**Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations (not to exceed 6000 words).**

The art of total synthesis of natural products paved the way from discovery of potent molecules for biomedical applications to creation of ground for innumerable fundamental and applied tangible advancements. Prof. Goswami has made significant contributions in this general area of organic synthesis in particular to the asymmetric synthesis of bioactive natural products and their analogues and evaluation of their role in pharmaceutical domain. His research efforts successfully cover the chemical synthesis of a broad spectrum of natural products which culminated the total synthesis of 38 natural products till the date and many of the synthesized molecules have been investigated for biological study. In particular, the development of synthetic routes for accessing novel natural products (***sunshinamide, beauveamide A thailandamide lactone***) and their analogues and finding of their biological implication is quite important in respect to applied medicinal chemistry point of view.

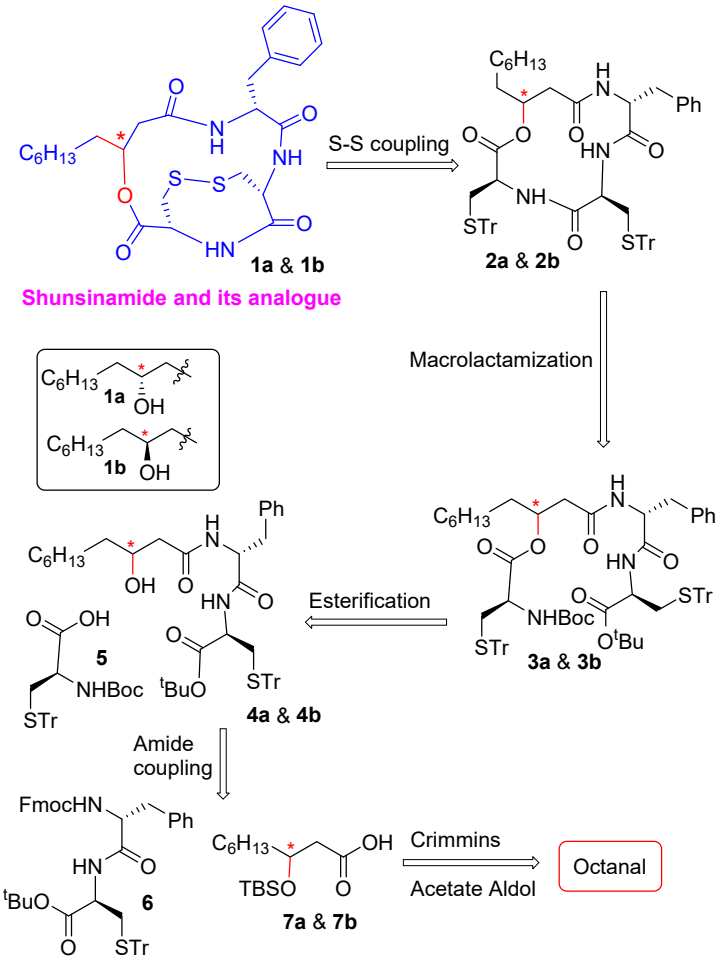
**(i) Sunshinamide and its Analogues and Evaluation of Their Anticancer Potential:**

Bicyclic natural products containing a disulfide linkage are an important class of molecules that exhibit a broad range of biological activities and pharmacological properties. Many of these natural products showed striking anticancer and immunosuppressant activities. Thus, chemical and biological study on this class of natural products is a subject of great importance. Disulfide bridged cyclodepsipeptide sunshinamide isolated in 2018 in minute amount which showed potent anticancer cytotoxicity to different human carcinoma cell lines. Structurally, it comprises of two cyclic scaffolds; one15-member and another 8-member. D-phenyl alanine and two consecutive L-cysteins are embedded in the peptide backbone whereas the nonpeptidic part is 3-hydroxy decanoic acid. The unique architectural features and promising bioactivity of sunshinamide drew attention to the scientific community.

The first synthetic route of sunshinamide is shown in Scheme 1. The stereochemistry of the hydroxy center in nonpeptidic segment of sunshinamide remained undisclosed during its isolation. Thus, we planned to synthesize both the possible stereoisomers **1a** and **1b** to compare them with the reported data of the isolated natural product. Compounds **1a** and **1b** could be constructed from compounds **2a** and **2b** by S-S bond formation. There are several possible sites in compounds **1a** and **1b** for macrocyclization. We relied on macrolactamization and planned to

disconnect compounds **2a** and **2b** between two cysteine residues to realize compounds **3a** and **3b** which could further be made by esterification of compound **5** with compounds **4a** and **4b**, respectively. Compounds **4a** and **4b** would be constructed separately from compounds **7a** and **7b**, respectively, by amide coupling with compound **6**. Compounds **7a** and **7b** could be synthesized from octanal using Crimmins acetate aldol as one of the key steps.

