BIOMOLECULES & THERAPEUTICS

Original Article
Biomol Ther 32(4), 492-498 (2024)

Anti-Melanogenic Activity of Undecylprodigiosin, a Red Pigment Isolated from a Marine *Streptomyces* sp. SNA-077

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Abstract

Bioassay and HPLC-UV guided fractionations of the crude extract of marine-derived *Streptomyces* sp. SNA-077 have led to the isolation of a red pigment, undecylprodigiosin **(1)**. The chemical structure of undecylprodigiosin **(1)** was revealed by the interpretation of NMR and mass spectroscopic (MS) data. Further, anti-melanogenic effects of undecylprodigiosin **(1)** were investigated. First, the melanin contents of undecylprodigiosin **(1)**-treated B16 cells were evaluated. Furthermore, undecylprodigiosin **(1)** significantly inhibited the key enzymes involved in melanogenesis, including tyrosinase, tyrosinase related protein-1 (TYRP-1), and dopachrome tautomerase (DCT). The mRNA and protein expression levels of Microphthalmia-associated transcriptian factor (MiTF), a critical transcription factor for tyrosinase gene expression, were also suppressed by undecylprodigiosin **(1)** treatment in B16 analyses. Collectively, our results suggest for the first time that undecylprodigiosin **(1)**, a potent component isolated from an extract of marine *Streptomyces* sp. SNA-077, critically exerts the anti-melanogenic ability for melanin synthesis.

Key Words: Undecylprodigiosin, *Streptomyces* sp., Red pigments, Marine natural products, Anti-melanogenic

INTRODUCTION

Melanin plays a role in protecting the skin from external stimuli such as ultraviolet B (UVB). However, hyperpigmentation of the skin can cause abnormal skin disorders such as spots and freckles, and also lead to unwanted cosmetic results (Swalwell *et al*., 2012). In particular, tyrosinase expression or activation is the key process for melanogenesis because tyrosinase oxidizes L-tyrosine to L-DOPA (3,4-dihydroxyphenylalanine), and then oxidizes L-DOPA to DOPA quinone, resulting in melanin production during melanogenesis (Hearing and Tsukamoto, 1991).

To solve this problem, many researchers have developed depigmenting agents from plants because bioactive materials isolated from natural plants have relatively low toxicity and high efficacy (Jeong *et al*., 2015; Kim *et al*., 2019; Lee *et al*., 2019). Recently, bioactive materials from microorganisms, and bio-active compounds produced by marine microorganisms are particularly investigated (Lim *et al*., 2021; Lee *et al*., 2022; Lim *et al*., 2022).

Marine microorganisms physically and chemically interact with their nearby microbes, which is thought to be driving factor in the production of bioactive secondary metabolites with medicinal potential (Mincer *et al*., 2002; Ranjani *et al*., 2016; Carroll *et al*., 2021; Kim *et al*., 2022). Marine microorganisms, especially marine derived-actinomycetes have been found as a significant source of structurally distinct secondary metabolites (Kala and Chandrika, 1993; Fenical and Jensen, 2006; Lam, 2006; Lee *et al*., 2022). Members of the genus *Streptomyces* have received increased interests among the numerous genera of marine actinomycetes (Udwary *et al*., 2007; Prieto-Davó *et al*., 2008; Dharmaraj, 2010; Ryu *et al*., 2021). *Streptomyces* possess remarkable capabilities for producing clinically valuable antibiotics. Although actinomycetes can also be isolated from marine environments, the bioactivity of natural products produced by these marine-derived strains has been less thoroughly explored (Fenical and Jensen, 2006). *Streptomyces*, which are Gram-positive bacteria with

Open Access **https://doi.org/10.4062/biomolther.2023.208**

Received Nov 28, 2023 **Revised** Jan 15, 2024 **Accepted** Feb 14, 2024
Published Online Apr 23, 2024

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a colony shape resembling hyphae (filamentous fungi), can produce metabolically active secondary metabolites, including antibiotics, antivirals, antifungals, antitumor agents, and immunosuppressants.

