Biocatalysis and Agricultural Biotechnology 17 (2019) 628–637

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Marine sponge alkaloid aaptamine enhances the anti-bacterial and anti-cancer activity against ESBL producing Gram negative bacteria and HepG 2 human liver carcinoma cells

Govindan Rajivgandhi, Seduraman Naveen kumar*, Govindan Ramachandran, Natesan Manoharan*

Department of Marine Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

ARTICLE INFO

Keywords:
Multi drug resistant bacteria
Aaptamine
Minimum inhibition concentration
Confocal laser scanning electron microscope
Liver cancer cells

ABSTRACT

Marine sponge alkaloid aaptamine is a promising anti-bacterial and anti-cancer drug against ESBL producing Gram negative bacteria and HepG 2 human liver carcinoma cell line. The antibacterial activity of aaptamine against selected ESBL producing Gram negative bacteria was inhibited at the concentration of 55 μg/mL. At the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) of 50 μg/mL, the aaptamine was significantly effective against all the ESBL positive uropathogens. Importantly, the inhibition was much high compared to other marine compounds through intracellular damage and morphological modification by confocal laser scanning electron microscope (CLSM) and scanning electron microscope (SEM). Further, the cytotoxic efficacy of aaptamine against HepG 2 cells was showed at 75 μg/mL very effectively and this concentration was indicated as IC_{50} dose. The morphological and intracellular nuclear damage of aaptamine treated HepG 2 cell was detected by florescence microscope using acridine orange/ethidium bromide (AO/EB) combination and Hoechst 33342 staining assays. The nuclear fragmentation of aaptamine treated HepG 2 cells were confirmed by expression of Caspase-9, Caspase-8, Caspase-3 cleavage, which provide the suggestion for the induction of intrinsic and extrinsic apoptosis pathways. In addition, the western blot analysis of appatamine treated HepG 2 cells also confirmed that the expression genes were damaged and the aaptamine showed dose and time dependent relationship. Hence, our findings suggest, the marine natural compounds aaptamine might be a potential alternative agents for ESBL producing uropathogens and human liver cancer therapy.

1. Introduction

Bacterial resistance is a major worldwide problem (Huang et al., 2018). In health care industry, the patients affected by multi drug resistant bacteria (MDR), the death rates are twice as high, expenses are twice as long and healthcare costs are significantly increased compared to infections with drug-sensitive strains (Dholvitayakhun et al., 2017; WHO, 2012). In particular, urinary tract infections (UTIs) are most predominant infections comprising 1/4th of all the infections (Kumar, Das, 2016). The global rate is estimated to be 250 million cases each year (Allam et al., 2017). The WHO reported recently as the fourth most common infection in USA and third common in India (Fallah et al., 2017; WHO et al., 2012). The emergence of MDR effects in UTI is acquired by improper prescription and uncontrolled use of antibiotics, inappropriate dosage and other co-expressed resistance mechanisms (Chen et al., 2012). The antimicrobial resistance pattern in Gram negative bacteria is increasing alarmingly worldwide (Rajivgandhi et al., 2014). These patterns include, among others, an increasing frequency of pathogens producing extended spectrum beta-lactamases (ESBLs) and carbapenemases including *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterobacter sp*. (Hayajneh et al., 2015; Satpathy et al., 2016). Among these patterns, ESBL producing GNB persist to be predominant in UTI, the synthesizing enzymes trigger the resistance against beta lactam antibiotics by hydrolysis of β-lactam ring, such as ampicillin, amoxicillin, third generation of cephalosporin and fluorroquinolones, trimethoprim (Rajivgandhi et al., 2014). Due to the defect, the bacteria retain susceptibility to very few antimicrobials (Molina-Quiroz et al., 2015). Therefore emerging discovery of new antibiotics is needed to neutralize the antibiotic resistance mechanisms in bacteria.

Cancer is the second most noncommunicable disease that recognized as one of the most problematic diseases to disclose as a result

* Corresponding author.
E-mail addresses: bionaveen14@gmail.com (S.N. kumar), biomano21@yahoo.com (N. Manoharan).

https://doi.org/10.1016/j.bcab.2019.01.007

Available online 07 January 2019
1878-8181/

of its high genetic heterogeneity and quick developing drug resistance (Li et al., 2016; Yan et al., 2018). According to the World Health Organization reports, liver cancer is the word's second most serious disease among the all cancers leading to the morbidity and mortality in the world (Wahab et al., 2014). Every year, two million peoples lost their lives, which is 25.10% of the total deaths occurred due to liver cancer (Ahamed et al., 2016). HepG 2 cells are polygenic infectious cells with multiplex mechanism and signaling pathways. Due to the multiplex mechanism, it is hard to treat successfully by single treatment modality. Therefore, development of new drugs to prevent the proliferation of liver cancer cells is facing great difficulties (Abdelfattah et al., 2016). To tackle this issue, marine sponge derived natural product will be provide great solution and alternative sources for cancer therapy (Majeeda et al., 2016). The interaction between marine natural compounds and cancer cells and their subsequent biomedical responses is one of the most valuable research fields in marine pharmacology (Rajivgandhi et al., 2016; Beygmoradi, Homaei, 2017).

Aaptamine, a benzo[de][1,6]naphthyridine group of marine sponge alkaloids isolated from the Japanese sponge *Aaptos* and characterized by Nakamura et al. (1982). Previously, various biological activities were reported from aaptamine, they are cytotoxic (Stuhldreier et al., 2015), antiviral (Larghi et al., 2009), antimicrobial (Yu et al., 2015), anti-parasitic (Ohizumi et al., 1984), α-adrenergic antagonistic (Gul et al., 2006), radical scavenging (Enrique et al., 2009), and anti-fouling activity (Mohamad et al., 2009). The biomedical application based active compounds aaptamine derivatives have been isolated from various marine sponges belonging to the genera *Aaptos*, *Suberites*, *Luffariella*, *Hymeniacidon*, *Suberea*, and *Xestospongia* (Shen et al., 1999; Arai et al.al., 2014). Among the genus, Aaptos has tremendous sources of novel aaptamine alkaloids, which still major interest in discovering new secondary metabolites (Calcabrini et al., 2017). These alkaloids have become an interesting focus for synthesis, structure activity relationship and anti-bacterial activity against ESBL producing bacteria, anti-cancer studies (Melander et al., 2016; Lee et al., 2017; Rajivgandhi et al., 2018f). Therefore, the present study proposes the anti-bacterial and anticancer effect of marine sponge compound aaptamine hostile to ESBL producing GNB and HepG 2 liver cancer cells.

2. Materials and methods

2.1. Materials

The marine sponge alkaloid drug aaptamine was procured from Labex Corporation, New Delhi (ALX-350-104-M001). All the ESBL identification discs were purchased from Himedia Laboratories, Mumbai, India. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), dulbecco's modified eagle's medium (DMEM), fetal calf serum (FCS), antibiotic and antimycotic solution were purchased from Sigma Aldrich, Mumbai, India. AO/EB, trypan blue, 6-diamidino-2-phenylindole (DAPI) and propidium iodide dyes were purchased from Thermo Fisher Scientific, India. The gene specific primers, reverse transcriptase enzyme, RNA isolation Kit were procured from Clone tech, Japan. The rabbit polyclonal antibodies against human Bcl-2, caspase 3 and 8, rabbit monoclonal antibody caspase-9 and mouse monoclonal antibody against p53 were purchased from Merck Germany.

2.2. Bacterial culture

The ESBL producing uropathogens *E. coli*, *P. mirabilis*, *P. aeruginosa*, *K. pneumoniae*, *Enterobacter* sp. were obtained from Medical Microbiology & Marine Pharmacology unit, Department of Marine Science, Bharathidasan University, Tamil Nadu, India. The ESBL producing ability of the selected uropathogens was reported in our previous paper by Rajivgandhi et al. (2018a).

2.3. Cell line used

Human hepatocellular liver *carcinoma* cell line (HepG 2) was procured from National Centre for Cell Science (NCCS), Pune, India. The cells were maintained in DMEM supplemented with streptomycin (100 UmL^{-1}), gentamycin (100 $mg\,mL^{-1}$) and 10% FBS at 37 °C in a humidified atmosphere of 95% with 5% CO_2.

2.4. Antibacterial activity

The anti-bacterial effect of aaptamine was evaluated against selected ESBL positive uropathogens *E. coli*, *P. mirabilis*, *P. aeruginosa* and *K. pneumoniae* and *Enterobacter* sp. by agar well diffusion assay (Lin et al., 2013). Briefly, the 24 h bacterial cultures were spread on MHA plates using sterile cotton swab. The sterile discs (5 mm) impregnated aaptamine with different concentration (5, 10, 25 and 50 μg/mL) was placed over the bacterial lawn. The ceftazidime, a third generation cephalosporin disc acted as a positive control for ESBL production. All the plates were incubated at 37 °C for 24 h. After incubation, all the plates were observed for the zone of inhibition around the disc and the zones were measured in diameter (mm).

