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).

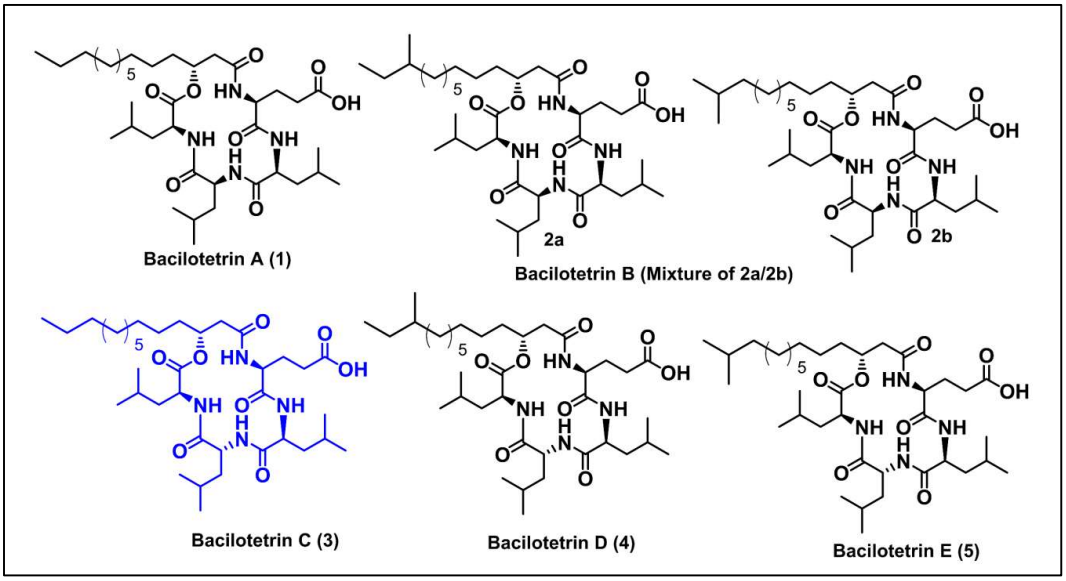
The art of total synthesis of natural products paved the way from discovery of potent molecules for biomedical applications to creation of ground for innumerable fundamental and applied tangible advancements. Prof. Goswami has made significant contributions in this general area of organic synthesis in particular to the asymmetric synthesis of bioactive natural products and their analogues and evaluation of their significance in pharmaceutical domain. His research efforts successfully cover the chemical synthesis of a broad spectrum of natural products which culminated the total synthesis of nearly 40 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 (***bacilotetrin C***, ***sunshinamide, thailandamide lactone***) and their analogues and finding of their biological implication is quite important in respect to applied medicinal chemistry point of view.

(i) Total Synthesis of Lipopeptide Bacilotetrin C: Discovery of Potent Anticancer Congeners Promoting Autophagy:

***ACS. Med. Chem. Lett, 2024, doi.org/10.1021/acsmedchemlett.4c00237*; A part has of this work entitled "*Autophagy Inducing Synthesized Cyclic Depsipeptides in Cancer Chemotherapy*" filed for Indian Patent (*Application No 202331077534 dated 14.11.2023*).**

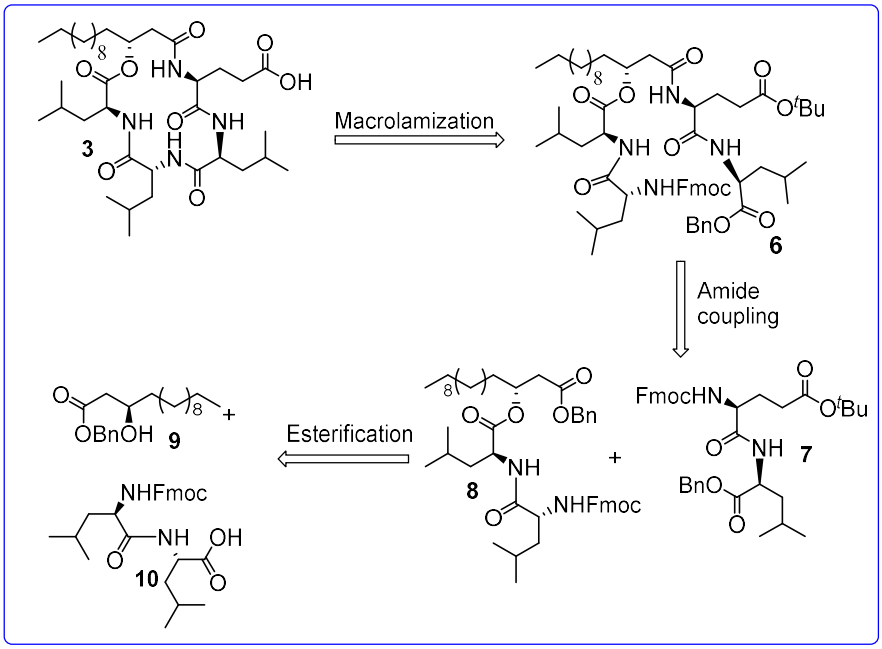
Dysregulation of autophagy is closely associated with many disorders, including cancer. Tuning autophagy by selecting specific regulatory molecules in its machinery can control different disease processes, making it a promising pharmacological target for drug discovery. The recent investigations revealed autophagy as a double-edged sword in the context of cancer therapy as it can have both tumor protective as well as tumor suppressive roles. The correct modulation of autophagy depending on the stage of malignancy considered as one of the novel approaches in cancer therapy. Thus, searching for novel molecule(s) in this direction is highly desirable. Natural products and their variants exhibited significant potential in this regard to facilitate the discovery of anticancer drugs for their lethal and selective actions. Shin and coworkers discovered a series of cyclic lipopeptides bacilotetrins A-E (**1-5**, Figure 1) from marine-derived bacterium *Bacillus subtilis*. Bacilotetrins A-B (**1-2**) were reported to exhibit moderate anti-MRSA activity [Minimum Inhibitory Concentration (MIC) ranges from 8 to 32 μg/mL][25], whereas bacilotetrins C-E showed anti-mycoplasma activity (MIC value of 31 μg/mL). We targeted bacilotetrin C (**3**) with an initial thought to explore its anticancer potential by investigating its in-depth structure activity relationship study, as peptide-based molecules are known to exhibit such potential effects.

Figure 1: Structures of bacilotetrin family of natural products.