Our research team has conducted studies to discover natural products with minimal cytotoxicity and potent anti-melanogenic properties from microbial extracts with established stability. Based on metabolite analysis of bacterial extracts using chemical analysis techniques and liquid chromatography-mass spectroscopy (LC-MS) and nuclear magnetic resonance (NMR)-based structural elucidation, skin-whitening active components such as deoxyvasicinone from *Streptomyces* sp. CNQ617, pseudoalteromone A from *Pseudoalteromonas* spp., acremonidin E from *Penicillium* sp. SNF123, and (-)-4-hydroxysattabacin from *Bacillus* sp. were identified (Kim *et al*., 2017; Kim *et al*., 2021; Lim *et al*., 2021; Lee *et al*., 2022). The aim of our ongoing research is to discover anti-melanogenic agents from microorganisms for use as constituents of cosmetic products.

In recent years, the utilization of natural pigments across diverse industrial sectors such as food, cosmetics, and healthcare has experienced notable growth (Numan *et al*., 2018; Koyande *et al*., 2019). These natural pigments present alternatives to synthetic colorants, known for their potential health hazards. Certain synthetic colorants have been removed from industrial use due to evidence of carcinogenicity and environmental concerns (Numan *et al*., 2018). Concurrently, the potential antimicrobial attributes of certain bio-dyes are emerging as promising options to address the emergence of antibiotic-resistant pathogens, which pose significant public health challenges. This has spurred the exploration of new bioactive molecules. Microorganisms offer distinct advantages for pigment production, including rapid growth in cost-effective culture media, short replication times, high growth rates, uncomplicated purification processes, and climate-independent production. Bio-dyes may also exhibit additional beneficial biological activities, including antioxidant, antiviral, antibacterial, and anticancer properties (Islan *et al*., 2022). For example, phycocyanin, a pigment-protein complex found in Cyanobacteria, Rhodophyceae, and Cryptophyceae, has demonstrated antitumor efficacy in treating various cancer types (Kannaujiya *et al*., 2019). Similarly, violacein, primarily synthesized by *Chromobacterium violaceum*, exhibits antiviral, antiprotozoal, anticancer, and antioxidant attributes (Durán *et al*., 2021).

In a previous study, the crude extract of SNA-077 culture broth was reported to inhibit melanogenesis by downregulating melanogenic proteins via inactivation of cAMP/PKA/CREB signaling (Lim *et al*., 2022). Furthermore, due to the red coloration of the extract, the possibility of a red-colored pigment produced by *Streptomyces* sp. SNA077 was hypothesized. To isolate pigments in the SNA-077 extract, a systematic fractionation process was performed. The peak observed at 535 nm with a retention time of 13.409 min in LC/MS spectral data was identified as a member of the prodigiosin alkaloid family of natural compounds. This classification was established using a robust match score of 998 based on comparison with an in-house spectral library (Supplementary Fig. 1).

Numerous natural red pigments have been identified within the biomass of various species, including *Pseudomonas*, *Serratia*, and *Streptomyces*. Notably, prodigiosin, a vibrant red tripyrrole bacterial pigment, belongs to the category of bioactive colored molecules derived from microbial fermentation. Prodigiosin is primarily produced by the human pathogen *Serratia marcescens* (Wasserman *et al*., 1960), as well as other species like *Alteromonas rubra*, *Pseudomonas magneslorubra*, *Hahella chejuensis* (Kim *et al*., 2007), *Colwellia psychrerythraea*, and Gram-positive actinomycetes such as *Streptoverticillium rubrireticuli*, *Streptomyces longisporus ruber* (Lim *et al*., 2022), *Streptomyces variegatus*, and *Streptomyces grisioviridis* (Kawasaki *et al*., 2009). Operating as a characteristic secondary metabolite during the bacterial idiophase, prodigiosin exhibits substantial significant therapeutic potential, including properties such as anticancer, antimalarial, antifungal, and antibacterial activities (Yip *et al*., 2021).

Recent reports underscore the multifaceted bioactivity of prodigiosin and its analogues, showcasing their effectiveness as biological control agents against harmful algae, cell growth regulators, and natural dyes (Lee *et al*., 2011). The combination of these bioactive properties with their distinctive red pigment characteristics opens up promising applications across various sectors, including medicine, biotechnology, and cosmetics.

In this study, we have isolated undecylprodigiosin from marine-derived *Streptomyces* sp. SNA-077, obtained from marine sediment collected off the coast of Yeosu, Korea. The chemical structure of the isolated compound was elucidated using UV, LRMS and NMR spectrum analysis. Additionally, we have demonstrated the anti-melanogenic activity of undecylprodigiosin **(1)**.

