CHAPTER 2

GENERAL INTRODUCTION

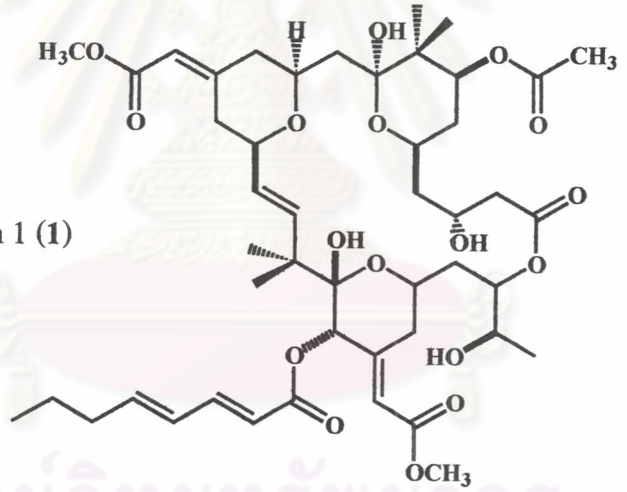
Secondary metabolite as a naturally produced substance does not play an explicit role in the internal economy of the organism that produces it.[3] It provides more specific and complex structure than primary metabolite which gives the low molecular weight compound, for example, carboxylic acid and amino acid. Furthermore, secondary metabolite is a continuous process from primary metabolite that is a photosynthesis of living organism. Natural products have been exploited by human for a long time for many purposes such as food, medicines, fragrances, pigments, pesticides, herbicides, fungicides and insecticides. In historical times, the secondary metabolites of terrestrial plants and animals have been studied since 1800s but the studies of natural products from marine sources were still limited. However, during the 1960s, the use of scuba diving technology have opened up areas of unexplored marine environments that enabled researchers to collect the marine organisms which led to the discovery of a broad spectrum of novel molecular structures. During the early investigations of the 1970s to today, the natural drug discovery from the world's oceans has already become valuable tools in biomedicine.[1-2]

2.1 Marine Natural Products

During the last 43 years, organic chemists have intensively studied the natural products in marine plants and animals, for example, sponges, coelenterates, alga, miscellaneous, echinoderms, tunicates, mollusc, bryozoans, microorganisms and phytoplankton. These organisms often provide novel chemical structures that have biological activities and use in pharmacology. Many of which are currently in clinical trials for the area of cancer treatments. Other compounds are being developed as an analgesic or inflammation.[4] (Table 2.1)

One of the bioactive compounds is bryostatin 1 (**1**) which is a macrocyclic lactone derived from the marine bryozoan *Bugula neritina* found in 1982.[5] Compound **1** was found to have activity against a variety of tumor cells both *in vitro* and *in vivo*. The important action of **1** works by down-regulating the expression of one or more protein kinase C (PKC) isoforms to achieve its cytotoxic effect.[6] Base on this activity, several phase I trials of bryostatin 1 in human have been completed and phase II trials are currently initiated. Ecteinascidin-743 (ET743, **2**), the most advanced compound from tunicate *Ecteinascidia turbinata*, is a cytotoxic tetrahydroisoquinoline alkaloid which binds to the minor groove of DNA. ET743 has shown a broad spectrum against human tumor cell lines *in vitro* and against human breast, melanoma and lung tumors *in vivo*. Compound **2** is especially effective against solid tumors such as sarcomas and breast cancer.[7-9]

Bryostatin 1 (**1**)



Ecteinascidin-743 (**2**)

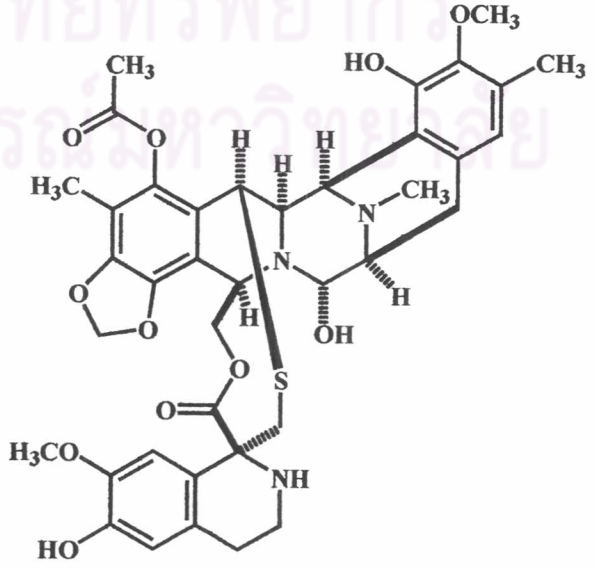


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Table 2.1 Selected Marine Natural Products Currently in Clinical Trials [10-11]

