The inhibitor of human bladder epithelial cancer cells from Indonesian marine sponge of *Petrosia* sp.

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**Abstract.** The investigation of bioactive compound from Indonesian marine sponge *Petrosia* sp. has been done on February 2019. The bioactive compound isolated based on bioassay-guide separation with several steps of chromatography. The bioactive compound known as C\(_{16}\)H\(_{25}\)NS determined by LCMS-ESI with molecular weight [M+H] + 263.42 m/z. The FTIR spectrum showd that the bioactive metabolite has alkane skeleton of hydrocarbon at 2982.58 cm\(^{-1}\) and isonitrile functional group at 2115 cm\(^{-1}\). The result of NMR interpretation showed that the bioactive compound know as 4-amorphene with decalin as hydrocarbon skeleton with additional isonitrile moiety. The rate of viability show scytotoxic against NBT-T2 cell.

1. **Introduction**

Human bladder cancer is a disease caused by abnormal cell development in the bladder tract. The world cancer institution reporteed that the mortality rate in 2018 around 9.6 million / person and will increase by 300% worldwide in 2030 [1]. That number is 70% in developing countries such as Indonesia. Bladder cancer can be caused by poor quality of life such as smoking. people who smoke 4 times more at risk of bladder cancer than non-smokers. Bladder cancer is a disease caused by abnormal cell development in the bladder tract [2]. The world cancer institution reports that the mortality rate in 2018 is 9.6 million / person and will increase by 300% worldwide in 2030. That number is 70% in developing countries such as Indonesia. Bladder cancer can be caused by poor quality of life such as smoking. people who smoke 4 times more at risk of bladder cancer than non-smokers [3-4].

Handling to treat bladder cancer has been carried out intensively by medical surgery, chemotherapy, radiotherapy, and administration of synthetic drugs such as M-CAVI (Methotrexate, Carboplatin, and Vinblastine) and GemCarbo have been done, but the results are still far from as...
expected. This is because the treatments has high risks on the economy and health. This is because medical expenses are very expensive and sufferers can experience side effects from the use of such methods such as nausea and vomiting, blackened skin on radiation-affected parts of the body, hair loss little by little, menstrual disorders in women and disorders of the number and quality of sperm in men [6-7].

Several studies reported that compounds produced from marine organisms have potential as anticancer such as Xestopongiamuta, Enyectyondelaubenfelsi, Spongionellapulchella and Raspaidaramosa from Indonesian marine sponges [7].

In this study, we focused on extraction, isolation, and characterization of secondary metabolites of Indonesian marine sponges Petrostia sp. which has biological activity as an anticancer bladder (NBT-T2 cell).

2. Experimental Section
2.1 Material
Elucidation of the structure using NMR JEOL ECA-500 MHz. The secondary metabolite sample from extraction was observed using the ESI-TOF-MS type Q-Tof Ultima (Waters Co., MA, A.S.A). Testing parts of the IR spectrum using the JASCO FT / IR-5300 tool. UV Spectrum Testing using a UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan). Chromatographic techniques for fraction separation using the OCC NP-SiO 2 type open chromatography column. Purification stage using HPLC RP-5C18. The active compound were analyzed by additional tools such as 96-well plate Biorad spectroscopy and biorad plate under CO 2 incubators. Biomaterial analysis using Duxorubicin 20 ug / mL solution, trypsin Mc-Coys media, blue trypan, human cells (NBT-T2), general glucose media, glucose deficiency media, phosphate saline buffer (PBS), Fetal Bovine Serum (FBS), ethyl acetate, n-hexane, aquadest, and Dulbecco modified Eagle's medium (DMEM).

2.2 Cell Culture Test and Toxicity
Bladder cancer cells (NBT-T2) cultivated on the Dulbeccomodified Eagle's medium (DMEM) medium using 10% FetalBovine Serum (FBS) as an inactive temperature additive and duxorubicin (20 μg / mL) in a humid area with a range in under 5% CO 2 and a temperature of 37°C. For nutrient starvation state, (NBT-T2) can be processed with media that lack Glucose (Basal Medium (25mM N- (2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid (HEPES )) (pH 7.4) plus 6.4 g / L NaCl, 700mg / L, NaHCO 3, 400mg / L, KCl, 265mg / L CaCl 2·2H 2O, 200mg / L MgSO 4·7H 2O, 125mg / L, NaH 2PO 4, 0.1 mg / L Fe (NO 3) 2·3H 2O, 15mg / L Phenolred, 10 mL vitamin (X100) (GIBCO, Carlsbad, CA, USA), 200mmol / L L-glutamine (GIBCO), 10% FBS filled with kanamycin 50mg / L dialysis: Addition of glucose and 10% FetalBovine Serum (FBS) with 2.0 g / L glucose (25mM) can serve as a toxicity test to determine differences in cell development in glucose deficient medium glucose [4-6].

