VOLUME 8 NOMOR 2 DESEMBER 2021 **ISSN 2548 – 611X**

JURNAL
BIOTEKNOLOGI & BIOSAINS INDONESIA

Homepage Jurnal: http://ejurnal.bppt.go.id/index.php/JBBI

A GLUTARIMIDE FROM THE INDONESIAN MARINE CYANOBACTERIUM *Oscillatoria* sp.

Glutarimida dari Sianobakteri Laut Indonesia *Oscillatoria* sp.

Viqqi Kurnianda[1], Khairunnisa[1], Sofyatuddin Karina[1], Sri Agustina[1], Nurfadillah Nurfadillah[2], Musri Musman[3]*
[1]Department of Marine Science, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh, 23111, Indonesia
[2]Department of Aquaculture, Faculty of Marine and Fisheries, Syiah Kuala University, Banda Aceh, 23111, Indonesia
[3]Department of Chemistry Education, Faculty of Teacher Training and Education, Syiah Kuala University, Banda Aceh, 23111, Indonesia
*Email: musrimusman@unsyiah.ac.id

ABSTRACT
In continuous concern of marine resources relating to molecular structures that have biological activity around the western part of the Indonesian Archipelago, a marine cyanobacterium species Oscillatoria *sp. was found in Sabang Island, Aceh. This study aimed at investigating the chemical and biological contents of* Oscillatoria *sp. The isolation of the target molecule was carried out based on bioassay-guided separation with several steps of chromatography. In addition, the evaluation of bioactivity was determined by the MTT assay protocol against the human pancreatic cancer Panc-1 cell line. The planar structure of the target molecule was determined by spectroscopic analysis. Results showed that the whole planar structure was identified as cycloheximide-4-acetate (**1**), a cycloimide class of molecule. Moreover, the relative stereochemistry was confirmed by nuclear overhauser effect (NOE) analysis. Therefore, the molecule was assigned as (2S*,4S*,6S*,7R*)-cycloheximide-4-acetate. The cytotoxicity evaluation of compound **1** showed IC_{50} value of 0.32 μM against the human pancreatic cancer cell.*

Keywords: *cyanobacterium, cycloimide, cytotoxicity,* Oscillatoria *sp., Panc-1*

ABSTRAK
Dalam perhatian berkelanjutan terhadap sumber daya laut yang berkaitan dengan struktur molekul yang memiliki aktivitas biologis di sekitar bagian barat Kepulauan Indonesia, spesies sianobakteria *Oscillatoria* sp. ditemukan di Pulau Sabang, Aceh. Penelitian ini bertujuan melakukan investigasi kandungan kimiawi dan biologis dari *Oscillatoria* sp. Isolasi molekul target dilakukan melalui metode pemisahan berdasarkan uji bioaktivitas dengan menggunakan beberapa tahapan kromatografi. Selain itu, evaluasi bioaktivitas ditentukan oleh protokol uji MTT terhadap sel kanker manusia Panc-1. Struktur planar dari molekul target ditentukan dengan analisis spektroskopi. Hasilnya, struktur planar dari senyawa target diidentifikasi sebagai sikloheksimida-4-asetat (**1**), kelompok molekul sikloimida. Selanjutnya, relatif stereokimia dikonfirmasi oleh analisis *nuclear overhauser effect* (NOE). Oleh karena itu, molekul tersebut ditetapkan sebagai (2S*,4S*,6S*,7R*)-sikloheksimida-4-asetat. Evaluasi sitotoksisitas pada senyawa **1** menunjukkan nilai IC_{50} sebesar 0.32 μM terhadap sel kanker pankreas.

Kata Kunci: *Oscillatoria* sp., Panc-1, sianobakteri, sikloimida, sitotoksisitas,

Received: *17 July 2021* ***Accepted:*** *28 October 2021* ***Published:*** *28 December 2021*

INTRODUCTION

Bacterial infection of *Escherichia coli* is one of the diseases of special concern in the medical world. So far, it has been reported that *E. coli* is very dangerous, may cause mortality and morbidity. In 2016, WHO reported that the mortality caused by the bacteria was about 9.6 million people/year (WHO 2017). Considering antibiotics are prone to causing resistance, marine resources are being investigated as possible alternatives. Many studies have reported that marine resources have bioactive molecules as antibacterial, anticancer, and antitumor (Arai et al. 2015, Khairunnisa and Kurnianda 2017, Kurnianda et al. 2019, Mane et al. 2021).

On the other hand, streptovitacins is a glutarimide class molecule produced by marine bacteria *Streptomyces* sp. which has been widely used as a source of drug development (Zhang et al. 2019, Jiang et al. 2021, Moghaddam et al. 2021). Currently, glutarimide is a well-known secondary metabolite that has biological activity as a eukaryotic translation inhibitor for protein synthesis (Schneider-Poetsch et al. 2010, Govindan and Lin 2021). This investigation on marine-associated microorganisms in Sabang Island found the member of glutarimide class molecules produced by cyanobacterium *Oscillatoria* sp.

