Scientific paper

Aaptamine, Isolated from the Bunaken National Park's Sponge, *Aaptos* sp., Promotes Cell Cycle Arrest and Induces Necrotic Cell Death of Colorectal Cancers

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Abstract

The sponge is one of the potential sources of bioactive compounds. Among them, the sponge *Aaptos* has been a promising source of leading drugs including aaptamine (**1**). This study investigated the aaptamine (**1**), spectroscopically determined, from *Aaptos* sp. collected from Bunaken National Park, Indonesia, as an anticancer agent, specifically targeting colorectal cancers (CRCs). Compound **1** showed potent cytotoxicity against DLD-1 and Caco-2 with IC_{50} values of 30.3 and 236.8 μg/mL, respectively. In addition, the exposure of compound **1** on those colorectal cancer cells could promote cell cycle arrest and induce necrotic cell death.

Keywords: Sponge, *Aaptos*, aaptamine, DLD-1, Caco-2, Necrotic

1. Introduction

Colorectal cancer is the third most common cause of cancer-related deaths.[1] In 2020, colorectal cancer (CRC) resulted in approximately 1.9 million new cases and 0.9 million fatalities globally. The frequency of CRC has increased in developing countries because of the shifting in lifestyle and diet towards Westernization.[2] According to GLOBOCAN (2020), Indonesia contributed 34.189 (8.6%) cases of colorectal cancer in a total of 396.914 cases consisting of 21.764 cases for men and 12.425 cases for women.[3] The elevated incidence of CRC in men may be attributed to a variety of factors. Unlike women, men appear to be more significantly impacted by environmental influences rather than genetic factors, including alcohol consumption, obesity, smoking, and bad dietary habits.[4] The substantial occurrence and fatality rates associated with CRC, coupled with the limitations of current treatments and preventive measures, underscore the pressing necessity for the exploration and development of new drugs.

Natural products hold the promise to be a source of new drugs with minimum side effects and improved compatibility with the human body.[5] Over the years, the potential of Marine Natural Products (MNPs) has attracted the attention of researchers. The immense expanses of the oceans and many levels of biodiversity in the marine environment make researchers eagerly explore novel active compounds from sponges.[6]

Sponges are marine invertebrates and have been recognized as a major source of bioactive compounds.[7] For example, eribulin mesylate from the sponge *Halichondria okadai* has been reported to exhibit potential clinical activity against pre-treated metastatic breast cancer cells.[8]

Over various genera of sponges, *Aaptos* has been known as a genus of sponges that produce bioactive compounds. *Aaptos* is a cosmopolitan marine sponge with a wide geographical distribution including Indonesian waters.[9] A unique bioactive compound identified from this genus is aaptamine, which exhibits various biological activities, including antibacterial,[10,11] antioxidant,[12] antiproliferative,[13] anti-mycobacterial,[9] α-adrenoceptor blocking,[14] sortase A inhibition, [15] cholinesterase inhibition,[16] antifungal,[17] anti-HIV,[17] and cytotoxic activities.[18]

Recently, Utkina and co-workers (2021) reported that isoaaptamine isolated from *A. aaptos* could reduce the expression of enzymes in human colorectal adenocarcinoma DLD-1 cells from 100% to 64% at a concentration of 5 μM.[19] Another bioactive compound, 3-([9-methylhexadecyl] oxy) propane-1,2-diol 2,2,3-dihydro-2,3-dioxoaaptamine, shows cytotoxic activity against lung cancer SK-LU-1, breast cancer MCF-7, liver cancer HepG2, and melanoma SK-Mel-2 with IC_{50} of 41.27 ± 2.63, 40.70 ± 2.65, 34.31 ± 3.43, and 36.63 ± 1.40 μM, respectively. [20]

In this study, we will report bioactive compounds isolated from the marine sponge *Aaptos* sp. as anticancer agents against colorectal cancer cells, DLD-1 and Caco-2. Herein, we also describe the mechanism of action of the active compounds present in this sponge.

2. Method

2. 1. Biological Material

The sponge was collected by scuba diving at a depth of 10-15 meters from Likuan 3, Bunaken Island, Bunaken National Park, North Sulawesi, Indonesia (N 01.60572 E 124.76818), in September 2022. The sample was immediately frozen and kept at the Genomics Laboratory, National Research and Innovation Agency (BRIN), Cibinong, West Java, Indonesia, until the extraction was performed. The specimen was identified by the analysis of morphology and the spicules which were identified as *Aaptos* sp.

