A marine isonitrile produced by Indonesian marine sponge of *Petrosia* sp. as an inhibitor of the human pancreatic cancer cells

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Abstract. The study of search the bioactive compound from Indonesian Marine Sponge *Petrosia* sp. has been conducted on February 2019. The bioactive compound isolated based on bioassay-guided separation with several steps of chromatography. The compound known as C₁₆H₂₅NS determined by LCMS-ESI with molecular weight [M+H]⁺ 263.42 m/z. The FTIR data showed that the functionality as hydrocarbon skeleton of alkane and isonitrile at 2982.58 cm⁻¹ and 2115 cm⁻¹, respectively. Furthermore, the NMR data confirm that the active compound knowns as 4-amorphene-10-isothiocyanate. The cytotoxic data indicate that the bioactive compound has activity against Panc-1 cell [IC₅₀ = 0.1 μg / mL].The secondary metabolite from Indonesian marine sponge of *Petrosia* sp. has potential activity against human pancreatic cancer cells.

1. Introduction
The pancreas is a large gland located in the digestive system. the pancreas produces hormones and insulin to maintain the stability of blood sugar levels in the body. The pancreas can have potential disorders caused by diet, diabetes, obesity, and smoking [1].

The World Health Organization (WHO) estimates that there are 18.1 million new cancer cases and 9.6 million deaths that occurred this year. WHO predicts that cancer will be the number one cause of death in the world. Pancreatic cancer is one of the human diseases with a high mortality rate in the world, around 216,000 people/year must struggle to survive. Pancreatic cancer is a disease caused by the growth of tumors that abnormal cancer cells appear in pancreatic tissue. There is a change in DNA in cells that mutate to multiply and grow rapidly. The cells then unite, become tumors that cause cancer, infect the
surrounding cells and spread to other parts of the body. Smoking can increase the risk of pancreatic cancer because toxins and dangerous chemicals can trigger the growth of cancer cells [2-3].

Nowadays, the cancer usually treated by employing surgery, chemotherapy, and radiotherapy. Chemotherapy is a treatment method in healing to reduce the risk of cancer growth. The treatments using chemotherapy and radiotherapy techniques combined with synthetic drugs have the high risks such as attacking normal cells, thrush, fatigue, nausea, vomiting, hair loss and can increase the risk of infection [4].

Based on research conducted by world experts revealing drugs derived from marine natural resources can be an alternative for medical purposes such as cancer treatment. Some studies reported that compounds produced from Indonesian marine sponges such as *Xestopongia muta*, *Endectyon delaubenfelsi*, *Spongionella pulchella* and *Raspailia ramosa* are potentially bioactive against pancreatic cancer [5-7].

2. Experimental Section

2.1 Materials

NMR Spectrum was analyzed using JEOL ECA-500 MHz. Extraction sample test using ESI-TOF-MS Q-Tof Ultima (Waters Co, MA, USA). IR Spectrum Analysis was carried out using the JASCO FT/IR-5300.

Column chromatography is used in the separation of samples containing active components with BW-200 Silica gel (Fuji Silysia, Aichi, Japan), Cosmosil 3C18-MS-II (10 mm id × 250mm, Nacalai Tesque) and Cosmosil ODS (βC18-OPT, Nacalai Tesque, Kyoto, Japan). The active compound was purified using HPLC (UV detector: L-4000H) and TLC Silica gel 60F254 chromatography (Merck Chemical, Darmstadt, Germany). The extraction results were analyzed by supporting devices such as biorad plates, biorad spectroscopy using 96-well plate. Toxicity test preparation was carried out using a CO2 incubator. Biological analysis used kanamycin 50 µg / mL solution, intermediate trypsin Mc-Coys, blue trypan, PANC-1 human cells, general glucose media, glucose deficient media, phosphate buffer saline (PBS), Fetal Bovine Serum (FBS), and Dulbecco media modified Eagle (DMEM) medium.