Compounds	Disease area	Phase of clinical trials
Dolastatin 10 (sea hare: *Dolabella auricularia*)	Cancer	II
LU103793[a] (sea hare: *Dolabella auricularia*)	Cancer	II
Bryostatin 1 (bryozoan: *Bugula neritina*)	Cancer	II
Ecteinascidin 743 (tunicate: *Ecteinascidia turbinata*)	Cancer	II/III
Halichondrin B (sponge: *Halichondria sp.*, etc.)	Cancer	preclinical
Squalamine lactate (shark: *Squalus acanthias*)	Cancer	II
Ziconotide (cone snail: *Conus magnus*)	Pain	III
Didemnin B (tunicate: *Tridemnum solidum*)	Cancer	II
Aplidine (tunicate: *Aplidium albicans*)	Cancer	I/II
KRN7000[b] (sponge: *Agelas mauritianus*)	Cancer	I
IPL 576,092[c] (sponge: *Petrosia contignata*)	Inflammation/asthma	I
Methopterosind (soft coral: *Pseudopterogorgia elisabethae*)	Inflammation/wound	I
Manoalide (sponge: *Luffariella variabilis*)	Inflammation/psoriasis	I
GTS-21[e] (marine worm: *Amphiporus lactifloreus*)	Alzheimer/schizophrenia	I

[a] Synthetic analogue of dolastatin 15; [b] Agelasphin analogue (α-galactosylceramide derivative); [c] Synthetic analogue of contignasterol (IZP-94,005); [d] Semisynthetic pseudopterosin derivative; [e] Also known as DMXBA, 3-(2, 4-dimethoxybenzylidene)-anabaseine

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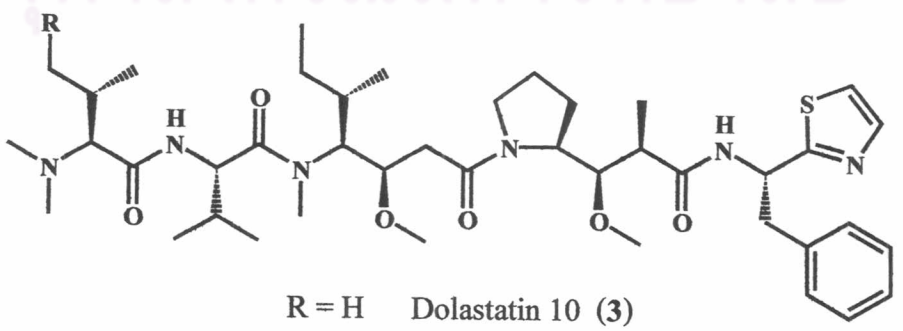
2.2 Natural Products Studied of the Marine Microorganisms