The retrosynthesis of bacilotetrin C (**3**) is delineated in Scheme 1 where compound **6** was selected as the macrolactamization precursor presuming to prepare by esterification of an acid of smaller dipeptide rather than a bulky tetrapeptide acid segment. Compound **6** could be prepared from the corresponding benzyl deprotected acid of compound **8** and Fmoc deprotected amine of dipeptide **7**. Compound **8** could further be constructed from dipeptide acid **10** and alcohol **9** by intermolecular esterification.

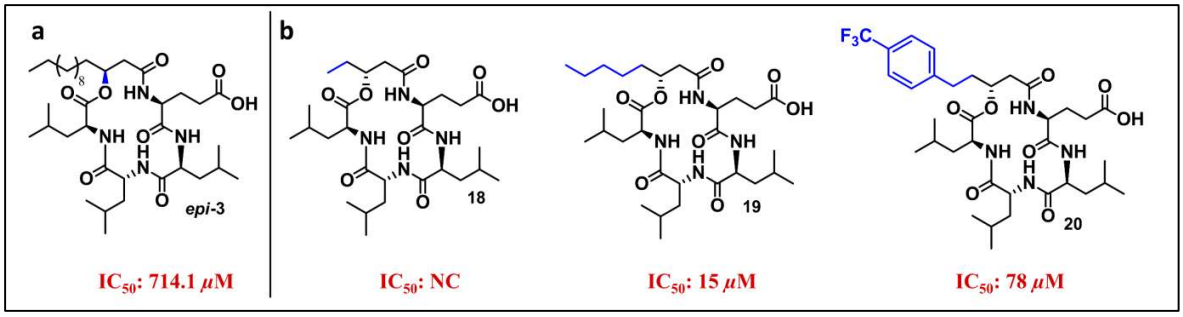
Scheme 1: Retrosynthesis of Bacilotetrin C.



Having the optimized synthetic route of bacilotetrin C in hand, detailed SAR study of bacilotetrin C was planned by altering the key structural features such as the long chain, the ring size and the sequence of amino acid residues in cyclic core. This was established primarily in the triple negative breast cancer cell line MDA-MB-231 possessing highly invasive and resistant properties along with other cell lines (MCF7, PC-3, HepG2).

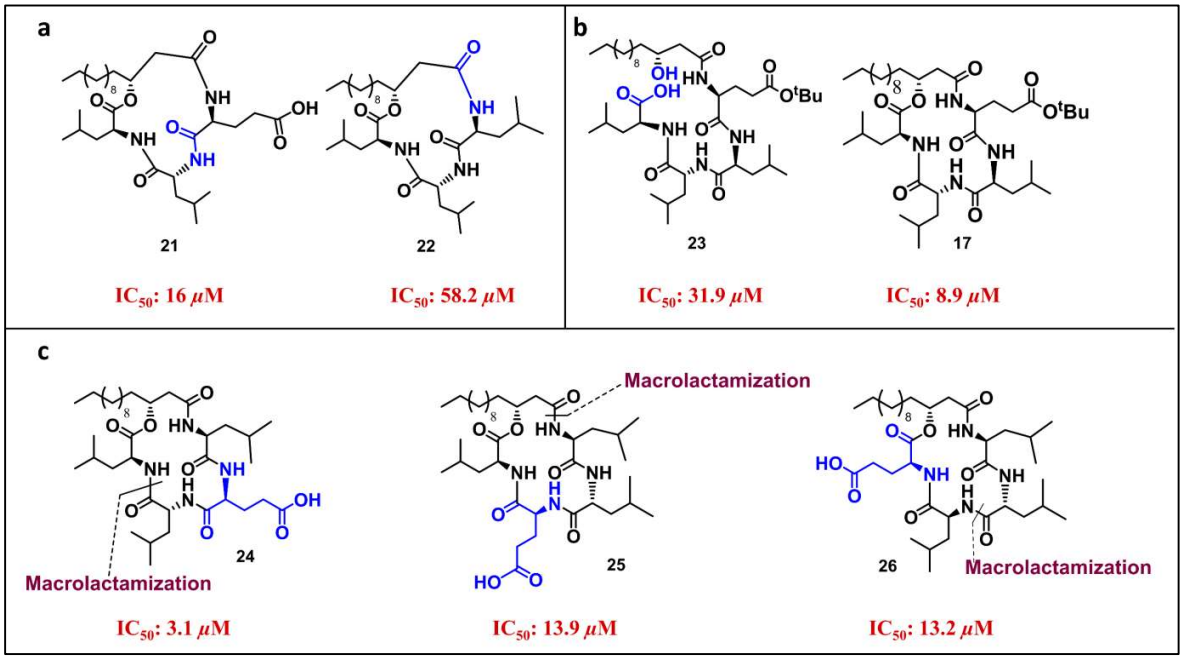
Firstly, the role of stereochemistry of *β*-hydroxy fatty acid in bacilotetrin C towards cytotoxicity was evaluated before exploring the effect of other segments of the molecule. Thus, compound ***epi*-3** (Figure 2a) was synthesized and evaluated for cytotoxicity. The moderate activity of the natural product was found to be abolished completely when it was tested in MDA-MB-231 cell line. The observed IC_{50} values were not encouraging in other tested cell lines too. Thus, the SAR study of bacilotetrin C was carried out keeping the stereochemistry of C-3 intact. We then sought to evaluate the effect of modification of the fatty acid chain. Thus, compounds **18**, **19** and **20** (Figure 2b) were prepared where the undecane chain of bacilotetrin C was altered to shorter ethyl, pentyl and hydrophobic ethylated trifluoro benzyl moieties, respectively.

Figure 2: Structures of bacilotetrin C analogues with their corresponding IC_{50} in MDA-MB-231 cell line: (**a**) Epimer with inverted stereochemistry at C-3 (***epi*-3**) (**b**) Analogues bearing modified *β*-hydroxy chain (**18-20**). NC = Not converged



Next, compounds **21-22** (Figure 3a) were planned to understand the effect of ring size as well as the role of L-leucine and L-glutamic acid, respectively. Simultaneously, compound **23** (Figure 3b) was conceived to know the significance of the cyclic core of the natural product towards cytotoxicity. The cytotoxicity of compounds **21-23**, along with tertbutyl ester precursor of bacilotetrin C (**17**) was evaluated. Compound **21** without L-leu residue showed almost similar effects as the natural product whereas compound **22** devoid of glu-residue was found to be quite ineffective. Interestingly, compound **17** exhibited a significantly improved cytotoxic effect compared to the natural product whereas its linear counterpart **23** was found to have poor activity in most of the cases. The results revealed that the ring structure and L-glutamic acid were essential for the cytotoxicity, and the activity was enhanced further when L-glutamic acid of bacilotetrin C was derivatized to ester. The L-glutamic acid of bacilotetrin C played a significant role in exhibiting cytotoxicity towards different cancer cell lines which prompted us to evaluate the positional effect of this residue before accessing different variants. Therefore, compounds **24-26** (Figure 3c) were synthesized. Interestingly, the positional effect of L-glutamic acid in the cyclic core was found prominent. Compound **24** exhibited a much more promising efficacy than bacilotetrin C, whereas compounds **25-26** had slightly better cytotoxic effects than the parent molecule.