**Scheme 1**: Retrosynthetic Analysis of Sunshinamide.




Both the targeted compounds **1a** and **1b** were synthesized and their spectroscopic data compared with the data for isolated natural product. $^{1}H$ and $^{13}C$ NMR data of compound **1a** were in very good agreement which clearly confirmed that the structures of isolated sunshinamide *i.e* compound **1a. The unknown center has been assigned.** The synthesized naturally occurring sunshinamide (**1a**) and its configurational isomer (**1b**) were evaluated for their *in vitro* cytotoxic

effects against MDA-MB-231 (human metastatic breast adenocarcinoma cell line), MCF7 (human breast adenocarcinoma cell line), HeLa (human cervical cancer cell line), and HepG2 (human liver cancer cell line) cells using MTT reduction assay. The effects of both of the compounds were also evaluated on non-cancerous cell lines like CHOK1 (Chinese hamster ovary cell line), WI38 (Human lung fibroblast cell line) to check whether they have differential cytotoxic effects on cancer and non-cancer cells. The results are shown in Table-1 which revealed that the synthesized compounds are selective towards cancer cell lines and possessed attractive cytotoxic activity.

**Table 1:** Evaluation of Cytotoxic Activities of Sunshinamide (**1a**) and Its Congener (**1b**) to Human Cancer and Noncancerous Cell Lines.

| **Compounds** | **$IC_{50}$ values in µM** | | | | | |
|---|---|---|---|---|---|---|
| | **MDA-MB-231** | **MCF-7** | **HeLa** | **HepG2** | **CHOK1** | **WI38** |
| **1a** | 0.11± 0.041 | 0.08±0.019 | 0.11±0.005 | 0.10±0.018 | 0.72±0.099 | 0.36±0.031 |
| **1b** | 0.10±0.087 | 0.09±0.013 | 0.13±0.017 | 0.13±0.20 | 0.47±0.008 | 1.05±0.645 |

Next, in order to study the mode of action behind their cytotoxic effects, we have treated the cancer cells with the above-mentioned compounds and systematically analyzed the mode of killing by measuring several cellular assays. Confocal microscopic examination of the cancer cells (MDA-MB-231) treated with the compounds and nuclear DNA stained with propidium iodide, showed typical apoptotic features, like fragmented nuclei, chromatin condensation, and formation of apoptotic bodies (**Figure 1A**). The present study also showed that the exposure of the synthesized compounds (**1a**, **1b**) to the cancer cells increased the activity of the caspase 3 (**Figure 1D**), which is known as the key signature regulator of the apoptotic process. To further confirm apoptosis, treated MDA-MB-231 cells were subjected to flow cytometric analysis to evaluate the fragmented apoptotic DNA. Exposure of the cancer cells with sunshinamide (**1a**) and its analogue (**1b**) caused significant accumulation of fragmented DNA at sub G0/ G1 phase of the cell cycle

(**Figures 1B, 1C**) which is also a characteristic feature of apoptosis. Overall, these results suggest that sunshinamide showed cytotoxicity by inducing apoptosis to the cancer cells.