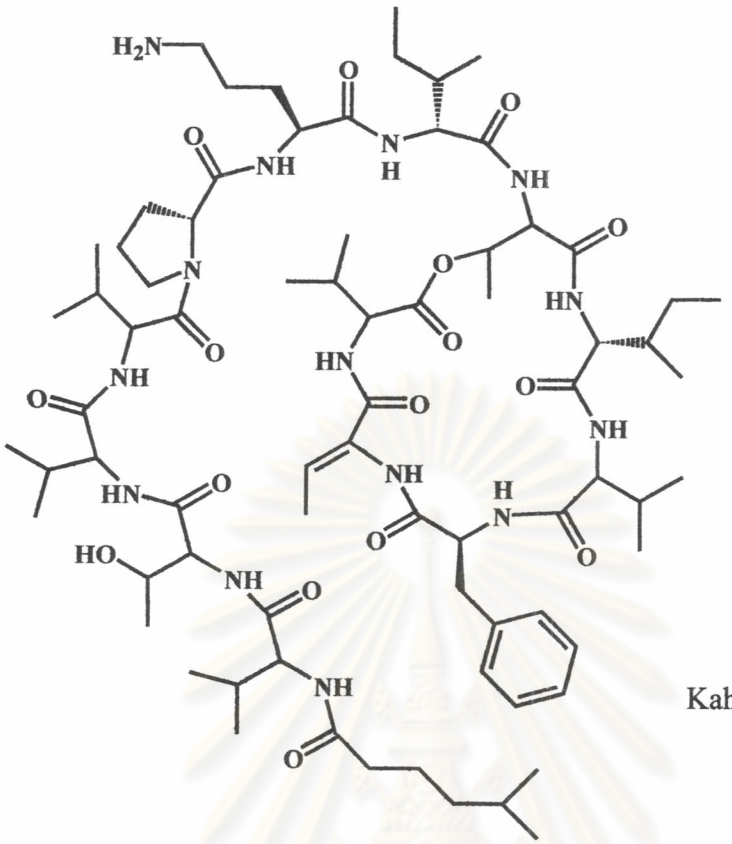
Most marine invertebrates particularly the filter feeders such as marine sponge are known to harbor microorganisms including bacteria, cyanobacteria and fungi within their tissues where they reside in the extra- and intra-cellular space. These microorganisms may constitute up to 40% of the biomass such as the Mediterranean sponges *Aplysina aerophoba*.[4] Some of microorganisms such as mat-forming cyanobacteria are also serve as general food sources for some marine invertebrates such as sea hare *Dollabella* sp. and *Stylocheilus* sp.

Several well-known marine chemicals were first discovered from marine invertebrate and later found that the true producers are the marine microorganisms. One of these is dolastatin 10 (**3**) which is a potent antitumor agent and is currently in the current phase II clinical trials. Compound **3** was originally isolated in a minute amount (28.7 mg each from 1000 kg wet) from the sea hare *Dolabella auricularia* in 1987.[12] Interstingly, a research group at the University of Hawaii discovered the structural analogue of **3**, symplostatin 1 (**4**) from the marine cyanobacterium *Symploca hydnoides*.[13] Later, dolastatin 10 (**3**) was also detected in a *Symploca* sp. These finding clearly indicated that dolastatin 10 found in *D. auricularia* is likely to be dietary-origin.[14] Similarly, kahalalide F (**5**), a cyclic depsipeptide which exhibits biological activity against AIDS OI pathogens *in vitro*[15] was first found from a sacoglossan mollusk, *Elysia rufescens*. Subsequent studies of the green alga *Bryopsis* sp. which *E. rufescens* feeds on was shown to elaborates kahalalides F and its acyclic analog, kahalalide G (**6**).[16]

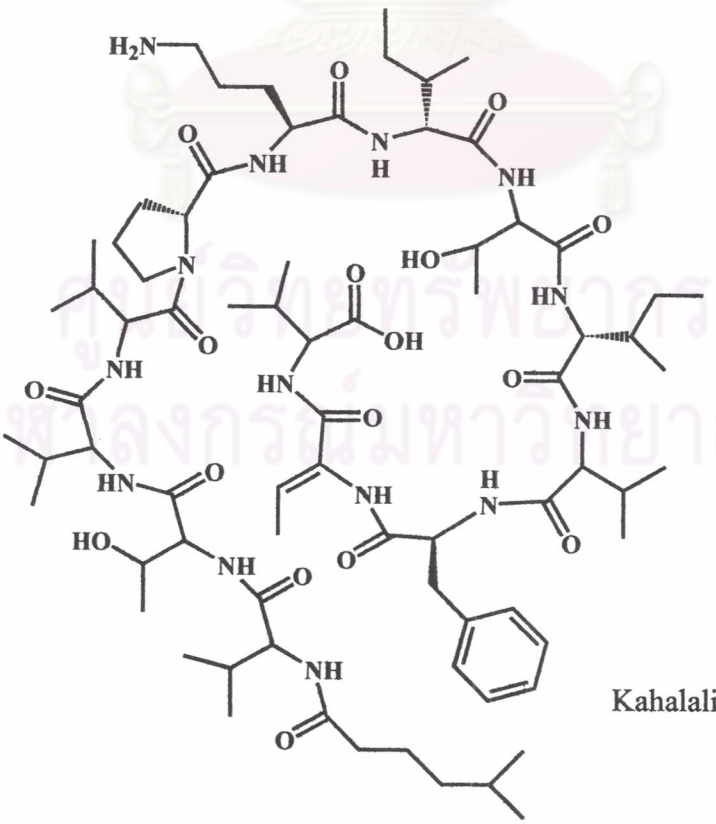


R = H Dolastatin 10 (**3**)