Cyanobacteria are found in a variety of habitats, such as terrestrial, marine, fresh, and brackish. Besides being symbiotic, they can be commensal or parasite as well. A wide variety of environmental conditions is suitable for them, such as extremes in temperature, light intensity, pH, and salinity (Martínez-Francés and Escudero-Oñate 2018, do Amaral et al. 2021). The investigation of *Oscillatoria* sp. revealed that there are many exciting bioactive compounds in the organisms, including oscillapeptin A, hapalindole A, minutissamide A, caylobolide B, anabaenopeptin E, and lyngbic acid (Demay et al. 2019, Carpine and Sieber 2021, Khalifa et al. 2021, Mutalipassi et al. 2021).

Nevertheless, only 40% of the compounds found in *Oscillatoria* sp. shows potent antimicrobial and anticancer properties due to their geography, it indicates that the species have unique mechanisms to adapt to their environment. On the other hand, it also introduces a unique metabolite in their body. To date, this species is known to useful as potential therapies for antimicrobial, antiviral, anti-inflammatory, antiprotozoal, anticancer, and proteinase-inhibiting agents (Gogineni and Hamann 2018, Mazur-Marzec et al. 2021). The identification of active compounds from freshwater and marine cyanobacterial strains is crucial for the development of a therapeutic approach for controlling various microbial pathogens and for the conduct of anticancer studies in unexplored locations.

As there is no reported paper on the natural products of marine cyanobacterium in Sabang Island, so far. It gives an opportunity to investigate the chemical and biological contents on the organisms. Herein, this work reports the chemical investigation together with the bioactivity of the *Oscillatoria* sp. against the Panc-1 cell.

MATERIALS AND METHODS

Location and time

Oscillatoria sp. (Figure 1) was collected by hand from a depth of 0.1-0.5 meters in a shallow sandy basin at Rubiah Island (5^o 52' 42" N; 95^o 15' 41" E), Sabang, Aceh, Indonesia in August 2014.

Experimental Section

1D and 2D data of nuclear magnetic resonance (NMR) were carried out from a JEOL 500 MHz spectrometer with the residual solvent for Methanol-d_4 (MeOD) at δ_H 3.31 ppm; δ_C 49.0 ppm as the reference. The

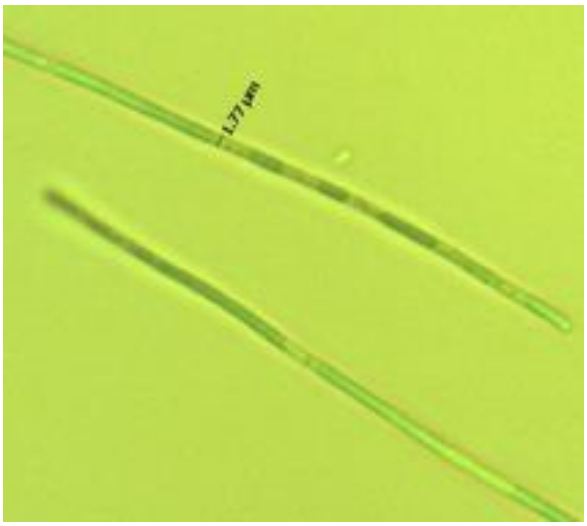

Figure 1. Microscope of ZEISS Axioscope 5 of *Oscillatoria* sp. with 500x magnification

high-resolution electrospray ionization of mass spectroscopy (HRESIMS) data was obtained from an Agilent 6230 TOF LC-MS. Optical rotation and fourier transform infrared (FTIR) data were recorded on a JASCO J-815 and JASCO FT/IR-6100 spectrometer, respectively. Purification of the active compound was applied for open column chromatography using reversed-phase silica gel (75C_{18}-OPN, Nacalai tesque), and the high-performance liquid chromatography (HPLC) system with a photodiode array (PDA) using $^{5}C_{8}$-MS (46 × 250 mm, Nacalai tesque).

Specimen of cyanobacterium *Oscillatoria* sp.

The specimen was kept frozen after filtered using a mesh bag to remove excrescent seawater. The species of *Oscillatoria* sp. was concluded by comparison with its characteristics (Geitler 1932). The voucher of *Oscillatoria* sp. was deposited at the Marine Chemistry Laboratory, Faculty of Marine and Fisheries, Syiah Kuala University, Indonesia (Voucher code VK-NP-12-5-14).

