An indole alkaloid produced by Indonesian's marine sponge *Raspailia ramosa* as an inhibitory of the panc-1 cell adapted to nutrient starvation

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Abstract. The bioactivity from Indonesian's Marine Sponge *Raspailia ramosa* has been done on July 2018. Bioactive compound isolated based on bioassay-guided separation with several steps of chromatography. The active metabolite compound of *Raspailia ramosa* has cytotoxic activity against PANC-1 cell with $IC_{50} = 10$ µg / mL. Based on NMR spectrum shows that the active compound has molecular formula $C_{16}H_{17}N_4Br$ determined by LCMS-ESI with molecular weight $[M+H]^+345.23$ m/z. The FTIR spectrum shows that metabolic metabolites have NH secondary amines 3435.56 cm-1 with CN imine fingerprint area (1637.27 cm-1), CH methyl (2853.39 cm-1), CH methylene (2769.64 cm-1) indicate as indole alkaloid. The active metabolite compound of *Raspailia ramosa* has cytotoxic activity against PANC-1 cellwith$IC_{50} = 10$ µg / mL. This compound has the potential activity for the drug model for commercial drug antimycin = 0.1 µg / mL.

1. Introduction

Pancreatic cancers possess a poor prognosis with less than 4% of five-year survival rate. Cancer is a disease caused by the destruction of the basic regulatory mechanisms of cell behavior because of an error or failure in the condition of cells that result in uncontrolled growth factors [1,2]. Cancer therapy can be done in various ways, ranging from the conventional nature of the surgery to the modern nature of the use of chemotherapy. However, chemotherapy has some disadvantages and harmful side effects, including causing damage to surrounding tissues and other organs, such as the stomach, liver, and kidney. Although radical resection rate seems to be improved lately because of sufficient pancreatic resection, extensive lymph nodes and retroperitoneal plexus dissection, and combined resection of major blood vessels; long-term survival rate is not as high as expected [3,4].

Induction of glucose-related protein 78 (GRP78) was found to be important for the adaptation of cancer cells to nutrient starvation, and these processes have attracted attention as drug targets for cancer chemotherapy [5,6,7].

Therefore, the objective of this study was to investigate the active compounds from Indonesian's Marine Sponge *Raspailia ramosa* against pancreatic cancer cell line in order to provide effective treatments of pancreatic cancers.

2. Materials and Methods

2.1 Materials

NMR Spectrum analysis was carried out using JEOL ECA-500 (^{1}H: 500 MHz, ^{13}C: 125 MHz). Extract samples were observed using ESI-TOF-MS type Q-Tof Ultima (Waters Co., MA, U.S.A). IR spectrum observation uses JASCO FT / IR-5300 tools. UV spectrum was studied using UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan). Open chromatography column type Silica gel BW-200 (Fuji Silysia, Aichi, Japan), Cosmosil 5C18-MS-II (10 mm id × 250mm, Nacalai Tesque) and Cosmosil ODS (75C18-OPN, Nacalai Tesque, Kyoto, Japan) are used for fraction separation. Further analysis using HPLC chromatography (UV detector: L-4000H) and TLC Silica gel 60F$_{254}$ (Merck Chemical, Darmstadt, Germany). The fraction extract obtained was analyzed with additional tools such as 96-well plastic plates Bioradic spectroscopy and biorad plate, preparation toxicity test using CO_2 incubator. Biomaterial analysis using Kanamycin 50 ug / mL solution, blue trypan, trypsin Mc-Coys medium, panc-1 human cell, glucose deficiency media, general glucose media, Phosphate buffer saline (PBS), Fetal Bovine Serum (FBS), ethyl acetate, n-Heksane, aquadest ethanol, methanol, and Dulbecco's modified Eagle's medium (DMEM).

