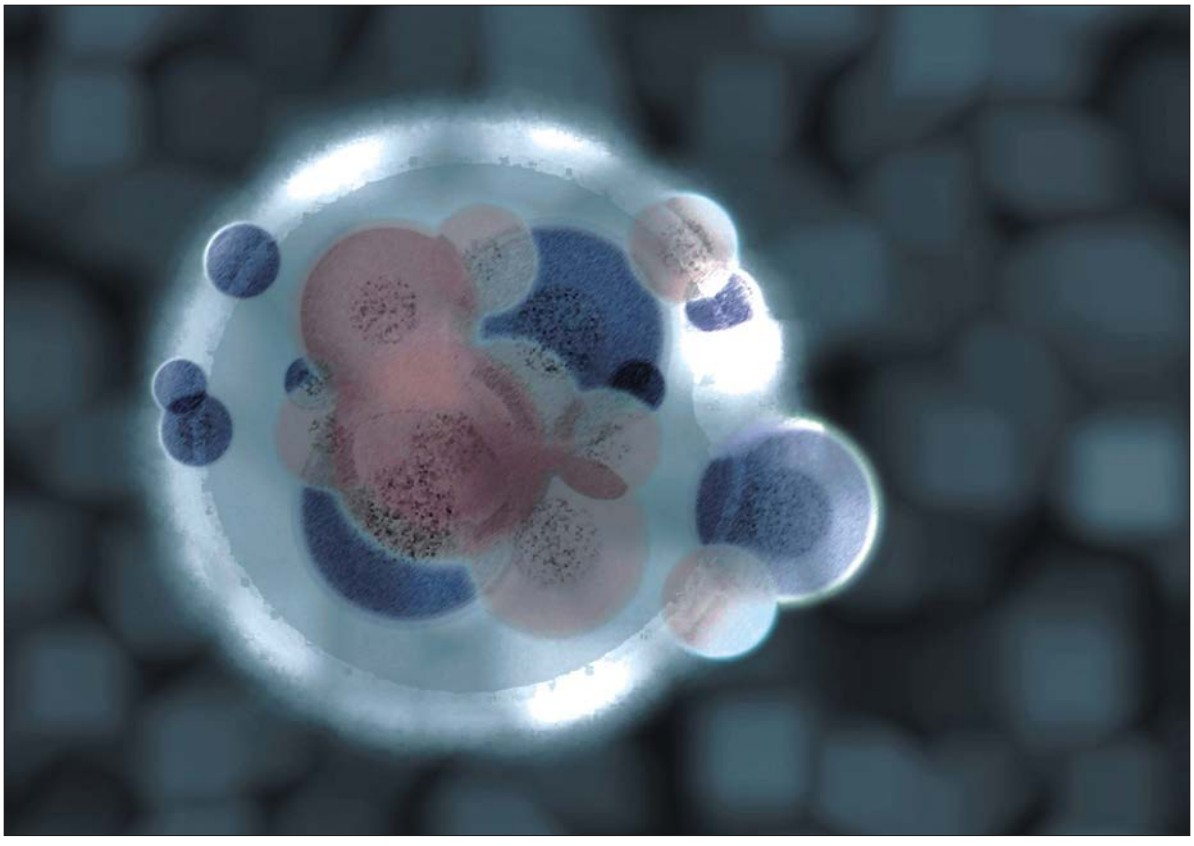


Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2016.30.36

Research Article

Apoptosis Induction of Bioactive Substance Theonellapeptolide 1d and 1-(Tetrahydro-4-Hydroxy-5-(Hydroxymethyl)Furan-2-yl)-5-Methyl Pyrimidine-2,4(1H,3H)-Dione Isolated from *Kaliapsis* sp. Sponge Collection from West Bali National Park Indonesia

[1]Erna P. Setyowati, [1]Umar Anggara Jenie, [1]Sudarsono and [2]L. Broto Kardono