Figure 3: Structures of bacilotetrin C analogues with their corresponding IC_{50} in MDA-MB-231 cell line: (**a**) Role of amino acids in core structure (**21**, **22**), (**b**) Comparison of linear (**23**) and cyclic form (**17**), (**c**) Positional effect of glutamic acid residue depicting the key steps involved in the synthesis of analogues **24**, **25** & **26**.

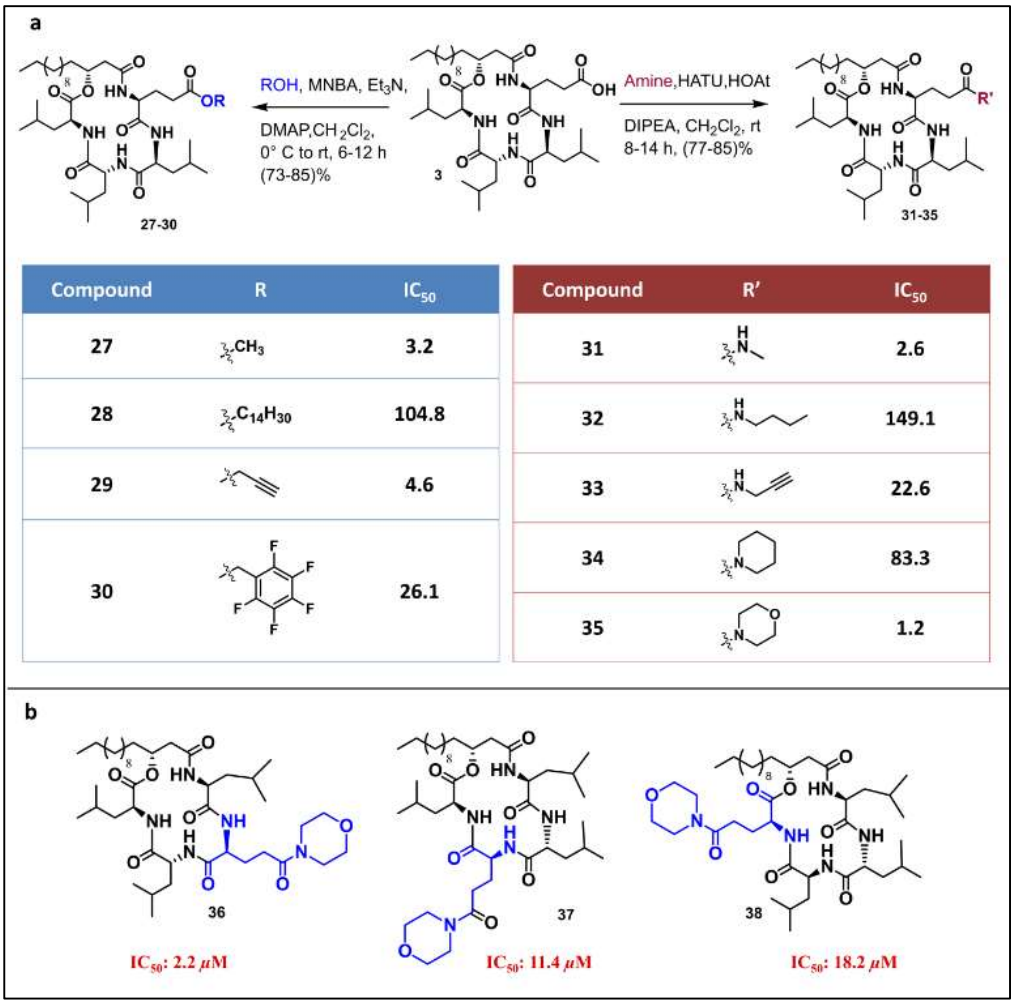


The interesting outcome of SAR studies inspired us to design new incarnations of bacilotetrin C via diversification of its architecture. *tert*-Butyl ester (**17**), the immediate precursor of bacilotetrin C, showed significant cytotoxicity compared to the natural product in some specific cancer cell lines which prompted us to investigate the effect of different ester and amide derivatives of L-glutamic acid counterpart of the molecule (Figure 4a). Therefore, esters of methyl (**27**), tetradecanyl (**28**), propargyl (**29**), pentafluoro benzyl (**30**) as well as amides of methyl (**31**), *n*-butyl (**32**), propargyl (**33**), piperidine (**34**) and morpholine (**35**) were taken into account. A variety of hydrophobic and hydrophilic counterparts were introduced to understand their role whereas the introduction of alkyne moiety was conceived as a click partner for probable conjugation in future course of study. The synthesis of esters was achieved from bacilotetrin C and the respective alcohols following the Shiina esterification condition whereas amides **31-35** were prepared from the parent natural product and the corresponding amines following peptide coupling conditions (Figure 4a). Interestingly, compounds **27** (methyl ester), **29** (propargyl ester) and **31** (methyl amide) exhibited better efficacy compared to the active analogue **17** whereas **35** (morpholine amide) was found to be the best with respect to all the other active analogues. The cytotoxic effects of tetradecane and fluorinated benzyl esters as well as *n*-butyl and piperidine amides were found to be less than the parent natural product.

The morpholine amide **35** was observed to possess a superior effect compared to the parent natural product which encouraged us to prepare the amide derivative of compounds **24-26**. Thus, morpholine amides **36-38** (Figure 4b) were synthesized. The cytotoxicity data revealed that amide **36** and its acid surrogate **24** had similar effects in all the tested cell lines whereas significant

improvement in activities of amides **37** and **38** was observed in certain cancer cell lines compared to their corresponding acids **25** and **26**, respectively.

Figure 4: Structures of bacilotetrin C analogues with their corresponding IC_{50} in MDA-MB-231 cell line: (**a**) Ester (**27-30**) and amide (**31-35**) derivatives of the natural product (**b**) Positional effect of morpholine amide of glutamic acid residue (**36-38**).