2. 2. Extraction

The extraction process was conducted according to Ahmadi et al. (2017).[21] In brief, the sample (80.94 g, wet) was chopped and soaked in methanol (MeOH) overnight. Thereafter, the filtrate was collected by filtration and the spent biomass was reextracted using fresh methanol. The extraction was performed for at least three times. After maceration, all filtrate was combined and concentrated by using a Rotary Vacuum Evaporator (Rotavapor Buchi R-300). Subsequently, the residue was partitioned exhaustively by using water and ethyl acetate (EtOAc) to give 388.97 mg of EtOAc fraction (SBM 066-1).

2. 3. Thin Layer Chromatography (TLC)

As guidance to identify the components present in the extract, a thin layer chromatography (TLC) was performed using TLC sheets of silica gel 60 F_{254} (Merck), with a mobile phase of n-hexane:EtOAc (7:3). Silica plates were exposed to UV light at 254 nm. Further, the plates were reacted using cerium sulfate $Ce(SO_4)_2$, ninhydrin, and Dragendorff's. The TLC plate was heated and examined to calculate the Rf value.

2. 4. Open Column Chromatography (OCC) by Silica

The SBM 066-1 (~250 mg) was fractionated by using open-column chromatography (OCC) with normal-phased silica (7500 mg) as a stationary phase. Several mobile phases were used and started consecutively with n-hexane, followed by dichloromethane (DCM), EtOAc, and MeOH. Every solvent was collected and gave a corresponding yield of 80.53 mg (SBM 066-1-1), 26.13 mg (SBM 066-1-2), 112.76 mg (SBM 066-1-3), and 55.86 mg (SBM 066-1-4).

2. 5. Spectroscopy Analysis

The sample was analyzed by using the Waters ACQUITY UPLC® H-Class System, which included an ACQUITY UPLC® HSS C_{18} column (11.8 μm, 2.1 Å, 100 mm) and coupled with the Xevo G2-S QTOF Mass Spectrometer, all from Waters, Beverly, MA, USA. The system utilized electrospray ionization (ESI) in a positive ion mode for the mass spectrometry. The elemental compositions were determined using the Waters MassLynx (v4.1) and Mestrenova software. Additionally, the compound type was compared to the PubChem database. Meanwhile, ^{1}H-NMR spectra were recorded by using BRUKER at 500 MHz for ^{1}H-NMR in CD_3OD.

2. 6. Purification using Sep-pak C_{18}

Further purification was conducted by applying 53 mg of SBM 066-1-4 on Sep-pak C_{18} and eluted with several eluent: 100% H_2O, 25% MeOH in H_2O, 50% MeOH in H_2O, 75% MeOH in H_2O, and 100% MeOH. Five fractions were yielded with amounts of 27.63 mg (SBM 066-1-4-1), 3.3 mg (SBM 066-1-4-2), 0.65 mg (SBM 066-1-4-3), 8.07 mg (SBM 066-1-4-4), and 13.35 mg (SBM 066-1-4-5) (Figure S1). Fraction SBM 066-1-4-1 was further identified to lead to an aaptamine compound with a purity of more than 90% (Figure S6).

2. 7. Cell Culture and Conditions

The study was conducted on two human colorectal cancer cells (DLD-1 and Caco-2) and normal cells (HEK293), all sourced from the American Type Culture Collection (ATCC, USA). These cell lines were grown in DMEM medium (Gibco, Germany) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich),1.5% Penicillin/Streptomycin (Sigma, Aldrich), and 0.5% fungizone (Sigma-Aldrich). The cells were maintained in a cell culture incubator set to 37°C with 5% CO_2 to create an optimal environment for their growth.

2. 8. MTT Assay

The colorectal cancer cells, DLD-1 and Caco-2, along with HEK293 as normal cells, were collected when they reached 70-80% confluence and then subjected to centrifugation at 1200 rpm at 21 °C for 5 minutes. The cells were enumerated using a microscope and a haemocytometer then were plated into a 96-well plate at a concentration of 1×10^4 cells per well. These plates were placed in an incubator at 5% CO_2 and 37°C for 24 hours to facilitate cell attachment. After this incubation period, non-adherent cells were carefully removed. The cells were then exposed to a 100 mg/mL sample (for screening purposes) and a series of dilutions (100, 50, 25, 12.5, 6.25, 3.125 mg/mL for IC_{50} purposes) of compound **1** and further incubated for 72 hours. All the samples were prepared by diluting them in DMSO and then in a culture medium. In this experiment, doxorubicin was used as a positive control with various concentrations corresponding to the sample's concentration. Following this incubation, MTT reagent (Sigma-Aldrich, Germany) (5 mg/mL) was introduced into each well and kept in the same incubation conditions (5% CO_2 and 37° C) for 4 hours. The mixture of MTT and media was subsequently discarded, and the purple formazan crystals were dissolved in sodium dodecyl sulfate (SDS) and further incubated in a dark environment. The absorbance at a wavelength of 570 nm was measured using Synergy HTX Multi-Mode Reader (Agilent Technologies, USA). All experiments were conducted in triplicate. The 50% inhibitory concentration (IC_{50}) of the samples was determined through statistical analysis. Cell viability was assessed using the following formula:

Cell Viability = (Abs Treated cells / Abs Untreated cells) × 100%

2. 9. Cell Cycle Assay

Cell cycle assay was conducted using a flow cytometer with a cell cycle staining kit from Abbkine (cat No. Cat #: KTA2020) according to the manufacturer's instructions. Briefly, colorectal cancer cells (DLD-1 and Caco-2) were harvested at 70–80% confluency before 5×10^4 cells/mL of DLD-1 cells and 1×10^4 cells/mL of Caco-2 cells were then seeded in a 6-well plate containing DMEM medium (10% FBS, 1.5% penicillin- streptomycin, 0.5% fungizone) and incubation was set to 5% CO_2 at 37 °C for 24 h. Cells were then treated with half of the compound **1** IC_{50} (μg/mL) in DLD-1 or Caco-2 cells (15.15 and 118.4 μg/mL, respectively) for 72 h. After that, cells were harvested to obtain pellets and then fixed with 70% ethanol at –20 °C overnight before being stained with PI staining solution. After 30 minutes of incubation at 37 °C in the dark, the cells were washed with PBS and analyzed with a BD Accuri™ C6 Plus Personal Flow Cytometer (BD Biosciences).

2. 10. Apoptosis Assay

Apoptosis assay was performed by Annexin V-FITC/PI staining using flow cytometry. After reaching confluency at 70–80%, a total of 5×10^4 cells/mL of DLD-1 cells and 1×10^4 cells/mL of Caco-2 cells were seeded in each well of a 6-well plate and then treated with half of the compound **1** IC_{50} (μg/mL) in DLD-1 or Caco-2 cells (15.15 and 118.4 μg/mL, respectively) for 72 h. Cells were then harvested using trypsin-EDTA before being stained with an Annexin-V-FLUOS staining kit (Roche, Zhangjian Hi-Tech Park, Shanghai, China) and incubated for 15 minutes in the dark. The cells were then analyzed with a BD Accuri™ C6 Plus Personal Flow Cytometer (BD Biosciences) within 30 min of staining.

3. Results And Discussion

3. 1. Cytotoxic Activities

The preliminary cytotoxic evaluation of the ethyl acetate fraction of *Aaptos* sp. (SBM 066-1) was evaluated against DLD-1, Caco-2, and HEK293 cell lines. In this study, the IC_{50} was observed at 20.91 and 21.64 μg/mL for DLD-1 and Caco-2, respectively. Meanwhile, the IC_{50} value of SBM 066-1 against HEK293 shows less cytotoxic with an IC_{50} value of 41.97 μg/mL (Figure S2, Table S1).

3. 2. Characterization of Major Compound (1) Contained in SBM 066-1 and its Purification

As a preliminary analysis, the characteristic compounds contained in fraction SBM 066-1 were conducted by using the TLC visualization method. The first visualization using UV light at 254 nm (short wavelength) showed the phosphorescent dark spot at Rf 0 (Figure S3a), cerium (IV) sulfate showed a brown spot in the polar region (Rf 0) and less polar region (Rf 0.7 and 0.8) (Figure S3b), Dragendorff's specific reagent showed an orange spot at Rf 0 (Figure S3c), and ninhydrin visualization analysis showed a purplish stain at Rf 0 (Figure S3d). Summarizing the TLC analysis, major compounds contained in fraction SBM 066-1 were characterized as alkaloid compounds bearing conjugated double-bonds and/ or aromatic rings, possess-

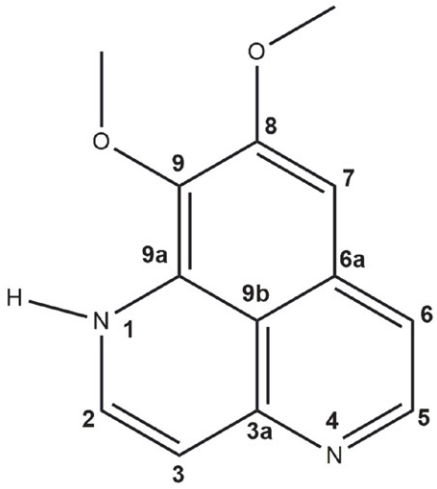

Figure. 1 The planar structure of aaptamine (**1**, SBM 066-1-4-1).

ing tertiary, primary, and/ or secondary amine moieties. These analyses elicited that the major compound contained in SBM 066-1 was similar to the characteristics of aaptamine.