2.5. Detection of MIC and MBC

The lowest inhibition concentration of the aaptamine was performed against the selected ESBL positive uropathogens was noticed as MIC by micro broth dilution method. Briefly, the exponential phase of ESBL positive bacterial cultures were diluted in tryptic soy broth (TSB) along with 5–50 μg/mL of aaptamine and incubated at 37 ºC for 24 h. After incubation, the microbial sedimentation was confirmed by visual verification, and the absorbance reading was measured at 570 nm using a ELIZA reader (Shimadzu, Japan). The MIC was considered as the lowest concentration of aaptamine that inhibited the entire growth of ESBL producing bacteria (Prasad et al., 2017). Further, an aliquot of 50 μl from MIC wells which showed no bacterial growth development were inoculated on muller hinton agar (MHA) without addition aaptamine. If the plate showed no bacterial growth was considered as MBC. The test results were considered for their anti-bacterial effect against ESBL positive uropathogens when either MIC or MBC value is equal or MBC was higher than MIC (Enrique et al., 2009). Both MIC and MBC were performed in triplicate. The percentage of MIC was calculated as,

$$Percentage\ of\ inhibition \frac{(\text{Control OD 570 nm} - \text{Test OD 570 nm})}{\text{Control OD 570 nm}} \times 100$$

2.6. Intracellular damage of aaptamine by CLSM

Detection of bacterial death due to the expose of aaptamine MIC in the treated ESBL positive *P. aeruginosa* and *K. pneumoniae* were analyzed by CLSM (Rajivgandhi et al., 2018b). After 12 h incubation, aaptamine treated bacterial pellet was recovered from centrifugation and washed with PBS. After, 10 mg/mL of acridine orange/ethidium bromide (AO/EtBr) was stained for 15 min under dark condition. The live or dead cells of the treated and untreated cells were analyzed by CLSM with 488 nm argon laser and a 500–640 nm band pass emission filter. The images was received using a Carl Zeiss CLSM 710 (Carl Zeiss, Jena,Germany) equipped with a 100× oil-immersion objective lens.

2.7. SEM

The morphological alteration of aaptamine in the ESBL positive uropathogens *P. aeruginosa* and *K. pneumoniae* were investigated by SEM based on the method of Mauthupandy et al., (2018). After MIC treatment, the pellets of the bacteria were collected from centrifugation and fixed with 4% glutaraldehyde and followed three times with

phosphate buffer saline (pH 7.3) for 4 h. The fixed cells were vaccum filtered by 0.1 mm polycarbonate membrane filters and subsequently, dehydrated in ethanol graded series (0%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 90% and 100%). Finally, the air dried samples were analyzed by SEM using an accelerating voltage of 20 kV (Model: VEGA3 TESCAN, Brno, Czech Republic).

2.8. Anti–cancer studies

2.8.1. Cytotoxicity assay

The cytotoxicity assay of HepG 2 cells was determined as previously reported method (Al-Hadi et al., 2017). Briefly, the HepG 2 cells at a density of 1×10^4 cells/well were seeded into a 96-well plate and incubated at 37 °C with 5% CO_2 and 95% humidity. After incubation, the aaptamine was added into the wells with various concentrations (10–100 µg/mL). After exposure, 20 µl of MTT (5 mg/mL in PBS) was added to each well and the plates were kept with undisturbed condition for 4 h at 37 °C with aluminum foil. After incubation, the supernatant was discarded and 100 µl of formazan crystals dissolved DMSO was added. The absorbance was measured at 570 nm using spectrophotometer (Shimadzu, Japan). The experiment was done in triplicates and used to calculate the mean. The percentage of cell death was calculated using the formula:

$$\text{Cytotoxicity}(\%) : \frac{\text{Mean OD of untreated cells(Control)} - \text{Mean OD of treated cells}}{\text{Mean OD of untreated cells(Control)}} \times 100$$

2.8.2. Detection of cellular morphology

The IC 50 dose of aaptamine against HepG 2 cell morphology was analyzed by florescence microscopy (Naveen Kumar et al., 2018). Briefly, the HepG 2 cells (5×10^{-5}) were seeded on the well of a 6-well plate, and incubated with IC_{50} dose of aaptamine at 37 °C with 5% CO_2 and 95% humidity. After incubation, the supernatant of the medium was aspirated and washed with PBS. Consequently 1: 1 ratio of 10 mg/mL^{-1} of AO (10 mg/mL^{-1}) and EB (10 mg/mL^{-1}) was added in the plate, and incubated for 5 min at 37 °C in the dark. The stained cells were examined under a fluorescence microscope (Carl Zeiss, Jena, Germany) with representative fields were captured at 40x magnification.

2.8.3. Hoechst 33342 staining

The damaged nuclear morphology of HepG 2 cells due to the effect of aaptamine IC_{50} dose was observed by florescence microscopy using Hoechst 33342 staining (Sthijns et al., 2017). Briefly, after treatment of the cells with IC_{50} dose of aaptamine in the 6 well plates was incubated at 37 °C for 24 h. After incubation, the cells were trypsinized and followed by centrifugation. After, the cells were washed twice with PBS for removal of excess growth. Consequently, the cells were stained with Hoechst 333421 staining reagent and incubated for 5 min at 37 °C in the dark. The morphological changes was determined under a fluorescence microscope (CarlZeiss, Jena, Germany) and representative fields were captured at 40x magnification.

2.8.4. DNA Fragmentation analysis

After 24 h treatment, the aaptamine treated HepG 2 cells of the DNA fragmentation was detected by agarose gel electrophoresis (Brzoska et al., 2015). Briefly, after treatment with IC_{50} dose of aaptamine, the HepG 2 cells (5×10^{-5}) were mixed with 200 µl lysis buffer at 55 °C for 24 h followed by addition of RNAse solution in the cells and again incubated at 37 °C for 2 h. The cells were then centrifuged at 5000 rpm for 15 min and supernatant was collected. After, phenol: chloroform (1:1 ratio) extraction was performed, followed by chloroform, isoamyl alcohol were added in the ratio of 1:24 ratio to precipitate the fragmented DNA. The obtained DNA was run on 1% agarose gel containing ethidium bromide (0.5 µg/mL). The gels obtained were photographed in Gel DocTM XR + (Bio-Rad, CA USA).

2.8.5. RNA isolation, cDNA synthesis and semi-quantitative PCR

After IC_{50} dose treatment, the total RNA was extracted with RNeasy™ mini kit with manufacturer's instructions (RNeasy Mini Kit (Cat# 74104 and 74106), QIAGEN, Hilden, Germany) (Mishra et al., 2014). Further, the cDNA was synthesized using 2 µg total RNA by iScript™cDNA Synthesis Kit from BIO-RAD. The reverse transcription polymerase chain reaction (RT-PCR) assays were performed using CFX96™ Real-Time PCR detection system (BIO-RAD). The cDNA was amplified with each gene such as p53 and Bcl-2, caspase-3, caspase-8 and caspase-9 and the reaction was carried out according to the manufacturer's instructions and determined by semi-quantitative RT-PCR. The sequences of specific primers were used for each gene study including the endogenous loading control of β-actin. PCR conditions were given as follows:94 °C for 5 min; 35 cycles at 94 °C for 1 min, 55–62 °C for 1 min, 72 °C for 1 min; and a final extension step of 72 °C for 10 min. The products were verified by agarose gel electrophoresis and visualized by staining with ethidium bromide as well. The intensity of β-actin gene expression served as an internal control.

2.8.6. Western blotting analysis

The HepG 2 cells were treated with IC_{50} concentrations of aaptamine and incubated at 95 °C for 48 h. After incubation, the cells were scraped from cell culture dish and washed thrice with PBS. Then, the cells were lysed with cell lysis buffer containing 20 mM Tris pH 7.5, 0.15 M NaCl, 1 mM EDTA, 1% NP-40, 1 mM DTT, 0.25 mM PMSF for 30 min on ice. Cell lysates were then centrifuged for 30 min at 4 °C, and the supernatant was collected. The extracted protein sample (60 µg total protein/lane) was added in the same volume of sample buffer and subjected to denaturation at 95 °C for 10 min, then electrophoresed on 10% SDS-PAGE. Finally the sample was transferred onto PVDF membrane. The PVDF membrane was soaked in TBS containing 50 g/L skimmed milk at room temperature for 2 h, followed by incubation with the primary antibodies p53, Bcl-2, caspase- 3, caspase- 8, caspase- 9, MMP- 2, MMP- 9 and β-actin (1:1000 dilution) respectively, at 4 °C for overnight. After that, incubated membrane was washed with TBST for 30 min and corresponding secondary antibody was added and again incubated at room temperature for 1 h. After incubation, the membrane was washed thrice with TBST for 15 min. The florescence emitted result was visualized with enhanced chemiluminescence (Amersham, Arlington Heights, IL) (Huang et al., 2016).

2.9. Statistical analysis

All the results of this study are expressed as mean ± SD. The statistical significances of differences between values were evaluated by one-way ANOVA. In the MTT assay, each experiment was performed in triplicate and statistically significant values were tested by Duncan's Test and p values < 0.05 were considered statistically significant.