[1]Faculty of Pharmacy, Gadjah Mada University, Jl. Sekip Utara, Yogyakarta, Indonesia
[2]Indonesian Institute of Science (LIPI), Jl. Gatot Subroto 10, Jakarta, Indonesia

Abstract

A study about cancer cell selectivity and apoptosis cell death had been conducted against 5M74 (theonellapeptolide 1d) and 1 (1-(tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-5-methylpyrimidine-2,4 (1H,3H)-dione). The 5M74 and 1 were isolated from *Kaliapsis* sp., sponge from West Bali National Park Indonesia. Cell selectivity was observed using heLa cell, myeloma cell, Raji cell and T47D cell. Apoptosis cell was conducted against the most sensitive cell with both isolates. The result were the 1 isolate was the most cytotoxic against myeloma cell because it had the lowest IC_{50} value namely 0.18 μg mL^{-1} while 5M74 isolate had the lowest IC_{50} value against Raji cell (7.8 μg mL^{-1}). The isolated cell death test result was the 1 isolate increased apoptosis against myeloma cell. Moreover, on 5M74 isolate also increased apoptosis against Raji cell.

Key words: Sponge, *Kaliapsis* sp., cell selectivity, apoptosis

Received: October 20, 2015 Accepted: December 21, 2015 Published: January 15, 2016

Citation: Erna P. Setyowati, Umar Anggara Jenie, Sudarsono and L. Broto Kardono, 2016. Apoptosis induction of bioactive substance theonellapeptolide 1d and 1-(tetrahydro-4-Hydroxy-5-(Hydroxymethyl)Furan-2-yl)-5-methyl pyrimidine-2,4(1H,3H)-dione isolated from *Kaliapsis* sp. sponge collection from West Bali National Park Indonesia. J. Biol. Sci., 16: 30-36.

Corresponding Author: Erna P. Setyowati, Faculty of Pharmacy, Gadjah Mada University, Jl. Sekip Utara, Yogyakarta, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

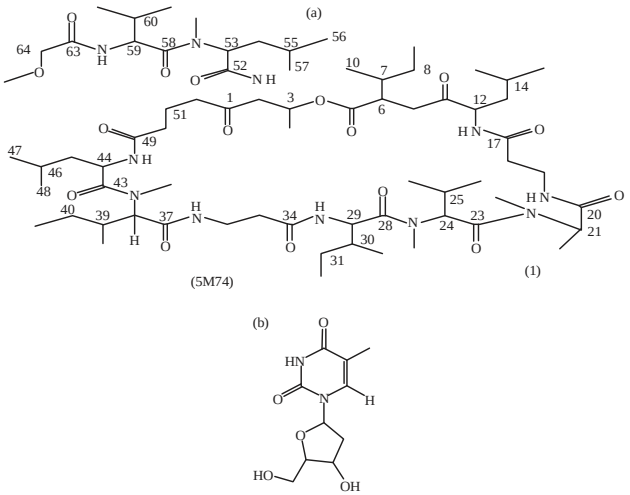
Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cancer is a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. If the spread is not controlled, it can result in death. Treatments include surgery, radiation, chemotherapy, hormone therapy, immune therapy and targeted therapy (drugs that specifically interfere with cancer cell growth) In 2015, about 589,430 Americans are expected to die of cancer or about 1,620 people per day. Cancer is the second most common cause of death in the US, exceeded only by heart disease and accounts for nearly 1 of every 4 deaths (ACS., 2015). According to Hanahan and Weinberg (2000), cancer was mostly characterized by able to provide its growth signal, insensitive to growth-inhibitory signals, evasion of apoptosis, potential to unlimited replication, angiogenesis and able to invade its surrounding tissue and metastasize.

The main cancer characteristic is uncontrolled cleavage because of cell cycle irregularity. Both external factors (growth factor) and internal factor (a series of kinase protein can induce cell cycle according to organism needs). Cell cycle inhibition cause the cell enter apoptosis phase. The process of programmed cell death or apoptosis, is generally characterized by distinct morphological characteristics and energy-dependent biochemical mechanisms. Apoptosis is considered a vital component of various processes including normal cell turnover, proper development and functioning of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death (Elmore, 2007). Apoptosis started by various signals that caused cell decay. The cell collapse and lost its common intercellular contact. Furthermore, chromatin considerated and became congested, cytoplasm became distended and the cell core fragmented into smaller apoptosis (Lockshin and Zakeri, 2001).