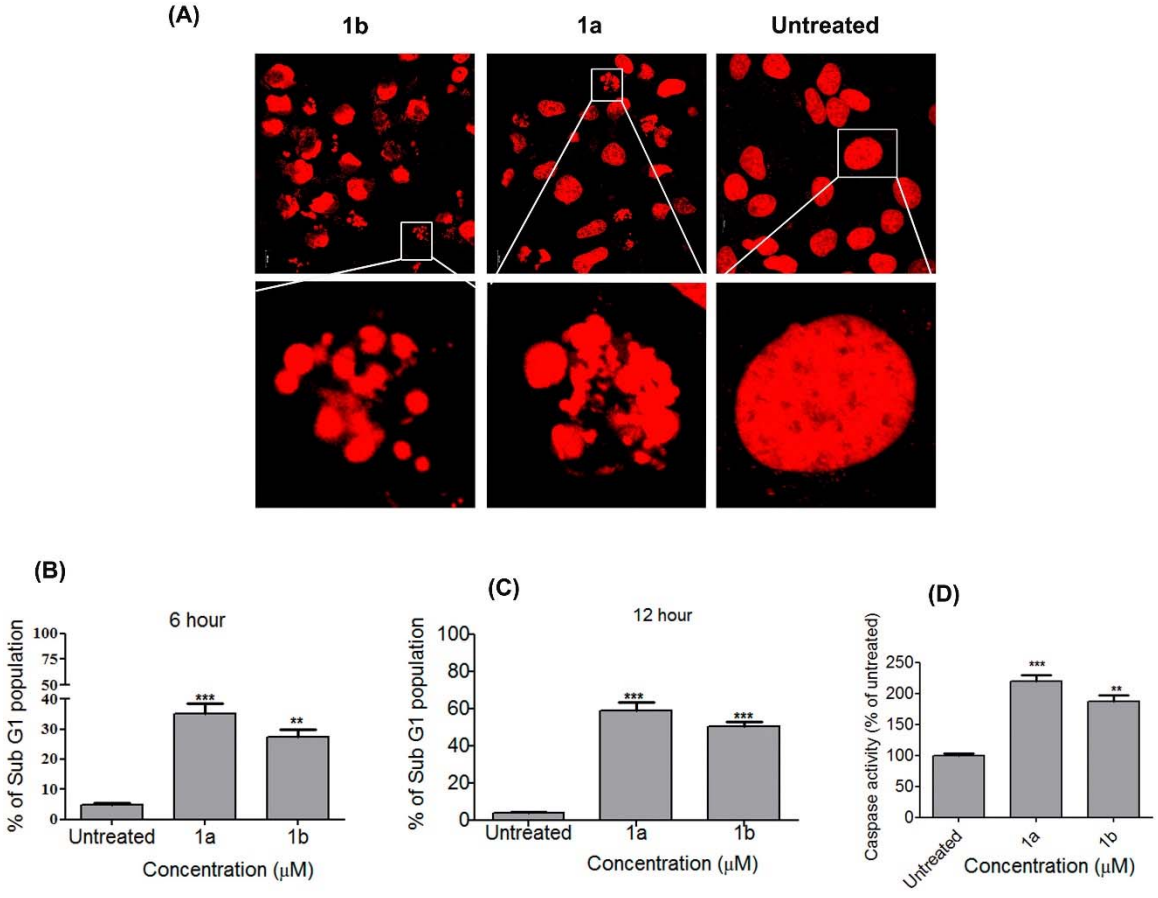



**Figure 1:** Induction of apoptosis by the synthesized compounds **1a** and **1b**

(A) MDA-MB-231 cells were incubated with the $IC_{50}$ doses of the compounds 1a and 1b for 6 h and then stained with PI. Images were captured by confocal microscopy. Images are representative of three independent experiments. (B) & (C) MDA-MB-231 cells were treated with the $IC_{50}$ doses of the compounds for 6 h and 12 h and subjected to cell cycle analysis by Flow cytometry, following staining with PI. The percentage of sub G1 phase was graphically represented. Values are expressed as the means± standard deviation (SD) of 3 independent experiments. (D) MDA-MB-231 cells were exposed to the $IC_{50}$ doses of the compounds and caspase 3 activities were accessed. Values are expressed as the means± standard deviation (SD) of triplicate samples. Scale bar is 10 μM.

Having the first chemical synthetic route of sunshinamide along with the initial cytotoxicity in hand (***Org. Lett. 2020**, 22, 1188-1192*), we turned out our attention towards its structure activity relationship study. A number of analogues of the potent natural product have been synthesized (**Figure 2**) and their cytotoxicity were screened recently against number of cancer cell line (**Table-2**). The data reveled the role of different units of the sunshinamide towards its anticancer effect.

The mechanism of action of the natural product and their variants have been evaluated which showed unique mode of action (***manuscript under preparation***). In overall, this study clearly indicates that shunshinamide and its analogues has very promising pharmaceutical relevance and left a huge scope for further development.