R = CH_3 Symplostatin 1 (**4**)



Kahalalides F (**5**)



Kahalalides G (**6**)

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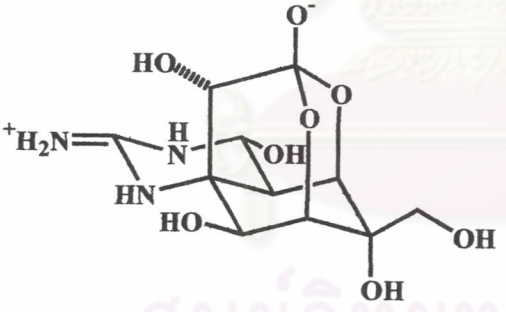
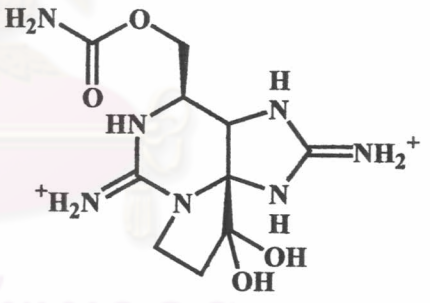
A neurotoxic alkaloid, tetrodotoxin (**7**), was first found in pufferfish of the family Tetraodontidae. Human who receive this poison may be completely paralyzed and in some cases completely lucid until shortly before death, depending on the amount of toxin consumed. Later studies have shown that many species of marine bacteria are the true producer of compound **7**. The wide distribution of tetrodotoxin in marine bacteria was underlined in a study by Simidu et al.[17], who identified the toxin in marine bacteria of the genera *Vibrio*, *Aeromonas*, and *Alteromonas*. Saxitoxin (**8**) and structurally related toxins, the causes of paralytic shellfish poison (PSP), were presented in several species of dinoflagellates[18] which studies have shown that they may acquire saxitoxin from bacteria rather than synthesize the toxins by themselves. PSP is a serious illness caused by eating shellfish contaminated with microorganism that contains a toxin harmful to human. Both of tetrodotoxin (**7**) and saxitoxin (**8**) are the specific sodium channel blockers and are the important compounds for the ion channel studies.[19]



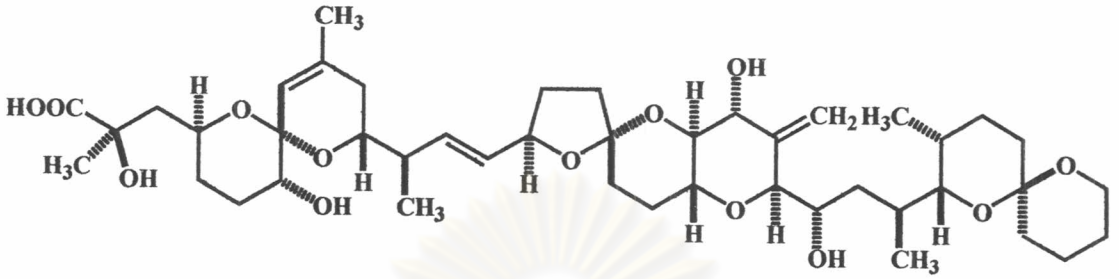
Tetrodotoxin (**7**)



Saxitoxin (**8**)

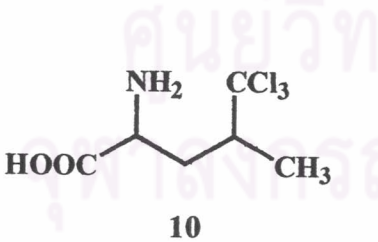
Okadaic acid (**9**), a polyether derivative of C38 fatty acid, is a very potent inhibitor of phosphatases-1 (PP1) and –2A (PP2A) that also stimulates the phosphorylation of many proteins. This compound has become a valuable powerful probe for studying regulatory phenomena and signal transduction pathways in eukaryotic cells.[20] Compound **9** was first discovered from the extracts of marine sponge *Halichondria okadai* and *H. melanodocia*.[21] Later, compound **9** was also

isolated from the marine dinoflagellate *Prorocentrum lima*[22] indicating that *P. lima* is likely to be the true producer of **9** since this sponge are known to harbor dinoflagellates in their extracellular space.