Sponge as the main material rich in bioactive substance invention that potential to produce anticancer bioactive substance (Belarbi *et al*., 2003). Hooper and Wiedenmayer (1994) stated that Australia, Indonesia and Papua New Guinea were the largest sponge producing area in the world. Research about sponge bioactive substance also represented that there were many sponge secondary metobalites should be able to develope as anticancer drug (Muller *et al*., 2004a, b). A previous study conducted by Setyowati *et al*. (2007, 2008, 2009) were showed that 5M74 isolate (theonellapetolide 1d) and 1 isolate (1-(tetrahydro-4-hydroxy-5-(hydroxymethyl) furan-2-yl)-5-methylpyrimidine-2,4 (1H,3H)-dione) successfully isolated from *Kaliapsis* sp. It had cytotoxic effect. In this study had already done a study about apoptosis effect of both substances.



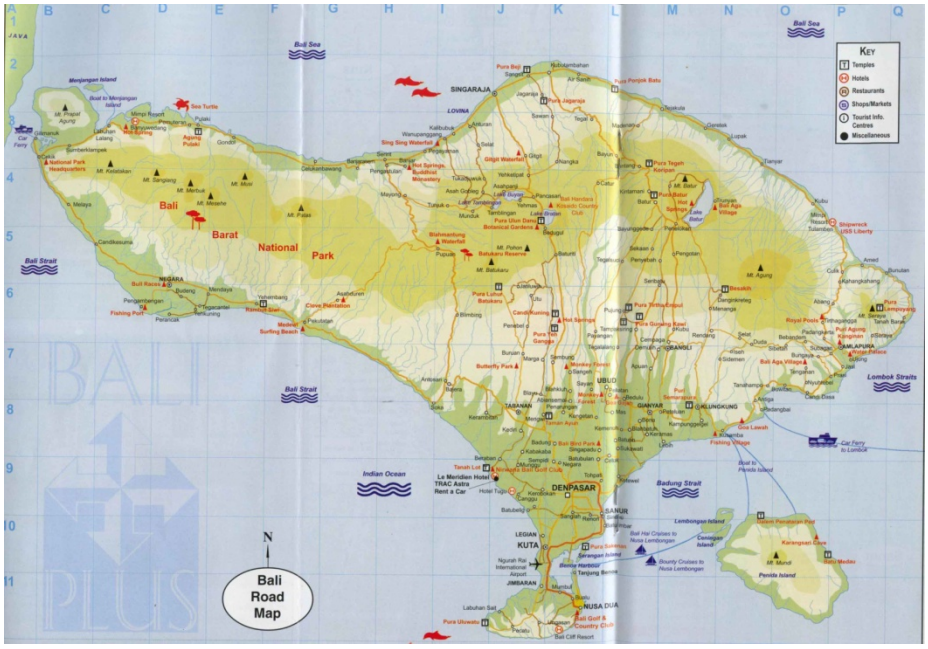
MATERIALS AND METHODS

Material: 1(1-(tetrahydro-4-hydroxy-5-(hydroxymethyl)furan-2-yl)-5-methyl pyrimidi ne-2,4 (1H,3H)-dione) and 5M74 (theonellapeptolide 1d) isolated from *Kaliapsis* sp. obtained from West Bali National Park, Indonesia (Fig. 1). Voucher specimen is kept at Department of Pharmaceutical Biology Faculty of Pharmacy UGM, Yogyakarta, Indonesia. Roswell Park Memorial Institute (RPMI) 1640 (sigma) media, Fetal Bovine Serum (FBS) 10% v/v (Gibco), Fungison 0.5%, penicillin-streptomycin 1% v/v (Gibco), doxorubicin hydrocholoride (ebewe/ebedoxo stock 10 mg 5 mL^{-1}), 3-(4,5-dimetiltiazol-2-il)-2-5-difenil tetrazoliumbromida (MTT) [sigma chemical Co).

