CHAPTER 1

INTRODUCTION

1.1 General introduction

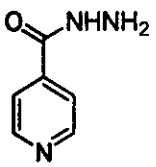
Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is currently considered among the most dangerous infectious diseases world-wide, and is one of the major AIDS-associated infections (Inderlied, 1999). According to the alarming data furnished by the World Health Organization (WHO), one-third of the world population is infected with *M. tuberculosis*, and there are approximately eight million new cases and more than two million deaths reported each year. In particular, three of the four highest-burden countries are in Southeast Asia. Besides that, Thailand is ranked among the top 22 TB-high burden countries, with 88,000 new cases in the year 2000 (Dye *et al.*, 1999; WHO, 2002).

Despite the availability of a vaccine (BCG) and effective chemotherapeutic agents against TB since 50 years ago, TB was ironically declared a global emergency in 1993 (Crofton, 1997). The prime factors contributing to such declaration are due to a high prevalence of TB in patients who have AIDS and to multi-drug resistant strains of mycobacteria, thus causing the number of patients infected with TB to increase world-wide (Glassroth, 2001).

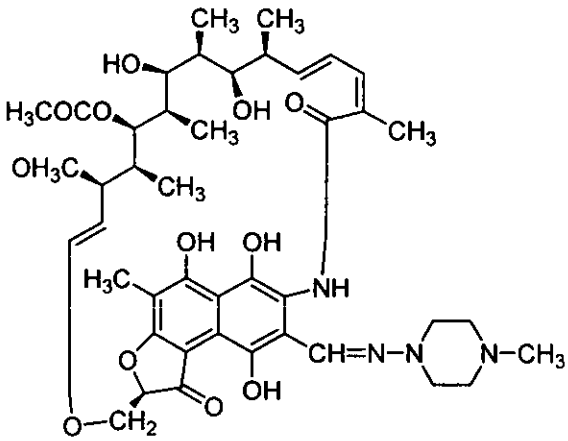
HIV infection has increased the incidence of TB by causing immunosuppression, which enables latent infection to clinically progress (Glassroth, 2001). There are approximately 10.7 million people having TB/HIV coinfection (0.18% of the world population), and 640,000 cases were associated with HIV infection (Dye *et al.*, 1999). Unlike other diseases associated with AIDS, the severe uniqueness of TB is that it can be spread by airborne transmission to adults and children who are not at risk of AIDS (Haas and Des Prez, 1995).

Resistance to the current antituberculosis drugs is another threatening problem. First-line drugs currently used in the treatment of TB include isoniazid, rifampin, pyrazinamide, ethambutol and streptomycin (Figure 1) (Sensi and Grassi, 1996). Short-course regimens using initially at least three first-line drugs are effective, and combination therapy has been well documented to reduce the emergence of *M. tuberculosis* strains that are resistant to individual agents. The major problems faced in TB control are poverty, thus leading to the lack of diagnosis and short in drug supply, and patients' failure to complete their course of drugs. As a result, multi-drug resistant (MDR) strains of *M. tuberculosis*, defined as strains with the resistance to at least isoniazid and rifampin, have been emerged (Duncan, 1997; Inderlied, 1999). There were approximately 3.2% of newly estimated TB cases world-wide that were MDR-TB in 2000 (Espinal, 2003). Second-line drugs, including ethionamide, cycloserine, kanamycin, capreomycin, amikacin, para-aminosalicylic acid and thiacetazone, which are less efficacious

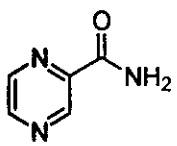
and/or more toxic than first-line ones, are obligated in such cases (Glassroth, 2001).



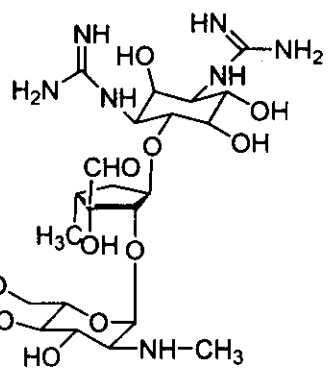
isoniazid



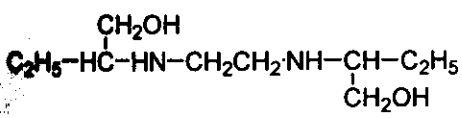
rifampin



pyrazinamide



streptomycin



ethambutol

Figure 1 First-line drugs for tuberculosis

In spite of the advance in computer-assisted drug design, molecular biology and gene therapy, there is still a pressing need for new drugs to counteract with multi-drug resistant tuberculosis. However, for over 30 years no antituberculosis agents with new mechanism of action have been developed. There have been a

number of practical obstacles in developing new antituberculosis agents. Among these is the lack of economic incentive due to the predominance of disease in the developing world. The very slow growth and highly contagious nature of *M. tuberculosis* have also discouraged the drug discovery effort (Cantrell, Franzblau and Fischer, 2001). Yet new drug discovery with new and different mode of actions is among urgent needs to control the spread of drug resistant strains as well as to lower the mortality rate of MDR-TB.