Further separation of SBM 066-1 on silica open column chromatography (OCC) gave four distinct fractions (SBM 066-1-1 – SBM 066-1-4). All the fractions were then tested against colorectal cancers (DLD-1) using an MTT assay at a final concentration of 100 mg/mL. Among the tested fractions, SBM 066-1-4 showed the highest activity with a % inhibition value of 83.3.

The LCMS/MS chromatogram (Figure S4) of SBM 066-1-4 showed a total of 13 peaks which is described in Table S2. The major peak, Rt 4.62 was selected for further analysis and presumed as the major compounds that are responsible for the anticancer activities in the fraction SBM 066-1-4. The peak at Rt 4.62 min, showed an *m/z* value at 229.0976 $[M+H]^+$ with chemical formula $C_{13}H_{13}N_2O_3$ (calcd for $C_{13}H_{13}N_2O_3$ 229.0977 (Δ – 0.1 ppm)). This compound was identically 99.20% with aaptamine and was supported by its mass-fragmentation patterns (Figure S5). Hence, SBM 066-1-4 was determined to contain aaptamine as one of the major compounds.

The ^{1}H-NMR-based metabolite profiling was then performed to identify the proton environments of compound 1 (Figure S6). There are distinct signals for two methoxy protons at δ_H 3.89 (s, 8-OCH_3) and δ_H 4.04 (s,