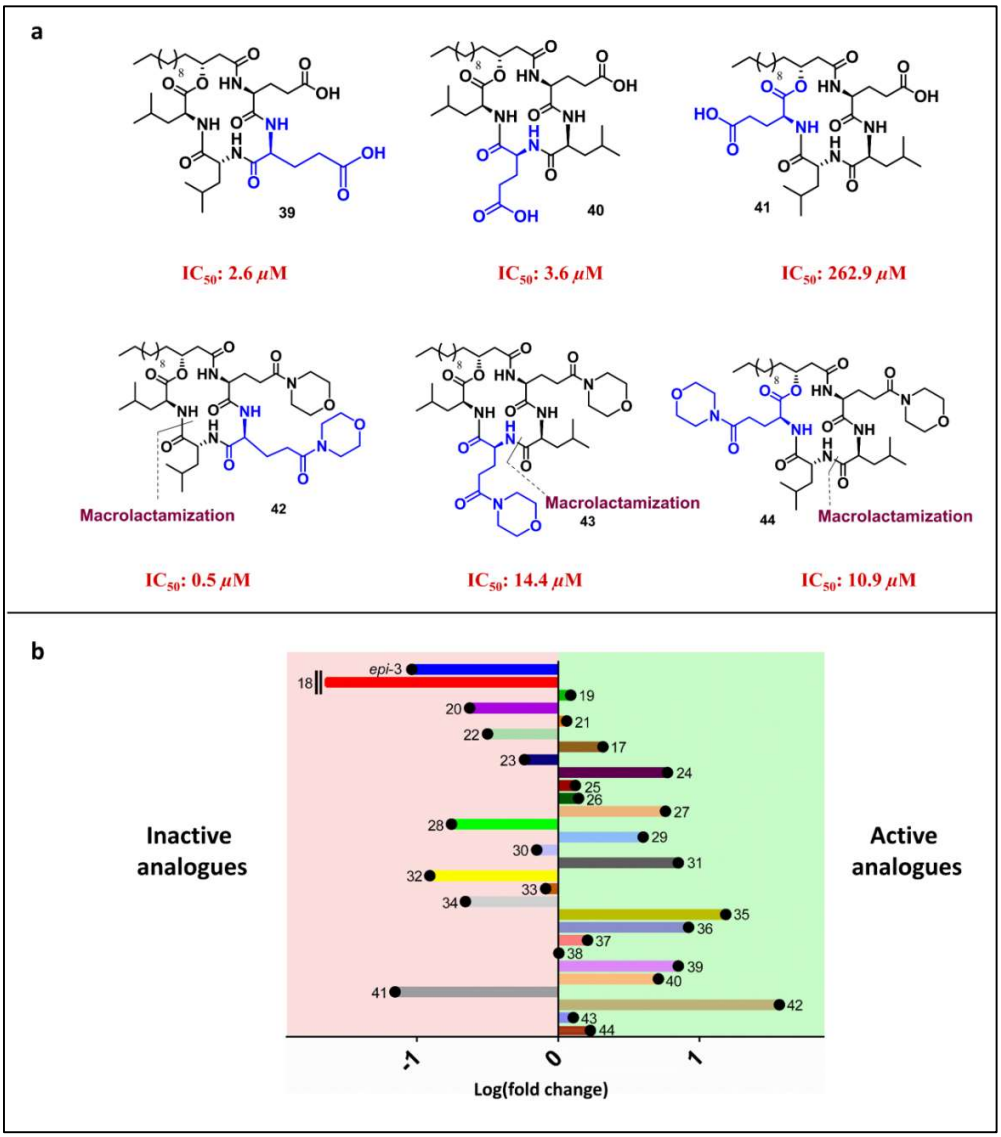
Compound	R	IC_{50}
27	CH_3	3.2
28	$C_{14}H_{30}$	104.8
29		4.6
30		26.1

Compound	R'	IC_{50}
31		2.6
32		149.1
33		22.6
34		83.3
35		1.2

Notably, the presence of L-glutamic acid in a cyclic scaffold was important to exhibit its cytotoxicity (**21** vs **22**) and the effect significantly varied with the position of L-glutamic acid (**24**) compared to the parent natural product. Thus, it was planned to introduce an additional L-glutamic acid sequentially in the place of leu residues of bacilotetrin C core to access compounds **39-41** (Figure 5a). Furthermore, their di-morpholine amides (**42-44**) were also designed as the incorporation of morpholine amide showed much improved effect compared to its acid counterpart in most of the cases (**35** vs **3**, **36** vs **24**, **37** vs **25**). Synthesis of these analogues was performed following our previously optimized route where the site of macrolactamization was chosen far from the bulky ^{t}Bu-protected glutamic acid residue.

Interestingly, the incorporation of glutamic acid residue in specific positions of the cyclic core (**39** & **40**) resulted in significant enhancement of cytotoxicity which was in good agreement with our hypothesis. Furthermore, compound **42**, the dimorpholine amide of **39** showed the best activity. However, compound **43**, the dimorpholine amide of **40** did not exhibit improved effect than its acid counterpart. Furthermore, compound **41** where the glutamic acid residues were far apart from each other did not show any encouraging cytotoxicity even though its di-morpholine amide (**44**) possessed much better efficacy.

Figure 5: (**a**) Structure of designed incarnations **39**, **40**, **41** and their corresponding dimorpholine amides **42**, **43**, **44** with their IC_{50} values in MDA-MB-231 cells. (**b**) Summary of potency of all analogues in MDA-MB-231 cells with respect to the natural product.



Summarizing, morpholine amide **35** showed the potent cytotoxic behavior, closely followed by the glutamic acid shifted isomer **24** and it was found that when these structural diversifications were combined (**42**), it turned out to be the most potent analogue having sub-micromolar activity in MDA-MB-231 cell line (Figure 5b). The morpholine amides **42** (most potent) and **35** (next to **42** in potency) along with parent bacilotetrin C (**3**) were evaluated for their mode of action in MDA-MB-231 cells.

After promising improvements in the cytotoxicity of the designed analogues, we became interested in investigating the mechanism of action of this family of compounds. Among the tested cell lines, MDA-MB-231, a triple negative breast cancer cell line, was selected for further study due to its highly invasive and resistant properties. The most active analogues (**35**, **42**) were incubated for a shorter time period with a chronic dose (IC_{50} x 2) in the preliminary experiments. Hoechst staining of the cells after compound incubation did not reveal fragmented nuclei in intact cells; morphologically, the cells showed shrunken and elongated shapes which is a sign of significant stress. Acridine Orange/EtBr staining for apoptosis[64] did not show EtBr permeabilized cells, in the number of acidic vesicles (orange staining) was observed (Figure 6). Mitochondrial membrane potential in the cells was also unaltered and associated markers of apoptosis, Bax, Bcl-2 and p53 showed a very random distribution without the characteristic responses (Bax/p53 upregulation, Bcl-2 downregulation). Hence, apoptosis was ruled out as the primary mode of cell death.