Extraction and isolation

The fresh material of *Oscillatoria* sp. (0.87 kg, wet weight) was extracted and partitioned with a volume of CH_2Cl_2-CH_3OH and CH_3OH-H_2O, respectively, to give a hydrophilic portion. The portion was subjected to a flash column reversed-phase silica gel (Cosmosil 75C_{18}–OPN) using a CH_3OH-H_2O mixtures to obtain polar constituents. The polar fraction was purified on normal-phase silica gel (Cosmosil 5C_8–MS) HPLC system using CH_3OH-H_2O (2-15) to give compound **1** (43.2 mg).

Compound **1**, colorless glass; $C_{17}H_{25}NO_6$; $[\alpha]_D^{20}$ +240 (*c* 0.5, $CHCl_3$); FTIR (thin film) ν_{max}: 3512, 2945, 2890, 2118, 1465 cm^{-1}; ^{1}H and ^{13}C NMR: see Table 1; HRESIMS *m/z* 304.17552 $[M+H]^+$ (calcd for $C_{17}H_{26}NO_6$, 304.17552).

Bioassay test

Panc-1 cell was cultured using Roswell Park Memorial Institute (RPMI) medium. The cell was incubated in a 96-well plate with 100 mL of the medium under 5% CO_2 at 37ºC for 24 h. After incubation, 1 mL of dimethyl sulfoxide (DMSO) solution of compound **1** was added and incubated for 48 h and the medium was removed by aspiration. Moreover, 100 mL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) solution was added and incubated for 3 h. After removing the solution, 100 mL of DMSO and the formazan was measured on a microplate reader at 570 nm.

Table 1. NMR data of compound **1**

No	δ_C	δ_H (mult. *J* in Hz)
1	211.7	
2	40.7	2.64 (1H, m)
3	45.2	1.74 (1H, t, 13.2)
		2.58 (1H, m)
4	80.7	
5	37.8	1.94 (1H, t, 13.3)
		2.67 (1H, m)
6	51.0	2.53 (1H, m)
7	65.5	4.10 (1H, ddd, 3.4; 3.4; 5.0)
8	51.0	2.52 (2H, m)
9	27.6	2.38 (1H, m)
10	36.5	2.35 (1H, m)
		2.74 (1H, m)
11	174.2	
12	N-H	
13	174.1	
14	38.0	2.64 (2H, m)
15	13.2	1.03 (3H, d, 6.4)
16	21.1	1.82 (3H, s)
17	170.7	
18	20.9	2.02 (3H, s)

Both ^{1}H and ^{13}C NMR were recorded in MeOD at 500 MHz and 125 MHz, respectively.

RESULTS AND DISCUSSION

The HRESIMS data indicated that the active compound **1** as a protonated ion with the molecular formula $C_{17}H_{25}NO_6$. The 1D and 2D NMR analysis (Table 1, Figures 6-10) showed three methyls (δ_H 1.82, 2.02, 1.03; δ_C 21.1, 20.9, 13.2), four carbonyls (δ_C 211.7, 174.2, 174.1, and 170.7), an oxymethine (δ_H 4.10, δ_C 65.5), three methines (δ_H 2.53, 2.64, 2.38; δ_C 51.0, 40.7, 27.6), and five methylenes (Table 1). A methyl doublet (δ_H 1.03, H-15) showed a heteronuclear multiple bond correlation (HMBC) to a carbonyl group (δ_C 211.7, C-1, Figure 9). On the other hand, a downfield-shifted methyl singlet (δ_H 1.82; H-16) has HMBC cross-peaks to H-16/C-3, H-16/C-4, and H-16/C-5 (Figure 9). The

correlation spectroscopy (COSY) of the methines at C-2/C-3 and C-5/C-6 confirmed the presence of a six-membered ring on this compound (Figure 10). With careful analysis, HMBC correlations at methine (δ_H 2.38, H-9) to two carbonyls (H-9/C-11 and H-9/C14) and COSY cross-peaks at H-9/H-10 and H-9/H-14 established the presence of glutarimide moiety (Figures 9-10). Further analysis by HMBC cross-peaks showed an acetoxymethyl at C-18 (δ_H 2.02, δ_C 20.9) which correlates with C-4 confirmed that

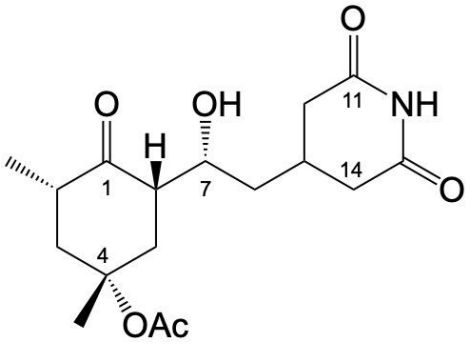

Figure 2. Cycloheximide-4-acetate (**1**)