3. Result

3.1. Anti-bacterial activity

The Aaptamine were showed excellent inhibition activity against selected ESBL positive uropathogens (Fig. 1). The maximum zone of inhibition 20 ± 0.5, 18 ± 0.5, 24 ± 0.5, 23 ± 0.5 and 24 ± 0.5 mm at 55 µg/mL was observed against *E. coli*, *P. mirabilis*, *P. aeruginosa*, *K. pneumonia* and *Enterobacter* sp. respectively. Whereas, the minimum zone of inhibition 6 ± 0.5, 3 ± 0.5, 3 ± 0.5, 2 ± 0.5 and 6 ± 0.5 mm at 25 µg/mL were also observed respectively (Fig. 1a, b, c, d, e). The positive control of ceftazidime exhibited no zone of inhibition

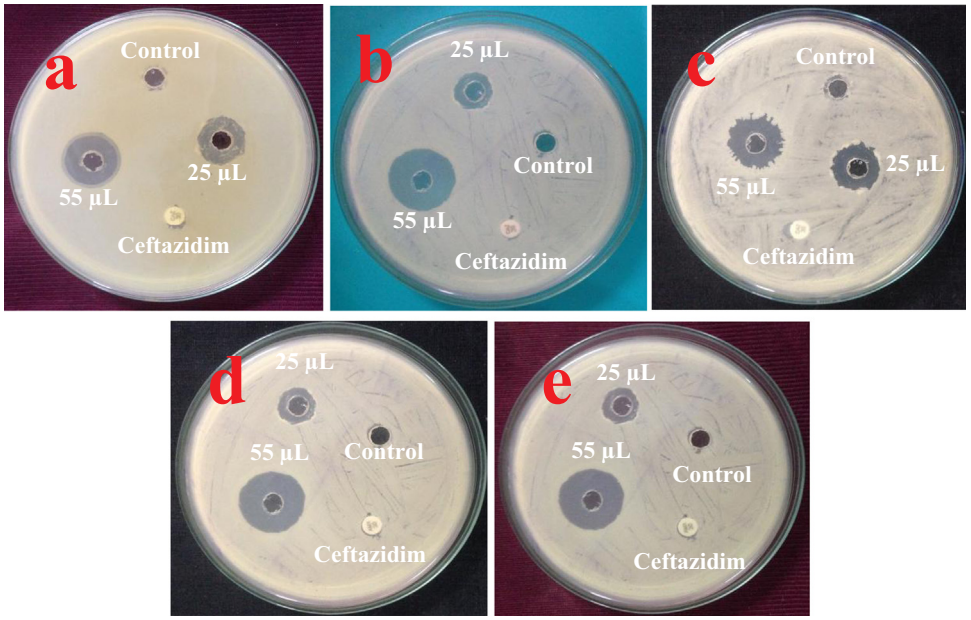

Fig. 1. Inhibition of ESBL producing pathogens by aaptamine. The minimum and maximum growth inhibition of selected ESBL positive uropathogens at lowest and highest concentration of aaptamine (a) *E. coli*, (b) *P. mirabilis*, (c) *P. aeruginosa*, (d) *K. pnumoniae*, and (e) *Enterobacter* sp.

along the tested concentration were observed. It confirms, the selected uropathogens were ESBL positive. However, the aaptamine was effectively inhibit the ESBL producing bacteria and has more bactericidal. Aaptamine is one of the potential natural inhibitor for bacteria which have remarkable biological properties (Jaworski et al., 2015). Our result was accordance with previous report of Yu et al. (2014) and Rajivgandhi et al. (2018c), the antibacterial activity of aaptamine has more bactericidal effect and acted as a drug resistant pathogen modifier. Previously, Pham et al. (2013) studied with aaptamine, and reported that the aaptamine is excellent candidates for future therapeutic applications. Hence, our result revealed that the aaptamine has antibacterial chemical molecules for inhibiting the ESBL producing bacteria very effectively.

3.2. MIC and MBC

After 24 h incubation, the in vitro inhibition effect of aaptamine against selected ESBL positive uropathogens were showed excellent inhibitory activity at 50 µg/mL. The MIC result showed that the ESBL positive uropathogens were partially susceptible at the lowest concentration 25 µg/mL while completely inhibited at the maximum concentration of 55 µg/mL. The rate of inhibition was 84, 82, 80, 79 and 77 against selected ESBL positive uropathogens respectively at same concentration (50 µg/mL). However, the increasing concentration of aaptamine was decreased the bacterial growth very effectively and the aaptamine was concentration dependent. It is cardinal evident that the aaptamine was more bactericidal even at 55 µg/mL concentration (Fig. 2.) and this concentration was selected as a MIC. In addition, MBC of aaptamine result was same as the MIC concentration for all the bacterial strains were observed clearly. The aliquot of MIC wells on MHA plates were exhibited absence of growth completely at 55 µg/mL (Fig. 3a, b, c, d and e). The result was revealed that the MIC growth was inhibited the bacteria same as MBC. Recently, Rajivgandhi et al. (2018d) reported that the viability of Gram negative bacterial growth was decreased at 100 µg/mL concentration by marine sponge derived actinomycetes. The reported result was showed double time higher than our result comparatively. The recent study of Tsukamoto et al. (2010) stated, the aaptamine has an excellent inhibitory activity against MDR bacteria and the inhibition percentage in the range of 55–89%. The supportive evidence of Abdul rashid et al. (2014) reported that the

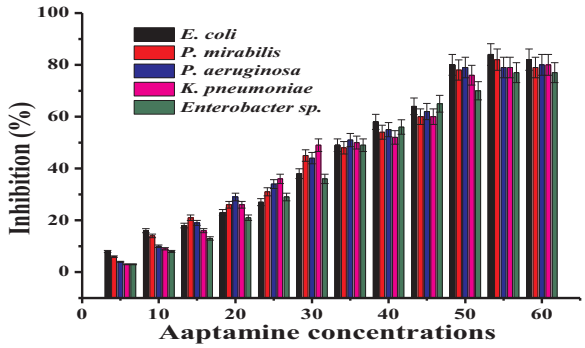

Fig. 2. MIC of aaptamine effect against selected uropathogens *E. coli, K. pnumoniae, P.aeruginosa, K. pnumoniae, Enterobacter* sp.). All the uropathogens were inhibited by aaptamine at the concentration of 55 µg/mL.

increasing concentration of aaptamine inhibit the fungi at 4–64 µg/mL. Hence, overall study revealed that the aaptamine was efficient antibacterial inhibitor against ESBL producing uropathogens.

3.3. CLSM

At the MIC dose of 50 µg/mL, the intracellular particles of selected uropathogens were damaged, and loosely deviated colonies were observed by CLSM. Whereas the strong adherent ability and depicted well development of tightly closed colonies on the control slides were observed (Fig. 4). The inhibition mechanism of aaptamine against selected uropathogens indicates, the aaptamine was excellent anti-bacterial drug against ESBL producing bacteria. After 12 h incubation, the 60% of cell death was observed followed by complete cell death were observed in 24 h due to the effect of aaptamine. The aaptamine is strongly attached to surface of the ESBL producing uropathogens receptors and were emitted red color images. It indicated that the bacterial cells were vigorously inhibited at MIC concentration. Whereas, the control strain were observed as green color images in CLSM represents live cells (Rajivgandhi et al., 2018e). The cellular damage of the bacterial architecture was clearly visible due to interaction of cells upon treatment of drug (Maruthupandy et al., 2018). Our result suggested that the selected ESBL positive uropathogens were resistant to most of the drugs;

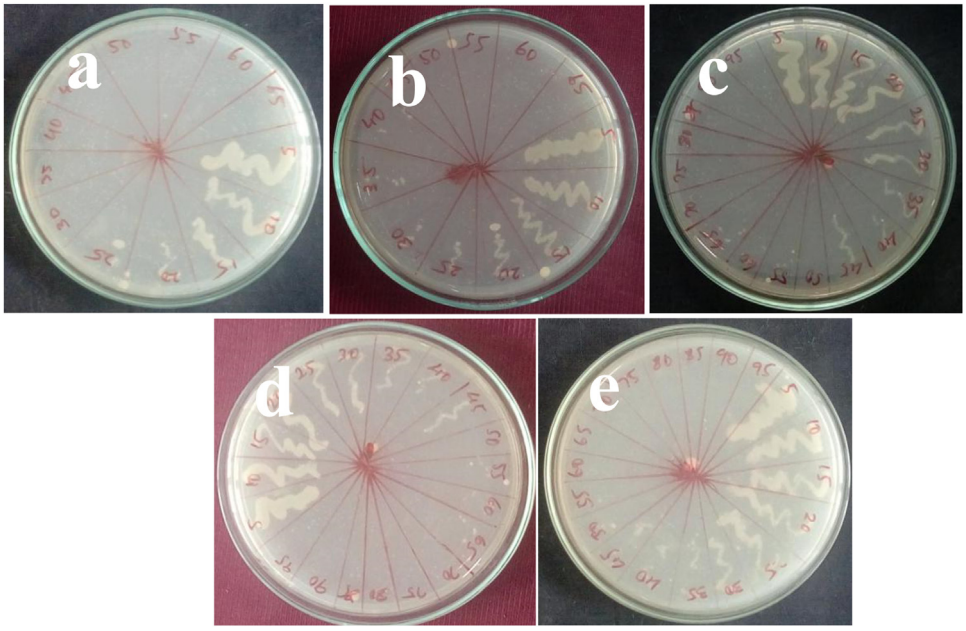

Fig. 3. MBC of aaptamine effect against selected uropathogens. All the aliquot inoculated cultures of MIC of MHA plates exhibited, same concentration of MIC. Here, *E. coli* (a), *K. pnumoniae* (b), *P.aeruginosa* (c), *K. pnumoniae* (d), *Enterobacter* sp.).