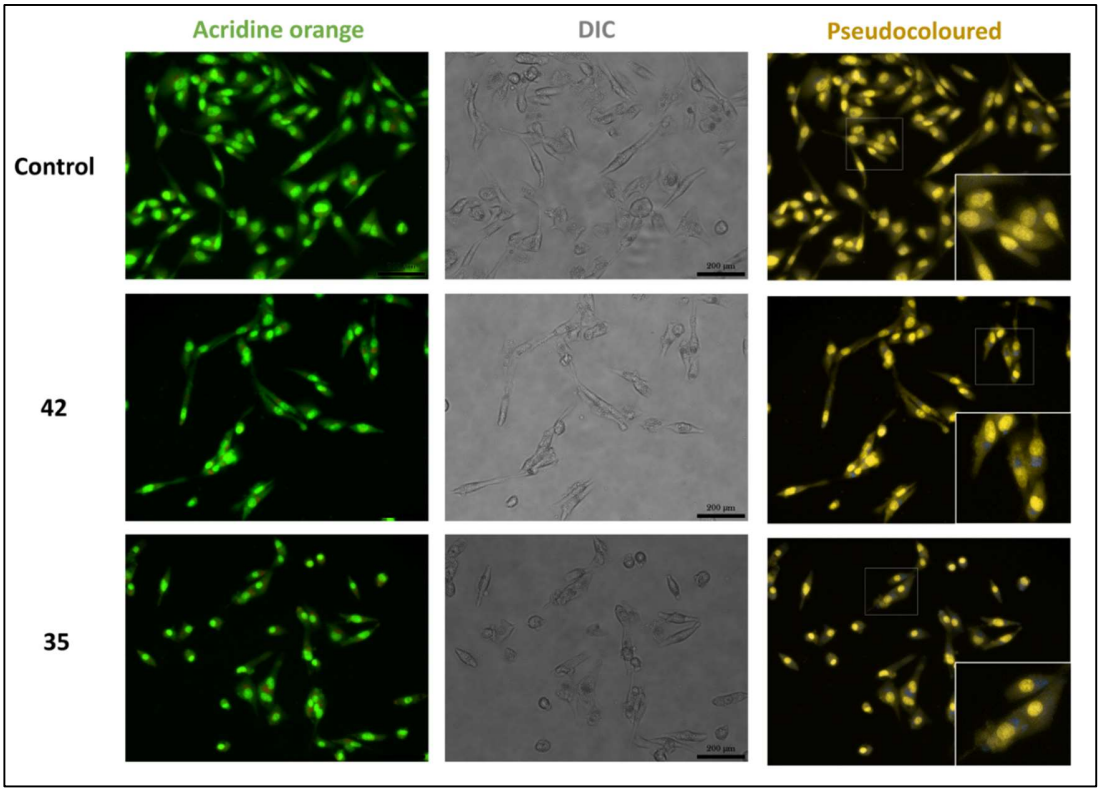


Figure 6. Initial studies for apoptotic activity: Acridine Orange staining of MDA-MB-231 cells after incubation with **35** (2.4 μM) and **42** (1 μM) for 24 h, scale bar 200 μm. Pseudo-colored images represent green as yellow and red as blue for better visualization.

Building on the initial observations with acridine orange, quinacrine, a standard fluorescent marker of acidic vesicles, was used to validate this result further. Treatment of compounds **35** and **42** resulted in bright puncta of quinacrine as opposed to the diffused fluorescence in the control cells, which indicated significant acidification of vesicles (Figures 7a & b). To determine whether this acidification promoted cell death, chloroquine was co-treated with **35** and **42** for 24 h and MTT assay was carried out. Chloroquine has been known to act as an endosomal buffering agent by preventing further acidification and maturation to lysosomes. Surprisingly, chloroquine significantly decreased the viability of **35**/**42** treated cells (Figure 7c).

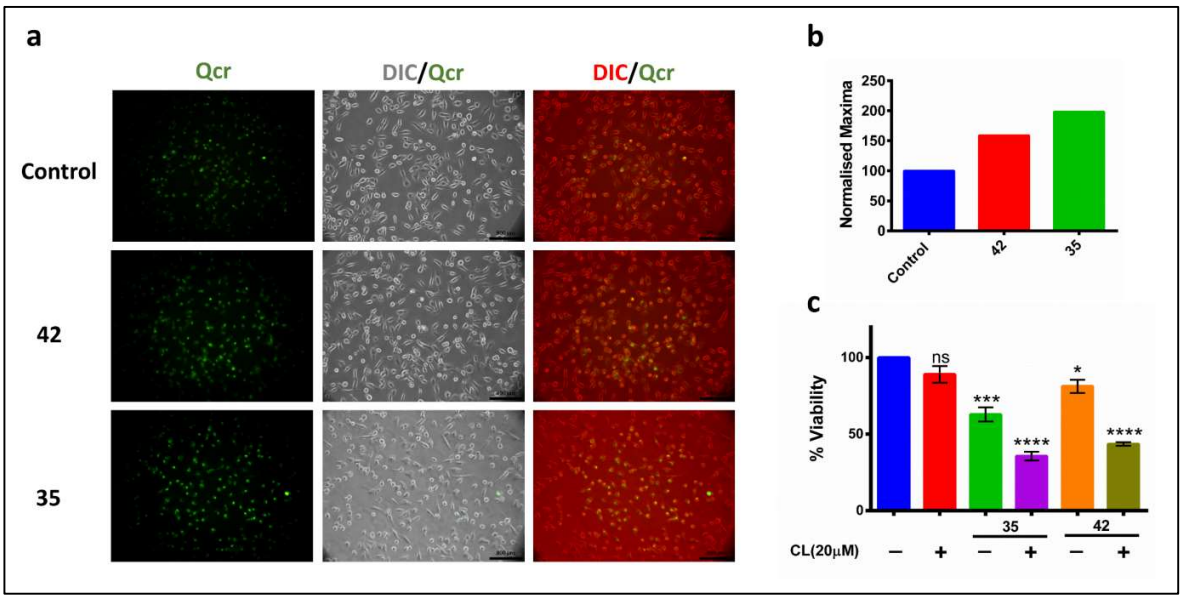


Figure 7. Studying the acidification of vesicles in MDA-MB-231: Cells were incubated with **35** (2.4 μM) and **42** (1 μM) for 24 h. (**a**) Fluorescence microscopic images of quinacrine staining. DIC channel in the merged image has been pseudo-colored to red for better visualization. (**b**) Normalized fluorescence maxima of quinacrine staining with respect to cell number. (**c**) MTT assay in presence of chloroquine (**CL**) after incubation with **35** (2.4 μM) and **42** (1 μM) for 24 h, n=3, mean ± SEM. Data was analysed using one way ANOVA (Dunnett's test) and values corresponding to $p<0.05$ were considered significant.