MATERIALS AND METHODS

General experimental procedures

The NMR spectra were acquired with a JEOL NMR spectrometer (JEOL Ltd., Tokyo, Japan) operating at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) in $CDCl_3$ (Cambridge Isotope Laboratories (CIL), Inc., Tewksbury, MA, USA) using a solvent signal as internal reference (δ_H 7.26 ppm and δ_C 77.2 ppm). The low-resolution LC/MS data were obtained on the Agilent Technologies (Santa Clara, CA, USA) 1260 quadrupole and Waters Corp (Milford, USA) Micromass ZQ LC/MS system using a reversed-phase column (Phenomenex, Torrance, CA, USA; Luna C18 (2) 100 Å, 50×4.6 mm, 5 μm) at a flow rate of 1.0 mL/min at the National Research Facilities and Equipment Center (NanoBioEnergy Materials Center) at Ewha Womans University (Seoul, Korea). Open column chromatography was performed using silica (40-63 μm, Merck silica gel 60, Kenilworth, NJ, USA) eluting with a gradient solvent of dichloromethane (CH_2Cl_2) and methanol (MeOH). Medium-pressure liquid chromatography (MPLC) was performed on Biotage-Isolera One system (SE-751 03, Biotage, Uppsala, Sweden) using Biotage SNAP KP-Sil. The fractions were purified via semi-preparative HPLC using a Waters 996 Photodiode Array Detector HPLC coupled with a reversed-phase Phenomenex Luna C18 (2) (100 Å, 250 nm×10 mm, 5 μm) column with a mixture of acetonitrile, methanol, and H_2O at a flow rate of 3.0 mL/min.

Collection and phylogenetic analysis of the SNA-077 strain

The marine-derived *Streptomyces*, strain SNA-077, was isolated from marine sediment collected off the coast of Yeosu, Korea and identified as *Streptomyces* sp. based on 16S

rRNA gene sequence analysis. The phylogenetic analysis revealed this strain showed 99.9% similarity to *Streptomyces coelicoflavus* based on the result of NCBI blast analysis of the partial 16S rDNA. The gene sequence data are available from GenBank (deposit # NR_041175).

Cultivation and extraction

Streptomyces strain SNA-077 was cultured in 80 L of 2.5 L Ultra Yield Flasks (Thomson Instrument Company, Oceanside, CA, USA) each containing 1 L of the medium (10 g/L of soluble starch, 2 g/L of yeast, 4 g/L of peptone, 34.75 g/L of sea salt dissolved in 1 L of distilled water) with agitation at 120 rpm and a temperature of 27°C. After 168 h, the culture broth was extracted with ethyl acetate (EtOAc; 80 L in total) to obtain 3.9 g of EtOAc extract.

Isolation of compound

The crude extract (3.9 g) of the SNA-077 strain was fractionated using silica open column chromatography with a step gradient of CH_2Cl_2 and MeOH (99/1, 98/2, 96/4, 95/5, 90/10, 80/20, 50/50, 0/100, v/v, 600 mL for each gradient) to produce fractions F1-F8. The first fraction (F1) was partitioned between water and chloroform ($CHCl_3$), with the chloroform phase containing all red pigments. The chloroform phase was separated using MPLC eluting with a mixture of n-hexanes:ethyl acetate (2:1 [v/v]). The MPLC fractions containing the desired compounds were pooled and separated with thin-layer chromatography (TLC) using a 95:5 (v/v) mixture of chloroform:methanol. The isolation of undecylprodigiosin was obtained using reversed-phase HPLC (Phenomenex Luna C-18 (2) 100 Å, 250×10 mm, 5 μm) at the absorption wavelength of 535 nm, utilizing mobile phases consisting of 0.1% trifluoroacetic acid (TFA) in water (solvent A) and acetonitrile/methanol (1:1; solvent B) to yield 7.8 mg undecylprodigiosin (**1**, t_R 15.6 min). The elution was performed using a gradient program at 3 mL/min with the following conditions: 0-30 min 70-100% solvent B (linear gradient), 30-35 min 100% solvent B, and 35-40 min 70% solvent B for column re-equilibration.