Nature is one attractive source of new therapeutic candidates as the tremendous chemical diversity is found in millions of species of both marine and terrestrial plants, animals and microorganisms. Despite major scientific and technological progresses in combinatorial chemistry, drugs derived from natural products, however, still make an enormous contribution to drug discovery today. Of the new approved drugs reported between 1983 and 1994, for examples, drugs of natural origins predominate (78%) in the area of antibacterials, whereas 61% of anticancer drugs are naturally-derived or are modeled on natural product parents (Cragg, Newman and Snader, 1996).

The oceans, covering more than 70% of the earth's surface, have been long known as the ecological habitat with a highly unique and wide-ranged biodiversity. Such uniqueness that earns marine biota the excellence candidacy as the producers of novel biologically active agents include the physical and chemical differences between the marine and terrestrial environments. Among these differences are the great density of the sea water, the reduced light permeation thus allowing

photosynthesis only in a narrow surface zone, and the skeleton of the biosynthetically starting materials, which are protein-dominated (as compared to the carbohydrate dominance in terrestrial plants). Besides these properties, the food chain in the marine environment is also far more complex than that in the terrestrial counterpart. These properties result in the abundance of filter-feeding sessile organisms, which serve as excellent substrata for epibionts and symbionts, therefore becoming the communities that are either absent or rare in terrestrial ecosystems (Scheuer, 1990). Furthermore, ecological stresses, including predation, competition for space, and fouling of the surface, lead to the evolution of unique secondary metabolites with various biological activities (Konig *et al.*, 1994). Altogether, these have proved to be beneficial to the discovery of drugs with greater efficacy and specificity for the treatment of several diseases than those currently used in clinic.

Since the first reports in 1951, marine plants, animals and microbes have already yielded more than 12,000 novel chemicals, with hundreds of new compounds still being discovered every year (Donia and Hamann, 2003). The isolation of two new unusual arabinonucleosides, spongothymidine and spongouridine from the sponge *Cryptotethia crypta* by Bergmann in 1950's led to the development of several nucleoside analogues, including ara-C as anticancer agent, and acyclovir as antiviral drug for *Herpes simplex* virus infections (Munro *et al.*, 1994). Currently, ara-C and acyclovir are the only marine-related compounds in clinical use. However, many marine natural products and their derivatives have successfully advanced to the stages of clinical trials, especially in the area of

chemotherapy (Table 1) (Munro *et al.*, 1999; Haefner, 2003). Additionally, the reviews by Mayer and Hamann (2002) reported a growing number of candidates that have been selected as promising leads for extended preclinical assessment.

Whereas most of natural products in clinical trials are aimed toward anticancer chemotherapy, the emerging drug resistance encountered in the infectious diseases also contributes to the interest in assessing marine natural products. There are many marine natural products that have been described for their potent antiinfective activities and show their potential toward clinically useful treatments (Mayer and Hamann, 2002; Donia and Hamann, 2003).

Among the marine organisms, sponges were the first marine invertebrate group that have been studied in search for new compounds (Bergquist, 1978). To date, sponges have yielded a great number of novel bioactive compounds. (Faulkner, 1995). The sponges, belonging to the phylum Porifera, are the most primitive group of multicellular animals existing as far back as Precambriam periods or approximately 600-700 million years ago (Allen, 1996). They are sedentary and feed on their food by filtering the microplanktons from sea water passing through the small holes on their bodies (Bergquist, 1978). To survive for such a long period of time, the sponges have had to fight off even more sophisticated predators and to compete for space by producing distasteful or otherwise deterrent chemicals. Interestingly, these chemicals are intrinsically bioactive and are therefore the compounds that researchers seek today as potential-

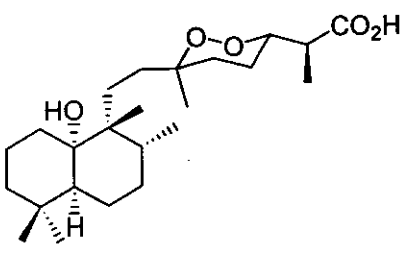
Table 1 Marine natural products and derivatives in clinical development