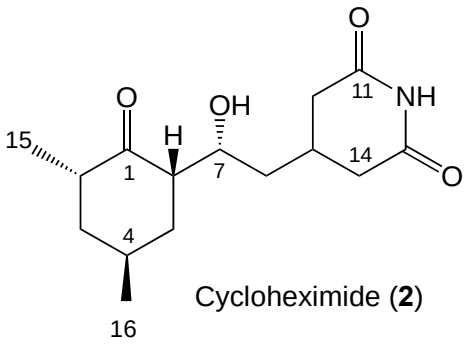

Figure 3. Cycloheximide (**2**)

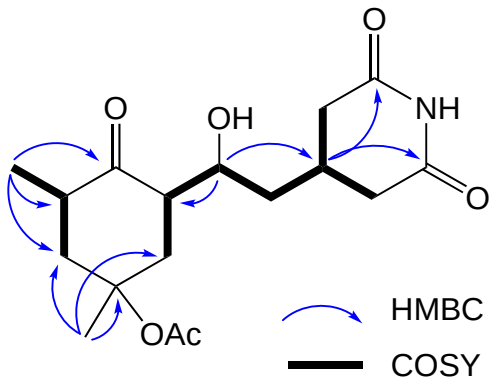

Figure 4. Key HMBC and COSY correlations of compound **1**

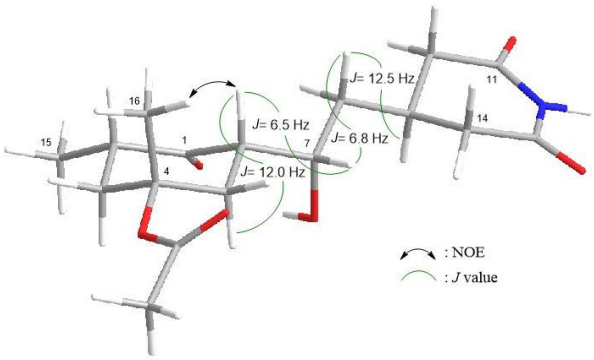

Figure 5. Key NOEs and decoupling study of compound **1**

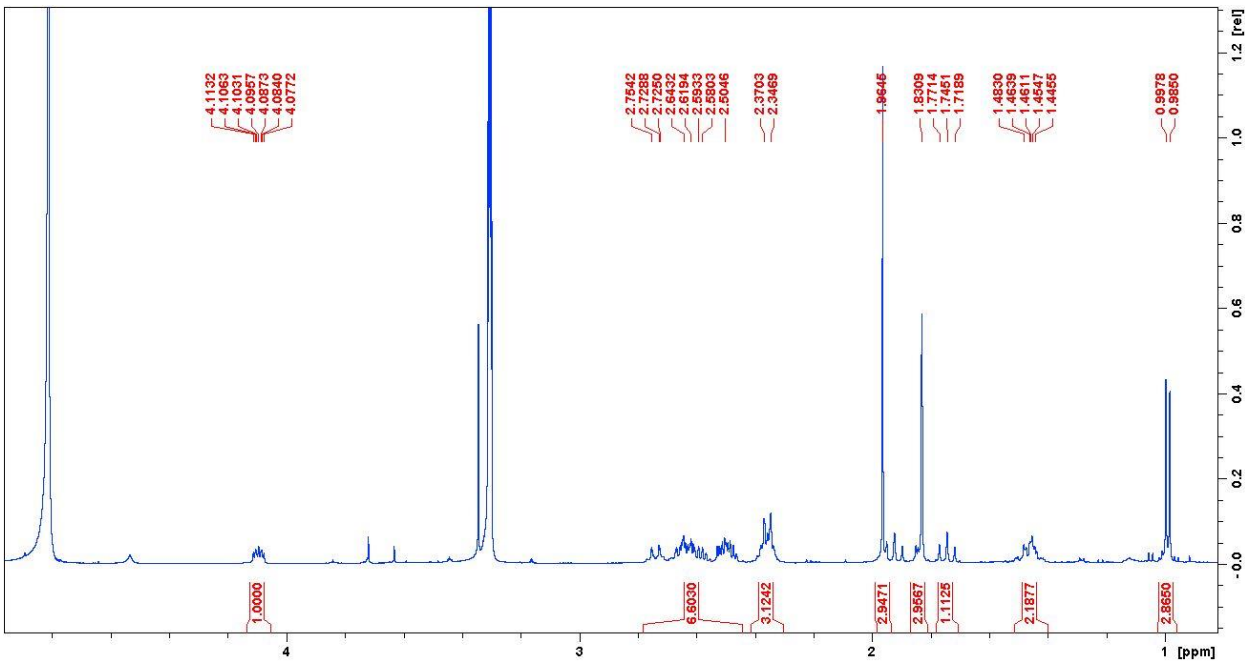

Figure 6. ^{1}H NMR spectra of compound **1** in MeOD.

acetoxy moiety was attached to C-4 (Figure 7). In the conclusion, the NMR data was close identical with those reported, cycloheximide (Figure 3) (Herr 1959, Schneider-Poetsch et al. 2010, Zhang et al. 2019).