Undecylprodigiosin **(1)**: red amorphous powder; ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 7.28 (1H), 7.01 (s, 1H), 6.97 (1H), 6.85 (1H), 6.37 (1H), 6.21 (1H), 6.10 (s, 1H), 4.02 (s, 3H, O-CH_3), 2.79 (s, 2H), 1.21-1.26 (m, 20H), 0.86, (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C 14.2 (CH_3), 22.8 (CH_2), 28.4 (CH_2), 29.2 (CH_2), 29.4-29.7 (CH_2), 32.0 (CH_2), 58.9 (O-CH_3), 93.7 (CH), 112.3 (CH), 112.7 (CH), 116.6 (CH), 118.8 (CH), 128.7 (CH), 130.1 (CH), 171.6 (qC); LRMS *m/z* 394.28 $[M+H]^+$

Cell culture

The B16 murine melanoma cell line was purchased from ATCC and cultured in Dulbecco's modified Eagle's medium (DMEM; Welgene, Gyeongsan, Korea) supplemented with 5% fetal bovine serum (FBS; ATCC, Manassas, VA, USA) and 1% penicillin-streptomycin mixture (PS; Lonza, Basel, Switzerland). B16 cells were cultured at 37°C in a humidified environment with 5% CO_2 and 95% air.

Cell viability assay

Cell viability was determined using Quanti-MAX™ WST-8 Cell Viability Assay Kit (Biomax, Seoul, Korea) according to the manufacturer's protocols. Cells were seeded in a 96-well plate at an appropriate concentration for 24 h. Next, the culture medium was replaced with fresh culture medium containing undecylprodigiosin diluted to the indicated concentrations. Following exposure for 72 h, cell viability was assessed by replacing the medium with the appropriate medium containing 10% WST-8 solution. The plate was incubated for 1 h and the absorbance was measured at 450 nm using a Microplate Reader (Bio Tek Instruments, Winooski, VT, USA). Cell viability was computed with the following general formula: cell viability (%)=optical density (450 nm) of the experimental group/optical density of the control group×100.

Measurement of melanin content

For melanin quantification, B16 cells were cultured for 24 h in 48-well plates. B16 cells were treated with the concentrations of undecylprodigiosin in the presence of α-MSH (0.1 μM) in phenol red-free cell culture medium. After 72 h, for measurement of intracellular melanin contents, 1N NaOH was added to each well, the plate was heated to 60°C for 30 min, the resulting lysate was aliquoted to a 96-well plate, and absorbance was determined at 405 nm. The intracellular melanin content was normalized with respect to the total protein amount. Melanin levels were calculated by comparison to the corresponding levels in the controls and shown as percentages. The measurement of extracellular melanin content was determined by measuring absorbance using media. Photography was used to document the color of culture media.

Tyrosinase activity assay

Tyrosinase activity was estimated by measuring the oxidase activity using mushroom tyrosinase or intracellular tyrosinase of B16 cells. The reaction mixture consisted of sodium phosphate buffer (0.1M), pH 6.8, distilled H_2O, and tyrosinase solution. After adding the undecylprodigiosin to a 96-well plate, the prepared reaction mixture was added. Kinetic spectrophotometric determination of the dopachrome level in the mixture was performed at 475 nm for at least 5 times per 10 min at an incubation temperature of 37˚C.

Western blot analysis

B16 cells were cultured in 6-well plates and treated with the indicated doses of undecylprodigiosin. The following day, cells were washed twice with cold PBS and total intracellular proteins were extracted in RIPA buffer 1X (Cell Signaling Technology, Danvers, MA, USA) supplemented with a 1:200 dilution of protease inhibitor cocktail III for 20 min on ice. After centrifugation at 13,000 rpm at 4°C, the supernatant was collected. A Pierce BCA Protein Assay Kit was used to estimate the protein quantity in extracts. Equal amounts of soluble proteins were resolved on 10% SDS-PAGE and transferred to a nitrocellulose membrane (BioRad, Hercules, CA, USA) in cold transfer buffer (25 mM Tris, 192 mM glycine, and 20% (w/v) MeOH) for 90 min at 280 mA. Membranes were blocked for at least 1 h with TBS 1X supplemented with 5% Blotting-Grade Blocker (BioRad) and incubated overnight at 4°C with primary antibodies diluted to the proper concentration (per the provided data sheet) in 1X TBS. Following overnight incubation, the membranes were further incubated with secondary antibodies and enhanced using a Clarity™ Western ECL substrate (ECL solution; BioRad). Images of the blotted membranes were captured with an iBright™ CL750 Imaging System (Invitrogen, Carlsbad, CA, USA). Equal loading was assessed by comparison to the endogenous actin protein.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