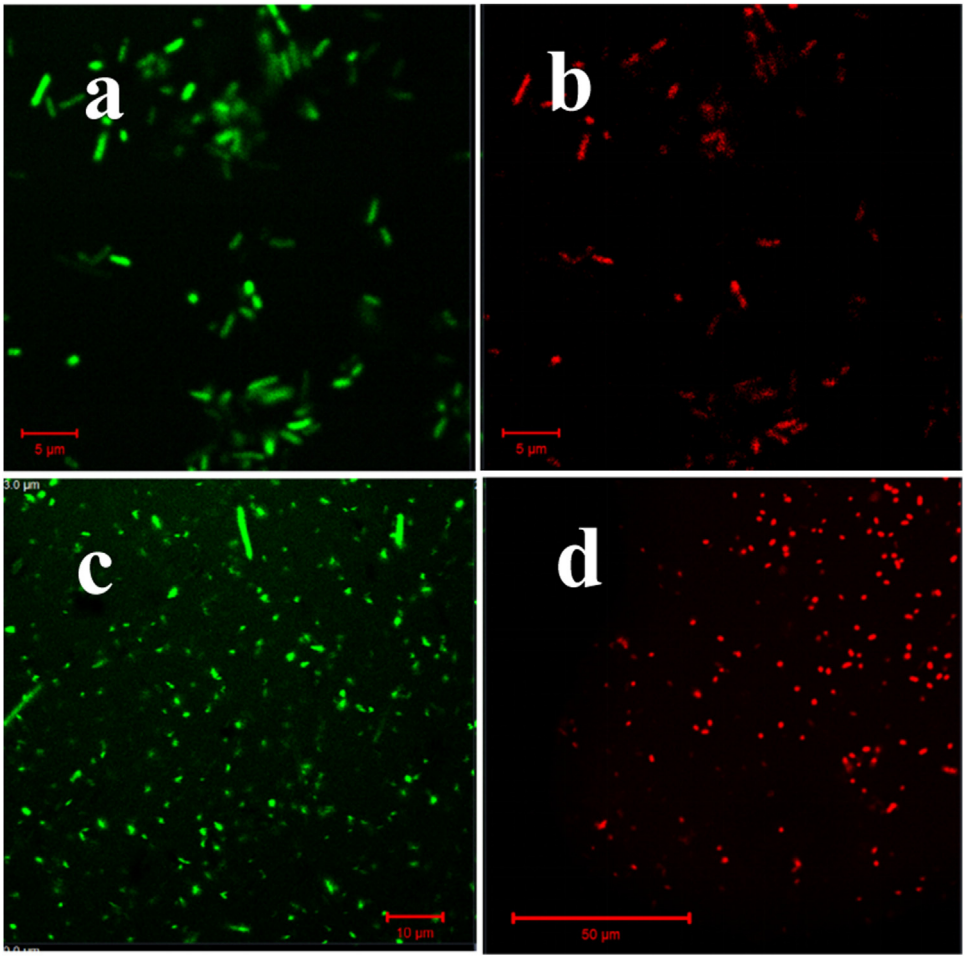

Fig. 4. CLSM images of ESBL producing *P. aeruginosa*, *K. pnumoniae* treated at MIC of aaptamine. The control (a, c) and treated (b, d) cells of *P. aeruginosa*, *K. pnumoniae* (Red color cells indicate dead cells and green color indicate live cells). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

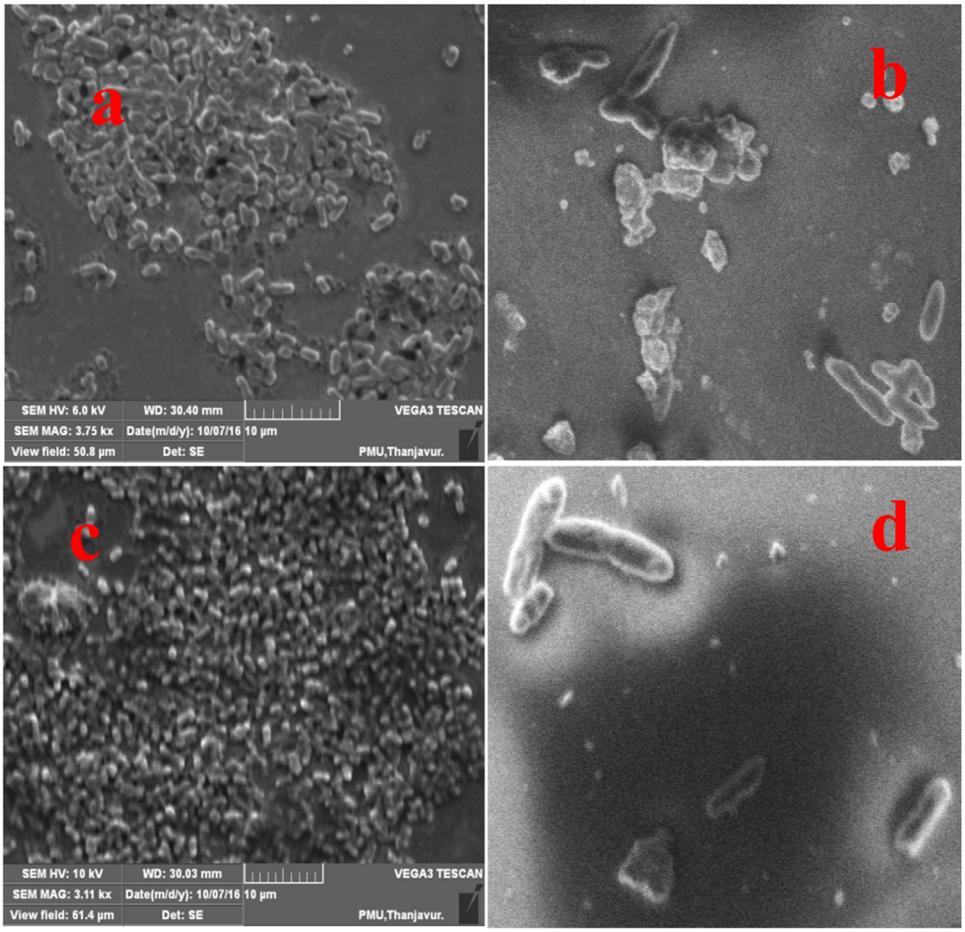

Fig. 5. MIC dose of aaptamine treated ESBL producing *P. aeruginosa*, *K. pnumoniae images* by SEM. The treated cells of *P. aeruginosa* and *K. pnumoniae* showed smooth colonies with morphological damage (b, d), whereas the control cells showed no morphological changes (a, c).

they were more sensitive to aaptamine. Hence, the result proved that the drug aaptamine has potential anti-bacterial effect against ESBL producing bacteria. The result was most accordance with earliest report of Stuhldreier et al., 2015 and showed aaptamine had more inhibition ability against MDR pathogens. Previously, Yu et al. (2014) suggested that CLSM result could be used to investigate the live and death cells of *E. coli* and *S. aureus* and revealed that the aaptamine penetrated in damaged and compromised cells properly. The wide spectrum of morphological differences and substantially reduction of thickness in the GNB surface architecture was observed due to the effect of aaptamine (Rajivgandhi et al., 2018g).

3.4. SEM

The morphological conversion of treated and control ESBL positive uropathogens at desired MIC concentration was showed in Fig. 5a, b, c, d. After dehydration, the treated samples were exhibited inconsistent of cellular content and damaged morphology with compromised cell leakage. The surface attachments of aaptamine in the cell membrane of uropathogens indicate, both the uropathogens were more sensitive to aaptamine. After treatment, cell membrane was agitated and alteration in morphological emphasize could leads to cell wall death/leakage of cellular content. The deposition of rumples and damage of membrane integrity of appatamine treated pathogens surface was observed (Rajivgandhi et al., 2018f). Whereas the control slide showed smooth morphology with tightly packed cells in their morphology. However, the SEM result was more evident, aaptamine as a potential cell wall damaging drug for ESBL producing uropathogens. Our result was in accordance with the previous report of Maruthupandy et al., 2018 and showed that the loss in viability was correlated to impairment of cell membrane integrity, result in the slight damaged cell walls of the strains by SEM analysis. The supportive evidence of Rajivgandhi et al., 2018f reported that the lowest concentration of marine sponge compounds were very effective against MDR pathogens. Hence, this study was highlighted that the aaptamine altered the sustained virulence pathway of several pathogenic bacteria could lead to combat the bacterial infections.