Equipment: Incubator (hera cell (heraeus) Kendro Laboratory Product Germany), Laminar air flow (Labconco purifier™ class II) inverted microscope (axiovert 25), hemocytometer (nebauer), sterile conical tube, microplate 96 well (Nuclon), tissue culture flask, ependorf, yellow tip and blue tip. Fluorescent microscope (zeiss MC 80).

Cell cytotoxic: The density cell $2x10^4$ cell/well (heLa, myeloma), 1×10^4 cell/well (Raji, T47D) in culture media (RPMI 1640) was distributed into 96 well plate inside incubator with 5% CO_2 flow at 37°C temperature during 24 h to adapt with the situation and stick on the bottom of the well. The media was taken on the next day and it washed by PBS then added with 100 µL culture media containing DMSO or methanol (solvent control) or sample, incubated for 48 h. At the end of incubation process, the culture media containing sample was discarded, then it was washed by 100 µL FBS. One hundred microliters culture media containing MTT 5mg mL^{-1} was added into each well. It were incubated for 4 h at 37°C. The living cell would react with MTT to form purple formazan

(a)



(b)

Fig. 1(a-b): Location of sponges in the Menjangan Island West Bali, Indonesia

crystal. After 4 h, the media containing MTT was discarded, washed by FBS (Fetal buffer saline) then added. Two hundred microliters isopropanol was added to dissolve the formazan crystal. Furthermore, the media was incubated at room temperature during 12 h then the absorbance was read by ELISA reader on 550 nm wave length.

Apoptosis observation: The cell (density 2×10^4cell mL^{-1}) were grown on the cover slips in 24 plate until 50-60% confluent. After that incubated with test compound for 48 h. The media was taken, then washed by PBS. Raised the cover slips containing cell, put it over the object glass and added with 10 µL 1X working solution acridine orange-ethidium bromide, allowed to stand for 15 min. Immediately observed under a fluorescent microscope.

RESULTS AND DISCUSSION

The inhibition curve of 5M74 isolate between % living cell and good concentration on heLa cell, Raji cell, myeloma cell or T47D cell (selectivity test against cancer cell 5M74 isolate) were showed on Fig. 2(a-d). The percentage of cell life profile against 5M74 and 1 isolate showed that cell life percentage on the fourth cell was decreased. The decrease of life percentage is greater in the presence of elevated levels of extract (dose dependent phenomenon).

The IC_{50} of the myeloma cell, T47D cell, hela cell and Raji cell were 10.3, 8.3, 16.5 and 7.8 µg mL^{-1}, respectively. It could be concluded that the highest cytotoxic effect of 5M74 isolate was on Raji cell.

The result of 5M74 isolate cytotoxic profile against each cell could be seen in Fig. 3. In the Fig. 3 was conducted on 25 µg mL^{-1} and incubated during 48 h (as death profile representation). There were significant changes in shape/ morphology for each cell (heLa, myeloma, Raji or T47D) between cell without treatment (control) and treatment cell. However, the result showed the signs of death cell had been found. It was concordant with cell toxicity parameter namely membrane damage, impaired synthesis and macromolecules degradation, metabolic capacity modification, as well as changes in cell morphology due to a test substance (Sladowski *et al*., 1993).

The 1 isolate selectivity test in the same way showed results as in the following Table 1.

Apoptosis induction: Apoptosis induction is one of the most desired effect from anticancer bioactive substance. On those following substances, apoptosis might be occured because the manifestation of cell cancer proliferation retardment.