**Figure 2:** Different Synthesized Analogues of Shunshinamide for SAR.

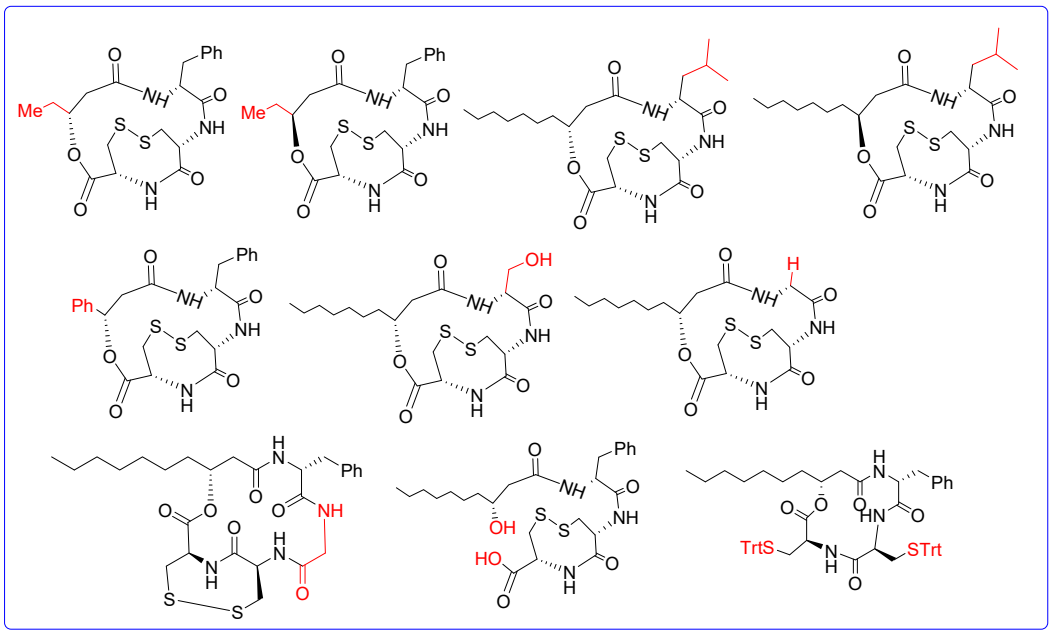


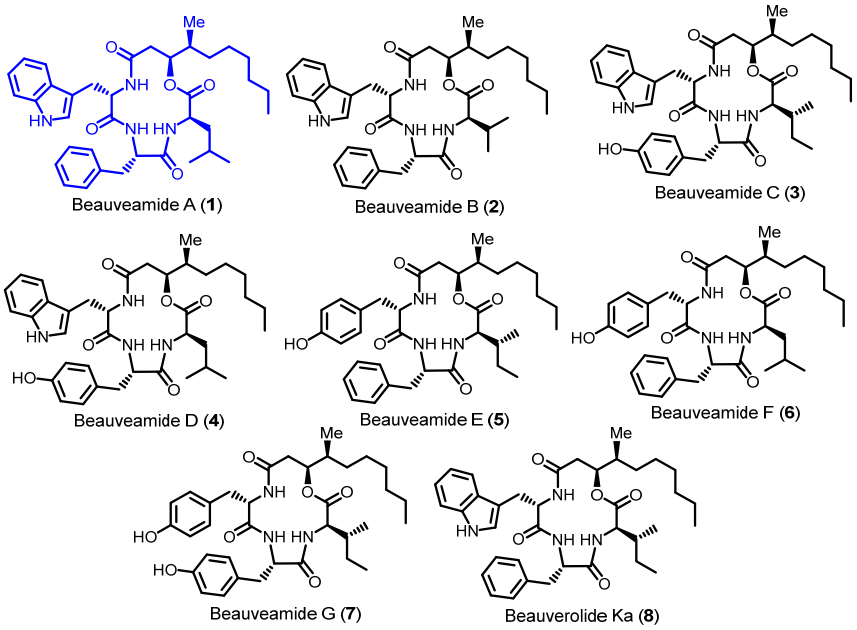
**Table 2:** Evaluation of Cytotoxic Activities of Different Analogues of Sunshinamide to Human Cancer and Noncancerous Cell Lines.

| **$IC_{50}$ value (nM)** | | |
|---|---|---|
| **MDA-MB-231** | **HeLa** | **CHOK1** |
| 12.94 | 7.68 | 85.01 |
| 11.42 | 4.77 | 81.03 |
| 10.29 | 9.16 | 71.04 |
| 8.258 | 8.68 | 66.29 |
| 14.20 | 9.80 | 90.86 |
| 41.38 | 38.14 | 155.1 |
| 26.03 | 14.36 | 123.6 |
| 13.54 | 6.04 | 86.33 |
| 13.85 | 6.66 | 85.18 |
| 22.87 | 14.38 | 111.4 |

