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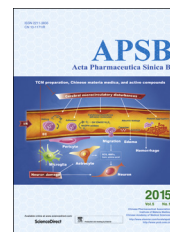


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ORIGINAL ARTICLE

# Chromopeptide A, a highly cytotoxic depsipeptide from the marine sediment-derived bacterium *Chromobacterium* sp. HS-13-94



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## KEY WORDS

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**Abstract** A bicyclic depsipeptide, chromopeptide A (**1**), was isolated from a deep-sea-derived bacterium *Chromobacterium* sp. HS-13-94. Its structure was determined by extensive spectroscopic analysis and by comparison with a related known compound. The absolute configuration of chromopeptide A was established by X-ray diffraction analysis employing graphite monochromated Mo  $K_{\alpha}$  radiation ( $\lambda=0.71073 \text{ \AA}$ ) with small Flack parameter 0.03. Chromopeptide A suppressed the proliferation of HL-60, K-562, and Ramos cells with average  $IC_{50}$  values of 7.7, 7.0, and 16.5 nmol/L, respectively.

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## 1. Introduction

The bacteria belonging to the genus *Chromobacterium* are known to be the main source of violacein, which shows important antitumor, antimicrobial, and antiparasitic activities<sup>1</sup>. *C. violaceum* is the most studied bacterium due to the isolation of romidepsin (**2**), which has been approved by the US FDA in 2009 for the treatment of refractory cutaneous T-cell lymphoma<sup>2</sup>. Romidepsin is a bicyclic depsipeptide composed of four amino acids (D-valine, D-cysteine, Z-dehydrobutyryne, L-valine) and (3*S*, 4*E*)-3-hydroxy-7-mercapto-4-heptenoic acid<sup>2</sup>. Inspired by this discovery, a series of depsipeptides structurally related to romidepsin, such as spiruchostatins, burkholdacs, and thailandepsins<sup>3–6</sup>, have been isolated and characterized.

In the course of our continuing search for new bioactive substances from marine microorganisms<sup>7–10</sup>, a bacterial strain, HS-13-94, belonging to the genus *Chromobacterium*, was recently isolated from the sediment collected from the East China Sea, Zhejiang Province, China. A chemical investigation of the whole culture of the bacterium HS-13-94 led to the isolation of a depsipeptide, chromopeptide A (**1**), which shares a common bicyclic framework as compound **2** but possessing an unusual trisulfide bond in the molecule. This paper describes the isolation, structural elucidation and cytotoxic activity of compound **1** (Fig. 1).

## 2. Results and discussion

Compound **1** was isolated as a colorless block crystal. Its molecular formula was deduced to be C<sub>24</sub>H<sub>36</sub>N<sub>4</sub>O<sub>6</sub>S<sub>3</sub> from the pseudo-molecular ion peak at *m/z* 595.1678 [M+Na]<sup>+</sup> (calcd. 595.1689) by HR-ESI-MS spectrum, indicating the presence of nine degrees of unsaturation. The IR spectrum showed absorption bands of amines and carbonyls at  $\nu_{\max}$  3350 and 1734 cm<sup>-1</sup>, respectively. The <sup>1</sup>H and <sup>13</sup>C NMR spectra (Table 1) showed diagnostic signals ascribable to five carbonyls ( $\delta_C$  171.1, 170.8, 169.7, 169.3 and 164.1) and four amide protons ( $\delta_H$  8.91, s; 8.16, d, *J*=4.3 Hz; 7.16, d, *J*=8.3 Hz; and 7.02, d, *J*=9.5 Hz), indicating the peptide nature of compound **1**. The presence of a tri-substituted double bond ( $\delta_C$  130.5 and 130.9;  $\delta_H$  6.44, d, *J*=7.1 Hz) and a disubstituted double bond ( $\delta_C$  129.9 and 131.2;  $\delta_H$  5.79, m and 5.73, brd, *J*=16.2 Hz) were easily recognized in the <sup>1</sup>H and <sup>13</sup>C NMR spectra. Five methyls, including one vinyl methyl ( $\delta_H$  1.61, d, *J*=7.1 Hz) and four secondary methyls ( $\delta_H$  1.05, d, *J*=6.8 Hz; 0.99, d, *J*=6.8 Hz; 0.90, d, *J*=6.8 Hz; and 0.88, d, *J*=6.8 Hz), were also observed in the <sup>1</sup>H NMR spectrum of **1**. These data account for seven of the required nine sites of unsaturation, implying compound **1** possesses two rings.