A previous report had attributed this type of chloroquine mediated enhancement of cell death in a compound showing autophagy; chloroquine blocked the induced autophagy flux and caused organelle stress leading to cell death. Hence, autophagy induction experiments were carried out. LC3-II and p62, which are commonly used markers of cellular autophagic induction, showed significant upregulation when incubated with **35**, **42** as well as **3** (Figure 8a). Since basal LC3-II levels may not represent the actual scenario due to continuous degradation, the autophagic flux was also observed on co-treatment with chloroquine as the more confirmatory study of compound activity. All three compounds showed enhanced autophagic flux with chloroquine with a much greater increase of LC3-II levels above the compounds/chloroquine alone (Figure 8b). Immunofluorescence of MDA-MB-231 cells after treatment with compounds also showed enhancement of cytosolic LC3-II puncta, confirming autophagy induction by this family of compounds (Figure 8c). However, autophagy induction is known to prevent cell death and is a

primary mechanism adopted by cancer cells to resist chemotherapeutics. More recently, it has been shown that Tat Beclin peptides lead to enhancement of autophagy and subsequent cell death, which is known as autosis. It was also shown that autosis induced cell death was partially rescued by 3-methyl adenine (**3-mA**), a PI3K (phosphoinositide 3-kinase) inhibitor, and it was also regulated by Na+/K+-ATPase.[34] Similar studies with **3-mA** and digoxin (**Dig**), an inhibitor of Na+/K+-ATPase, were carried out with **42**, the most potent analogue. It was found that cell death was partially rescued by both the inhibitors, suggesting the involvement of a similar pathway in case of **42** (Figure 8d). We also explored the selective action of the best analogues **35** and **42** by carrying out cell viability studies in HEK-293 (human embryonic kidney) cell line and compared it with MDA-MB-231. It was found that the activity was similar in both cell lines at low doses; though this combination of cell lines belongs to different organs, this result ruled out any exceptional toxicity against the normal cells which is usually the case with small molecule chemotherapeutics after the development of chemoresistance.

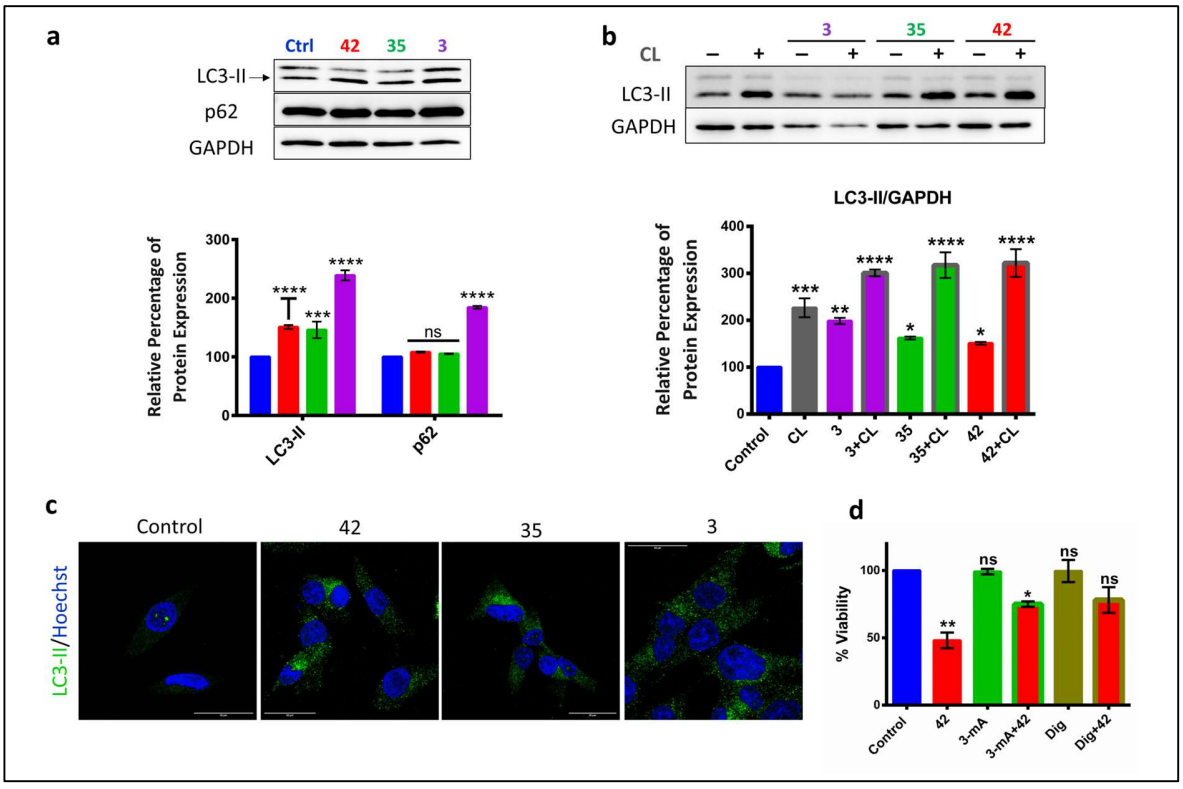


Figure 8. Autophagic induction in MDA-MB-231 cells; Cells were incubated with **3** (37 μM), **35** (2.4 μM) and **42** (1 μM) for 24h. (**a**) Western Blots of lipidated LC3 (LC3-II) and p62 after incubation with the compounds for 24 h, n=3, mean±SEM. (**b**) Western Blot of LC3-II for studying the autophagic flux in presence of chloroquine (**CL**, 20 μM), n=3, mean±SEM. (**c**) Confocal images of LC3-II localization in cells with nuclear staining agent Hoechst. Scale bar 30 μm. (**d**) MTT assay of **42** (1 μM) in combination with 3-methyl adenine (**3-mA**, 500 μM) and Digoxin (**Dig**, 500 nM) after 24 h of incubation, n=3, mean±SEM. Data was analysed using one way ANOVA (Dunnett's test) and values corresponding to $p<0.05$ were considered significant.