## (i) Beauveamide A and its Analogues and Evaluation of Their Anticancer Potential:

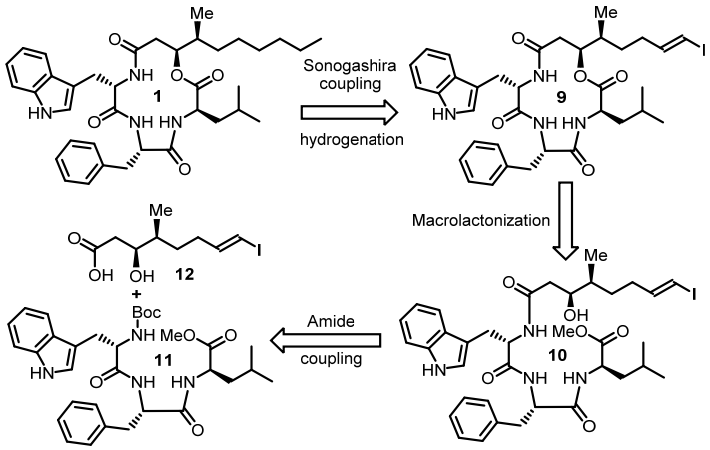
Cyclodepsipeptides are a large family of natural products having diverse architectural features. Many of them possess potent pharmaceutical and agrochemicals values which attracted researchers worldwide to envisage the chemical synthesis of this class of natural products. During the search of novel secondary metabolites from Endolichenic fungi, a family of seven new cyclotetradepsipeptides beauveamides A−G (**1**-**7**) along with the known cyclodepsipeptide beauverolide Ka (**8**) (Figure 1) have been discovered in 2021. Structurally, beauveamides A−G (**1**-**7**) are 13-membered macrocycles bearing a common fatty acid moiety, 3-hydroxy-4-methyldecanoic acid (HMDA). There are three amino acids in the peptide segment which varies among the members. Beauveamides A (1) and B (**2**) exhibited protective effects on mouse auditory cell line (HEI-OC1) in micromolar concentration whereas beauveamides A (**1**), D (**4**) and E (**5**) stimulated the glucose uptake in cultured rat L6 myoblasts. Bioactivities, natural scarcity, interesting structural features, together with our interest towards natural product chemistry prompted us to embark on the chemical synthesis of beauveamide A (**1**), the most active member of this family, where the peptide part is comprised with D-leucine, L-phenyl alanine and L-tryptophan.

**Figure 1:** Chemical Structures of Beauveamides A-G (**1**-**7**) and Beauverolide Ka (**8**).



The retrosynthetic analysis of beauveamide A (**1**) is emanated in Scheme 1 where a late-stage functionalization approach has been adopted to diversify the synthetic route. The target natural product could be synthesized from the key precursor **9** following Sonogashira coupling followed by hydrogenation. Macrolactonization approach could be planned to access compound **9** from the corresponding seco acid derived from compound **10** which in turn could be prepared from tripeptide **11** and acid **12** using amidation chemistry.

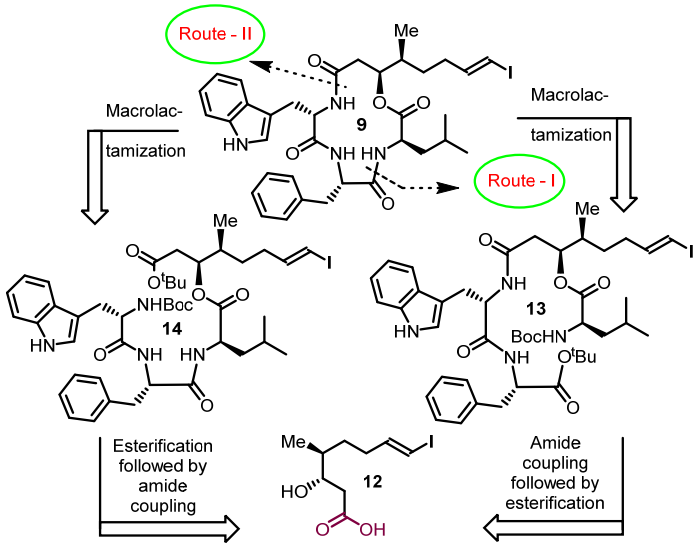
**Scheme 1**: Retrosynthesis of Beauveamide A (**1**).




We have successfully prepared intermediate **10** in a number of steps using modern chemical arsenals but our efforts for macrolactomization to access precursor **9** was not successful. It compelled us to look for other strategy.

An alternative strategy for the synthesis of key intermediate **9** is depicted in Scheme 2 where we relied on macrolactamization approach. There are three sites for macrolactamization. To search for a shorter synthetic path, we planned to test the feasibility of this strategy in two possible sites using the intermediates **13** (route-I) and **14** (route-II) which could further be synthesized from acid **12** and dipeptide of L-tryptophan-L-phenyl alanine and D-leucine using amidation and esterification chemistry, respectively.