3.5. Anti–cancer studies

3.5.1. Cytotoxicity assay

The cytotoxic evaluation of aaptamine against HepG 2 cell development was identified by MTT assay and the result was showed with decreased growth of Hep G 2 at increasing concentration of aaptamine after 24 h (Fig. 6). A dose dependent inhibition of the cell viability was exhibited in aaptamine treated HepG 2 cells. Among the various concentrations of aaptamine, 78% cell death was indicated at 105 µg/mL and the viability of remained cells were 33% against HepG 2 cells. About 15 µg/mL of aaptamine indicated significant cell death of 13% and about 75 µg/mL of aaptamine indicated 52% cell death against HepG 2 cell were also observed after treatment with 24 h incubation. Whereas the control cells of untreated HepG 2 cells were showed no any changes in their growth development (Fig. 6a). However, the cytotoxicity assay result revealed that the obvious cell proliferation was induced in HepG 2 cell lines at 75 µg/mL. This concentration (75 µg/mL) was fixed as IC_{50} concentration for further study. The result was suggested that the aaptamine is a promising drug for inhibiting the growth of HepG 2 cancer cells. Recently, sponge derived alkaloid

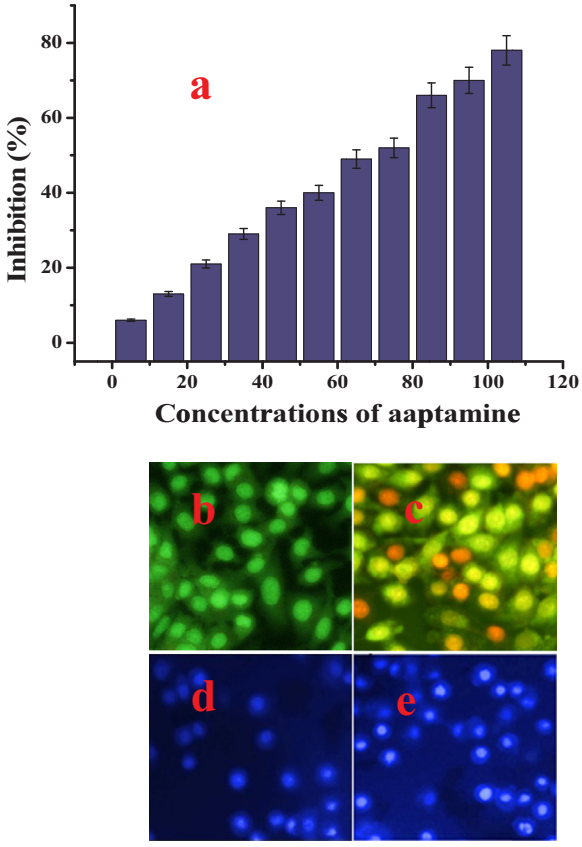

Fig. 6. Cytotoxicity effect of aaptamine against HepG 2 lung cancer cell lines. (a). The cell death (50%) was observed at 75 µg/mL of aaptamine and chosen for further study. Apoptotic cells of control (b, d) and aaptamine treated (c, e) of HepG 2 cells, observed under florescence microscope using AO-EB dual staining and Hoechst 33342 staining method respectively.

fascaplysin had remarkable cell proliferation against A549 lung cancer cells at increasing concentration (Naveen kumar et al., 2018). Among these, many anticancer drugs obtained from various marine sources exhibited excellent cytotoxic activity against different cancer cells (Gul et al., 2006). Previously, Ohizumi et al. (1984) reported that the biological sponge derived compounds had been worked against various biological activities including antioxidant, enzymatic inhibition, antiviral, anti-microbial, anti-parasitic, cytotoxic and antifouling very effectively. Based on the above result, the aaptamine has anti-cancer and related anti-bacterial activity against ESBL producing bacteria and cancer cells. The previous report of aaptamine against bacteria and cancer cells were mentioned in Table 1.

3.5.2. Morphological damage assays

The morphological and cellular alteration of aaptamine treated HepG 2 cells were showed cell shrinkage and chromatin condensation due to the induction of apoptosis (Fig. 6). After treatment with IC_{50} dose of aaptamine, the differentiation between the viable, apoptotic and necrotic cells were observed under fluorescent microscope using AO/EB staining (Fig. 6c). The control images of AO/EB staining revealed, a mature and well attached HepG 2 cells were observed with green color (Fig. 6b). Whereas, the treated cells were showed morphologically damaged and dispersed cell structures at IC_{50} concentration. In result, yellow color florescence images of the cells were suggested, the early apoptosis cells, whereas red color florescence image indicated late apoptosis, which highly observed in the aaptamine treated HepG 2 cells. After 24 h treatment of aaptamine, the necrosis (orange nucleus cells) was also observed in HepG 2 cell lines.

Further, the nucleus damage of the aaptamine treated HepG 2 cells were captured by using florescence microscope using Hoechst 33342 staining at IC_{50} dose of aaptamine. After 24 h incubation, the cells were modified with shrink and became round and then the number of cells decreased. The arrangement of the cells were loose and not adhered well, finally most of cells were coated in the medium. The viable cells were bright blue (Fig. 5d), while the apoptotic cells were red (Fig. 5e) as distinguished by Hoechst 33342. After treatment with IC 50 dose, the number of death cells was increased when compared with untreated control cells.

3.5.3. DNA fragmentation analysis

The investigation of IC_{50} concentration of aaptamine induced endonuclease cleavage products of apoptosis was visualized by DNA laddering technique. This assay involves extraction of DNA from lysed homogenous cells followed by gel electrophoresis. This is the confirmation method for analysis the characteristic feature of apoptosis (Yuan et al., 2015). Induction of apoptosis could be confirmed due to the irregular reduction in size of cells, in which the cells are reduced and shrunken, and lastly DNA fragmentation. In the result of Fig. 7 indicate, the clear fragmented DNA ladders were observed in aaptamine treated HepG 2 cells (due to the cell death), whereas untreated control cells did not exhibit any prominent DNA ladders on agarose gel. Previous study of Enrique et al. (2009) reported that the both cancer and noncancerous cell lines treated with aaptamine exhibited shrinkage of cell structures were observed in DNA ladder of the gel. The attachment of aaptamine inside the nucleus of HepG 2 cells could affect the DNA multiplication and cell division. The marine sponge derived aaptamine has more anti-cancerous properties with DNA damage effect chromosomal aberrations, errors in chromosome segregation and formation of sister chromatic exchanges (Mohamad et al., 2009). The supportive evidence of Shen et al. (1999) reported, the marinespong derived aaptamine have more anti-cancerous properties with DNA damage vis reactive oxygen species and activating P53 related proteins. Hence, our result evidenced that the cell death was observed in aaptamine induced HepG 2 cells through apoptosis.

Table 1
Previous reports of aaptamine against bacteria and cancer cells.

	Inhibition effect of aaptamine against various bacteria and cancer cells			
	Test Sample	**MIC**	**IC50**	**References**
1.	*Pseudomonas aeruginosa,Eschericia coli* and *Salmonella typhi.*	100 mg/mL	–	[Cita et al. (2017)]
2.	C. albicans, C. parapsilosis, C. neoformans, T. rubrum, and M. gypseum.	32, 64, 32, 4, and 16 µg/mL	–	[Yu et al., 2014]
3.	*Staphylococcus aureus, Escherichia coli, Candida albicans* and *Aspergillus niger* and cytotoxic activities.	21,93, and 65.02, and 178.22 µg/mL,	–	[Abdillah, Zuraiha (2014)]
4.	leukemia (L1210) and colon (Colon38)	–	250 µg/mL	[Bowling et al. (2008)]
5.	HeLa, SNU-C4, SK-MEL-28, andMDA-MB-231	–	0.5; 1.0; 2.0 ??M)	Dyshlovoy et al., 2014
6.	HeLa, K562, MCF-7, and U937 cell lines	–	0.90–12.32 µM	Gan et al. (2015)
7.	*Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter* sp. and HepG 2 cell line	55 µg/mL	75 µg/mL	Present Study

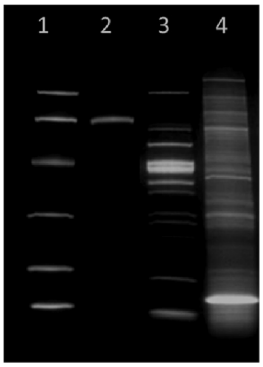

Fig. 7. DNA fragmentation of HepG2 exposed to aaptamine. Fragmentations of genomic DNA in HepG2 cells were treated with IC50 of aaptamine for 48 h. DNA laddering formation was viewed on ethidium bromide-stained gel (1%) and photographed by UV illumination. 1) molecular marker; 2) intact DNA of untreated cells; 3) 15 μg/mL 4) 75 μg/mL of aaptamine treated cells.