The presence of three amino acids (one cysteine and two valines) was established by extensive interpretation of the 2D NMR spectra of **1** (Fig. 2). Moreover, detailed analysis of the two-dimensional NMR spectra allowed for the identification of the other two fragments, dehydrobutyryne and acyl-(3*S*, 4*E*)-3-hydroxy-7-mercapto-4-heptenoic acid. All these elaborated subunits were connected mainly by HMBC correlations of the carbonyl carbon of one amide acid residue with the amide protons of the neighboring residue, and the carbonyl carbon signals in each amino acid unit were deduced by their HMBC correlations with the  $\alpha$  or  $\beta$  proton of their respective amino acids (Fig. 2), whereas the ester bond between the second valine and the hydroxyl in acyl-heptenoic acid group were deduced from the downfield chemical shift of C-3 ( $\delta_C$  71.6) (Table 1).

Subtraction of the atoms present in the above elaborated macrocyclic core from the molecular formula of **1** indicated that only two sulfide atoms remain unassigned. Bearing in mind the unsaturation degrees of **1**, the two sulfides have to connect with the sulfide atom of a cysteine, forming a trisulfide bridge between the two appendages at C(7) and the  $\beta$  carbon in the cysteine. As a consequence, the planar structure of chromopeptide A was thus constructed (Fig. 1), which was in good agreement with its molecular composition and nine degrees of unsaturation.

To secure the assigned structure and also to determine its absolute configuration, a suitable crystal of chromopeptide A, obtained from CHCl<sub>3</sub>/MeOH (1:1, *v/v*), was subjected to X-ray diffraction analysis (Fig. 3). The X-ray result not only completely agreed with the proposed structure but also allowed unambiguous assignment of its absolute configuration with absolute structure parameter 0.03(7) based on the final refinement on the Mo *K $\alpha$*  data, and due to the existence of heavy sulfur atom in **1**. Thus, the structure of **1**, including the absolute configuration, was confidently assigned.

A systematic literature review revealed that the structure of **1** was very similar to that of FR 901228 (romidepsin, **2**)<sup>11</sup>. In fact, the main difference between them was that a disulfide bond in **2** was replaced by a trisulfide bridge linkage in **1**. It is worthwhile to note that, although the structure of **1** had been mentioned in a previous report<sup>12</sup>, it actually describes a method for the preparation of **2**. To the best of our knowledge, this is the first report of the full NMR data assignment and X-ray structure of **1**.

The cytotoxic activities of **1** against tumor cell lines including HL-60 (acute promyelocytic leukemia), K-562 (chronic myelogenous leukemia), and Ramos (Burkitt's lymphoma) were evaluated by CCK-8 assay as described previously<sup>13,14</sup>. The results showed that **1** significantly suppressed the proliferation of HL-60, K-562, and Ramos cells, with average IC<sub>50</sub> values of 7.7, 7.0 and 16.5 nmol/L, respectively, which is comparable to that of **2**. Since the structure of **1** is very similar to that of **2**, an HDAC (histone deacetylase) inhibitor approved by the US FDA, we are currently evaluating the ability of **1** to target and inhibit HDACs.

## 3. Conclusions

In summary, chromopeptide A (**1**), a new depsipeptide structurally related to the known clinically used drug romidepsin, was isolated from a deep-sea-derived bacterium *Chromobacterium* sp. HS-13-94. Chromopeptide A exhibited potent cytotoxic activities against HL-60, K-562, and Ramos cell lines. In light of the observation that there is structural similarity between chromopeptide A and romidepsin, the biosynthetic pathway for chromopeptide A should probably be the same as that of romidepsin<sup>15</sup>. However, formation