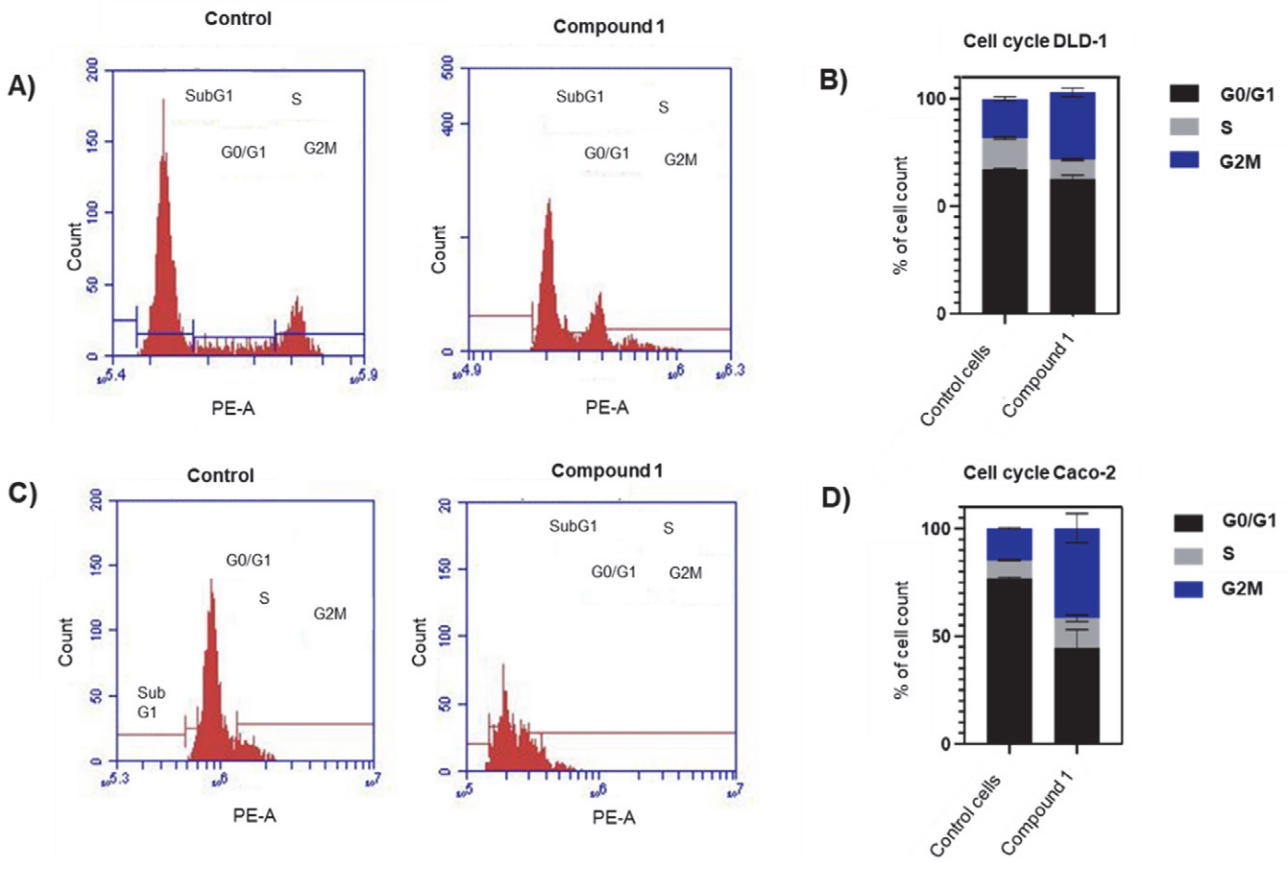

Figure 2. The effect of compound **1** on cell cycle distribution against DLD-1 and Caco-2 colorectal cancer cells (A) The DLD-1 cell cycle histogram (B) The cell distribution analysis of DLD-1 cells cycle (C) The Caco-2 cell cycle histogram (D) The cell distribution analysis of Caco-2 cell cycle. Both cell lines were treated with a 10 µg/mL concentration of compound **1** for 72h and stained with PI to analyze cell distribution by flow cytometry. Vertical bars represent the standard deviation of means (SD) (n = 3).

9-OCH_3), two pairs of coupled protons at δ_H 7.78 (d *J*= 7.5 Hz, H-2), δ_H 6.35 (d, *J*= 7.5 Hz, H-3), and δ_H 7.59 (d, *J*= 7.5 Hz, H-5) and δ_H 6.88 (d, *J*= 7.5 Hz, H-6). In addition, a single proton signal at δ_H 8.19 indicated a proton signal of H-7 (Table S3). These signals supported the chemical shifts for aaptamine.

3. 3. Further Isolation of aaptamine (1)

The major compounds in SBM 066-1-4 were further purified using Sep-pak C_{18} to give a single compound aaptamine (**1**, SBM 066-1-4-1, 27.63 mg, Figure 1).

3. 4. Compound 1 Induces Cell Cycle Arrest on Colorectal Cancer Cells

The examination of several stages of the cell cycle is essential to get valuable knowledge about the mechanisms that govern cellular growth and division. To investigate cell cycle arrest, the treated colorectal cancer cells were analyzed by flow cytometry at the different cell cycle phases (G0/G1, S, and G2/M) on DLD-1 and Caco-2 cells. On the DLD-1 colorectal cancer cells, compound **1** treatment increased cell accumulation mainly in the G2/M phase (Figure 2A and 2B), from 18.42% (control cells) to 31.28% (compound **1**).

Meanwhile, on the Caco-2, the compound **1** administrations have increased in the S phase from 8.66% in the control cells to 13.81%. Moreover, compound **1** also increases the cell accumulation in the G2/M phase from 14.82% (control cells) to 41.80% (compound **1**) (Figure 2C and 2D). Overall, the exposure of compound **1** on Caco-2 colorectal cancer cells induced cell cycle arrest because of the accumulation in the S phase and G2/M phase that influences the synthesis DNA process and inhibits the mitosis process in colorectal cells.

Overall, based on the cell cycle study, the G1 phase (%) was deficient, meanwhile, the G2/M phase (%) was increased in colorectal cancer cells (DLD-1 and Caco-2) (Figure 2B and 2D). Thus, the exposure of compound **1** on colorectal cancer cells DLD-1 and Caco-2 could relatively induce cell cycle arrest. These results also suggest that compound **1** inhibits the cell cycle of DLD-1 colorectal cancer cells through different mechanisms than Caco-2 colorectal cancer cells, specifically interfering with the preparatory phase of mitosis. Some anticancer drugs cause cell death by interfering with the cell cycle processes, including inhibiting the phase of mitosis.[22] As a result, a mi-