The selective NOEs were assigned to determine the relative configuration of compound **1**. A positive nuclear overhauser effect (NOEs) was observed on Me-16/H-6 indicating 1,3–diaxial orientation on them (Figure 5). On the other hand, the irradiation of an oxymethine proton at H-7 showed negative NOEs to H-6 indicate H-7 and H-6 located in the opposite orientation. As compound **1** has a chair conformation, decoupling study was used to confirm the stereochemistry. The axial position on H-6 was confirmed by 1,3–diaxial orientation to Me-16. Moreover, as the coupling constant values $J_{6\text{-}7}$ = 6.5 Hz was observed, it suggested gauche and antiperiplanar conformation on H-7 (Figure 5). Therefore,

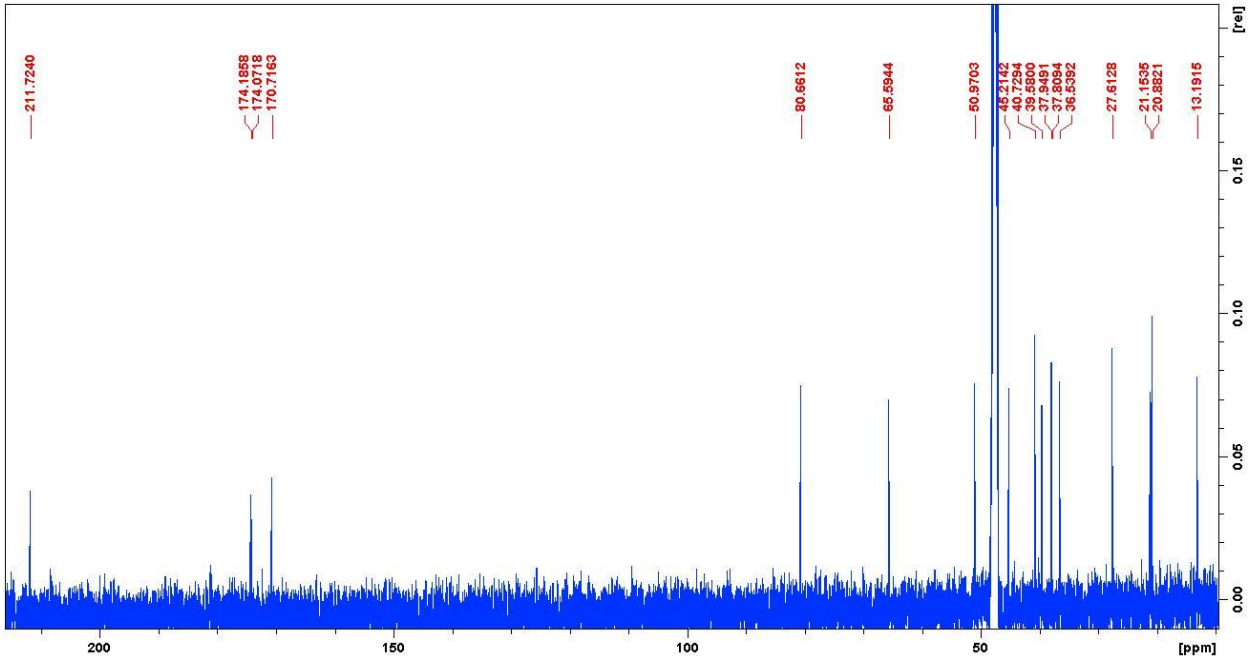

Figure 7. ^{13}C NMR spectra of compound **1** in MeOD

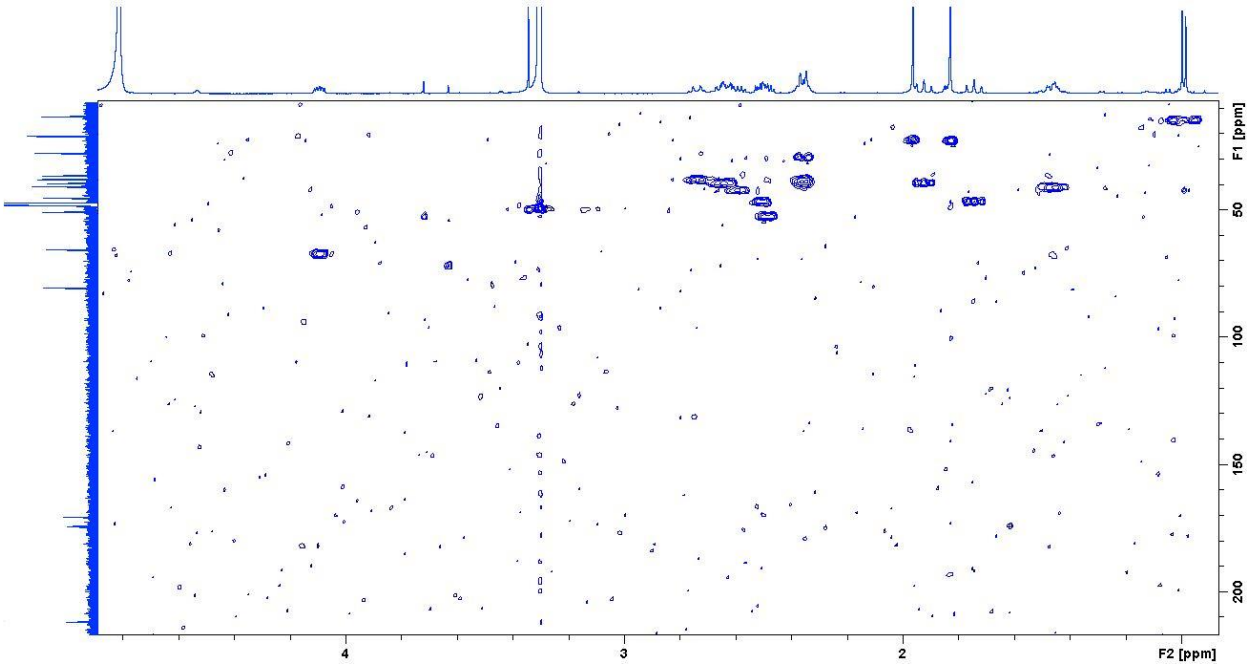

Figure 8. HSQC NMR spectra of compound **1** in MeOD

compound **1** was assigned as 2*S**, 4*R**, 6*S**, 7*S**-cycloheximide-4-acetate (Figure 1).

The evaluation of cytotoxicity showed that compound **1** showed the biological activity with an IC_{50} value of 0.32 μM against the Panc-1 cell. Compound **1** is a well-known secondary metabolite for inhibition of eukaryotic translation. Since compound **1** produced by cyanobacterium *Oscillatoria* sp. from Sabang Island was found, it may give further information about the resource of strong active metabolites. The cultivation of *Oscillatoria* sp. provides an opportunity to increase the amount of this compound. Although compound **1** has been synthesized, it does not rule out the possibility that there are derivatives of this compound that have a potent activity than compound **1**.

It has been identified that compound **1** binds the ribosome and inhibits eEF2 mediated translocation from the ribosome to the eukaryotic translation elongation phase. Interestingly, this compound allows one complete translocation cycle to proceed