Use of DMEM with 10% FBS to observe cells (NBT-T2) for 24 hours. The media to be replaced so that it can regulate nutritional deficiencies is used for regular glucose. Samples that have been dissolved and incubated under conditions of 5% CO 2 at 37 °C are carried out after 12 hours of incubation. WIM-8 Colorimetric Reagents detect cell proliferation. IC50 values can be determined by interpolated growth inhibition curves. Based on differences in IC50 values in the general glucose medium and glucose Deficient Medium to determine the results of selectivity in anti-proliferation (S.I) activity [8].

3. Extraction and Isolation
Sponge of Petrostia sp. (100 g) was cut into small parts and extracted for 72 hours using methanol solvent. The extract was dried and evaporated using a rotary evaporator 8.2 g. Then, Petrostia sp. (8.2 g) was partitioned using EtOAc: H 2O (1: 1 v / v) to produce EtOAc fraction (3.69 g), and H 2O fraction (4.51 g). The results of cytotoxic showed that the EtOAc fraction has bioactivity against NBT-T2 cell [3.69 g (IC 50 = 43 μg / mL)]. The results of the EtOAc fraction (3.69 g) were separated with open
column chromatography (OCC NP-SiO2) using n-Hexane and EtOAc gradient solvent systems obtained 5 fractions. The first fraction [14.5 mg (IC50 = 15 μg / mL)] was purified using the HPLC OPN-5C18 column with methanol: H2O (10: v / v) obtained 7 fractions. The first fraction had the highest cytotoxic effect on NBT-T2 cells [10.2 mg (IC50 = 0.4 μg/mL)].

4. Results and Discussion

4.1 Structure Identification

The compound known as 4-amorpene-10-isothiocyanate has the molecular formula C16H25NS determined by LCMS-ESI with molecular weight [M+H]+ 263.42m/z. The FTIR data confirm that the active metabolite has C-H alkane at 2982.58 cm\(^{-1}\) and N functional groups 2115 cm\(^{-1}\) indicate that the active compound has nitrogen atom as indicate isonitrile functional group[5].

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

The proton NMR showed four 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 two methyl signals at 0.91 (d, 3H, 6.5 Hz) and 0.99 (d, 3H, 6.4 Hz) indicate that the CH\(_3\) attach at the same quaternary carbon (C-11). The quaternary carbon at 137.0 ppm suggest that the sp\(^3\)hybridizide of active compound bounded on sp\(^2\) hybridized as unsaturated skeleton. Then, the signal of sp\(^3\) quaternary carbon appears at 61.2 ppm indicate the electronegative atoms attach at C-10. The FTIR data confirm that the electronegative atoms is isonitrile moiety (NCS). The sp\(^2\)carbon in NCS system cannot observed due to the relaxation time of sp\(^2\) hybridized takes long time to resonances and send the signals to detector [13].

![Planar structure and chair conformation of bioactive compound](image-url)
The correlation of COSY and HMBC showed that methyl group has cross-link with quartenary carbon (137 ppm, q) at position C15/C4 indicate that methyl group attach on carbon sp² [5]. The active compound has two 6-membered ring moiety which is known as sesquiterpene in the decalin skeleton with cis configuration [13].

Figure 2. COSY and HMBC spectrum from active compound

Figure 3. 4-amorphene isothiocyanate

The bioactivity against human bladder epithelial cancer cells in glucose deficiency medium from the Indonesian’s marine sponge Petrosia sp. shows potential antiproliferative which is determine by linear interpolation of the viability curve [5-8].

Figure 4. Cytotoxic of Indonesian's Marine Sponge Petrosia sp. against NBT-T2 cell
5. Conclusion
The active metabolite from Petrosia sp has cytotoxic activity against NBT-T2 cell with IC\textsubscript{50} = 0.4 μg/mL.

Acknowledgement
Thanks to Department of Marine Science for supporting of collecting sponges in Sabang island; and Laboratory of Marine Natural Products, Faculty of Science, University of the Ryukyus for supporting of isolation and structure determination.

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