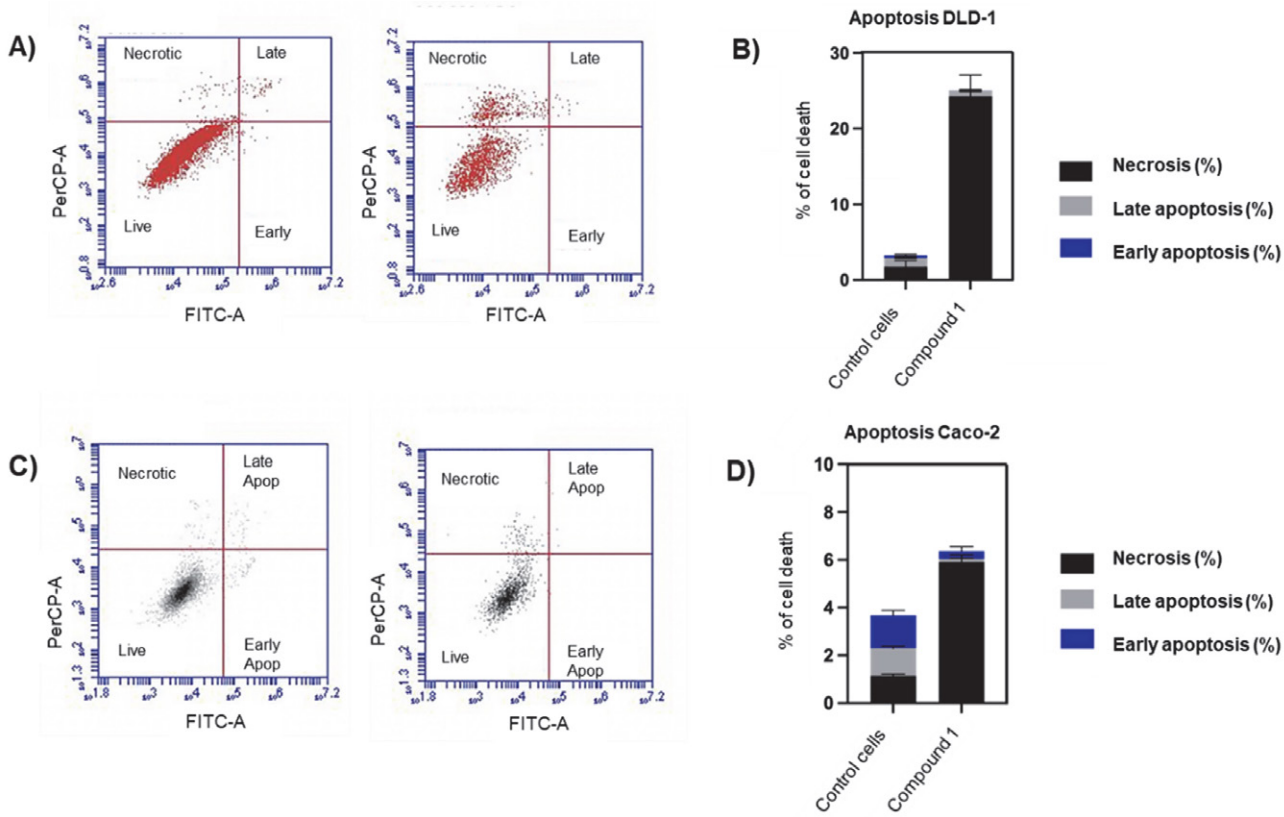

Figure 3. The effects of compound **1** treatment on apoptosis in DLD-1 and Caco-2 colorectal cancer cells (A) The DLD-1 cells death histogram (B) The cell distribution analysis of DLD-1 cell death (C) The Caco-2 cells death histogram (D) The cell distribution analysis of Caco-2 cell death. Both cell lines were treated with 10 µg/mL of compound **1**, stained with Annexin V, PI, and performed flow cytometry analysis. Vertical bars represent the standard deviation of means (SD) (n = 3).

totic catastrophe might happen, an oncosuppresive mechanism causing DNA damage and deformation of the microtubules associated with G2/M phase arrest. The characteristics or features of mitotic catastrophe are similar to necrosis where the cell size amplifies, and the DNA degrades in the first 24 h.[23]

3. 5. Cells Apoptosis Induction

An investigation into apoptosis, the regulated process of cell death, is crucial for the development of anticancer drugs that can specifically induce apoptosis in cancer cells, ultimately resulting in enhanced patient outcomes. Annexin V-FITC/PI double staining was conducted to confirm whether the effects of compound **1** on the cytotoxicity of DLD-1 and Caco-2 colorectal cancer cells were related to apoptosis or necrotic cell death, In both cells. the treatments of compound **1** at 10 μg/mL for 72 h show that the apoptotic cell population decreased, compared with the untreated cell population (Figure 3). In contrast, the necrotic cells demonstrated an increasing population from 1.75% and 1.13% (untreated cells) to 24.25% and 5.91% (compound **1**) in DLD-1 and Caco-2 cells, respectively. This result concludes that the compound could induce necrotic instead of apoptotic cell death in DLD-1 and Caco-2 colorectal cancer cells.

3. 6. The activity of Compound 1 against CRCs

To understand how compound **1** affects cell viability, this experiment assessed the cytotoxic activity by measuring cellular metabolic activity with MTT by converting a yellow dye into a purple formazan product. After treatment for 72 hours, a strong reduction was observed in doxorubicin-treated cells with 0% in DLD-1 and HEK293 and a 20% decline in Caco-2 cells (Figure 4A). A significant decrease in the number of viable cells was observed in