Compound	Source	Chemical class	Disease area	Status
Compounds targeting ion channels				
Ziconotide	Cone snail	Peptide	Chronic pain	Phase III
AM336	Cone snail	Peptide	Chronic pain	Phase I/II
GTS21	Nemertine worm	Anabaseine-derivative	Alzheimer's disease Schizophrenia	Phase I/II
Compounds targeting enzymes				
Methionine aminopeptidase inhibitors				
LAF389	Sponge	Amino acid derivative	Cancer	Phase I
Protein kinase inhibitors				
Bryostatin-1	Bryozoan	Polyketide	Cancer	Phase II
PLA_2 inhibitors				
OAS1000	Soft coral	Diterpene-pentoseglycoside	Wound healing Inflammation	Phase I/II
Microtubule-interfering agents				
Dolastatin-10	Sea slug	Peptide	Cancer	Phase II
ILX651	Sea slug	Peptide	Cancer	Phase I
Cemadotin	Sea slug	Peptide	Cancer	Phase II
Discodermolide	Sponge	Polyketide	Cancer	Phase I
HTI286	Sponge	Tripeptide	Cancer	Phase I
DNA-interactive agents				
Yondelis™	Sea squirt	Isoquinolone	Cancer	Phase II/III
Oxidative stress inducers				
Aplidin™	Sea squirt	Cyclic depsipeptide	Cancer	Phase II
Lysosomotropic compounds				
Kahalalide F	Sea slug/alga	Cyclic depsipeptide	Cancer	Phase I
Immunostimulatory agents				
KRN7000	Sponge	β-galactosylceramide	Cancer	Phase I
Calcium-binding protein antagonists				
Squalamine lactate	Shark	Aminosteroid	Cancer	Phase II
Compounds with unknown mechanism of action				
IPL512602	Sponge	Steroid	Inflammation Asthma	Phase II

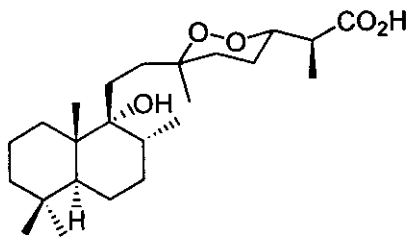
note; produced after Haefner (2003).

medicines (Faulkner, 1995). Furthermore, the filtration of sea water makes sponges a great reservoirs of the metabolites from marine microorganisms. Besides, the colonies of sponges also serve as symbiotic systems, in which large number of epibionts and symbionts such as bacteria and other microorganisms reside in a unique association. Consequently, unusual metabolites that were produced by the microorganisms can be found in sponges (Konig and Wright, 1996). It is thus not surprising that many marine natural products from sponges are highly active in many pharmacological assays.

Although Thailand's territorial waters, covering approximately more than 400,000 km^2, are one of the world's greatest biological diversified marine habitats (Allen, 1996), the researches in marine natural products are yet rather new to the Thai researchers. To date, there have been only a handful studies about the bioactive compounds from Thai marine organisms. For instances, Tanaka *et al.* (1993) reported the isolation of two new norsesterterpene peroxides, mycaperoxides A **(1)** and B **(2)**, from the Thai sponge *Mycale* sp. collected from Sichang Island, Chonburi. Both compounds exhibited cytotoxicity against P-388, A-549 and HT-29 tumor cell lines (IC_{50} 0.5-1 μg/mL).

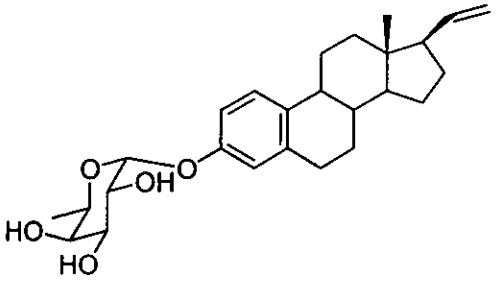


1

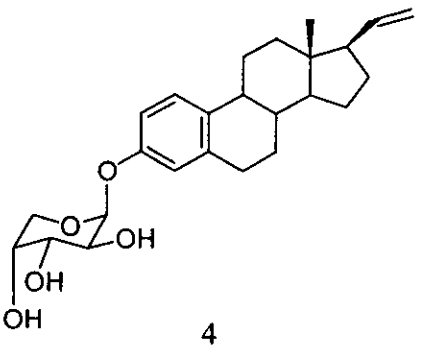


2

Kittakoop *et al.* (1999) reported the isolation of two new norpregnane glycosides, 19-norpregna-1,3,5(10),20-tetraen-3-*O*-α-fucopyranoside **(3)** and 19-norpregna-1,3,5(10),20-tetraen-3-*O*-β-arabinopyranoside **(4)**, from the Thai soft coral *Scleronephthya pallida* collected from Phuket. 19-Norpregna-1,3,5(10),20-tetraen-3-*O*-α-fucopyranoside exhibited moderate antimalarial (EC_{50} against *Plasmodium falciparum* 1.5 μg/mL) and cytotoxic (EC_{50} against BCA-1 breast cancer, 10 μg/mL) activities.