To determine the expression levels of mRNAs encoding melanogenesis-related proteins, B16 cells were cultured in 6-well plates and treated with the indicated doses of undecylprodigiosin. After 24 h, cells were washed twice with cold PBS and total RNA was extracted from cell lysates using the TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Complimentary DNA was synthesized from total mRNA using the GoScript™ Reverse Transcription System (Promega, Madison, WI, USA) and a thermal cycler (T-100; BioRad). The relative expression level of target cDNA was detected using the CFX Connect Optics Module (BioRad) and iQ SYBR® Green Supermix (BioRad). All results were normalized with respect to the mRNA level of β-actin. The specific primers were purchased from BioRad.

Statistical analysis

Data were expressed as the mean standard deviations (SDs) and statistical significance was analyzed using the Student's t-test. *P*-values <0.05 were considered statistically significant.

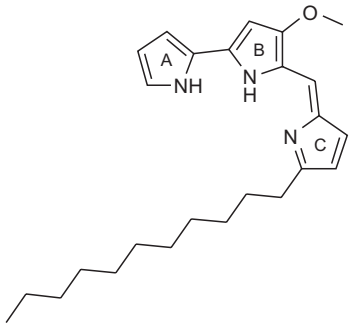

Fig. 1. Chemical structure of undecylprodigiosin **(1)**.

RESULTS

Identification of undecylprodigiosin

Compound **1** was obtained as red amorphous powder with an ionic peak of *m/z* 394.28 $[M+H]^+$ on LRMS. The NMR spectrum of compound **1** displayed proton signals at δ_H 7.28 (1H), 7.01 (s, 1H), 6.97 (1H), 6.85 (1H), 6.37 (1H), 6.21 (1H), 6.10 (s, 1H), 4.02 (s, 3H, O-CH3), 2.79 (s, 2H), 1.21-1.26 (m, 20H), and 0.86 (s, 3H) and carbons at δ_C 14.2, 22.8, 28.4, 29.2, 29.4-29.7, 32.0, 58.9, 93.7, 112.3, 112.7, 116.6, 118.8, 128.7, 130.1, and 171.6. Based on interpretation of the MS and 1D NMR spectroscopic data and comparison of the NMR data to previous reports (Bikash *et al*., 2021), compound **1** was identified as undecylprodigiosin.

Undecylprodigiosin, distinguished by its vibrant deep red color, is synthesized by various microorganisms, particularly *Streptomyces* spp. and related actinobacteria (Fürstner, 2003). Three pyrrole rings, designated as A, B, and C, compose the structure of prodigiosin and its analogs. The A and B rings form a bipyrrole unit and a dipyrrin linkage connects the B and C rings. A methylene bridge connects the monopyrrole moiety (C ring) to the methoxy-substituted bipyrrole moiety (A and B rings) (Yip *et al*., 2021). The tricyclic configuration of prodigiosin is shown in Fig. 1.

Anti-melanogenic efficacy of undecylprodigiosin in B16 cells

Prior to evaluating the anti-melanogenic efficacy of undecylprodigiosin in B16 cells, the cytotoxic concentration was assessed using undecylprodigiosin-treated B16 melanoma cells. Undecylprodigiosin was treated to B16 cells for 72 h. In Fig. 2A, undecylprodigiosin showed slight cytotoxicity at 194 nM. Thus, all experiments were performed at concentrations < 100 nM in the present study. Next, the anti-melanogenic efficacy of undecylprodigiosin in α-MSH-treated B16 cells was confirmed. The indicated concentrations of undecylprodigiosin were treated with α-MSH and incubated for 72 h. Lastly, melanin contents at both the extracellular and intracellular levels were analyzed. As shown in Fig. 2B, undecylprodigiosin treatment decreased the extracellular melanin levels in a dose-