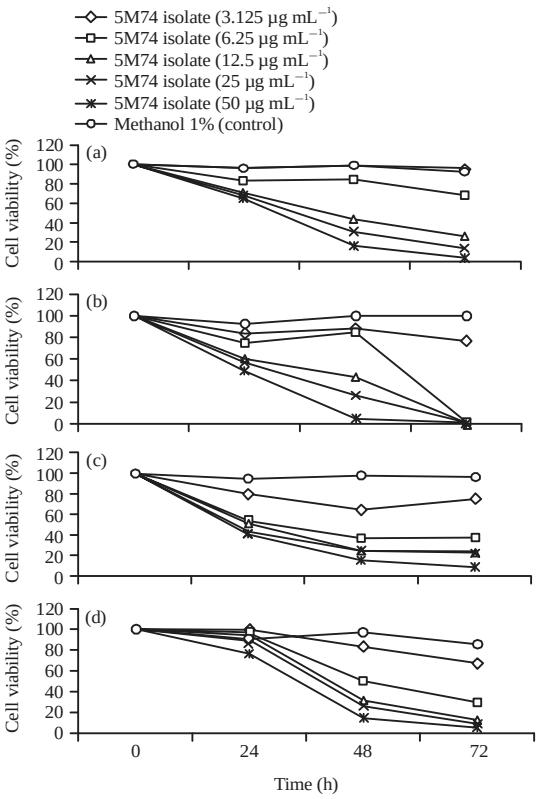


Fig. 2(a-d): (a) HeLa cell (b) Raji cell (c) Myeloma cell and (d) T47D (life percentage profile vs 5M74 isolate concentration)

Table 1: Cytotoxic effect (IC_{50} ug mL^{-1}) of 1 isolate against various cancer cells (48 h incubation)

		IC_{50} from various cancer cells (µg mL^{-1})			
No.	Substance name	Myeloma cell	T47D cell	HeLa cell	Raji cell
1.	1 isolate	0.18	7.9	6.9	5.8

Double staining was conducted using acridine orange and ethidium bromide methods to show death cell apoptosis mechanism from 1 isolate against myeloma cancer cell or 5M74 isolate to Raji cancer cell. Double staining method was used for showing differences between death cell due to necrosis or apoptosis (Wickenden *et al*., 2003).

In double staining method will visually looked cells which undergoing apoptosis would occur shrinkage and condensation of DNA and became fragmented to form apoptotic bodies. The damaged DNA would be entered by acridine orange and ethidium bromide so it turned out red-orange. The death cell because of necrosis would experience lysis but not fragmentation. The double staining result could be shown in Fig. 4 (5M74 isolate) and Fig. 5 (1 isolate).