2.2 Cell Culture and Toxicity Test

PANC-1 was cultured on Dulbecco medium modified Eagle (DMEM) media with the addition of 10% bovine fetal serum (FBS) and kanamycin (50 µg / mL) under humidity level of less than 5% CO2 at 37 °C. For glucose negative conditions, PANC-1 was cultivated in Medium deficiency glucose (Basal Medium (25mm N-(2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) plus 4.6g / L NaCl, 700mg / L, NaHCO3, 400mg / L, KCl, 265mg / L CaCl2,2H2O, 200mg / L MgSO4.7H2O, 125mg / L, NaH2PO4, 0.1mg / L Fe (NO3) 3.9H2O, 15mg / L, phenolred, 10ml / 1 vitamin (X100) solution (Gibco, Carlsbad, CA, USA), 200mmol / L L-glutamine solution (Gibco), 10% FBS contained in 50mg / L (kanamycin) analyzed [8-9]. Glucose with 10% Fetal Bovine Serum (FBS) and 2.0 g / L glucose (25mm) is useful for the determination of different toxicity tests in cell activity when the condition is deficient in glucose [10-12].

PANC-1 cells were observed for 24 hours using DMEM with a concentration of 10% FBS. Medium Regular Glucose or Fat Glucose is used in the media to be replaced so that it can regulate nutritional hunger. After 12 hours of incubation, the sample was dissolved and incubated under 5% CO2 at 37 °C [13,14]. WTS-8 Colorimetric Reagent used for cell proliferation. The growth curve used for determine the IC50 value based on the results of the selectivity of general and intermediate glucose anti-proliferation (SI) activity [15-6]
3. Extraction and Isolation

*Petrosia* sp. (8.2 g dry weight) extracted using acetone 3 times and partitioned using EtOAc: H₂O (1: 1 v/v) for 72 hours to produce EtOAc fraction (3.69 g), and H₂O fraction (4.51 g). The results of the viability from EtOAc portion shows the activity against Panc-1 [3.69 g (IC₅₀ = 28.5 μg / mL)]. Then, the EtOAc (3.69g) fraction was fractionated using an open column chromatography (OPN-C₁₈) with methanol : H₂O (10:0.5) produce 5 fractions. Furthermore, the first fraction [14.5 mg (IC₅₀ = 5 μg / mL)] was purified with OPN-5C₁₈ using methanol : H₂O (5:2) + TFA 0.1% solvent system to obtain 7 fractions. The activity of the first fraction [10.2 mg (IC₅₀ = 0.1 μg / mL)] showed the cytotoxic against Panc-1 cells.

4. Results and Discussion

The active compound (white amorphous) has the molecular formula C₁₆H₂₅NS determined by LCMS-ESI with molecular weight [M+H]⁺ 263.42m/z. The FTIR spectrum shows that the active metabolite has functional groups 2982.58 cm⁻¹ indicate as alkane skeleton of hydrocarbon and nitrogen vibration at 2115 cm⁻¹ indicate as isonitrile functional group [19,21]. The results from NMR data showed that the active compound as 6-membered ring of hydrocarbon skeleton contain isonitrile moiety [7].

| No | ¹³C  | ¹H   | J in Hz |
|----|------|------|---------|
| 1  | 48.4 (d) | 1.48 | m |
| 2  | 23.9 (t) | 1.82 | m |
|    |         | 2.15 | ddd (14.4, 8.2, 2.1) |
| 3  | 27.3 (t) | 1.96 | m |
|    |         | 2.34 | m |
| 4  | 137.0 (s) |      |     |
| 5  | 118.3 (d) | 5.30 | brs |
| 6  | 35.8 (d) | 2.64 | brs |
| 7  | 42.1 (d) | 1.45 | m |
| 8  | 22.8 (t) | 1.36 | dq (13.1, 13.1, 12.9, 2.8) |
|    |         | 1.67 | m |
| 9  | 42.6 (t) | 1.58 | dt (11.2, 10.2, 1.0) |
| 10 | 61.2 (s) |      |     |
| 11 | 28.5 (d) | 1.71 | m |
| 12 | 21.5 (q) | 0.99 | d (6.4) |
| 13 | 20.4 (q) | 0.91 | d (6.5) |
| 14 | 28.8 (q) | 1.77 | s |
| 15 | 24.4 (q) | 1.42 | s |

