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NOTE

NC1101, a novel tetrahydropyrimidine-containing bleomycin analog from *Streptomyces verticillus* var. *pingyangensis* n. var.

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The bleomycins (BLMs) are a family of glycopeptide anticancer antibiotics isolated from a fermentation broth of *Streptomyces verticillus*.[1] *Streptomyces verticillus* var. *pingyangensis* n. var., isolated from a soil sample collected in Pingyang, Zhejiang Province, China, produces a complex of antitumor antibiotics belonging to the BLM family.[2] We investigated it and discovered NC1101, a new analog of BLM with a tetrahydropyrimidine ring in the terminal amine.

The strain was maintained on an agar slant consisting of glucose 1.0%, soluble amylum 1.0%, peptone 0.5%, agar 2.0% and NaCl 0.5%, pH 7.5, incubated for 7 days at 28 °C. Seed medium and producing medium shared the same components; that is, soluble amylum 2.5%, glucose 0.5%, soybean meal 3.5%, KH_2PO_4 0.1%, $ZnSO_4$ 0.05% and $CuSO_4$ 0.01%, pH 6.5. The slant was transferred to 250 ml Erlenmeyer flasks containing 50 ml of seed medium, and then they were incubated at 28 °C on a rotary shaker at 220 r.p.m. for 2 days. A total of 10 ml of the seed medium was transferred to a 500-ml Erlenmeyer flask containing 100 ml of the producing medium. The fermentation was carried out at 29 °C for 7 ~ 8 days on a rotary shaker at 220 r.p.m. Paper-disk agar diffusion assay using *Bacillus subtilis* was adopted to measure the antibiotic activity.

Fermentation broth (20 l) was adjusted to pH 2 ~ 3 with oxalic acid and filtered. The filtrate was charged on a 122 resins (H^+ form, 2 l) column, and then sequently eluted with distilled water (3 l) and 0.3 M HCl (6 l). The active eluents were pooled and adjusted to pH 7, then 0.2% (w/v) $CuSO_4$ was added. The solution was desalted on a resin column. The active fractions were eluted with water-acetone (90:10, v/v) mixture containing 0.01 M HCl, then combined and evaporated *in vacuo*. The resulting solution was chromatographed on a column of CM-Sephadex C-25 (NH_4^+ form, 300 ml), and eluted with a stepwise gradient of NH_4Cl solution (0.1–0.6 M). The first blue fraction was desalted, and subjected to a CM-Sephadex C-25 column (NH^{4+} form, 100 ml) again, then eluted with 0.05 M NH_4Cl. As a result, copper-chelated NC1101 was purified. The solution was desalted, concentrated and lyophilized. The resulting blue powder (126 mg) was treated with dithizone to give copper-free compound NC1101 (98 mg).

Copper-free NC1101: white powder; UV λ_{max}^{MeOH}, nm: 233 ~ 235(sh.) (24 460), 293 ~ 295 (16 423); ESI-MS, mz^{-1} 1507.27 $[M^+H]^+$, 754.46 $[M+2H]^{2+}$, 503.67 $[M+3H]^{3+}$. UV spectra were collected by SHIMADZU UV-2550 (SHIMADZU, Tokyo, Japan), mass spectra were obtained from Thermo LTQ XL MS instrument (ThermoFisher Scientific, San Jose, CA, USA).

NC1101 showed a typical UV spectrum similar to that of BLM. Ninhydrin reaction of NC1101 was non-typical. Sakaguchi reaction was negative, indicated the absence of guanidine in the structure of NC1101, which could not be a compound of BLM B family. The molecular mass of copper chelated NC1101 was 1569.66 on the basis of ESI-MS data, which gave a molecular ion at mz^{-1} 784.83 $[M+Cu]^{2+}$ and a quasi-molecular ion at mz^{-1} 523.67 $[M+Cu+H]^{3+}$. The fragment ions at mz^{-1} 682.42 $[M+Cu$ –Carbamylmannosyl $+H]^{2+}$ and 602.25 $[M+$Cu-Carbamylmannosyl-Gulosyl $+H]^{2+}$ revealed that the typical glycannic part of BLMs was contained. In the ^{13}C NMR spectrum, the two anomeric carbon signals at δ_C 100.6 (G-1) and δ_C 100.7 (M-1) pointed to the same conclusion.