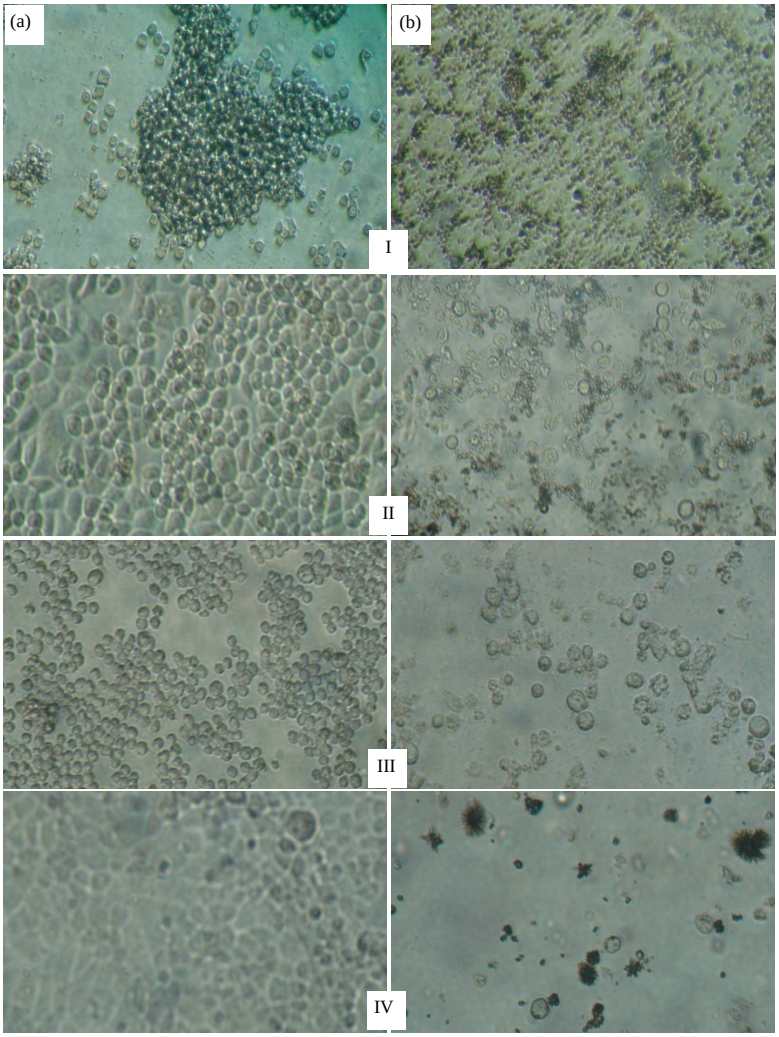


Fig. 3(a-b): (a) living cell (control) and (b) Death cell (cell with treatment), Cytotoxic test result profile of 5M74 isolate to cancer cells at 25 μg mL^{-1} concentration and 48 h incubation (I = Raji cell, II = Myeloma cell, III = Hela cell, iV = T47D cell)

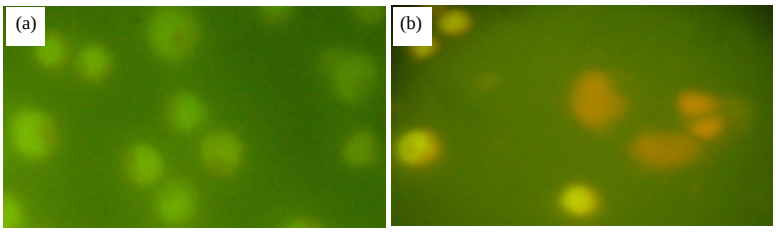


Fig. 4(a-b): (a) Cell control magnification 200x zoom 3x and (b) Test result magnification 200x (IC_{50}) zoom 3x Apoptosis induction observation using double staining on control Raji cell (A) and treatment of 7.8 μg mL^{-1} tested aqueous treatment (B)

On the control cell, the living cell was found as the green one (I). On the treatment, most cells underwent apoptosis at an early stage where the cells were still green (II), it can be seen the DNA fragmentation and chromatin condensation in the nucleus. In the final stages of apoptosis (III), would form orange apoptotic bodies as ethidium bromide-acridine orange staining. According to Fig. 4 and 5 could be seen that 5M74 and 1 isolate, respectively could induce Raji (5M74 isolate) and myeloma (1 isolate) cell death through apoptosis process (suicide program). It was shown by the formation of apoptotic bodies which were fraction of the cell's DNA. Those bodies were red (orange) because entered by acridin orange and ethidium bromide.

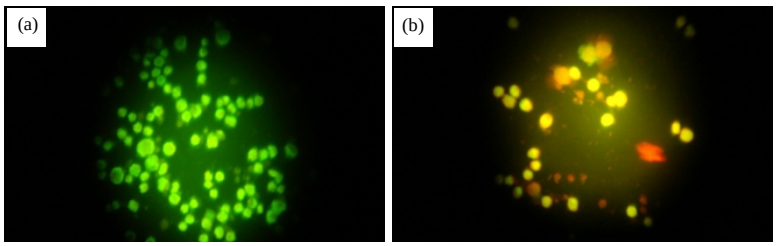


Fig. 5(a-b): (a) Cell control magnification 200x and (b) Test result maginification 200x (½ IC_{50}), apoptosis inductuion observation using double staining on control Myeloma cell (A) and treatment of 0,09 µg mL^{-1} tested aqueos

