Supporting information

Tripartin, a Histone Demethylase Inhibitor from a Bacterium Associated with a Dung Beetle Larva

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General Experimental Procedures

Optical rotation was measured on a Jasco P-2000 polarimeter with a 1 cm path length cell. IR spectra were recorded on a Jasco FT/IR-4200 spectrometer. UV absorption spectra were acquired with a Hitachi U-3010 UV/VIS spectrophotometer. Electrospray ionization (ESI) low resolution mass spectra were recorded on an Agilent Technologies 6130 quadrupole mass spectrometer coupled with an Agilent Technologies 1200 series HPLC. High resolution mass spectra were obtained by a chemical ionization (CI) GC-MS, a Jeol, JMS-700 high resolution mass spectrometer with Agilent Technologies 6890 series GC at the National Center for Inter-University Research Facilities. 1D and 2D NMR spectra, including ^1H - ^1H COSY, HSQC, HMBC, and ROESY experiments, were collected on a JEOL, JNM-LA300 spectrometer operating at 300 MHz (^1H) and 75 MHz (^{13}C) and a Bruker Avance 500 spectrometer operating at 500 MHz (^1H) and 125 MHz (^{13}C) with chemical shifts given in ppm (δ). Semi-preparative HPLC was performed by using a Gilson 305 pump with a Gilson UV/VIS-155 detector. Flash column chromatography (40 mm diameter $\times$ 100 mm height, YMC-Gel ODS-A 12 nm S-75 μm) was performed by applying vacuum with a Gast pump. X-ray crystallographic data were acquired on a Rigaku R-AXIS RAPID diffractometer using graphite-monochromated Mo K α radiation ($\lambda = 0.71075 \text{ \AA}$).

Biomaterial and Isolation of the Bacterial Strain (SNC023)

The bacterial (SNC023) strain was isolated from a larva of the dung beetle *Copris tripartitus*. The larva of the dung beetle was taken from Korea Rural Development Administration, in May, 2010. A larva of the dung beetle was dissected with a sterilized surgery knife and scissors. The comminution of the larva (weight : 1 g) was diluted in 5 mL of sterilized deionized water and was spread on chitin-based agar, actinomycete isolation agar, A1+C, Czapek Dox agar with cycloheximide (100 mg/ L) and YPG agar with penicillin (150 mg/ L). The bacterial strain (SNC023) was isolated on chitin-based agar (chitin 4 g, K₂HPO₄ 0.75 g, MgSO₄·7H₂O 0.5 g, KH₂PO₄ 3.5 g, FeSO₄·7H₂O 10 mg, MnCl₂·4H₂O 10 mg, ZnSO₄·7H₂O 10 mg, and agar 15 g in distilled 1 L water).

Isolation of Ribosomal DNA, Gene Amplification, and Sequencing

Isolation of the chromosomal DNA of bacterial strain SNC023 was performed using a G-spinTM Genomic DNA extraction Kit. The 16S rDNA sequence data were acquired in

COSMO co, Ltd. Through the 16S rDNA analysis SNC023 is most closely related to *Streptomyces diastaticus* (99% identity), identifying the strain as *Streptomyces* sp.

Cultivation and Extraction

The bacterial strain (SNC023) was cultivated in twenty 2.8 L Firnbach flasks each containing 1 L of YPM medium (4 g mannitol, 2 g yeast extract, 2 g peptone in 1 L of distilled water) at 30 °C with shaking at 180 rpm. After inoculation of the bacterium (SNC023), time-course analysis was performed once every two days to monitor the production of secondary metabolites. On day 8, the whole culture (20 L) was extracted with 40 L of ethyl acetate. The ethyl acetate layer was concentrated *in vacuo* to yield 10 g crude extract.

Isolation and Purification of Tripartin (1)

The crude extract was fractionated using flash column chromatography by a step gradient elution with water and methanol (20%, 40%, 60%, and 80% methanol in water and 100% methanol) to yield five subfractions. The 40% methanol fraction was dried *in vacuo* and separated by preparative reversed-phase HPLC (Phenomenex Luna 5 μ m C₁₈(2) 250 $\times$ 21.20 mm column, flow rate 10 mL/min, UV 254 nm detection) under the gradient condition (30% - 65% aqueous methanol over 50 min) to obtain 7.0 mg of tripartin (**1**). Tripartin eluted at 34 min.