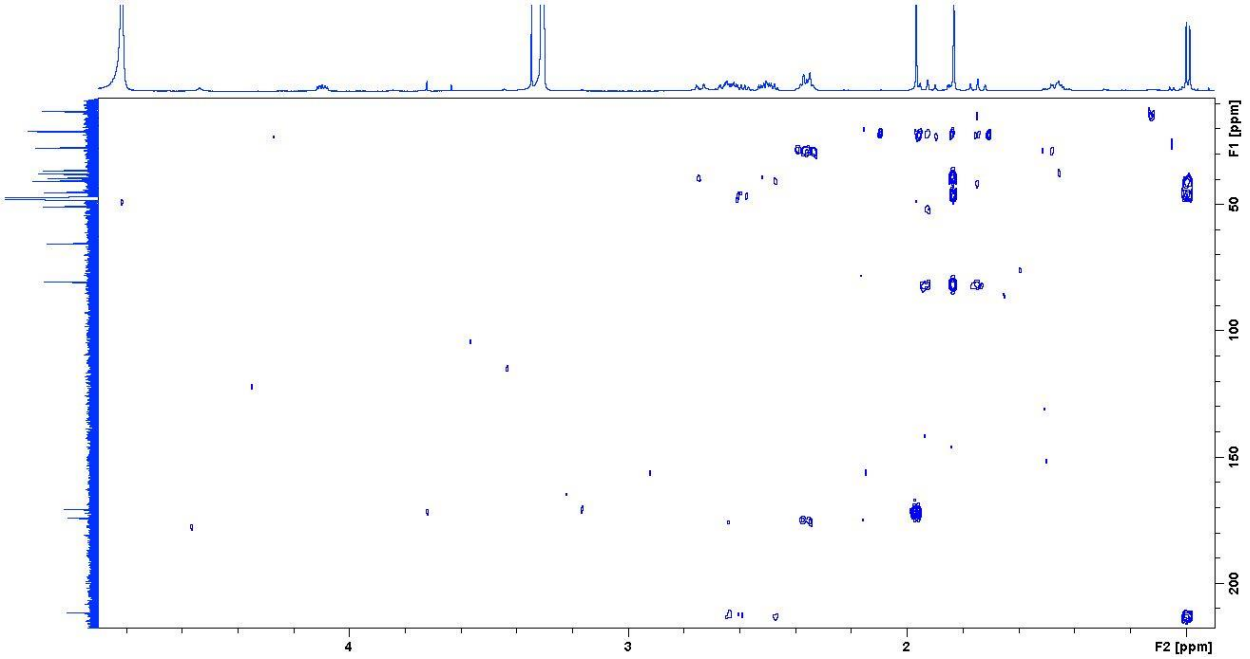

Figure 9. HMBC NMR spectra of compound **1** in MeOD

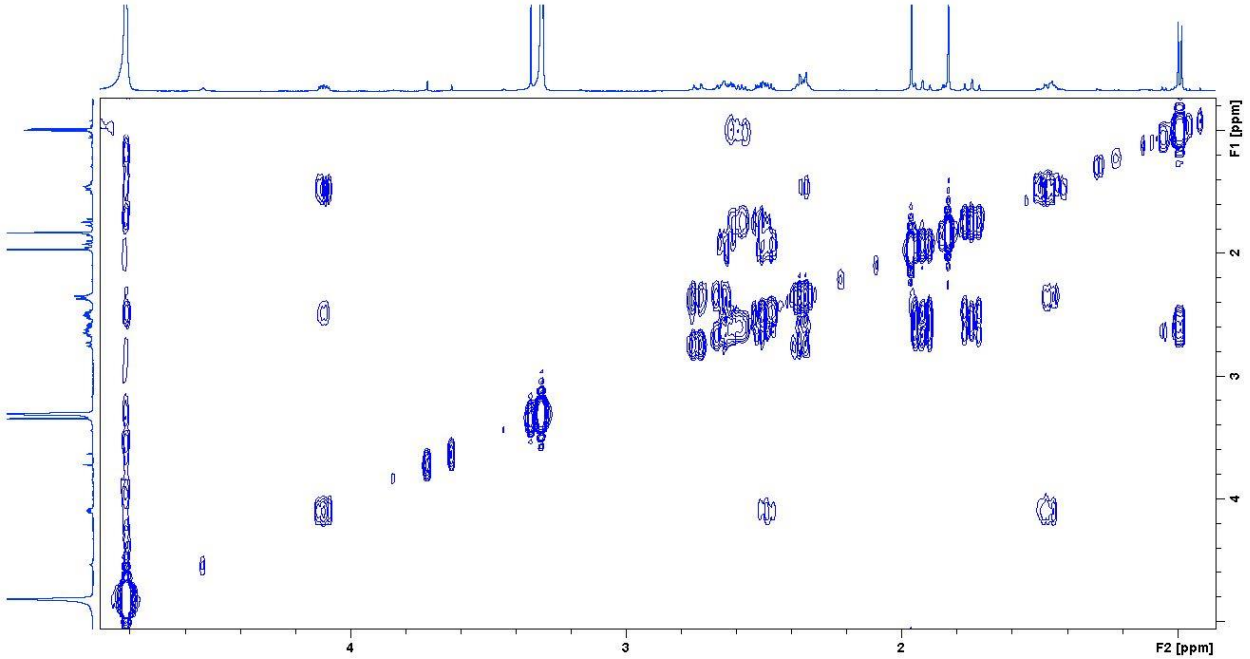

Figure 10. COSY NMR spectra of compound **1** in MeOD

before halting any further elongation. It also indicated that compound **1** requires an E-site-bound deacylated tRNA for activity (Schneider-Poetsch et al. 2010).

CONCLUSION

During an expedition in Sabang Island, cycloheximide-4-acetate (**1**) from marine cyanobacterium *Oscillatoria* sp. was found. The presence of compound **1** was confirmed by NMR, FTIR and HRESIMS data. Moreover, cytotoxicity showed potent biological activity with an IC_{50} value of 0.32 μM against the Panc-1 cell.

ACKNOWLEDGMENT

This project was supported by the Laboratory of Natural Products, Graduate School of Pharmaceutical Sciences, Osaka University, Japan.

REFERENCES

Arai M, Kamiya K, Pruksakorn P, Sumii Y, Kotoku N, Joubert J-P, Moodley P, Han C, Shin D, Kobayashi M (2015) Anti-dormant mycobacterial activity and target analysis of nybomycin produced by a marine-derived *Streptomyces* sp. Bioorg Med Chem 23: 3534-3541. doi: 10.1016/j.bmc.2015.04.033

Carpine R, Sieber S (2021) Antibacterial and antiviral metabolites from cyanobacteria: Their application and their impact on human health. Curr Res Biotechnol 3: 65-81. doi: 10.1016/j.crbiot.2021.03.001