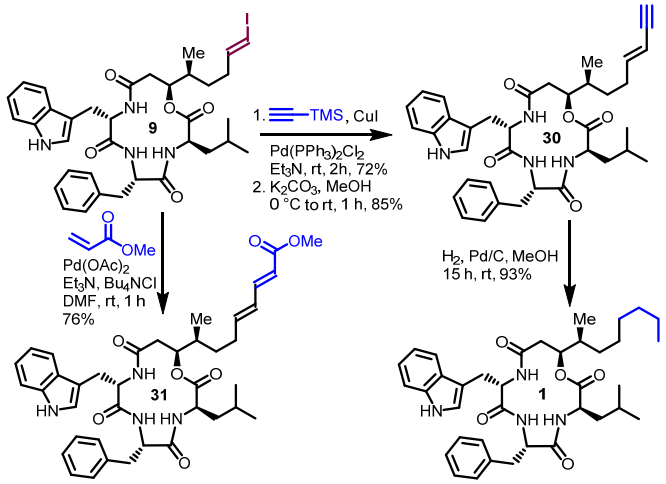
**Scheme 2:** Alternative Retrosynthetic Approach for Vinyl Iodide **9**.




Notably, the macrolactamization via intermediate **14** (route-II) was found much efficient compare to intermediate **13** (route-I) as the esterification with the dipeptide segment was found the determining step.

A late-stage functionalization approach was implemented where vinyl iodide **9** was subjected to Sonogashira coupling using TMS-acetylene to furnish compound **30** which was finally hydrogenated to obtain beauveamide A (**1**, Scheme 3). The initial success in the diversification of vinyl iodide **9** following Sonogashira coupling prompted us to test the feasibility of Heck coupling. Thus, vinyl iodide **9** was treated with methyl acrylate following Heck protocol to access compound **31** in very good yield.

**Scheme 3:** Completion of Total Synthesis of Beauveamide A (**1**) and its Congeners



Cyclodepsipeptides based natural products are known to exhibit anticancer activities, which made us curious to evaluate the anticancer efficacies of beauveamide A and its analogues **30** and **31** as no such literature precedents were available in this direction for this family of cyclodepsipsptides. The *in vitro* cytotoxic effects of the synthesized compound **1** and its congeners **30** and **31** were determined by MTT assay using MDA-MB-231 and HeLa cancer cells lines. Results suggested (**Table 1**) that the cytotoxicity of compound **31** is better than beauveamide A (**1**) whereas compound (**30**) was found least active.

**Table 1**: Estimation of Cytotoxic Potentials of Beauveamide A (**1**) and its Congeners (**30**, **31**) Against Cancer Cell Lines

| | **IC50 (µM)** | |
|---|---|---|
| | MDA-MB-231 | HeLa |
| Beauveamide A (**1**) | 16.15 | 13.60 |
| Compound **30** | 25.70 | 21.17 |
| Compound **31** | **9.56** | **7.55** |
| Doxorubicin | 7.96 | 5.52 |

Next, the mode of killing of cancer cells by all the synthesized compounds was evaluated using flow cytometric analysis (Figure 2). The cell cycle of the MDA-MB-231 cells was measured. All the synthesized compounds have shown a significant increase of DNA content at the sub G0/G1 position which is considered the signature peak for apoptosis. The untreated control set has very insignificant (5.6%) DNA content at SubG0/G1 whereas 45.6%, 33.5%, and 53.7%, 60.5% DNA contents were observed with a distinct apoptotic peak for beauveamide A **(1)**, compounds **30**, **31** and doxorubicin, respectively. FACS analysis was found consistent with the MTT assay where the extent of inducing apoptosis of compound **31** was comparable the known chemotherapeutic drug doxorubicin. The adopted late-stage functionalization approach diversified the synthesis. Interestingly, one of the synthesized analogues showed better anticancer efficacy compare to the parent natural product. Notably, the modification of HMDA part of beauveamide A reflected in its bioactivity. This study left an open space to explore different beauveamide A variants by coupling variety of suitable partners with the key vinyl intermediate **9** to understand their structure activity relationship study. Related study is in progress in our laboratory (*Org. Lett. 2022, 39, 7113-7117*).