Okadaic acid (**9**)

Futhermore, recent evidence have shown that the bryozoan *B. neritina* which is the well-known source of macrocyclic lactones bryostatins could be of the bacterial origin.[23] As a last example, the marine sponge *Dysidea* sp. are known to harbor cyanobacterium *Oscillaoria* sp. and also the source of many unique trichloromethyl group-containing metabolites such as compounds **10**, dysidin (**11**), and isodysidin (**12**).[24-25] Many researchers had long suspected that these metabolites are biosynthesized by *Oscillaoria* sp. In 1995, Gerwick and coworkers isolated barbamide (**13**),[26] a peptide containg a trichloromethyl group from the marine cyanobacterium *Lyngbya majuscula*. This discovery clarifies the origin of this series of compounds with such functional group from the marine sponge *Dysidea* sp..



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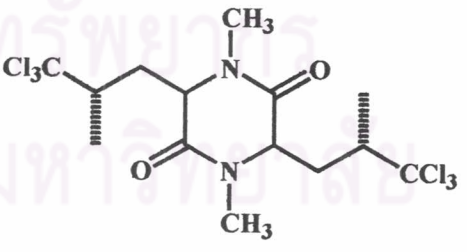

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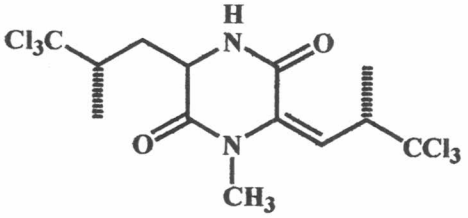

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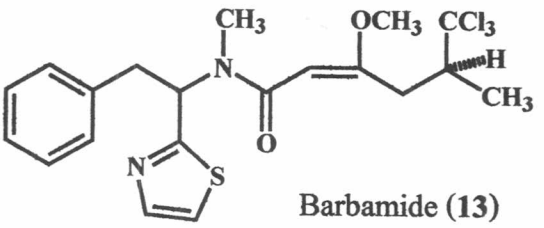
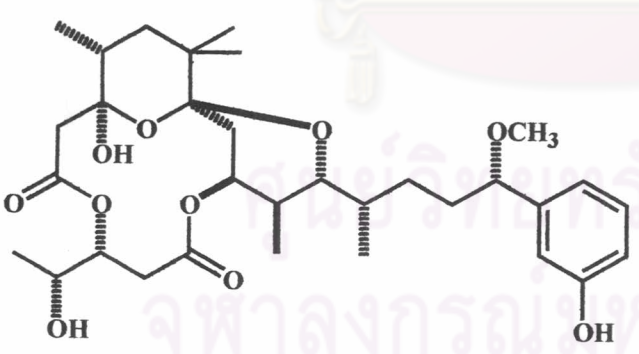


Barbamide (**13**)

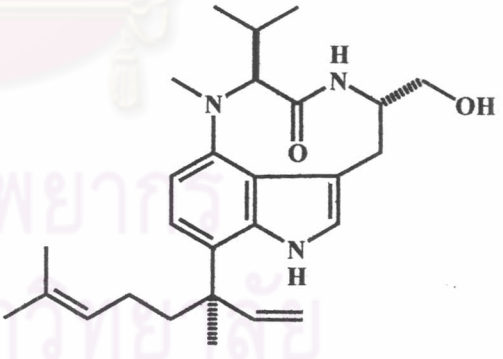
Marine microorganisms are a treasure source of useful natural products. The marine microalga are the best known for their production of many toxins. Especially the cyanobacteria, the group of these microalga, have been the richest in this regard. Because chemical variation is common in microalgae, the focus of this thesis aims to search the bioactive compounds from cyanobacteria, *Lyngbya majuscula*.