Demay J, Bernard C, Reinhardt A, Marie, B (2019) Natural products from cyanobacteria: Focus on beneficial activities. Mar Drugs 17: 320. doi: 10.3390/md17060320

do Amaral SC, Monteiro PR, Neto JDSP, Serra GM, Gonçalves EC, Xavier LP, Santos AV (2021) Current knowledge on microviridin from cyanobacteria. Mar Drugs 19: 17. doi: 10.3390/md19010017

Geitler L (1932) Cyanophyceae. *In*: Kryptogamen-Flora von Deutschland, Österreich und der Schweiz. Rabenhorst L (Ed) Ed 2 Vol 14, pp. 673-1196. Akademische Verlagsgesellschaft, Leipzig

Gogineni V, Hamann MT (2018) Marine natural product peptides with therapeutic potential: Chemistry, biosynthesis, and pharmacology. Biochim Biophys Acta 1862: 81–196. doi: 10.1016/j.bbagen.2017.08.014

Govindan K, Lin WY (2021) Ring opening/site selective cleavage in N-acyl glutarimide to synthesize primary amides. Org Lett 23: 1600-1605. doi: 10.1021/acs.orglett.1c00010

Herr RR (1959) Structures of the Streptovitacins. J Am Chem Soc 81: 2595-2596. doi: 10.1021/ja01519a077

Jiang L, Huang P, Ren B, Song Z, Zhu G, He W, Zhang J, Oyeleye A, Dai H, Zhang L, Liu X (2021) Antibacterial polyene-polyol macrolides and cyclic peptides from the marine-derived *Streptomyces* sp. MS110128. Appl Microbiol Biotechnol 105: 4975-4986. doi: 10.1007/s00253-021-11226-w

Khairunnisa, Kurnianda V (2017) Bioactivity from Indonesian's marine sponge *Xestospongia* sp. as antibacterial resistance *Escherichia coli*. Nat Prod Chem Res 5: 1000265. doi: 10.4172/2329-6836.1000265

Khalifa SAM, Shedid ES, Saied EM, Jassbi AR, Jamebozorgi FH, Rateb M, Du M, Abdel-Daim MM, Kai GY, Al-Hammady MA, Xiao J, Guo Z, El-Seedi HR (2021) Cyanobacteria—From the oceans to the potential biotechnological and biomedical applications. Mar Drugs 19: 241. doi: 10.3390/md19050241

Kurnianda V, Faradilla S, Karina S, Agustina S, Ulfah M, Octavina C, Syahliza F, Ramadhan MR, Purnawan S Musman M (2019) Polyoxygenated diterpene produced by the Indonesian marine sponge *Callyspongia* sp. as an inhibitor of the human pancreatic cancer cells. Microbiol Indones 13: 70-74. doi: 10.5454/mi.13.2.5

Mane PC, Sayyed SAR, Kadam DD, Shinde MD, Fatehmulla A, Aldhafiri AM, Alghamdi EA, Amalnerkar DP, Chaudhari RD (2021) Terrestrial snail-mucus mediated green synthesis of silver nanoparticles and in vitro investigations on their antimicrobial and anticancer activities. Sci Rep 11: 13068. doi: 10.1038/s41598-021-

92478-4
Martínez-Francés E, Escudero-Oñate C (2018) Cyanobacteria and microalgae in the production of valuable bioactive compounds. Microalgal Biotechnol 6: 104-128. doi: 10.5772/intechopen.74043
Mazur-Marzec H, Cegłowska M, Konkel R, Pyrć K (2021) Antiviral cyanometabolites—A review. Biomolecules 11: 474. doi: 10.3390/biom11030474
Moghaddam HS, Shahnavaz B, Makhdoumi A Iranshahy M (2021) Evaluating the effect of various bacterial consortia on antibacterial activity of marine *Streptomyces* sp. AC117. Biocontrol Sci Technol 31: 1-19. doi: 10.1080/09583157.2021.1940865
Mutalipassi M, Riccio G, Mazzella V, Galasso C, Somma E, Chiarore A, de Pascale D, Zupo V (2021) Symbioses of Cyanobacteria in marine environments: Ecological insights and biotechnological perspectives. Mar Drugs 19: 227. doi: 10.3390/md19040227
Schneider-Poetsch T, Ju J, Eyler DE, Dang Y, Bhat S, Merrick WC, Green R, Shen B, Liu JO (2010) Inhibition of eukaryotic translation elongation by cycloheximide and lactimidomycin. Nat Chem Biol 6: 209-217. doi: 10.1038/nchembio.304
WHO (2017) Global Health Observatory, World Health Organization. https://www.who.int/data/gho/data/themes/topics/topic-details/GHO/world-health-statistics. Accessed 12 April 2017
Zhang D, Yi W, Ge H, Zhang Z, Wu B (2019) Bioactive streptoglutarimides A–J from the marine-derived *Streptomyces* sp. ZZ741. J Nat Prod 82: 2800-2808. doi: 10.1021/acs.jnatprod.9b00481