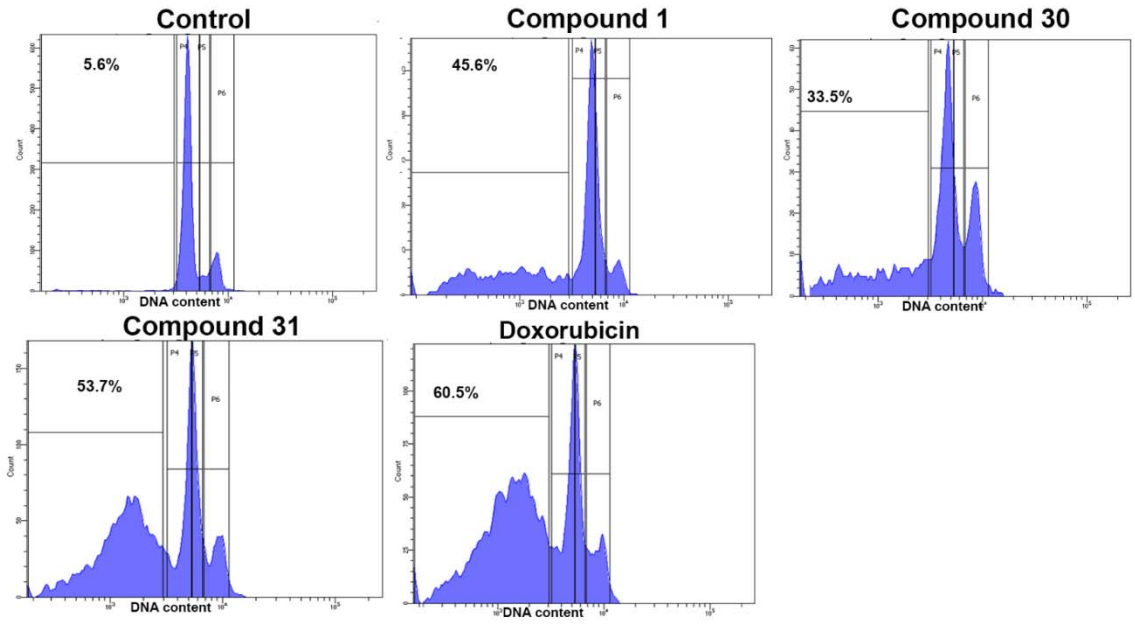


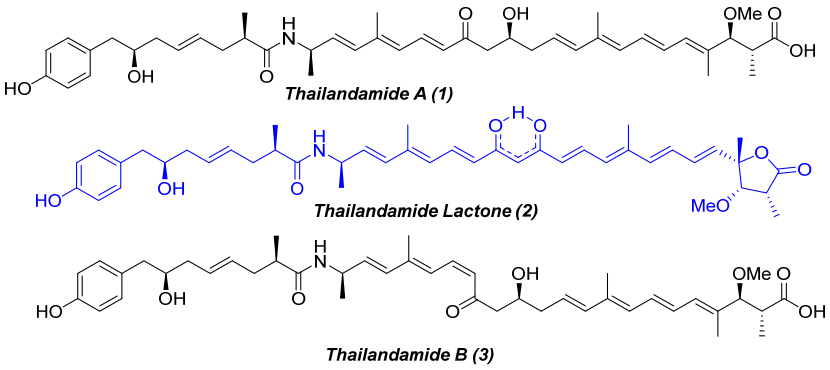

**Figure 2**: Compounds reduce cancer cell viability by triggering apoptosis. The apoptotic cell population was measured by flow cytometry analysis. MDA-MB-231 cells were treated with 25µM of Compounds (**1**, **30**, and **31**) for 48 h, stained with PI, and cell cycle analysis was performed in the flow cytometer. The percentage of apoptotic cells is mentioned.

## (iii) Thailandamide Lactone and its Antibacterial Potential:

Natural products served as important sources for powerful therapeutics against pathogenic microbes by virtue of their lethal and selective action. However, there is an increasing tendency of these microbes to develop resistance against currently available antibiotics which is quite alarming for the modern medicine. Many of the marketed drugs either becoming less effective or completely inactive to nullify their effects which necessities the discover new therapeutics to stay a few steps ahead for our sustainability. Researchers worldwide engage to look into the potential methods to discover new antibiotic(s). One such promising method is exploring the biosynthetic potential of microorganisms. In many organisms, the majority of genes potentially coding for the biosynthesis of secondary metabolites remain dormant and awakening of these silent clusters using elicitors would have a profound impact on drug discovery. During the mining of genome of *Burkholderia thailandensis*, a bacterium isolated from rice paddies in the central and northeastern Thailand, in 2008, a labile polyene polyketide thailandamide A was isolated (**1**, Figure 1) in very minute quantity. In order to understand the thailandamide biosynthesis further, the silent *tha* PKS-NRPS

gene cluster of *B. thailandensis* was activated through manipulation of a quorum sensing (QS) regulatory system which alter dramatically the metabolic profile of the mutant. This resulted the isolation of structurally challenging new polyketide thailandamide lactone (**2**, Figure 1) in 2010 which was not detectable in wild type broth initially. Later, another unstable metabolite thailandamide B (3, Figure 1), a geometrical isomer of thailandamide A was identified. Broad biological screening of thailandamide A reveled its selective and potential inhibitory activity against different pathogenic Gram-positive and Gram-negative bacteria with specific mode of action. However, the antibacterial activity of thailandamide lactone and thailandamide B remained undisclosed. Highly challenging architectural features, natural scarcity, lack of synthetic route encouraged us to envisage the total synthesis of thailandamide lactone. Structurally thailandamide lactone is a linear polyene polyketide where a tetraene conjugated with a γ-butyrolactone is fused with a conjugated triene through an enolized dione moiety. There are six asymmetric centers among which one is quaternary and a phenolic moiety embedded to another terminal of the molecule.