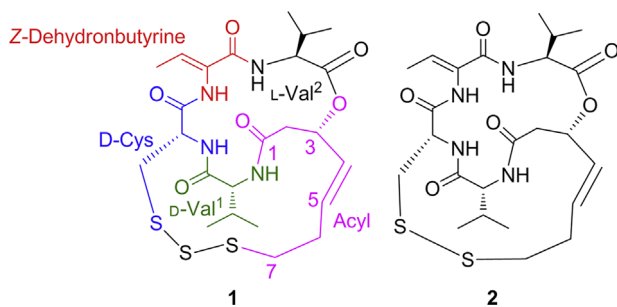
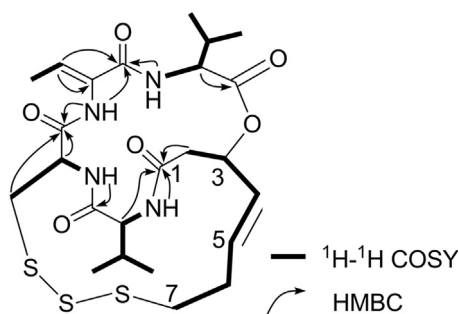


Figure 1 Structures of **1** and **2**.

**Table 1**  $^1\text{H}$  (400 MHz) and  $^{13}\text{C}$  NMR (100 MHz) data for chromopeptide A (**1**) in  $\text{DMSO-}d_6$ .

Fragment	Position	$\delta_{\text{H}}$ , mult. ( $J$ in Hz)	$\delta_{\text{C}}$ , mult.	Fragment	Position	$\delta_{\text{H}}$ , mult. ( $J$ in Hz)	$\delta_{\text{C}}$ , mult.
Acyl	1		171.1, C	D-Cys	NH	7.02, d (9.5)	
	2	2.58, dd (12.9, 6.4) 2.84, m	39.4, $\text{CH}_2$		$\alpha$	5.19, m	49.9, CH
	3	5.53, m	71.6, CH		$\beta$	2.82, m 3.83, dd (14.8, 3.0)	47.9, $\text{CH}_2$
	4	5.73, brd (16.2)	131.2, CH	Dehb	CO		169.3, C
	5	5.79, m	129.9, CH		NH	8.91, s	
	6	2.55, m	29.3, $\text{CH}_2$		$\alpha$		130.5, C
	7	2.96, m 3.23, m	40.2, $\text{CH}_2$		$\beta$	6.44, q (7.1)	130.9, CH
D-Val <sup>1</sup>				$\gamma$	1.61, d (7.1)	13.4, $\text{CH}_3$	
	NH	8.16, d (4.3)		CO		164.1, C	
	$\alpha$	3.89, dd (5.6, 4.6)	62.1, CH	L-Val <sup>2</sup>	NH	7.16, d (8.3)	
	$\beta$	2.19, m	29.2, CH		$\alpha$	4.45, dd (8.2, 4.6)	57.7, CH
	$\gamma$	0.99, d (6.8) 1.05, d (6.8)	19.7, $\text{CH}_3$ 19.8, $\text{CH}_3$		$\beta$	2.22, m	31.5, CH
CO		170.8, C	$\gamma$		0.88, d (6.8) 0.90, d (6.8)	19.5, $\text{CH}_3$ 18.7, $\text{CH}_3$	
				CO		169.7, C	

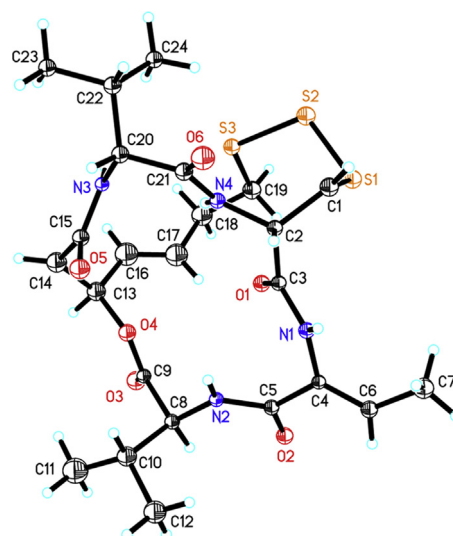
**Figure 2** Selected key COZY and HMBC correlations of **1**.

of the trisulfide bond in chromopeptide A is still unclear and further experimental investigation is needed. Moreover, in connection with the mode of action of FR 901228 (romidepsin, **2**)<sup>16</sup>, chromopeptide A may mediate antitumor activity through the same molecular mechanism. Further studies should be conducted to both understand its true biosynthetic pathway and to evaluate its use as an HDAC inhibitor.