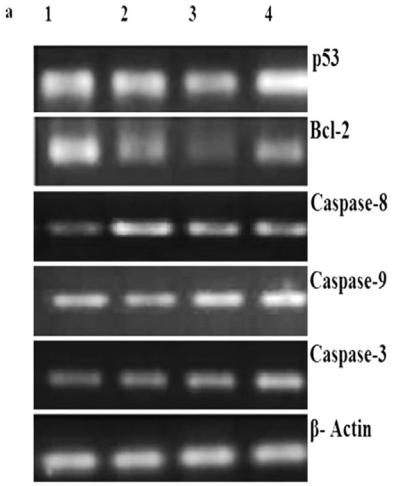

Fig. 8. Effect of IC50 dose of aaptamine on mRNA expression levels in HepG 2cells by RT-PCR using genes BCL2, p53 and caspase 3, 8, 9. 1) Control, 2). Positive control, 3). 15 μg/mL 4). 75 μg/mL of aaptamine treated cells.

3.5.4. RT-PCR analysis

The differential gene expression pattern of p53, Bcl-2, caspase-3, 8 and 9 compared with an internal control β-actin were showed in (Fig. 8). Previously, Arai et al., 2014 reported that the aaptamine could trigger many genes for involved in apoptosis including p53, Bcl-2, caspase-3, 8 and 9. It effectively compared with loading control of β-actin, which is predominantly modulating the metabolic pathways. It helped the activation of proapoptotic BCL-2 family protein and mitochondrial membrane interference, leading to activation of the mitochondrial apoptotic pathway. Hence, we found, the effect of aaptamine altered the apoptotic pathway by proapoptotic and antiapoptotic genes. After 24 h incubation, the aaptamine treated HepG 2 cell was showed with gene expression. Previous report of Calcabrini et al. (2017) documented that the aaptamine altered the HepG 2 cell cycle, proliferation and induced oxidative stress, which accompanied by elevated levels of p53. The treated cells were showed increased expression of p53, caspase-3, 8 and 9 and down regulating the gene of BCL-2. Our result was most accordance with earliest study of Stuhldreier et al. (2015) and Larghi et al. (2009), that the aaptamine has the induced ability in P53 and other gene expressions in most of the cell lines including HepG 2, A549, and LNaP. Hence, the result was more evident that the aaptamine has anticancer properties and exhibited better inhibition efficiency against HepG 2 cells.

3.5.5. Western blotting analysis

Generally, anticancer activity of the marine sponge compound aaptamine reveals, their anti-proliferative effects were observed from cancer cells apoptosis (Dyshlovoy et al., 2016). The apoptosis process was depends on the two different types of the major pathways including the proapoptotic intrinsic pathways which started by expanded volume of ROS generation to the scarcity of MMP and the anti-apoptotic extrinsic pathway which involves interaction of ligand and plasma membrane receptors (Calcabrini et al., 2017; Dyshlovoy et al., 2014). In this study, the expression level of p53 was increased due to the DNA fragmentation cell cycle control and apoptosis after the treatment of aaptamine. Both the pathways lead to activation of specific key proteinase including initiator caspase-3, caspase-8 and caspase-9. Effectors caspases performed apoptotic modification in cells was observed by degradation of nuclear DNA, various cytoskeleton proteins and formation of apoptotic bodies which effectively leads to apoptosis. The increase of P53 was arrested in theG2/M phase in HepG 2 liver cancer cell was clearly observed after treatment of aaptamine. In addition, the anti-apoptotic protein BCL-2 was enhanced the aaptamine effect via down regulating pathway. This important pathway of down regulation was activated the mitochondrial membrane permeation followed by activation of caspase cascade. Particularly, the result indicated that the availability of caspase role within the cancer cells were essential for the drug to induce apoptosis. For this reason, we found the significant up-regulation of proapoptotic proteins such as caspase-3, caspase-8 and caspase-9 during the treatment with aaptamine (Fig. 9). In this study, Caspase-8 acts as an initiator caspase during the treatment with marine compound by activating the Fas-TNF receptor ligand interaction (Ashkenazi and Dixit, 1998; Waring and Mullbacher, 1999; Magnusson and Vaux, 1999). Behind, the release of cytochrome c into the cytosol leads to the activation of procaspase-9 to activate the downstream caspase-3 (Chai et al., 2008; Koehler et al., 2013). In this process, procaspase-9 binds to the cytosol due to the effect of confirmation changes towards the recruitment of apoptosis (Li et al., 1997). The result was proved that the caspase-3 acts a dominant executioner caspase to induce cell death. In the treatment result, the induction of apoptosis in the HepG 2 liver cancer cell line was triggered in both pathways by treatment of aaptamine. Hence, our result was suggested that the marine natural products are alternative drugs for inhibiting the HepG 2 cell lines.(Fig. 10)

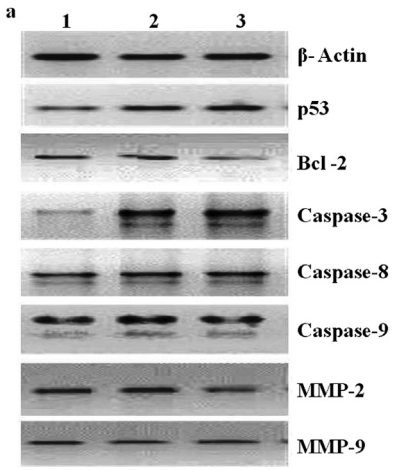

Fig. 9. Effect of IC50 dose of aaptamine on caspase 3, 8 and 9 protein expression as determined by Western blot analysis in HepG 2 cell line. 1) Control, 2). positive control 3).3). 15 μg/mL 4). 75 μg/mL of aaptamine treated cells. β-actin was used as the control. The experiment wa conducted in triplicate.

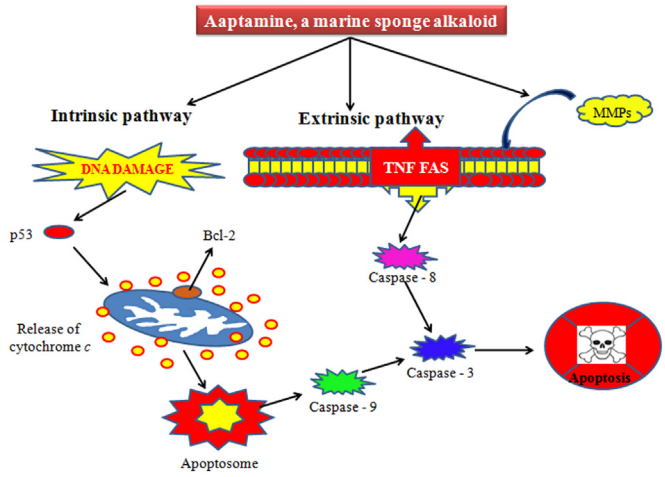

Fig. 10. Possible mechanism involved in the aaptamine induced apoptosis against HepG2 cancer cell line.

4. Conclusion

The marine habitat is an excellent source for valued species that need to be explored. In particular, marine sponge is a important species has tremendous biological material used for various biomedical applications. The findings of present study suggested that the marine sponge is one of the promising candidates as identification of new drug. The marine sponge alkaloid aaptamine proved its ability against ESBL producing bacteria and HepG 2 liver cancer cells by various in vitro studies. The identified result confirmed that aaptamine can use as antibacterial inhibitor as well as anticancer agents. To that end, further in-vivo studies need to be carried out before recommending the clinical use of aaptamine.

Acknowledgement

The work was supported by University Research Fellowship (2013-2016) funded by Ref. No-05441/URF/K7/2013) Bharathidasan University, Tiruchirappalli-24 for inhibition of ESBL studies. The author would like to acknowledge the Bharathidasan University for utilize the facility of Confocal Laser Scanning Electron Microscope (CLSM) (DST-PURSE - Sanction Order No- SR/FT/LS-113/2009).

Conflict of interest

All authors declare no conflict of interest.