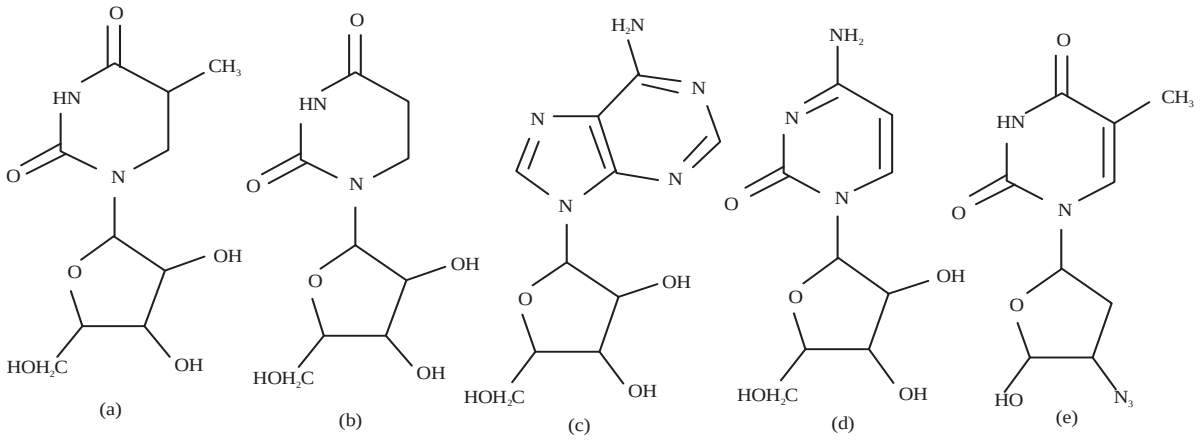


Fig. 6(a-e): Sponge nucleoside structures

Tetrahydro-4-hydroxy-5-(hydroxymethyl) furan-2-yl)-5-methyl pyrimidine-2,4(1H,3H)-dione (1 isolate) is a nucleoside compound that have a cytotoxic effect against T47D cell and can induce apoptosis. Nucleoside compounds primarily derived from the sponge has long been known to be a compound that have effect of cytotoxic. Spongotimidine (Fig. 6a) and spongouridine (Fig. 6b) were some strong tumor inhibitor nucleoside isolated from *Cryptotethia crypta* sponge. Following this finding, sponge become potential source for marine bioactive secondary metabolites. This compound led the researchers to synthesized the analogs, namely, Ara-A (Fig. 6c, Vidarabin®, Vidarabine Thilo®) and Ara-C (Fig. 6d, Cytarabine, Alexan®,Udicil®), which were found enhancing the antiviral. Further development of the analogs, the 3′-azido-3′ deoxythymidine (Fig. 6e, AZT, zidovudin) is currently being used to treat cancer and AIDS (acquired immune defisiency syndrome) (Setyowati *et al*., 2008).

Cyclodepsipeptide 5M74 isolates is comprised of a series of various amino acid. Ussually, the structural characteristics of depsipeptides include various unusual amino acid residues which may be responsible for their bioactivity. Moreover, protein hydrolysates formed by the enzymatic digestion of aquatic and marine by-products are an important source of bioactive peptides. Purified peptides from these sources have been shown to have cytotoxic effect on several human cancer cell lines such as heLa, AGS and DLD-1. These characteristics imply that the use of peptides from marine sources has potential for the prevention and treatment of cancer and that they might also be useful as molecular models in anticancer drug research (Suarez-Jimenez *et al*., 2012).

Nine new cyclodepsipeptides, Homophymines, B-E and A1-E1, isolated from the sponge *Homophymia* sp. have shown very potent cytotoxic activity with IC50 values in the nM range. This activity has been reported against several human cancer cell lines (Andavan and Lemmens-Gruber, 2010; Zampella *et al*., 2008) with moderate selectivity against human prostate (PC3) and ovarian (OV3) carcinoma. Homophymines A1-E1, which possesses the 4-amino-6-carbamoyl-2,3-dihydroxyhexanoic acid residue, exerts stronger potency than the corresponding A-E compounds which possess the same residue present in its carboxy form Zampella *et al*. (2008).