We assigned the ^{13}C NMR signals by comparison with ^{13}C NMR spectrum data of NC0604 in the previous paper,[4] and by analysis of ^1H-^1H COSY, HMQC, HMBC, DEPT spectra of NC1101. NMR spectra were recorded in D_2O with Varian VNS-600 spectrometer (Varian, San Francisco, CA, USA). The signals of the carbons constituting the kernel structure of NC1101 were consistent with those of NC0604 and the difference between NC1101 and NC0604 was in the terminal amine. The numbering of the parts in the NC1101 molecule follows the convention used in the previous paper[5] as shown in Figure 1. The ^{13}C NMR spectral data of the kernel structure of NC1101 were tabulated in Table 1 in comparison with those of NC0604.[4]

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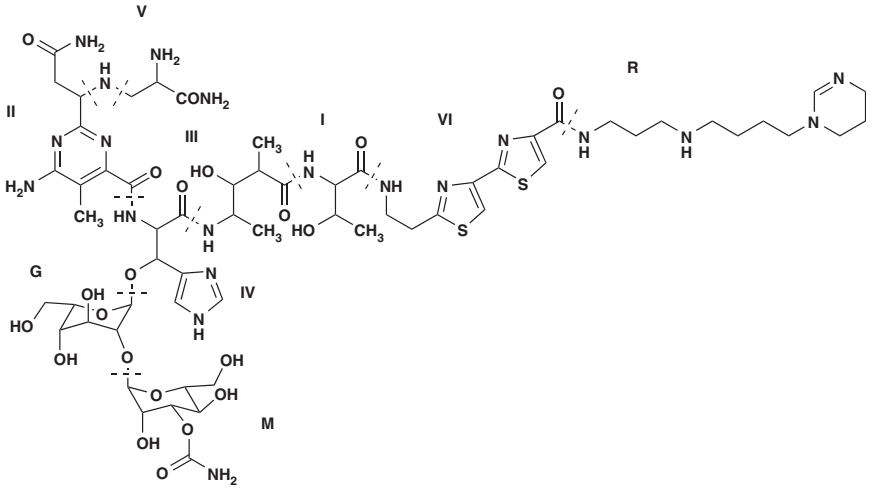

Figure 1 Structure of NC1101.

Table 1 ^{13}C NMR data of NC1101 (600 MHz, D_2O)

	^{13}C shift (p.p.m.)				^{13}C shift (p.p.m.)	
Assignment	*NC1101*	*NC0604*	*Assignment*		*NC1101*	*NC0604*
I						
CO	174.8	175.9	G	1	100.6	100.3
β-CH	69.9	69.8		2	72.8	73.1
α-CH	62.0	61.9		3	70.5	70.7
CH_3	21.8	21.7		4	72.0	71.9
II						
S-CO	179.0	179.0		5	70.0	69.8
R-CO	170.5	170.5		6	63.3	63.1
2	168.2	168.2	M	CO	160.9	160.7
4	167.4	167.4		1	100.7	101.0
6	154.8	155.0		3	77.2	77.1
5	115.0	115.1		5	76.5	76.4
α-CH	62.6	62.6		2	71.2	71.1
β-CH_2	43.0	43.0		4	67.5	67.6
CH_3	13.8	13.7		6	63.8	63.7
III						
CO	180.3	180.3	VII(R)	(CO)	—	177.5
β-CH	77.2	77.1		a	39.2	39.1
γ-CH	50.6	50.7		b	28.7	28.6
α-CH	45.7	45.4		c	48.1	48.0
γ-CH_3	17.3	17.5		d	50.0	49.8
α-CH_3	15.2	14.8		e	26.8	25.7
IV						
CO	171.3	171.9		f	25.4	25.5
2	138.9	139.8		g	57.0	49.7
4	135.2	137.7		h	46.2	46.1
5	121.0	120.5		i	20.9	33.3
β-CH	75.3	75.9		j	39.9	—
α-CH	59.5	59.8		CH	155.2	—
V						
CO	173.8	174.8				
α-CH	55.2	55.6				
β-CH_2	49.8	50.3				