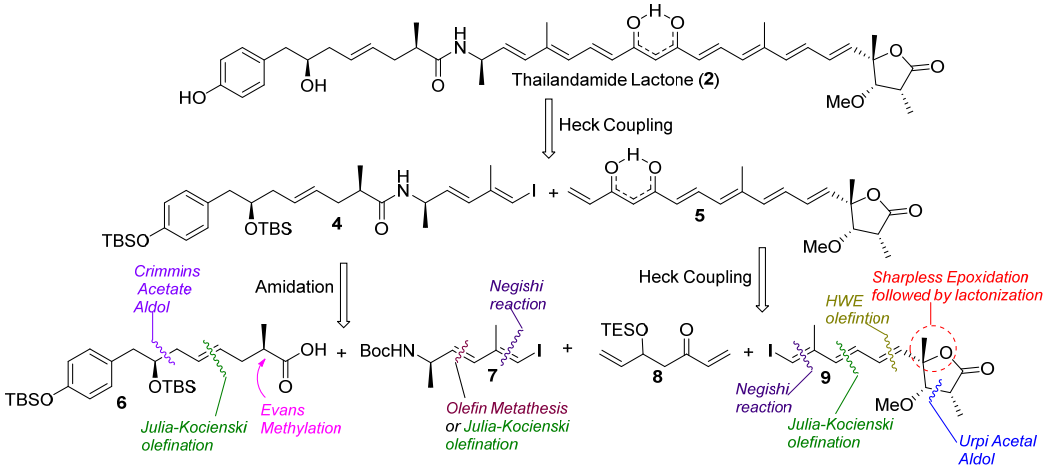
**Figure 1**: Chemical Structures of Thailandamide Family of Natural Products




Retrosynthetic analysis of thailandamide lactone (**2**) is emanated in Scheme 1 where we envisioned that the target molecule could be constructed from vinyl iodide **4** and 1,3-dione encapsulated polyene **5** adopting intermolecular Heck coupling as the key step. Vinyl iodide **4** could further be made from compounds **6** and **7** by amide coupling. Compound **6** could be assembled using Crimmins acetate aldol, Julia-Kocienski olefination and Evans methylation as the key steps whereas compound **7** could be access utilizing cross olefin metathesis or Julia-Kocienski olefination and Negishi reaction as the pivotal steps. On the other hand, keto-alkene **5** could be prepared from intermediates **8** and **9** using Heck coupling whereas compound **9** could be

synthesized using Negishi reaction, Julia-Kocienski olefination, HWE olefination, Urpi acetal aldol, Sharpless asymmetric epoxidation and subsequent $\gamma$-lactonization as the salient steps.

**Scheme 1**: Retrosynthetic Analysis of Thailandamide lactone (**2**)




By solving many synthetic hurdles, we first have developed the chemical route for the total syntehsis thailandamide lactone. Having thailandamide lactone in hand, we then have screened its antibacterial activity against different nonpathogenic and pathogenic Gram-positive bacteria like *Bacillus subtilis* (PY79), *Bacillus megaterium* (2G), *Staphylococcus aureus* as well as Gram-negative bacteria like *Vibrio cholerae* (N16961), *Enteropathogenic Escherichia coli* (EPEC e2348/69), *Escherichia coli* (MC1061)] which revealed its moderate to good antibacterial activity (Table-1). The efficacies of thailandamide lactone even against Gram-negative strains were found good. Importantly, our modular strategy would be amenable to thailandamide A, another member of this family as well as the structurally simplified designed analogues for further exploration towards antibacterial study. (***Chem. Sci., 2022, 13, 13403-13408***).

**Table 3**: Antimicrobial Activities of Thailandamide Lactone Against Different Bacterial Strains

| Staining type | Strains | MIC (µg/ml) |
|---|---|---|
| **Gram Negative** | *V. cholerae* (N16961) (pathogenic) | 71.3 |
| | EPEC (e2348/69) (pathogenic) | 71.3 |
| | *E. coli* (MC1061) | 53.5 |
| **Gram Positive** | *B. subtilis (PY79)* | 57.0 |
| | *B. megaterium* (2G) | 53.5 |
| | *S. aureus* (pathogenic) | 89.1 |

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