In summary, the first total synthesis of bacilotetrin C was achieved from commercially available dodecanal in six longest linear steps with an overall yield of 21.3%. The developed strategy culminated in total **twenty-nine synthetic analogues** of the parent molecule following structure activity relationship study. Tuning of lipophilicity, variation of ring structure as well as the change in sequence of amino acids were taken into consideration in course of designing of the analogues whose cytotoxic activities were evaluated against different human cancer cell lines. Notably, a number of analogues showed promising effect compared to the natural product and the di-morpholine amide **42** was found as the best active variant. The mode of induction of cell death by the potent analogues was studied in breast cancer cell line (MDA-MB-231) where the apoptotic hallmarks were missing with an increased acidification of endo-lysosomal compartments. Studies in combination with chloroquine revealed that aggravated autophagy induction led to the cell death and involvement of a possible autosis pathway was confirmed by using relevant inhibitors (**3-mA** & **Dig**). To the best of our knowledge, analogues **35** and **42** were discovered as much shorter and more potent compared to the other peptides known to induce autophagy. The presence of additional L-glutamic acid in the specific position of cyclic scaffold of bacilotetrin C was found to be crucial for activity which further was improved by transmuting to the corresponding morpholine amide. ***Remarkably, the study provided a solid platform for accessing novel molecules affecting autophagy which were seeded from the total synthesis of moderately active anticancer natural lipopeptide bacilotetrin C***.

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

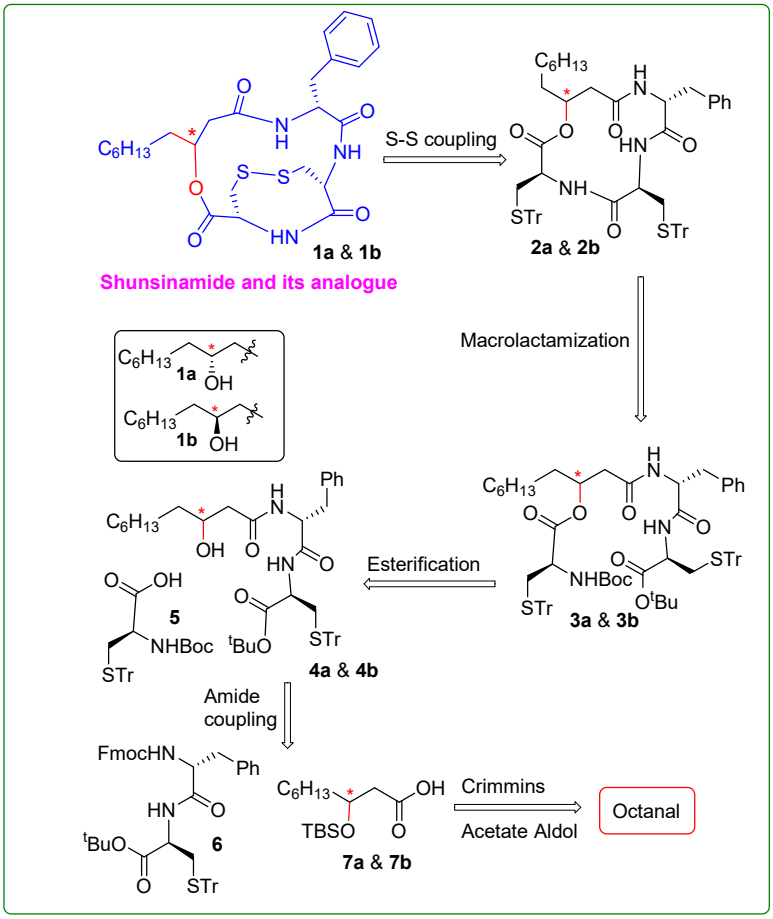
Org. Lett. 2020, 22, 1188-1192. An extension of this study resulted a potential outcome which is under consideration for publication as "***Decoding the Multifaceted Structure−Activity Relationship of Sunshinamide for Breast Cancer Eradication through Concurrent Modulation of Apoptotic and Ferroptotic Pathways via TrxR1 and Gpx4 Inhibition.***"

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.

Having the first chemical synthetic route of sunshinamide along with the initial cytotoxicity in hand, 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 moieties of the sunshinamide towards its anticancer effect.

Furthermore, in-depth biological activity demonstrated the effectiveness of sunshinamide in reducing tumor growth by inducing both ER stress-mediated apoptotic and non-apoptotic modes of cell death. The dual efficacy of sunshinamide in modulating both apoptotic and ferroptotic pathways positions it as a promising candidate for breast cancer therapy, addressing the challenge of chemoresistance associated with apoptosis evasion. This study sheds light on the multifaceted mechanisms underlying sunshinamide-induced cancer cell death and underscores its potential as a prominent contender in the ongoing battle against breast cancer. ***In overall, this study clearly indicates that shunshinamide and its analogues has very promising pharmaceutical relevance and left a huge scope for further development.*** (***this part of work is under consideration for publication and details biological data has not been provided***).