## 4. Experimental

### 4.1. General procedure

Optical rotations were measured on a Perkin-Elmer polarimeter 343. UV spectrum was recorded on a Mariner System 5304 Spectrometer. IR spectrum was determined on a Nicolet 5700 FT-IR Microscope Spectrometer (FT-IR Microscope Transmission).  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were acquired on a Bruker DRX-400 spectrometer. The chemical shifts ( $\delta$ ) were reported in ppm, and coupling constants ( $J$ ) were given in Hz. The ESI-MS and HR-ESI-MS data were recorded on the Bruker APEX III 7.0T spectrometer. Macroporous resin (SP850 and HP20ss, Mitsubishi Chemical Industries Ltd.) was used for column chromatography. Reversed-phase HPLC was performed on an Agilent 1100 liquid chromatography equipped with a Diode Array Detector at 210 nm. A semi-preparative Agilent ZORBAX-XDB column [250 mm  $\times$  9.4 mm, 5  $\mu\text{m}$ ] was employed for the purifications. Pre-coated silica gel GF<sub>254</sub> plates (Qing Dao Hai Yang Chemical Group Co.,

**Figure 3** The X-ray crystallographic structure for **1**.

Ltd., Qingdao, China) were used for analytical thin-layer chromatography (TLC). All solvents used were of analytical grade (Shanghai Chemical Reagents Co., Ltd.).

### 4.2. Bacterial material

The bacterial strain HS-13-94 was isolated from sediment collected from the East China Sea, Zhejiang, China and deposited in the China General Microbiological Culture Collection Center with the accession number CGMCC No. 6247. It was initially identified as *Chromobacterium* species based on its morphological characters and 16S rDNA sequence as described in previous literature<sup>17</sup>.

### 4.3. Fermentation and isolation

The bacterial strain was cultured on slants of agar (10 g tryptone, 5 g yeast extract, 10 g NaCl, 15 g agar per liter of tap water) at 37  $^{\circ}\text{C}$  for 3 days. The seeds from the above mature slant culture was inoculated into 1 L Erlenmeyer flask containing 200 mL

sterile seed liquid medium containing 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl, and cultured on a rotary shaker (250 rpm) at 30 °C for 12 h. Fermentation was performed in 50 L fermentor containing 30 L culture medium (4% glucose, 2% starch, 2% peptone, 0.5% NaH<sub>2</sub>PO<sub>4</sub>, and 0.1% valine). The fermentor was inoculated with 3% of the seed culture and maintained on a 100 rpm rotary shaker at 30 °C for 2 days. Upon harvest, the filtrate of 30 L of fermentation culture was fractionated by column chromatography over the macroporous adsorbent resin SP850 (Mitsubishi Chemical, Japan) (acetone/H<sub>2</sub>O, 0%, 25%, and 65%, v/v). The fractions with similar TLC profiles were combined and separated on a column of macroporous resin HP20ss (acetone/H<sub>2</sub>O, 25%, 40%, and 50%, v/v) to obtain three fractions. The third fraction was extracted with EtOAc to give the crude extract, which was further purified by HPLC (MeOH/H<sub>2</sub>O v/v=65:35) to afford compound **1** (*t*<sub>R</sub> = 13.4 min, 27 mg).

#### 4.4. Characterization of **1**

Colorless crystal, [ $\alpha$ ]<sub>D</sub><sup>20</sup> = -84.6° (*c* = 0.026, EtOH); IR  $\nu_{\max}$  (KBr) cm<sup>-1</sup>: 3350, 2962, 2929, 1734, 1699, 1669, 1520, 1507, 1457, 1258, 1080. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) and <sup>13</sup>C NMR (100 MHz, DMSO-*d*<sub>6</sub>) spectroscopic data are shown in Table 1. HR-ESI-MS *m/z* 595.1678 [M+Na]<sup>+</sup> (Calcd. for C<sub>24</sub>H<sub>36</sub>N<sub>4</sub>O<sub>6</sub>S<sub>3</sub>Na 595.1689).