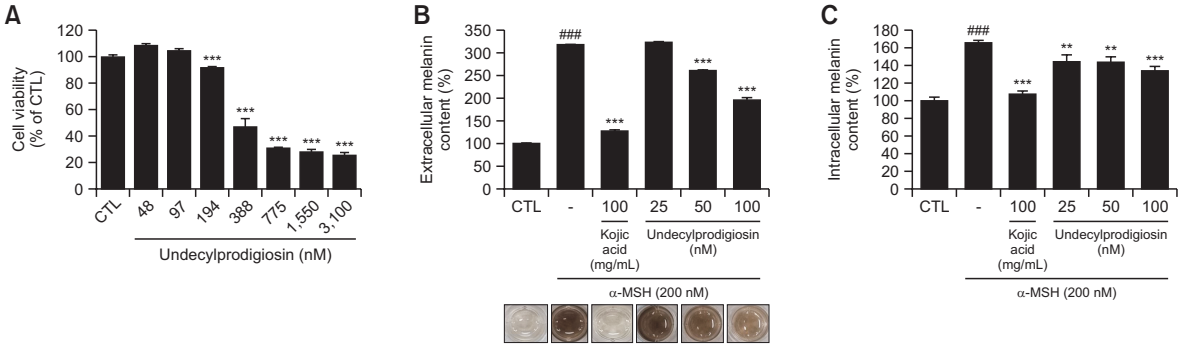

Fig. 2. Anti-melanogenic Efficacy of undecylprodigiosin in B16 Cells. (A) Effect of undecylprodigiosin on B16 cell viability. B16 cells were treated with the indicated concentrations of undecylprodigiosin for 72 h, then the cell viability was estimated using CCK-8 assays. (B, C) Effects of undecylprodigiosin and kojic acid, a positive compound, on extracellular and intracellular melanin content in α-MSH-stimulated B16 cells treated with the indicated concentrations of undecylprodigiosin for 72 h. Melanin content was determined by measuring absorbance at 405 nm and normalizing values to protein quantity, determined using a protein assay kit. Photographs show colors of medium in each well following treatment with α-MSH and undecylprodigiosin. Results are presented as means ± SD of three independent experiments, expressed as a percentage relative to the control group ($^{###}p$<0.001 vs. control (non-treated) group; $^{**}p$<0.01, $^{***}p$<0.001 vs. α-MSH-treated group).