REFERENCES

ACS., 2015. Cancer facts and figures 2015. American Cancer Society, Atlanta, GA., USA. http:// www.cancer.org/acs/groups/content/@editorial/documents/document/acspc-044552.pdf.

Andavan, G.S.B. and R. Lemmens-Gruber, 2010. Cyclodepsipeptides from marine sponges: Natural agents for drug research. Mar. Drugs, 8: 810-834.

Belarbi, E.H., A.C. Gomez, Y. Chisti, F.G. Camacho and E.M. Grima, 2003. Producing drugs from marine sponges. Biotechnol. Adv., 21: 585-598.

Elmore, S., 2007. Apoptosis: A review of programmed cell death. Toxicol. Pathol., 35: 495-516.

Hanahan, D. and R.A. Weinberg, 2000. The hallmarks of cancer. Cell, 100: 57-70.

Hooper, J.N.A. and F. Wiedenmayer, 1994. Zoological Catalogue of Australia, Volume 12: Porifera. CSIRO, Melbourne, Australia, ISBN-13: 978-0643056862, Pages: 624.

Lockshin, R.A. and Z. Zakeri, 2001. Programmed cell death and apoptosis: Origins of the theory. Nat. Rev. Mol. Cell Biol., 2: 545-550.

Muller, W.E., H.C. Schroder, M. Wiens, S. Perovic-Ottstadt, R. Batel and I.M. Muller, 2004a. Traditional and modern biomedical prospecting: Part II-the benefits: Approaches for a sustainable exploitation of biodiversity (secondary metabolites and biomaterials from sponges). Evidence-Based Complement. Altern. Med., 1: 133-144.

Muller, W.E.G., R. Batel, H.C. Schroder and I.M. Muller, 2004b. Traditional and modern biomedical prospecting: Part I-the history: Sustainable exploitation of biodiversity (sponges and invertebrates) in the Adriatic Sea in Rovinj (Croatia). Evidence-Based Complement. Altern. Med., 1: 71-82.

Setyowati, E.P., U.A. Jenie, Sudarsono, B. Kardono, R. Rahmat and E. Meiyanto, 2007. [Isolation of cytotoxic substance from Kaliapsis sponge]. Indonesian J. Pharm., 18: 183-189, (In Indonesian).

Setyowati, E.P., U.A. Jenie, Sudarsono, L.B.S. Kardono and R. Rahmat, 2008. Identification of cytotoxic constituent of indonesian sponge *Kaliapsis* sp. (Bowerbank). Pak. J. Biol. Sci., 11: 2560-2566.

Setyowati, E.P., U.A. Jenie, Sudarsono, L.B.S. Kardono and R. Rahmat, 2009. Theonellapeptolide Id: Structure identification of cytotoxic constituent from *Kaliapsis* sp. sponge (bowerbank) collected from West Bali Sea Indonesia. J. Biol. Sci., 9: 29-36.

Sladowski, D., S.J. Steer, R.H. Clothier and M. Balls, 1993. An improved MIT assay. J. Immunol. Methods, 157: 203-207.

Suarez-Jimenez, G.M., A. Burgos-Hernandez and J.M. Ezquerra-Brauer, 2012. Bioactive peptides and depsipeptides with anticancer potential: Sources from marine animals. Mar. Drugs, 10: 963-986.

Wickenden, J.A., M.C.H. Clarke, A.G. Rossi, I. Rahman, S.P. Faux, K. Donaldson and W. MacNee, 2003. Cigarette smoke prevents apoptosis through inhibition of caspase activation and induces necrosis. Am. J. Respir. Mol. Biol., 29: 562-570.

Zampella, A., V. Sepe, P. Luciano, F. Bellotta and M.C. Monti *et al*., 2008. Homophymine A, an anti-HIV cyclodepsipeptide from the sponge *Homophymia* sp. J. Org. Chem., 73: 5319-5327.