References

Abdelfattah, M.S., Elmallah, M.I.Y., Hawas, U.W., El-Kassema, L.T.A., Gamal Eid, M.A., 2016. Isolation and characterization of marine-derived actinomycetes with cytotoxic activity from the Red Sea coast. Asian Pac. J. Trop. Biomed. 6, 651–657.
Abdillah, S., Zuraiha, F., Syarmalina, 2014. Evaluation of antibacterial activities of secondary meta bolites produced by aaptos suberiptoides associated bacteria. OSR J. Pharm. Biol. Sci. 9, 34–38.
Abdul rashid, F.N.A.I.N., Asari, A., Mohamad, H.A., Mohd, S.M., 2014. Synthesis and antibacterial study of aaptamine derivatives. Asian J. Chem. 26 (20), 6903–6907.
Ahamed, M., Akhtar, M.J., Khan, M.A.M., Alhadlaq, H.A., Alshamsan, A., 2016. Cobalt iron oxide nanoparticles induce cytotoxicity and regulate the apoptotic genes through ROS in human liver cells (HepG 2). Colloids Surf. B Biointerfaces 148, 665–673.
Al-Hadi, M., Periasamy, V.S., Athinarayanan, J., Al-Khalifa, A.S., Alshatwi, A.A., 2017. Extraction of ultrafine carbon nanoparticles from samooli bread and evaluation of their in vitro cytotoxicity in human mesenchymal stemcells. Proc. Biochem. 52, 250–258.
Allam, A., Maigre, L., Vergalli, J., Dumont, E., Cinquin, B., Alves de Sousa, R., Pajovic, J., Pinet, E., Smith, N., Herbeuval, J.P., Réfrégiers, M., Artaud, I., Pagès, J.M., 2017. Microspectrofluorimetry to dissect the permeation of ceftazidime in Gram-negative bacteria. Sci. Rep. 7 (1), 986.
Arai, M., Han, C., Yamano, Y., Setiawan, A., Kobayashi, M., 2014. Aaptamines, marine spongean alkaloids, as anti-dormant mycobacterial substances. J. Nat. Med. 68 (2), 372–376.
Ashkenazi, A., Dixit, V.M., 1998. Death receptors: signalling and modulation. Science 281, 1305–1308.
Beygmoradi, A., Homaei, A., 2017. Marine microbes as a valuable resource for brand new industrial biocatalysts. Biocatal. Agric. Biotechnol. 11, 131–152.
Bowling, J.J., Pennaka, H.K., Ivey, K., Wahyuono, S., Kelly, M., Schinazi, R.F., Valeriote, F.A., Graves, D.E., Hamann, M.T., 2008. Antiviral and anticancer optimization studies of the DNA-binding marine natural product aaptamine. Chem. Biol. Drug. Des. 71, 205–215.
Brzoska, K., Meczynska-Wielgosz, S., Stepkowski, T.M., Kruszewski, M., 2015. Adaptation of HepG 2 cells to silver nanoparticles-induced stress is based on the pro-proliferative and anti-apoptotic changes in gene expression. Mutagenesis 30 (3), 431–439.
Calcabrini, C., Catanzaro, E., Bishayee, A., Turrini, E., Fimognari, C., 2017. Marine sponge natural products with anticancer potential: an updated review. Mar. Drugs 15 (10), 1–17.
Chai, W.S., Zhu, X.M., Li, S.H., Fan, J.X., Chen, B.Y., 2008. Role of Bcl-2 family members in caspase-3/9-dependent apoptosis during *Pseudomonas aeruginosa* infection in U937 cells. Apoptosis 13, 833–843.
Chen, C.Y., Chen, Y.H., Lu, P.L., Lin, W.R., Chen, T.C., Lin, C.Y., 2012. *Proteus mirabilis* urinary tract infection and bacteremia: risk factors, clinical presentation, and outcomes. J. Microbiol. Immunol. Infect. 45 (3), 228–236.
Cita, Y.P., Suhermanto, A., Radjasa, O.K., Sudharmono, P., 2017. Antibacterial activity of marine bacteria isolated from sponge Xestospongia testudinaria from Sorong. Papua Asi. Pac. J. Trop. Biomed. 7, 450–454.
Dholvitayakhun, A., Trachoo, N., Narkkong, N., Cushnie, T.P.T., 2017. Using scanning and transmission electron microscopy to investigate the antibacterial mechanism of action of the medicinal plant Annona squamosa Linn. J. Herbal. Med. 7, 31–36.
Dyshlovoy, S.A., Fedorov, S.N., Shubina, L.K., Kuzmich, A.S., Bokemeyer, C., Amsberg, G.K., Honecker, F., 2014a. Aaptamines from the marine sponge *aaptos* sp. display anticancer activities in human cancer cell lines and modulate AP-1-, NF-B-, and p53-dependent transcriptional activity in mouse JB6 Cl41 Cells. BioMed. Res. Intern. 469309, 1–7.
Dyshlovoy, S.A., Tabakmakher, K.M., Hauschild, J., Shchekaleva, R.K., Otte, K., Guzii, A.G., Makarieva, T.N., Kudryashova, E.K., Fedorov, S.N., Shubina, L.K., Bokemeyer, C., Honecker, F., Stonik, V.A., von Amsberg, G., 2016. Guanidine alkaloids from the marine sponge *Monanchora pulchra* show cytotoxic properties and prevent EGF-induced neoplastic transformation in vitro. Mar. Drugs 14 (7), 1–10.
Enrique, L., Larghi, Marı´a, L., 2009. Bohn, Teodoro S. Kaufman. Aaptamine and related products. Their isolation, chemical syntheses,and biological activity. Tetrahedron 65, 4257–4282.
Fallah, F., Yousefi, M., Pourmand, M.R., Hashemi, A., Nazari Alam, A., Afshar, D., 2017. Phenotypic and genotypic study of biofilm formation in *Enterococci* isolated from urinary tract infections. Microb. Pathog. 108, 85–90.
Gan, J.H., Hu, W.Z., Yu, H.B., Yang, F., Cao, M.X., Shi, H.J., Kang, Y.F., Han, B.N., 2015. Three new aaptamine derivatives from the South China Sea sponge Aaptos aaptos. J. Asian Nat. Prod. Res. 17, 1231–1238.
Gul, W., Hammond, N.L., Yousaf, M., Bowling, J.J., Schinazi, R.F., Wirtz, S.S., de Castro Andrews, G., Cuevas, C., Hamann, M.T., 2006. Modification at the C9 position of the marine natural product isoaaptamine and the impact on HIV-1, mycobacterial, and tumor cell activity. Bioorg. Med. Chem. 14, 8495–8505.
Hayajneh, W.A., Hajj, A., Hulliel, F., Sarkis, D.K., Irani-Hakimeh, N., Kazan, L., Badal, R.E., 2015. Susceptibility trends and molecular characterization of Gram-negative bacilli associated with urinary tract and intra-abdominal infections in Jordan and Lebanon: smart 2011–2013. Int. J. Infect. Dis. 35, 56–61.
Huang, C.H., Chiu, C.H., Chen, I.W., Hung, S.Y., Lin, C.W., Hsu, B.R., Huang, Y.Y., 2018. Antimicrobial resistance and outcomes of community-onset bacterial bloodstream infection in patients with type 2 diabetes. J. Glob. Antimicrob. Resist. 18 (30155-3).
Huang, Y.P., Hung, C.M., Hsu, Y.C., Zhong, C.Y., Wang, W.R., Chang, C.C., Lee, M.J., 2016. Suppression of breast cancer cell migration by small interfering RNA delivered by polyethylenimine-functionalized graphene oxide. Nanoscale Res. Lett. 11 (1), 1–8.
Jaworski, S., Sawosz, E., Kutwin, M., Wierzbicki, M., Hinzmann, M., Grodzik, M., Winnicka, A., Lipika, L., Wadyga, K., Chwalibog, A., 2015. In vitro and in vivo effects of graphene oxide and reduced graphene oxide on glioblastoma. Int. J. Nanomed. 10, 1585–1596.
Kumar, S., Das, A.P., 2016. Molecular identification of multi drug resistant bacteria from urinarytract infected urine samples. Microb. Pathog. 98, 37–44.
Koehler, B.C., Scherr1, A.L., Lorenz, S., Urbanik, T., Kautz, N., Elssner, C., Welte, S., Bermejo, J.L., Jager, D., Bergkamen, H.S., 2013. Beyond cell death – antiapoptotic Bcl-2 proteins regulate migration and invasion of colorectal cancer cells in vitro. PLoS One 8, 1–12.
Larghi, E.L., Bohn, M.L., Kaufman, T.S., 2009. Aaptamine and related products. Their isolation, chemical syntheses, and biological activity. Tetrahedron 65, 4257–4282.
Lee, Y., Phat, C., Hong, S.C., 2017. Structural diversity of marine cyclic peptides and their molecular mechanisms for anticancer, antibacterial, antifungal,and other clinical applications. Peptides 95, 94–105.
Li, C., Huang, Q., Xiao, J., Fu, X., You, L., Liu, R.H., 2016. Preparation of Prunella vulgaris polysaccharide-zinc complex and its antiproliferative activity in HepG 2 cells. Int. J. Biol. Macromol. 91, 671–679.
Li, P., Nijhawan, D., Budihardjo, I., Srinivasula, S.M., Ahmad, M., Alnemri, E.S., Wang, X., 1997. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 91, 479–489.
Lin, M.C., Hui, C.F., Chen, J.Y., Wu, J.L., 2013. Truncated antimicrobial peptides from marine organisms retain anticancer activity and antibacterial activity against multi-drug-resistant *Staphylococcus aureus*. Peptides 44, 139–148.
Magnusson, C., Vaux, D.L., 1999. Signalling by CD95 and TNF receptors: not only life and death. Immunol. Cell Biol. 77, 41–46.