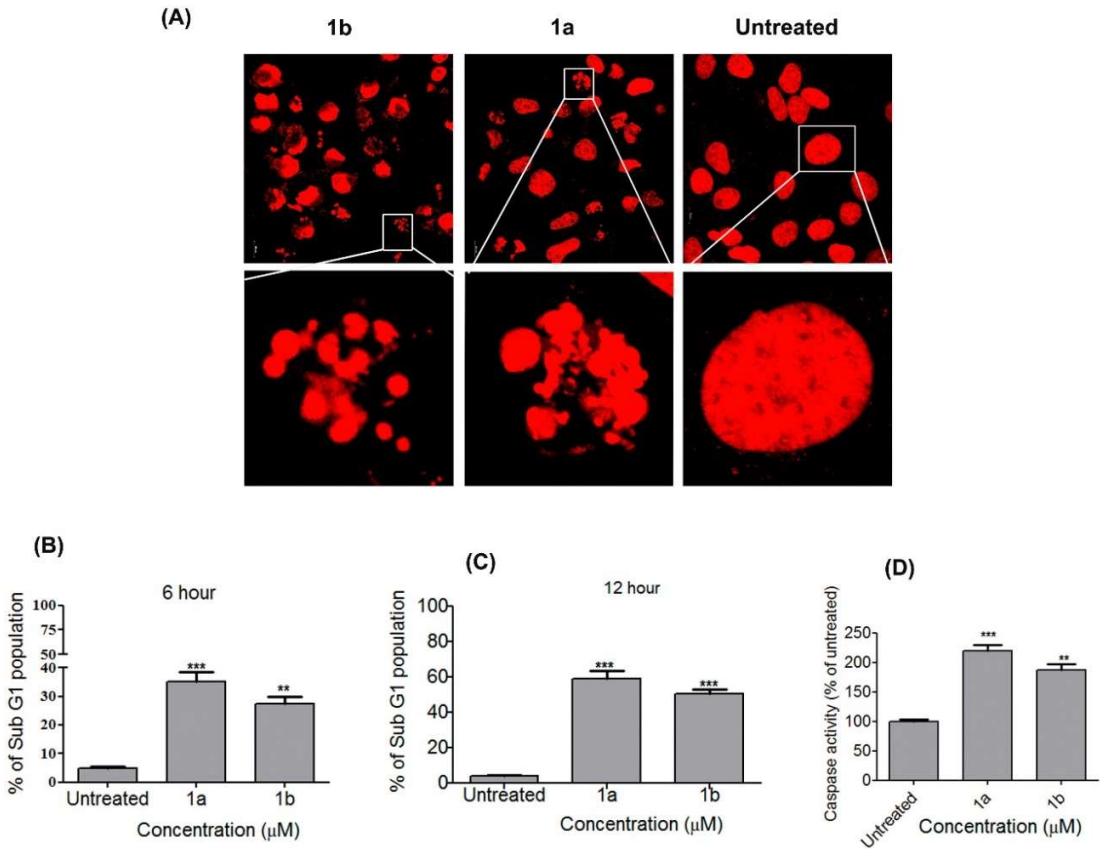


Figure 1: Induction of apoptosis by the synthesized compounds **1a** and **1b**

(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.

Figure 2: Different Synthesized Analogues of Shunshinamide for SAR.

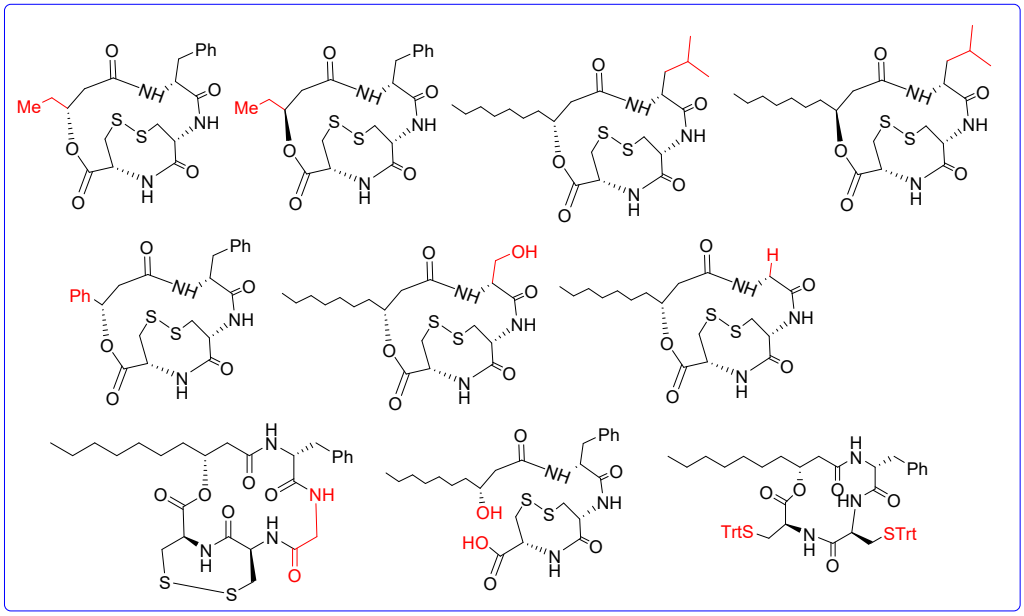


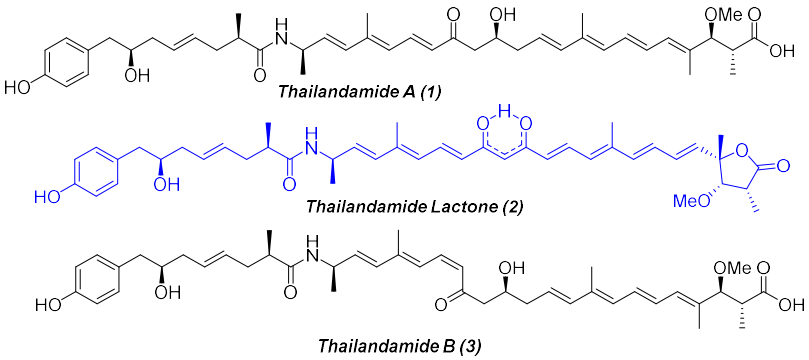
Table 2: Evaluation of Cytotoxic Activities of Different Analogues of Sunshinamide to Human Cancer and Noncancerous Cell Lines.

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

(iii) Thailandamide Lactone and its Antibacterial Potential: (***Chem. Sci., 2022, 13, 13403-13408***).

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.

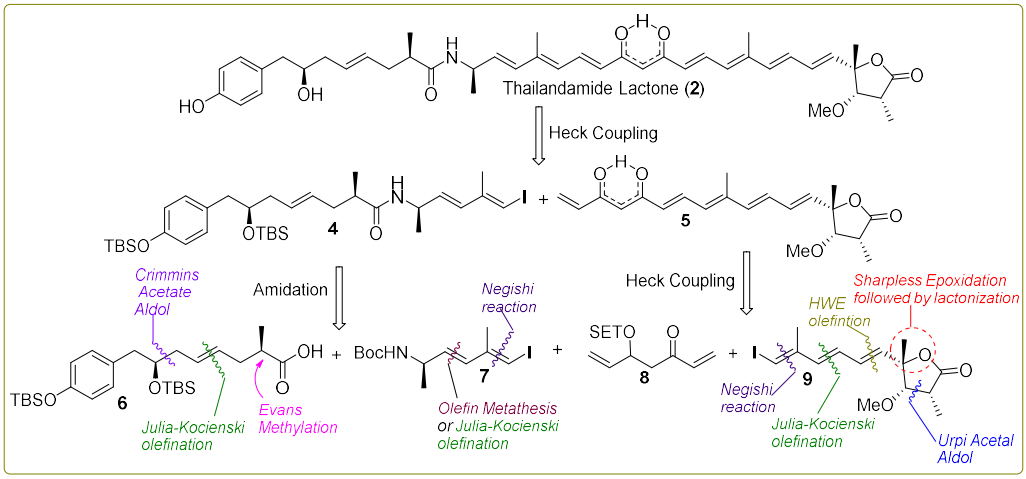
Figure 1: Chemical Structures of Thailandamide Family of Natural Products



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 *γ*-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.

Table 1: 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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