2.2 Cell Culture and Toxicity Test

Dulbecco's modified Eagle's medium (DMEM) cultivated PANC-1 with the use of 10% fetal bovine serum (FBS) as an additive with an inactivity temperature and kanamycin (50μg / mL) in a humid place with a range below 5% CO_2 and temperature 37°C. with a nutritional deficiency situation, PANC-1 can be processed with Glucose deficient Medium (Basal Medium (25mM N- (2-hydroxyethyl) piperazine-N'-2-ethanesulfonic acid (HEPES) buffer (pH 7.4) plus 6.4g / L NaCl , 700mg / L, NaHCO$_3$, 400mg / L, KCl, 265mg / L CaCl$_2$.2H$_2$O, 200mg / L MgSO$_4$.7H$_2$O, 125mg / L, NaH$_2$PO$_4$, 0.1mg / L Fe (NO$_3$) .9H$_2$O, 15mg / L Phenolred, 10mL / l vitamin solution (X100) (GIBCO, Carlsbad, CA, USA), 200mmol / L L-glutamine solution (GIBCO), 10% FBS filled with kanamycin 50mg / L which has been dialyzed. The addition of glucose which is 10% Fetal Bovine Serum (FBS) with 2.0 g / L glucose (25mM) can function as a toxicity test to determine differences in cell development when glucose is glucose deficiency medium [8,9,10,11].

Using DMEM with 10% FBS monitored PANC-1 cells for 24 hours. On the media to be replaced so that it can adjust the nutrient deficiency used Ordinary Glucose Medium. Samples that have been dissolved and incubated in moist conditions of 5% CO_2 at 37°C are carried out after 12 hours of incubation [12,13,14]. Colorimetric Reagents WTS-8 detects cell proliferation. IC50 values can be determined by interpolated growth inhibiting curves. Based on differences in IC50 values in general glucose media and Deficient Medium glucose to determine the results of selectivity on anti-proliferation activity (S.I).

2. 3 Extraction and Isolation

Sponge (*Raspailia ramosa*) weighing 100 g is cut into small parts and extracted for 72 hours using methanol solvent. The extract results were dried and evaporated obtained 62.50 g methanol crude. Then, *Raspailia ramosa* (42.2 g) partitioned using *n*-Hexane: EtOAc: EtOH (1: 1: 1 v / v) produce *n*-Hexane fraction (0.79 g), EtOAc fraction (15.26 g), and EtOH fraction (26.15 g). Based on viability results showed that EtOH fraction has activity [26.15 g (IC$_{50}$ = 50 μg / mL)]. Ethanol fraction (26.15 g) fractionated using an open column of chromatography (OPN-C18) with DCM: EtOH: 0.1% TFA gradient produced 8 fractions. The fifth fraction [6.73 g (IC$_{50}$ = 35 μg / mL)] purified using the RP-18 MPLC column (MeCN: MeOH) gradient obtained 11 fractions. The seventh fraction has cytotoxic [2.46 g (IC$_{50}$ = 20 μg / mL)] and repurified using HPLC RP-18 column (MeCN: H$_2$O gradient)

produce 3 fractions. The activity of the second fraction [0.08 g (IC_{50} = 10 μg / mL)] showed cytotoxic activity against PANC-1 cell.

3. Results and Discussions

Compound 1 (yellow amorphorus) which has been isolated known to has the molecular formula $C_{16}H_{17}N_4Br$ determined by LCMS-ESI with molecular weight $[M+H]^+$ 345.23 m/z. The spectrum of FTIR shows that the active metabolite has N-H secondary amines 3435.56 cm^{-1} with the fingerprint region of C-N imine (1637.27 cm^{-1}), C-H methyl (2853.39 cm^{-1}), C-H methylene (2769.64 cm^{-1}). Interpretation of data indicates that the active metabolite compound as an alkaloid [15,16].

The ^{1}H spectrum (Table 1) showed two CH methylene signals (δ_H = 7.35, 5.30 ppm). The proton signals from the amine groups (δ_H = 3.30 ppm) indicates as a tertiary amine. Based on the data indicates that the compound as the indole alkaloid skeleton [17,18].

The ^{13}C spectrum (Table 1) showed different neighborhood. The signal of C8 and C10 show that the same neighborhood as singlet signal (δ_C = 121.9 ppm). The signal of quartz carbon detected at C2, C4, C5, C6, C7, C8, C10, C11, C12 (δ_C = 114.9, 137.0, 123.0, 110.8, 126.4, 121.9, 121.9, 158.5, 152.0 ppm). Based on the interpretation of data indicate that the active metabolite as indole alkaloid [19,20].

