br d (15.0)	35.0 (t)	35.3 (t)
	2.14 dd (4.8, 3.6)	2.13 dd (4.0, 15.0)		
9			77.0 (s)	77.0 (s)
10	3.27 d (18.8)	3.20 d (18.5)	33.4 (t)	33.2 (t)
	2.96 d (18.2)	3.00 d (18.5)		
10a			134.4 (s)	134.5 (s)
11			155.9 (s)	155.6 (s)
11a			111.4 (s)	111.5 (s)
12			186.8 (s)	186.9 (s)
12a			135.6 (s)	135.6 (s)
13			211.9 (s)	212.9 (s)
14	2.44 s	2.42 s	24.8 (q)	24.8 (q)
4-OCH_3	4.11 s	4.09 s	56.7 (q)	56.8 (q)
1'	5.50 d (2.8)	5.49 br s	101.1 (d)	101.6 (d)
2'	1.85 dd (13.6, 4.0)	1.75 m	32.1 (t)	32.3 (t)
	1.63 m			
3'	3.27 m	3.15 m	48.1 (d)	44.3 (d)
4'	3.54 br s	3.59 br s	79.3 (d)	80.1 (d)
5'	4.16 m	4.14 br q (6.4)	65.1 (d)	65.8 (d)
6'	1.30 d (6.2)	1.27 d (6.4)	16.9 (q)	17.0 (q)
1"	4.73 t (5.3)	4.72 t (5.4)	107.6 (d)	107.4 (d)
2"	1.94 m	1.89 m	44.0 (t)	44.5 (t)
3"	4.11 m	4.03 m	64.5 (d)	64.7 (d)
4"	1.26 d (6.2)	1.23 d (6.0)	23.6 (q)	23.5 (q)
5"	3.92 m	3.53 dq (8.1, 6.0)	79.0 (d)	79.0 (d)
6"	3.66 d (9.8)	3.76 d (8.1)	54.4 (d)	94.3 (d)
7"	1.31 d (5.2)	1.21 d (6.0)	21.2 (q)	21.1 (q)
8"			117.5 (s)	

[a]Recorded in $CDCl_3$.
[b]Recorded in $CDC1_3$-CD_3OD (2:1).

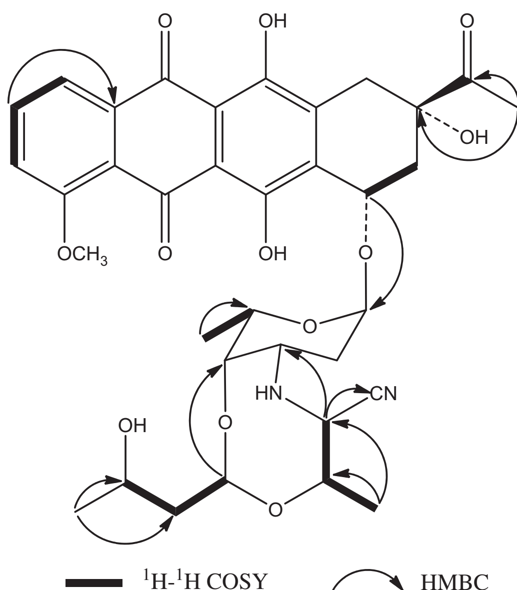

Figure 2 Key ^{1}H-^{1}H COSY and HMBC correlations of compound **1**.

NMR techniques including ^{1}H-^{1}H COSY, HMQC and HMBC, the placement of the cyano-group on C-6" was definitively determined by the key correlations described below, and shown in Figure 2. The doublet methyl proton H_3-7" (δ_H 1.31) is correlated to H-5" (δ_H 3.92), which in turn is correlated to H-6" (δ_H 3.66), as is evident in the ^{1}H-^{1}H COSY spectrum. Furthermore, the correlated signal from H-6" to a cyano-group carbon resonance (δ_C 117.5) was observed in the HMBC spectrum. As a consequence, the structure of **1** was established to be 6"-cyano-6"-deoxy-TAN-1120 as shown in Figure 1. The relative configuration of **1** was attempted to be assigned as that of TAN-1120 based on the similarities of NMR data and optical values between **1** and TAN-1120.

The cytotoxicity of **1** was assayed for growth-inhibition activity *in vitro* against two human tumor cell lines, human hepatocellular liver carcinoma cells HepG2 and human lung tumor cells A549, according to the CCK8 colorimetric method, as reported in our previous papers[10,11] using doxorubicin and daunorubicin as positive controls. As a result, the bioassay showed that **1** has stronger cytotoxicity against the two tumor cell lines than doxorubicin and daunorubicin (Table 2).

Table 2 Cytotoxic activity of 1, doxorubicin and daunorubicin against selected human tumor cell lines

	IC50 (μM)		
Compounds	*1*	*Doxorubicin*	*Daunorubicin*
A549	0.0559	1.47	1.28
HepG2	0.0368	0.401	0.410

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This research work was financially supported by the National Natural Science Foundation of China (31471809).

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