2.3 Natural Products from the Marine Cyanobacterium *Lyngbya majuscula*

The marine cyanobacterium (blue-green algae) *Lyngbya majuscula* has proven to be a rich source of chemically diverse classes of bioactive secondary metabolites. Ecologically, when bloomed, *L. majuscula* can smother seagrass that is a vital food source for dugongs and turtles. Some strains of *L. majuscula* cause many health problems to people who come in contact with it such as dermatitis, eye irritation, asthma and gastrointestinal inflammation. For example, two toxins that have been found to be the major cause of dermatitis in ocean swimmers are debromoaplysiatoxin (**14**) and lyngbyatoxin A (**15**). Both compounds **14** and **15** toxins are tumor promoters by binding to phorbol esters receptors leading to the activation of protein kinase C.[27-29]



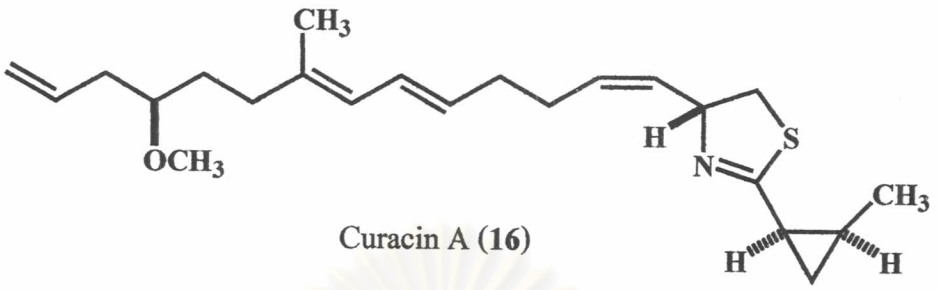
Debromoaplysiatoxin (**14**)



Lyngbyatoxin A (**15**)

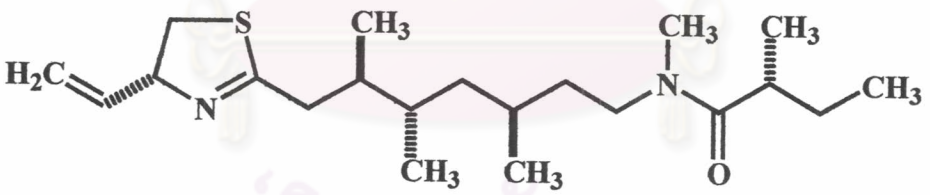
Other than toxins, the marine cyanobacterium *L. majuscula* is also known for its ability to produce a great diversity of biological active and unique secondary metabolites. Curacin A (**16**) is a metabolite, which was cytotoxic to brine shrimp (LC_{50} = 0.0025 μg/ml) and several cell-based assays, was obtained from a collection

of *L. majuscula* from Curacao.[30-32] Compound **16** was synthesized and observed to be a novel tubulin binding agent.[33]

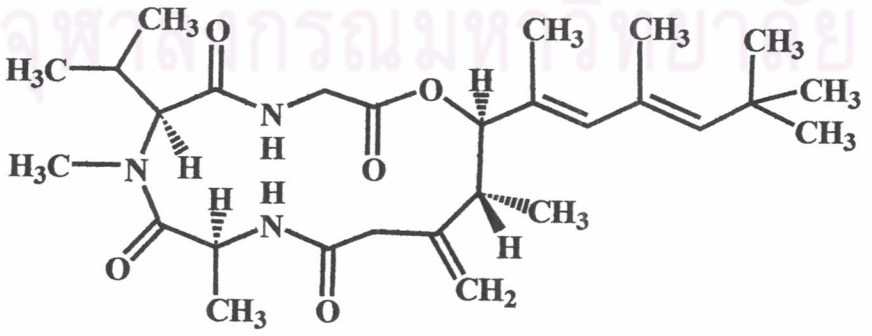


Curacin A (**16**)