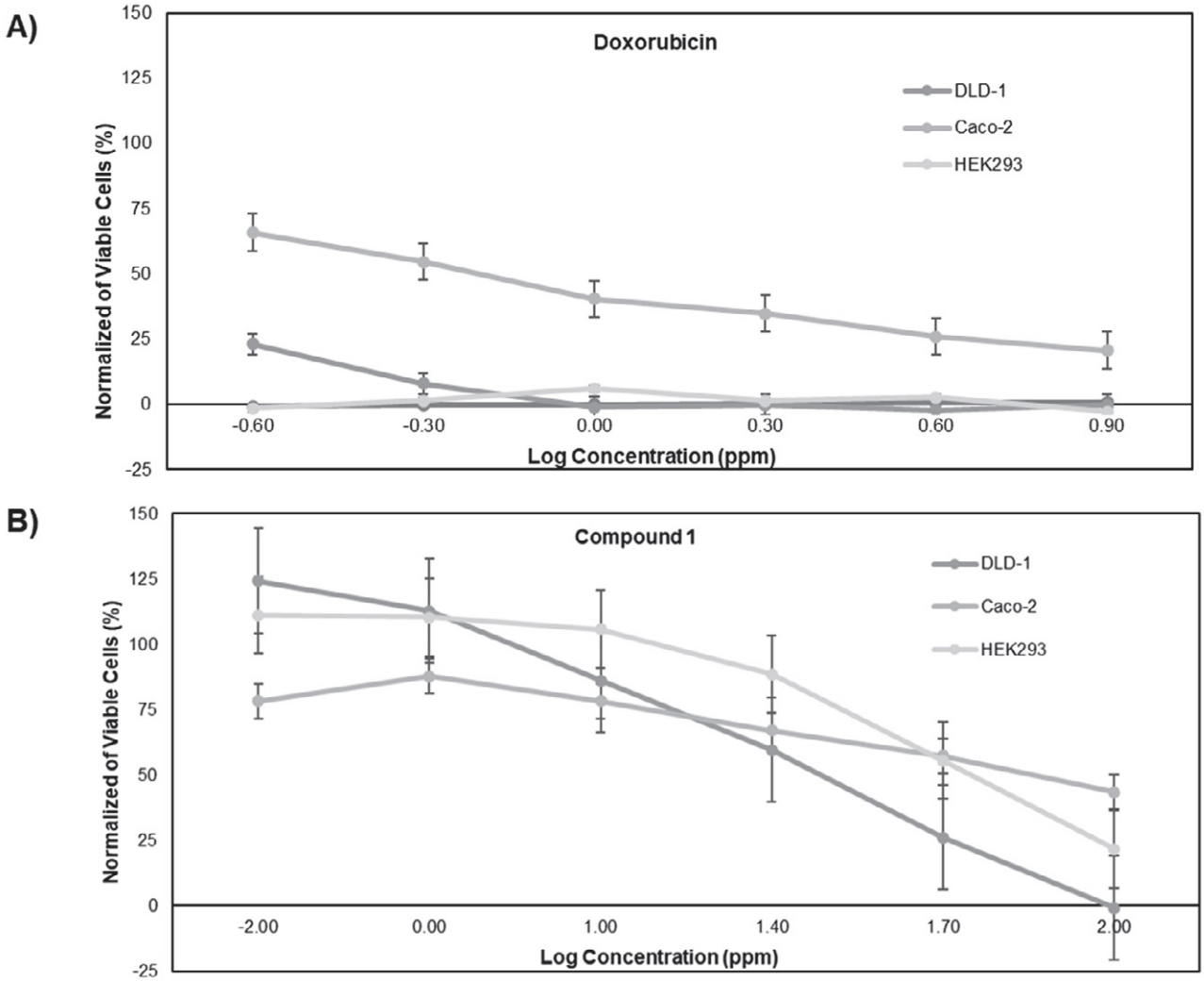

Figure 4. The viability of doxorubicin and compound **1** on DLD-1, Caco-2, and HEK293 cells. (A) The viability of doxorubicin-treated cells (B) The viability of compound **1**-treated cells. Both were measured by using an MTT assay.

Table 1. The IC_{50} values of compound **1** against several cell lines.

No	Cell lines	Compound	IC_{50} (μg/mL)	Selectivity Indeks (SI)
1.	DLD-1	Aaptamine (**1**)	30.3	4.03630363
		Doxorubicin	0.1	0.055
2.	Caco-2	Aaptamine (**1**)	236.8	0.51646959
		Doxorubicin	1.6641	0.00330509
3.	HEK293	Aaptamine (**1**)	122.3	–
		Doxorubicin	0.0055	–