Table 1 (Continued)

	^{13}C shift (p.p.m.)			^{13}C shift (p.p.m.)	
Assignment	*NC1101*	*NC0604*	*Assignment*	*NC1101*	*NC0604*
VI					
CO	166.4	166.3			
2	165.7	165.5			
2′	173.6	173.4			
4	151.7	151.6			
4′	149.8	149.7			
5	128.0	127.9			
5′	122.0	121.9			
β-CH_2	42.0	41.9			
α-CH_2	34.9	34.8			

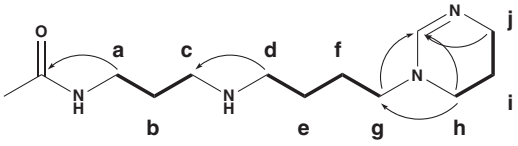

Figure 2 1H-1H COSY (bold lines) and selective HMBC (arrows) coorelations of the terminal amine.

The DEPT spectrum showed 1 methane and 10 methylenes in the terminal amine of NC1101. According to ^{1}H-^{1}H COSY and HMQC spectra, the connections of these methylenes were identified. The sequence from δ_H 3.56 (R-a) to δ_H 3.17 (R-c) through δ_H 2.07 (R-b), from δ_H 3.47 (R-h) to δ_H 3.37 (R-j) through δ_H 2.06 (R-i), and the correlated signals of δ_H 3.13 (R-d) and δ_H 1.74 (R-e), δ_H 1.74 (R-e) and δ_H 1.79 (R-f), δ_H 1.79 (R-f) and δ_H 3.51 (R-g) indicated the presence of three aliphatic chains that were confirmed by the HMBC spectrum.

In the HMBC spectrum, the crossing peaks between δ_H 3.17 (R-c) and δ_C 50.0 (R-d), δ_H 3.13 (R-d) and δ_C 48.1 (R-c), δ_H 3.51 (R-g) and δ_C 46.2 (R-h), δ_H 3.47 (R-h) and δ_C 57.0 (R-g) demonstrated the sequence of the three aliphatic chains (Figure 2). The methane proton

Table 2 Cytotoxicity of NC1101 (IC_{50}, μM)

Cell	*MCF7/DOX*	*HepG2*	*HeLa*	*HCT116*
NC1101 (a)	1.59	1.36	2.60	0.90
Bleomycin (b)	2.25	2.96	4.22	1.55
Ratio (b/a)	1.42	2.18	1.62	1.72

signal at δ_H 7.95 (R-CH) showed coupling to δ_C 57.0 (R-g), δ_C 46.2 (R-h) and δ_C 39.9 (R-j), the double-bond carbon signal at δ_C 155.2 (R-CH) showed coupling to the protons at δ_H 3.51 (R-g), δ_H 3.47 (R-h) and δ_H 3.37 (R-j). The nitrogen atom attachments to R-g, R-h and R-j, which were revealed by the ^{13}C chemical shift of these carbons, demonstrated the presence of a nitrogen-containing heterocyclic ring in the terminal amine. Furthermore, the linkage of the terminal amine and the bleomycinamide was established with the correlated signal of δ_H 3.56 (R-a) and δ_C 166.4 (VI-CO). Eventually, the structure of the terminal amine was determined, and the ^1H-^1H COSY and selective HMBC correlations were indicated in Figure 2. The assignments for carbon signals of the terminal amine of NC1101 were shown in Table 1.

The cytotoxicity of NC1101 against four strains of tumor cells was evaluated with MTT assay.[6] NC1101 exhibited growth-inhibitory activity toward human tumor cells slightly higher than that of BLM (Table 2).

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