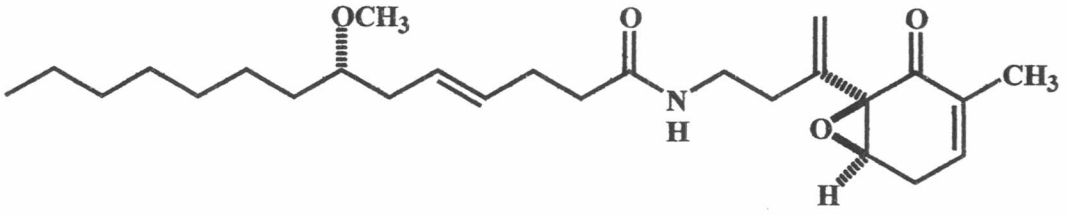
The bioactive compounds are potently the ichthyotoxic effect include kalkitoxin (**17**) (LC_{50} = 700 nM)[34] and antillatoxin (**18**) (LC_{50} = 0.05 μg/ml).[35] Furthermore, the Caribbean cyanobacterium *L. majuscula* provide the mildly ichthyotoxic compound, malyngamide H (**19**) (LC_{50} = 5 μg/ml).[36] Kalkitoxin (**17**) is an exquisitely toxic compound to fish, brine shrimp, sea urchin egg and a primary cell culture of rat neurons. Moreover, pharmacological investigations showed antillatoxin (**18**) to be highly toxic to a primary culture of rat cerebellar granule neurons (LC_{50} = 20.1 nM).[37]



Kalkitoxin (**17**)

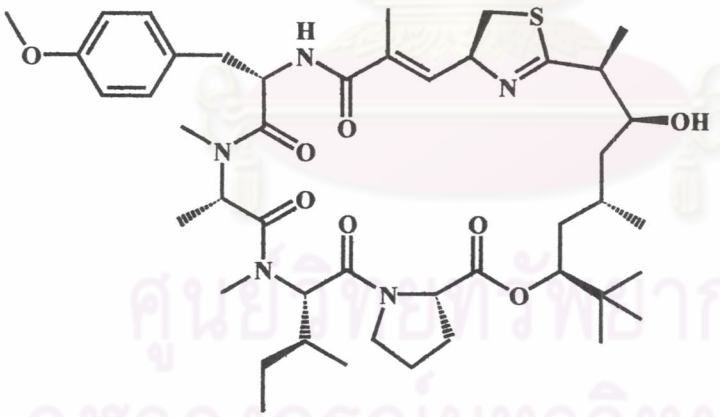


Antillatoxin (**18**)

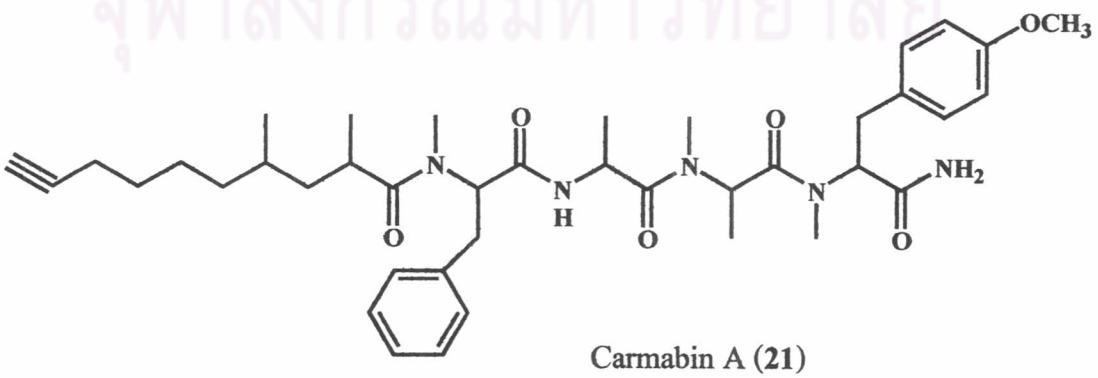


Malyngamide H (**19**)