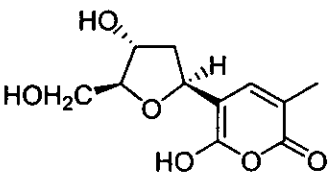


3

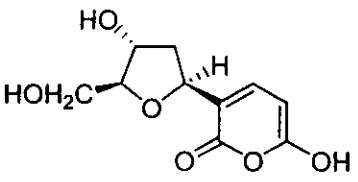


4

Watanadilok *et al.* (2001) reported the isolation of two unusual hydroxypyran-2-ones, tetillapyrone **(5)** and nortetillapyrone **(6)**, from the Thai sponge *Tetilla japonica* collected from Captain Yuth beach, Chonburi.



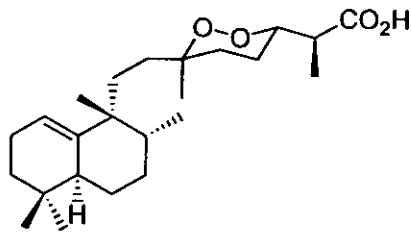
5



6

Most recently Phuwapraisirisan *et al.* (2003) reported the isolation of a new norsesterterpene peroxide, mycaperoxide H **(7)** from the Thai sponge *Mycale* sp.

collected from Sichang Island, Chonburi. This compound showed cytotoxic activity (IC_{50} 0.8 μg/mL against HeLa cells).



7

In our pilot study, we found that the extracts from several sponges collected from Koh-Tao, Suratthani, showed various potent biological activities including antimicrobial, cytotoxic and antituberculosis. Among these, the methanolic extract from a brown sponge, later identified as *Brachiaster* sp., exhibited potent anti-tuberculosis activity (MIC 12.5 μg/mL). These results along with the increasing prevalence and drug resistance of tuberculosis led to the initiation of a research project in search of new antituberculosis agents. The main objectives of this investigation are as the followings;

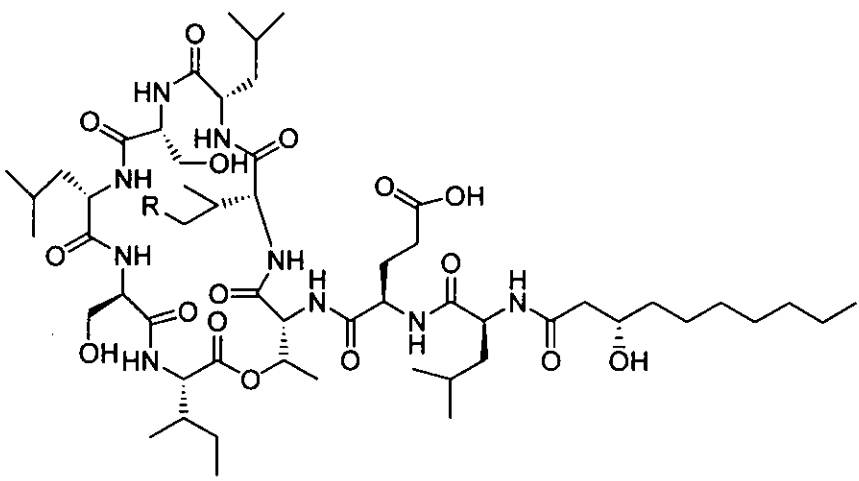
(i) to isolate antituberculosis constituents from the Thai sponge *Brachiaster* sp.,

(ii) to identify and elucidate the chemical structures of the isolated compounds, and

(iii) to propose the basic structure-activity relationship of the isolated compounds.

1.2 Marine natural products as antituberculosis agents

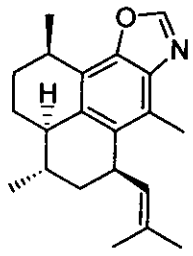
Whereas a large number of antimycobacterial agents from plant species were reported (for example, see review by Newton, Lau and Wright, 2000), to date there are a few reports regarding compounds with *in vitro* antituberculosis actitvity from the marine origins. The first report was the isolation of two cyclic depsipeptides, massetolide A **(8)** and viscosin **(9)**, from the cultures of two *Pseudomonas* species isolated from a marine alga and a tube worm, respectively. The two compounds exhibited antituberculosis activity against *M. tuberculosis* with MICs of 5-10 and 10-20 μg/mL, respectively (Gerard *et al.*, 1997).