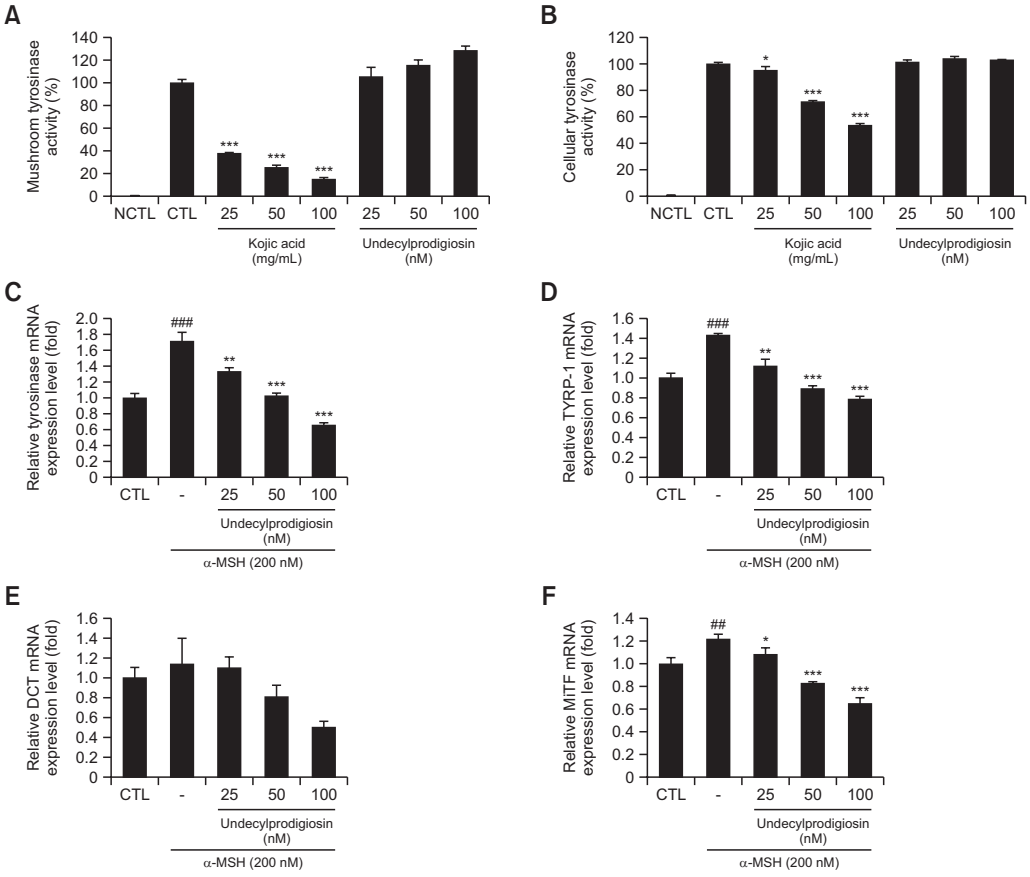

Fig. 3. Inhibitory effect of undecylprodigiosin on the mRNA expression of melanogenic molecules. (A) Mushroom tyrosinase activity and (B) cellular tyrosinase activity were measured spectrophotometrically at 475 nm as indicated in the materials and methods. Results are presented as means ± SD of three independent experiments, expressed as a percentage relative to the control group. (C-F) mRNA expression levels of melanogenic molecules were determined by real-time PCR analysis in B16 cells. Cells were treated with the indicated concentrations of undecylprodigiosin for 24 h. The mRNA levels of the genes encoding tyrosinase, TYRP-1, DCT and MiTF were normalized to that of β-actin ($^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 vs. control (non-treated) group; $^{*}p$<0.05, $^{**}p$<0.01, $^{***}p$<0.001 vs. a-MSH-treated group).

dependent manner. Especially, 100 nM undecylprodigiosin significantly inhibited melanin synthesis in B16 cells. In addition, a brightened color of culture media was clearly observed. Using the cell lysates, undecylprodigiosin also significantly reduced the intracellular melanin levels in a dose-dependent manner (Fig. 2C). Kojic acid was used as a positive control and significantly decreased melanin amounts in both extracellular and intracellular melanin contents.

Inhibitory effect by undecylprodigiosin on the expression of melanogenic proteins in B16 cells

Next, the underlying molecular mechanism of undecylprodigiosin for anti-melanogenesis was investigated. First, mushroom tyrosinase activity assay was used to determine whether undecylprodigiosin affects tyrosinase activity. However, undecylprodigiosin did not show any inhibitory effects on mushroom tyrosinase activity (Fig. 3A). Similar results were obtained using murine B16 cell lysates instead of mushrooms (Fig. 3B). However, kojic acid clearly suppressed mushroom and cellular tyrosinase activity. The results showed that undecylprodigiosin was not involved in inhibiting tyrosinase activity for melanogenesis. Therefore, the effect of undecylprodigiosin on suppressing mRNA expression of tyrosinase rather than its activation was investigated. Undecylprodigiosin was treated to α-MSH-treated B16 cells for 24 h. Notably, undecylprodigiosin suppressed tyrosinase gene expression in a dose-dependent manner in α-MSH-treated B16 cells (Fig. 3C). In addition, the mRNA expression levels of tyrosinase-related protein (TYRP)-1 and dopachrome tautomerase (DCT), the melanogenic enzymes for melanin synthesis, were analyzed. Undecylprodigiosin clearly inhibited mRNA expression of TYRP-1 but not DCT (Fig. 3D, 3E). In particular, 100 nM undecylprodigiosin suppressed both tyrosinase and TYRP-1 expression below the control group (CTL) level and also showed a significant decrease in DCT expression. Finally, the expression level of microphthalmia-associated transcription factor (MiTF), a key transcription factor for the tyrosinase expression, was significantly downregulated by undecylprodigiosin treatment in B16 cells (Fig. 3F).

Finally, the protein expression levels of tyrosinase, TYRP-1, DCT, and MiTF were confirmed. In Fig. 4A, undecylprodigiosin inhibited tyrosinase protein expression as well as mRNA expression in α-MSH-treated B16 cells. In particular, tyrosinase appeared with multiple bands between 50-80 kDa, because tyrosinase proteins undergo post-translational modifications such as glycosylation or alternative splice variants. In Fig. 4B,