Tripartin (1)

White powder, $[\alpha]_D^{20}$ -109 (c 0.05, MeOH), UV (MeOH) $\lambda_{\max}$ (log ϵ) 217 (3.38) nm, 268 (2.77) nm, 330 (2.42) nm, IR (neat) $\nu_{\max}$ 3360, 3195, 2924, 2853, 1659 cm⁻¹, For ¹H (500 MHz) and ¹³C (125 MHz) NMR data, see Table 1, HR-CI mass spectrometry obsd [M+H]⁺ at *m/z* 262.9873, (calcd for C₁₀H₉Cl₂O₄ [M+H]⁺ 262.9878).

X-ray Crystallographic Data of Tripartin (1)

Colorless platelet crystal, $C_{12}H_{14}Cl_2O_5S_1$, $M_r = 341.21$, triclinic, space group P-1 (#2), $a = 8.1107(12)$ Å, $b = 9.1475(16)$ Å, $c = 11.4039(19)$ Å, $\alpha = 106.487(5)^\circ$, $\beta = 103.627(4)^\circ$, $\gamma = 104.156(4)^\circ$, $V = 743.1(2)$ Å³, $T = 23.0$ °C, $2\theta_{\max} = 54.9^\circ$, $Z = 2$, $D_{\text{calcd}} = 1.525$ g/cm³, crystal dimensions = $0.200 \times 0.100 \times 0.050$ mm, $F_{000} = 352.00$, The final R_1 value is 0.0449 ($wR_2 = 0.1268$) for 3358 all reflections [$I > 2.00\sigma(I)$].

Crystallographic data for tripartin (1) have been deposited to the Cambridge Crystallographic Data Centre. The deposition number is CCDC 923572. Copies of the data are available free of charge via the Internet at <http://www.ccdc.cam.ac.uk>. CCDC 12 Union Road Cambridge CB2 1EZ UK (Tel: +44 (0)1223-762911).

Acid Extraction of Histones

Acid extraction of histones was performed as described previously.¹ Briefly, HeLa cell pellets were suspended in 10 volumes of PBS and centrifuged at $200 \times g$ for 10 min. Cells were then suspended with five volumes of hypotonic lysis buffer [10 mM Tris-Cl (pH 8.0), 1.5 mM $MgCl_2$, 1 mM KCl, 1 mM DTT, and 1 mM phenylmethylsulfonyl fluoride] and 0.4 N H_2SO_4 at a final concentration of 0.2 M and subsequently lysed on ice for 30 min. After centrifugation at $16,000 \times g$ for 10 min at 4 °C, the cell supernatant fraction that contained acid-soluble proteins was retained. Trichloroacetic acid was added to the supernatant up to 33% and the samples were incubated on ice overnight. Proteins were pelleted by centrifugation at $16,000 \times g$ for 10 min at 4 °C and washed 4 times with ice cold acetone with centrifugations at $16,000 \times g$ for 5 min at 4 °C. The histone pellet was air-dried at room temperature for 20 minutes and dissolved in an appropriate volume of ddH₂O.

Western Blot Analysis

Acid extracted proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)/immunoblotting with antibodies recognizing methylated

histones H3K9 and histone H3. Protein samples were separated along with molecular weight markers (Bio-Rad, Hercules, CA, USA) in 15% polyacrylamide gels. Gels were transferred onto 0.45- μ m nitrocellulose membranes (Schleicher and Schuell). Species-specific immunoglobulin G-horseradish peroxidase (IgG-HRP) secondary antibodies were purchased from Bio-Rad. Blots were developed with chemiluminescent substrate (Amersham International, Buckinghamshire England), and autoradiography was performed by utilizing X-OMAT film (Kodak).

References

- (1) Shechter, D.; Dormann, H. L.; Allis, C. D.; Hake, S. B. *Nat. Protoc.* **2007**, 2, 1445.

Figure S1. The ^1H NMR spectrum of tripartin (**1**) in $\text{DMSO}-d_6$ (300 MHz)

Figure S2. The ^{13}C NMR spectrum of tripartin (**1**) in $\text{DMSO}-d_6$ (75 MHz)

Figure S3. The COSY NMR spectrum of tripartin (**1**) in DMSO- d_6 .

Figure S4. The HSQC NMR spectrum of tripartin (**1**) in DMSO- d_6 .

Figure S5. The HMBC NMR spectrum of tripartin (**1**) in DMSO-*d*₆.

Figure S6. The proposed biosynthesis of tripartin (**1**).