DLD-1, Caco-2, and HEK293 cell lines, reaching 0%, 43%, and 21%, respectively (Figure 4B). Thus, the effect of compound 1 on DLD-1, Caco-2, and HEK293 cell viability after 72 hours is dose-dependent, with higher concentrations leading to greater reductions in cell viability. These data also demonstrated that compound **1** is toxic to DLD-1 cells with an inhibitory concentration (IC_{50}) of 30.3 μg/mL, 236.8 μg/mL, and 122.3 μg/mL on DLD-1, Caco-2, and HEK293 cells, respectively (Table 1). However, in comparison to doxorubicin as a control, the IC_{50} value of doxorubicin was higher than compound **1**. The IC_{50} values of doxorubicin were 0.1 μg/mL, 1.6641 μg/mL, and 0.0055 μg/mL on DLD-1, Caco-2, and HEK293 cells, respectively (Table 1). Moreover, compound **1** exhibited dose-dependent cytotoxicity across both CRCs with particularly strong effects in DLD-1 cells with a selectivity index (SI) of 4.03, noticeably higher than Caco-2 cells with a selectivity index of 0.51. The SI results suggested that compound **1** preferentially targeted DLD-1 cells over Caco-2 cells, with an SI greater than doxorubicin (SI > 3). Taken together, these data indicated that the cytotoxic effect of compound **1** was both cell-type and dose-dependent.

4. Conclusions

Aaptamine, a marine alkaloid derived from the sponge *Aaptos* sp. has shown promising anticancer properties in various studies. This study demonstrated that the extract of *Aaptos* sp. (SBM 066-1) is the potential source of anticancer against CRCs (DLD-1 and Caco-2), and considerably less cytotoxic against normal cells HEK293. Further isolation and purification of the bioactive compound from the EtOAc extract of this sponge (SBM 066-1) led to the discovery of aaptamine (compound **1**). The LCMS/MS analysis and ^{1}H-NMR data showed a characteristic signal for a specific characteristic of **1** at *m/z* 229.0976 and aromatic rings at δ_H 7.78 (d, *J* = 7.5 Hz, H-2), δ_H 6.35 (d, *J* = 7.5 Hz, H-3), and at δ_H 7.59 (d, *J* = 7.5 Hz, H-5) as well as at δ_H 6.88 (d, *J* = 7.5 Hz, H-6) ppm. Then, the study reveals that compound **1** exhibits cytotoxicity against DLD-1 and Caco-2 cells with IC_{50} values of 30.3 and 236.8 μg/mL. Compound **1** shows potential as a cytotoxic agent against CRCs. Meanwhile, previous research [24] was mainly concentrated on an aaptamine-rich fraction, which exhibited a higher cytotoxic effect on DLD-1 colorectal cancer cell viability (IC_{50} value 9.597 μg/mL) compared with NIH-3T3 murine fibroblast cells (IC_{50} value 12.23 μg/mL). Both papers revealed that aaptamine could be promoted as a potent anticancer agent, and its ability to target DLD-1 cells selectively makes it a promising candidate for further research and potential development for colorectal cancer treatment. Notwithstanding, this current research discovered that aaptamine could relatively induce necrotic cell death and promote cell cycle arrest in both DLD-1 and Caco-2 cells. This study revealed that compound **1** could relatively induce necrotic cell death and promote cell cycle arrest. Aaptamine was found to interfere with the cell cycle of CRCs. Specifically, it causes arrest at the G2/M phase, preventing the cells from proceeding to mitosis, and thereby inhibiting their proliferation. Unlike apoptosis, which is a programmed and controlled form of cell death, aaptamine induces necrosis in CRCs, which causes traumatic cell death, leading to the release of cellular contents and triggering inflammation in the tumor microenvironment. The study suggests that aaptamine's cytotoxic and necrotic effects are mediated through oxidative stress, mitochondrial dysfunction, and the activation of specific signaling pathways related to cell cycle control and cell death. Overall, aaptamine demonstrated selective toxicity toward DLD-1 cells, with an SI greater than 3. This selectivity is particularly notable due to its ability to induce necrotic cell death and arrest the cell cycle, making it a promising candidate for targeted therapies in CRCs. Further research is required to fully understand its molecular mechanisms and to evaluate its efficacy in vivo.

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Conflicts of interest

The authors report no conflicts of interest.

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Povzetek

Spužva je eden od potencialnih virov bioaktivnih spojin. Med njimi je spužva *Aaptos* obetaven vir spojin vodnic, vključno z aaptaminom (**1**). V tej študiji je bil spektroskopsko določen aaptamin (**1**) iz spužve *Aaptos* sp., nabrane v narodnem parku Bunaken v Indoneziji, in proučevan kot protirakavo sredstvo, usmerjeno predvsem proti raku debelega črevesa in danke. Spojina **1** je pokazala močno citotoksičnost proti DLD-1 in Caco-2 z vrednostmi IC50 30,3 oziroma 236,8 μg/ml. Poleg tega lahko dodatek spojine **1** celicam kolorektalnega raka povzroči zastoj celičnega cikla in nekrotično celično smrt.