Majeeda, S., Abdullaha, M.S., Nandab, A., Ansaria, M.T., 2014. In vitro study of the antibacterial and anticancer activities of silver nanoparticles synthesized from Penicillium brevicompactum(MTCC-1999). J. Taib. Uni. Sci. 10, 614–620.
Maruthupandy, M., Rajivgandhi, G., Muneeswaran, T., MingSong, T., Manoharan, N., 2018. Biologically synthesized zinc oxide nanoparticles as nanoantibiotics against ESBLsproducing gram negative bacteria. Microb. Pathog. 121, 224–231.
Melander, R.J., Liu, H.B., Stephens, M.D., Bewley, C.A., Melander, C., 2016. Marine sponge alkaloids as a source of anti-bacterial adjuvants. Bioorg. Med. Chem. Lett. 26, 5863–5866.
Mishra, D.K., Creighton, C.J., Zhang, Y., Gibbons, D.L., Kurie, J.M., Kim, M.P., 2014. Gene expression profile of A549 cells from tissue of 4D model predicts poor prognosis in lung cancer patients. Int. J. Cancer 134 (4), 789–798.
Mohamad, H., Rashid, Z.M., Shaari, K., Latip, J., Nordin, Md, Lajis, H.J., Abd. Manaf, Ali, 2009. Antibacterial and DPPH free radical-scavenging activities of methanolic extracts of *Aaptos* sp. (Marine Sponges). J. Trop. Agric. Sci. 32 (1), 43–50.
Molina-Quiroz, R.C., Silva, C.A., Molina, C.F., Leiva, L.E., Reyes-Cerpa, S., Contreras, I., Santiviago, C.A., 2015. Exposure to sub-inhibitory concentrations of cefotaxime enhances the systemic colonization of *Salmonella Typhimurium* in BALB/c mice. Open Biol. 5, 150070.
Nakamura, H., Kobayashi, J., Ohizumi, J., Hirata, Y., 1982. Isolation and structure of aaptamine, a novel heteroaromatic substance possessing α-blocking activity from the sea sponge Aaptos aaptos. Tetrahedron Lett. 23, 5555–5558.
Naveen Kumar, S., Rajivgandhi, G., Manoharan, N., 2018. Cytotoxicity effect of marine Sponge Alkaloid, Fascaplysin on HepG 2 Hepatocellular carcinoma cell. Front. Lab. Med. 2, 1–10.
Ohizumi, Y., Kajiwara, A., Nakamura, H., Kobayashi, J., 1984. α-Adrenoceptor blocking action of aaptamine, a novel marine natural product, in vascular smooth muscle. J. Pharm. Pharmacol. 36, 785–786.
Pham, C.D., Hartmann, R., Muller, W.E., de Voogd, N., Lai, D., Proksch, P., 2013. Aaptamine derivatives from the Indonesian sponge *Aaptos suberitoides*. J. Nat. Prod. 76, 103–106.
Prasad, K., Lekshmi, G.S., Ostrikov, K., Lussini, V., Blinco, J., Mohandas, M., Vasilev, K., Bottle, S., Bazaka, K., Ostrikov, K., 2017. Synergic bactericidal effects of reduced graphene oxide and silver nanoparticles against Gram-positive and Gram-negative bacteria. Sci. Rep. 7 (1), 1591 (8).
Rajivgandhi, G., Maruthupandy, M., Manoharan, N., 2018a. Detection of TEM and CTX-M genes from ciprofloxacin resistant *Proteus mirabilis* and *Escherichia coli* isolated on urinary tract infections (UTIs). Microb. Pathog. 121, 123–130.
Rajivgandhi, G., Muneeswaran, T., Maruthupandy, M., Ramakritinan, C.M., Saravanan, K., Ravikumar, V., Manoharan, N., 2018b. Antibacterial and anticancer potential of marine endophytic actinomycetes *Streptomyces coeruleorubidus* GRG 4 (KY457708) compound against colistin resistant uropathogens and A549 lung cancer cells. Microb. Pathog. 125, 325–335.
Rajivgandhi, G., Maruthupandy, M., Muneeswaran, T., Anand, M., Manoharan, N., 2018c. Antibiofilm activity of zinc oxide nanosheets (ZnO NSs) using *Nocardiopsis* sp. GRG1 (KT235640) against MDR strains of gram negative *Proteus mirabilis* and *Escherichia coli*. Proc. Biochem 67, 8–18.
Rajivgandhi, G., Maruthupandy, M., Ramachandran, G., Priyanga, M., Manoharan, N., 2018d. Detection of ESBL genes from ciprofloxacin resistant Gram negative bacteria isolated from urinary tract infections (UTIs). Front. Lab. Med. 2, 5–13.
Rajivgandhi, G., Ramachandran, G., Maruthupandy, M., Saravanakumar, S., Manoharan, N., 2018e. Antibacterial effect of endophytic actinomycetes from marine algae against multi drug resistant gram negative bacteria. Exam. Mar. Biol. Oceanogr. 1 (4), 1–8.
Rajivgandhi, G., Senthil, R., Ramachandran, G., Maruthupandy, M., Manoharan, N., 2018f. Antibiofilm activity of marine endophytic actinomycetes compound isolated from mangrove plant *Rhizophora mucronata,* Muthupet Mangrove Region, Tamil Nadu. India J. Terr. Mar. Res. 2 (4), 1–7.
Rajivgandhi, G., Vijayan, R., Kannan, M., Santhanakrishnan, M., Manoharan, N., 2016. Molecular characterization and antibacterial effect of endophytic actinomycetes *Nocardiopsis* sp. GRG1 (KT235640) from brown algae against MDR strains of uropathogens. Bioact. Mater. 1, 140–150.
Rajivgandhi, G., Vijayan, R., Maruthupandy, M., Vaseeharan, B., Manoharan, N., 2018g. Antibiofilm effect of *Nocardiopsis* sp. GRG 1 (KT235640) compound against biofilm forming Gram negative bacteria on UTIs. Microb. Pathog. 118, 190–198.
Rajivgandhi, G., Vijayarani, J., Kannan, M., Murugan, A., Vijayan, R., Manoharan, N., 2014. Isolation and identification of biofilm forming uropathogens from urinary tract infection and its antimicrobial susceptibility pattern. Int. J. Adv. Life Sci. 7 (2), 352–363.
Satpathy, S., Kumar, S., Sen, S., Pattanaik, S., Raut, 2016. Review on bacterial biofilm: an universal cause of contamination. Biocatal. Agric. Biotechnol. 7, 56–66.
Shen, Y.C., Lin, T.T., Sheu, J.H., Duh, C.Y., 1999. Structures and cytotoxicity relationship of isoaaptamine and aaptamine derivatives. J. Nat. Prod. 62, 1264–1267.
Sthijns, M.M., Thongkam, W., Albrecht, C., Hellack, B., Bast, A., Haenen, G.R., Schins, R.P., 2017. Silver nanoparticles induce hormesis in A549 human epithelial cells. Toxicol. Vitr. 40, 223–233.
Stuhldreier, F., Kassel, S., Schumacher, L., Wesselborg, S., Proksch, P., Fritz, G., 2015. Pleiotropic effects of spongean alkaloids on mechanisms of cell death,cell cycle progression and DNA damage response (DDR) of acutemyeloid leukemia (AML) cells. Cancer Lett. 361, 39–48.
Tsukamoto, S., Yamanokuchi, R., Yoshitomi, M., Sato, K., Ikeda, T., Rotinsulu, H., Mangindaan, R.E., de Voogd, N.J., van Soest, R.W., Yokosawa, H., 2010. Aaptamine, an alkaloid from the sponge *Aaptos suberitoides*, functions as a proteasome inhibitor. Bioorg. Med. Chem. Lett. 20, 3341–3343.
Wahab, R., Siddiqui, M.A., Saquib, Q., Dwivedi, S., Ahmad, J., Musarrat, J., Al-Khedhairy, A.A., Shin, H.S., 2014. ZnO nanoparticles induced oxidative stress and apoptosis in HepG 2 and MCF-7 cancer cells and their antibacterial activity. Colloids Surf. B Biointerfaces 117, 267–276.
Waring, P., Mullbacher, A., 1999. Cell death induced by the Fas/Fas ligand pathway and its role in pathology. Immunol. Cell Biol. 77, 312–317.
WHO, 2012. The Evolving Threat of Antimicrobial Resistance: Options for Action. World Health Organization, Geneva.
Yan, S., Hu, F., Hong, X., Shuai, Q., 2018. Water-soluble ferrocene complexes (WFCs) functionalized silica nanospheres for WFC delivery in HepG 2 tumor therapy. Mater. Sci. Eng. C Mater. Biol. Appl. 90, 397–406.
Yu, H.B., Yang, F., Sun, F., Li, J., Jiao, W.H., Gan, J.H., Hu, W.Z., Lin, H.W., 2014a. Aaptamine derivatives with antifungal and anti-HIV-1 activities from the South China Sea sponge *Aaptos aaptos*. Mar. Drugs 12, 6003–6013.
Yu, J., Zhang, L., Liu, Q., Qi, X., Ji, Y., Kim, B.S., 2015. Isolation and characterization of actinobacteria from Yalujiang coastal wetland, North China. Asian Pac. J. Trop. Biomed. 5, 555–560.
Yuan, Y., Zhang, Y., Liu, B., Wu, H., Kang, Y., Li, M., Zeng, X., He, N., Zhang, G., 2015. The effects of multifunctional MiR-122-loaded graphene-gold composites on drug-resistant liver cancer. J. Nanobiotechnol. 13, 1–15.