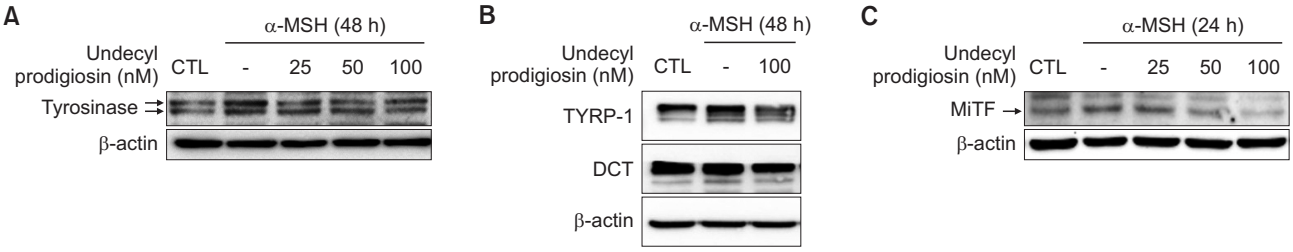

Fig. 4. Inhibitory effect of undecylprodigiosin on the protein expression of tyrosinase, TYRP-1, DCT, and MiTF. (A, B) Protein expression levels of tyrosinase, TYRP-1, and DCT were determined by Western blot analysis in α-MSH-treated B16 cells. Cells were treated with the indicated concentrations of undecylprodigiosin for 48 h. (C) Protein expression level of MiTF was determined by Western blot analysis in α-MSH-treated B16 cells. Cells were treated with the indicated concentrations of undecylprodigiosin for 24 h. Equal amounts of proteins were resolved by SDS-PAGE on 10% gels and detected using tyrosinase, TYRP-1, DCT, and MiTF antibodies; β-actin was detected as a control for equal loading.

TYRP-1 and DCT protein level were slightly reduced by 100 nM undecylprodigiosin treatment. MiTF protein level was also decreased by undecylprodigiosin in a dose-dependent manner in α-MSH-treated B16 cells (Fig. 4C). Based on the above data, undecylprodigiosin appeared to exert anti-melanogenic effects through the reduction of tyrosinase expression involved in MiTF inhibition.

DISCUSSION

The prodiginine group to which prodigiosin belongs, consists of structural isomers that share a tripyrrole core with various alkyl chains. Prodigiosin and its isoform pigments such as nonylprodigiosin, undecylprodigiosin, metacycloprodigiosin, norprodigiosin, and roseophilin have been reported to exhibit significant biological properties including antibacterial, antimalarial, immunosuppressive, anticancer, and antioxidant activities (Lee *et al*., 2011).

Undecylprodigiosin, a well-studied bioactive compound (Tsao *et al*., 1985; Malpartida *et al*., 1990), is produced by specific microorganisms, particularly certain strains of *Streptomyces* bacteria. This compound has been thoroughly investigated for its potential therapeutic properties, including antitumor, immunosuppressant, antifungal, and antimalarial activities (Stankovic *et al*., 2014; Bikash *et al*., 2021). This comprehensive examination of prodigiosin's performance across diverse applications provides robust support for its potential as a versatile and valuable compound in the fields of medicine, biotechnology, and cosmetics.

Our previous study demonstrated the significant inhibition on melanin synthesis of the extracts of *Streptomyces* sp. SNA077 (Lim *et al*., 2022). Following these results, the anti-melanogenic efficacy of undecylprodigiosin, a compound isolated from *Streptomyces* sp. SNA077, was anticipated. In current study, the anti-melanogenic efficacy of undecylprodigiosin was evaluated for the first time using B16 murine melanoma cells. The inhibitory effect of undecylprodigiosin and the extracts of *Streptomyces* sp. SNA077 on melanogenesis was both clearly shown. In addition, the molecular biological mechanisms that inhibited tyrosinase, TYRP-1, DCT, and MiTF expression in α-MSH-treated B16 cells were also similar. Although extracts of *Streptomyces* sp. SNA077 downregulate melanogenic proteins by inactivating the cAMP/PKA/CREB signaling pathway, the signaling pathway for the anti-melanogenic effects of undecylprodigiosin remains unexplored. To unravel the signaling pathway of undecylprodigiosin in melanocytes, an in-depth further investigation is imperative. Additionally, the anti-melanogenic efficacy of undecylprodigiosin will be further investigated using a 3D human equivalent skin tissue model in upcoming studies. This study suggests that undecylprodigiosin could be as the main component for the anti-melanogenic effect of the extracts of *Streptomyces* sp. SNA077 and have the potential to be used in the future as a treatment for pigmentary disorders or as a raw material for whitening cosmetics.

ACKNOWLEDGMENTS

This research was funded by the National Research Foundation of Korea grant funded by the Korean Government (Ministry of Science and ICT; no. 2021R1A4A2001251 to S.-J.N.), and also supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. NRF-2022R1A2C1011848).

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