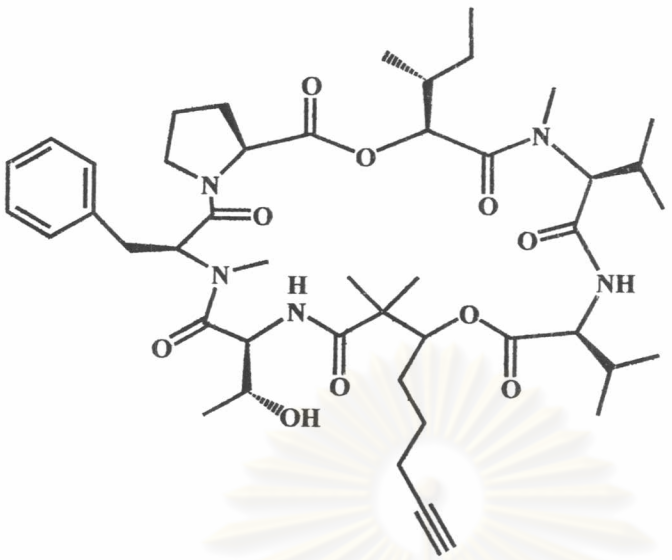
L. majuscula have proven to be an exceptional source of novel potential pharmaceuticals such as antillatoxin (**18**),[35] apratoxin A (**20**),[38] and kalkitoxin (**17**).[34] Interestingly, secondary metabolites isolated from marine cyanobacteria are predominantly lipopeptides.[39] Carmabin A (**21**),[40] georgamide (**22**),[41] pitipeptolide A (**23**),[42] and yanucamides (**24-25**)[43] represent a new group of lipopeptides from marine cyanobacteria. Each of these natural products possesses the lipid residue with terminal acetylene, which have shown to be biosynthetic signatures of cyanobacterial metabolites.



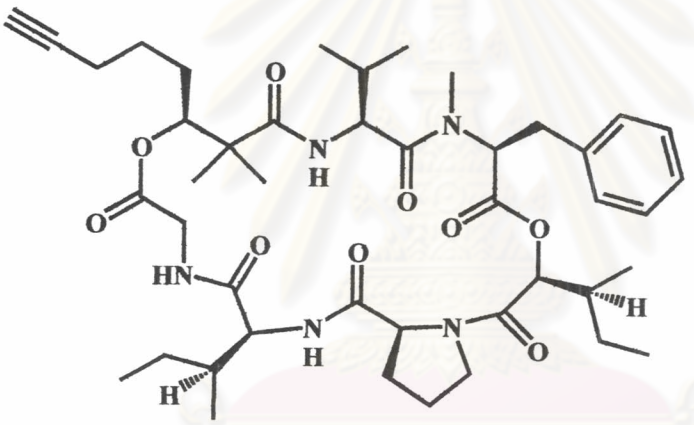
Apratoxin A (**20**)



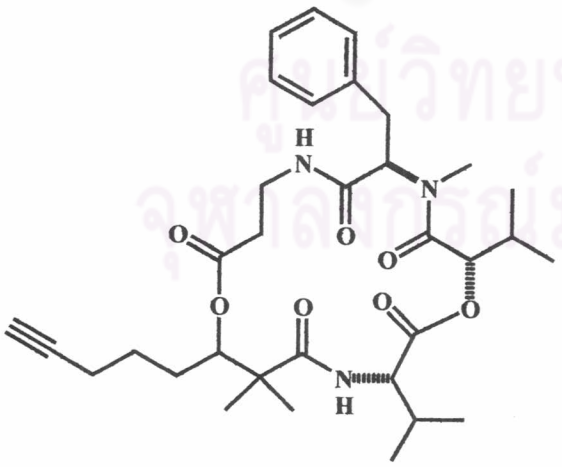
Carmabin A (**21**)



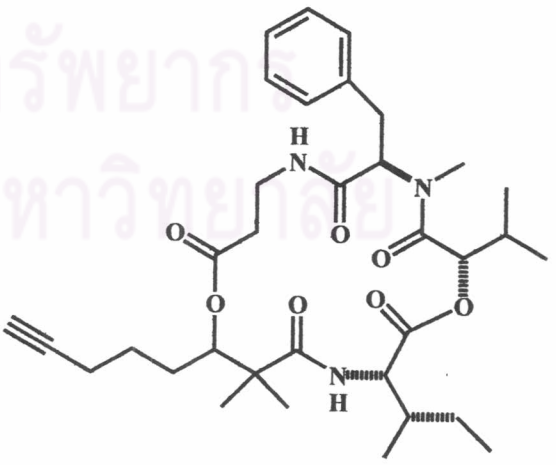
Georgamide (**22**)



Pitipeptolide A (**23**)



Yanucamides A (**24**)



Yanucamides B (**25**)

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