The ¹H NMR data showed that the characteristic of methyl group signals at 0.91 (d, 3H, 6.5 Hz), 0.99 (d, 3H, 6.4 Hz), 1.77 (3H, s) dan 1.42 (3H, s). The methyl signals at 0.91 (d, 3H, 6.5 Hz) and 0.99 (d, 3H, 6.4 Hz) indicated that the CH₃ attached at the same position on quaternary carbon (C-11). The quaternary carbon sp² appeared at 137.0 ppm confirm that the active compound as unsaturated hydrocarbon [7-9]. Furthermore, the characteristic signal of quaternary carbon sp³ at C-10 appeared at 61.2 ppm indicated the isonitrile moiety (NCS) attached at C-10 which confirmed by functional group from IR data [7].
Figure 1. HMBC and COSY correlation of active compound

The analysis of HMBC and COSY data showed the cross peak between C10/H-14 indicated that methyl group from C-14 attached into C-10 as quaternary carbon. The $^1$H and $^{13}$C signal of C-10 undergo from sp$^3$ hybridized in upfield shifted to downfield shifted due to NCS moiety attached in C-10. Further analysis showed that the active compound has two 6-membered ring moiety which known as decalin hydrocarbon skeleton [5-7]. Then, determination of the absolute configuration indicated that the active compound assigned as cis configuration [9-10].

Figure 2. 4-amorphene isothiocyanate

The bioactivity test against human pancreatic cancer cell in glucose deficiency medium from the Indonesian marine sponge *Petrosia* sp. shows the potential inhibitor which determined by linear interpolation of the viability curve. The decalin isonitrile indicated has an antiproliferative activity in glucose deficiency medium [7].
5. Conclusion
The active metabolite from Petrosia sp. has bioactivity against pancreatic cancer cell \([\text{IC}_{50} = 0.1 \mu\text{g/mL}]\). The compound which known as 4-amorphene-10-isothiocyanate has potential for a new drug models against compared by commercial drug duxorubicin \([\text{IC}_{50} = 20 \mu\text{g/mL}]\).

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References
[1] Cancer Information Service (National cancer Institute, USA) http://www.nci.nih.gov
[2] WHO, 2019. Global Health Observatory. http://www.who.int.
[3] NCI, 2016. Cancer.gov. Pancreatic Cancer—for patients.
[4] NCI, 2019. Type of Cancer Treatment. Melalui http://cancer.gov.
[5] V Kurnianda, A Mardiah, S Karina, S Agustina, M Ulfah, C Octavina, F Syahliza, M R Ramadhan, S Purnawan, *IOP Conf. Ser.: Earth Environ. Sci*, 2018, 216, 012032.
[6] V Kurnianda, F Syahliza, S Karina, S Agustina, M Ulfah, C Octavina, A Mardiah, M R Ramadhan and S Purnawan, *IOP Conf. Ser.: Earth Environ. Sci*, 2018, 012041.
[7] V Kurnianda, M R Ramadhan, S Karina, S Agustina, M Ulfah, C Octavina, A Mardiah, F Syahliza and S Purnawan, *IOP Conf. Ser.: Earth Environ. Sci*, 2018, 216, 012042.
[8] Kubota T., Nishi T., Fukushima E., Kawabata J., Fromont J., Kobayashi J., (2007)*Tetrahedron Lett.*, 48, 4983–4985
[9] Romuald C., Busseron E., Coutrot F., J, Org. Chem, 2010, 75, 6516–6531
[10] Morimoto Y., Kitao S., Okita T., Shoji T, Org. Lett. 2003, 5, 2611–2614
[11] Coombs J. R., Zhang L., Morken J. P., J. Am, Chem. Soc, 2014, 136, 16140–16143
[12] Patwardhan A. P., Thompson D. H, Org. Lett. 1999, 1, 241–244
[13] Yamamoto S, Kawasaki G, Yamada S, Oral Oncol, 2011, 47:855–860
[14] Shah AN, Summy JM, Zhang J, Park SI, Parikh NU and Gallick GE, Ann Surg Oncol, 2007, 14:3629–3637
[15] Zeng W, Chen X, Ma Y, Anticancer Res, 2014, 34:117–123
[16] Donadelli M, Costanzo C, Beghelli S, Biochim Biophys Acta, 2007, 1773:1095–1106