#### 4.5. Crystal data for **1**

Compound **1** was crystallized from CHCl<sub>3</sub>/MeOH (v/v = 1:1) to give colorless block. Crystal data: 2(C<sub>24</sub>H<sub>36</sub>N<sub>4</sub>O<sub>6</sub>S<sub>3</sub>)·CHCl<sub>3</sub>, *M* = 1260.89, trigonal, *a* = 14.6553(14) Å, *b* = 14.6553(14) Å, *c* = 28.089(3) Å,  $\alpha$  = 90.00°,  $\beta$  = 90.00°,  $\gamma$  = 120.00°, *V* = 5224.6 (9) Å<sup>3</sup>, *T* = 133(2) K, space group *P*3(2), *Z* = 3, 13559 reflections measured, 12196 independent reflections (*R*<sub>int</sub> = 0.039). The final *R*<sub>1</sub> values were 0.0681 (*I* > 2 $\sigma$ (*I*)). The final *wR*(*F*<sup>2</sup>) values were 0.1889 (*I* > 2 $\sigma$ (*I*)). The final *R*<sub>1</sub> values were 0.0740 (all data). The final *wR*(*F*<sup>2</sup>) values were 0.1888 (all data). The structure was solved by direct methods (SHELXS97) and refined by using full-matrix least-squares difference Fourier techniques. All non-hydrogen atoms were refined anisotropically, and all hydrogen atoms were placed in idealized positions and refined as riding atoms with their related isotropic parameters. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC 1019172. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033 or E-mail: deposit@ccdc.cam.ac.uk).

#### 4.6. Antitumor bioassays

For cytotoxicity measurements, stock epothilonoside solutions were prepared at 100 mg/mL in dimethyl sulfoxide and stored at -20 °C. Acute promyelocytic leukemia HL-60, chronic myelogenous leukemia K-562, and Burkitt's lymphoma Ramos were obtained from the Institute of Biochemistry and Cell Biology, Shanghai, Chinese Academy of Sciences. All cell lines were routinely cultured in Dulbecco's modified Eagle's medium containing 10% calf serum at 37 °C for 4 h, in a humidified atmosphere of 5% CO<sub>2</sub>. The adherent cells at their logarithmic growth stage were digested and inoculated onto a 96-well culture plate at a density of 1.0 × 10<sup>4</sup>/well for the determination of proliferation. Test samples

**Table 2** The antiproliferative activities of chromopeptide A (**1**) and romidepsin (**2**).

Compound	IC <sub>50</sub> (nmol/L)		
	HL-60	K-562	Ramos
<b>1</b>	7.7 ± 4.0	7.0 ± 1.0	16.5 ± 3.5
<b>2</b>	3.3 ± 3.2	6.7 ± 0.6	11.0 ± 2.8

ranging from 0.1–100 mg/mL in 100 mL were added to cells in triplicate wells, and incubation was continued for 72 h. Coloration substrate from cell counting kit-8 (CCK-8, Dojindo, Kumamoto, Japan), was added to the medium, followed by further incubation for 3 h. Absorbance at 450 nm with a 600 nm reference was measured. Media and dimethyl sulfoxide control wells in which the compound was absent were included in all the experiments in order to eliminate the influence of dimethyl sulfoxide. The inhibitory rate of cell proliferation was calculated by the formula (1) (where OD stands for optical density)

$$\text{Growth inhibition(\%)} = [\text{OD}_{\text{control}} - \text{OD}_{\text{treated}}] / \text{OD}_{\text{control}} \times 100 \quad (1)$$

The cytotoxicity of compound on tumor cells was expressed as IC<sub>50</sub> values (the drug concentration reducing the absorbance in treated cells by 50%, with respect to untreated cells) and was calculated by the LOGIT method (Table 2).

#### Acknowledgments

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