Table 1. ^{1}H and ^{13}C (500 MHz) data NMR (CDCl$_3$)

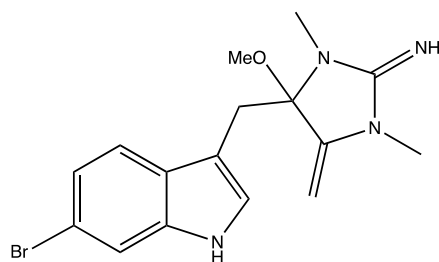| Position	δ_H	δ_C	Mult. (J), int
1	7.35	123.2 (d)	m, 1H
2	-	114.9 (s)	-
3	5.30	114.4 (d)	m, 1H
4	-	137.0 (s)	-
5	5.31	123.0 (s)	m, 1H
6	-	110.8 (s)	-
7	-	126.4 (s)	-
8	2.92	121.9 (s)	m, 1H
9	-	36.6 (t)	-
10	-	121.9 (s)	-
11	-	158.5 (s)	-
12	2.98	152.0 (s)	m, 1H
13	3.30	52.4 (q)	m, 3H
14		84.4 (t)	m, 1H
15	3.67	31.8 (q)	m, 3H
16	3.04	25.7 (q)	m, 3H

Figure 1. Structure of Active Compound.

Observation of cytotoxic effects in glucose deficiency medium using cell proliferation detected by WST-8 colorimetric reagents. Based on IC_{50} values the active compound from *Raspailia ramosa* has potential viability in glucose deficiency medium.Cell proliferation was detected by WST-8 colorimetric reagents. The value of IC_{50} is determined by the linear interpolation of the growth-survival curve. Based on IC_{50} values indicate differences in viability in glucose deficiency medium and general glucose medium. The active-compound had anti-proliferative activity in glucose deficiency medium but did not show activity in general glucose medium.
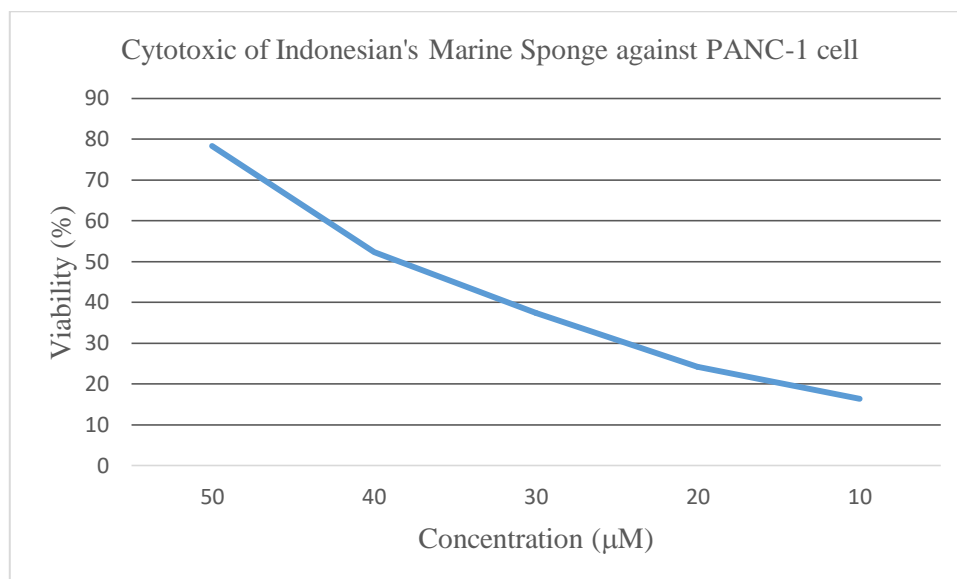

Figure 2. Cytotoxic of Indonesians Marine Sponge against PANC-1 cell

4.Conclusion

The active metabolite from *Raspailia ramosa* has cytotoxic activity against PANC-1 cell with IC_{50} = 10 μg/mL. The compound has a potential activity to alternative drug models against commercial drug antimycin = 0.1 μg/mL.

Acknowledgements

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