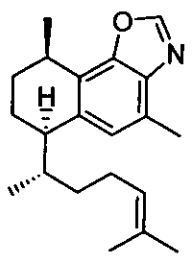
8 : R = CH_3 9 : R = H

Pseudopteroxazole **(10)** and seco-pseudopteroxazole **(11)**, the benzoxazole diterpene alkaloids isolated from the West Indian gorgonian *Pseudopterogorgia elisabethae*, respectively induced 97 and 66% growth inhibition in *M. tuberculosis* $H_{37}Rv$ at a concentration of 12.5 μg/mL without substantial toxic effect. (Rodriguez *et al.*, 1999). Additionally, erogorgiaene **(12)**, a serrulatane diterpene

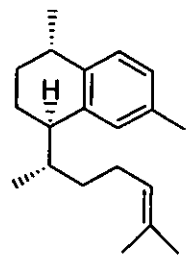
isolated from the same West Indian gorgonian, induced 96% growth inhibition in *M. tuberculosis* $H_{37}Rv$ at a concentration of 12.5 μg/mL (Rodriguez and Ramirez, 2001). It was proposed that the benzoxazole moiety is not essential for antituberculosis activity, as demonstrated by erogorgiaene.



10

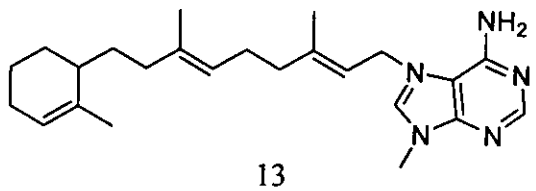


11



12

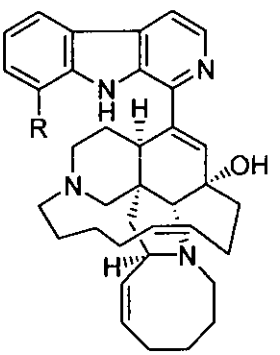
Agelasine F **(13)**, a monocyclic diterpenoid with a 9-methyladeninum unit isolated from the Philippine sponge *Agelas* sp. inhibited some drug-resistant strains of *M. tuberculosis* with MIC of 3.13 μg/mL (Mangalindan *et al.*, 2000).



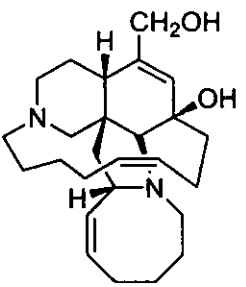
13

Manzamine A **(14)** and (+)-8-hydroxy-manzamine A **(15)**, two members of the unique β-carboline alkaloids, exhibited potent antituberculosis activity against *M. tuberculosis* $H_{37}Rv$ (MIC 1.53 and 0.91 μg/mL, respectively) (Yousaf *et al.*, 2002). These alkaloids were first isolated from sponge *Haliclona* sp. (Sakai and Higa, 1986) and *Pachypellina* sp. (Ichiba, Corgiat and Scheuer, 1994). Its presumed biogenetic precursor, ircinol A **(16)**, which does not possess the β-

carboline moiety, also exhibit the same activity at an MIC of 1.93 μg/mL. Ircinol A represents a useful candidate for *in vivo* assessment toward *M. tuberculosis* treatment, since it shows lower cytotoxicity and less structural complexity than other manzamine-type alkaloids (Yousaf *et al.*, 2002; Donia and Hamann, 2003).



14 ; R = H
15 ; R = OH

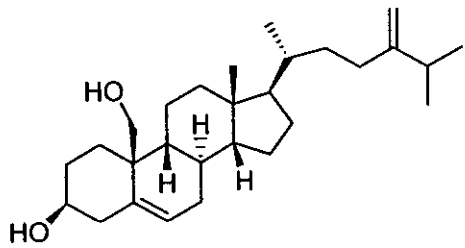


16

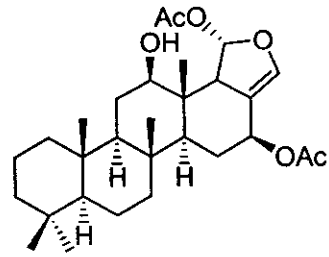
El Sayed *et al.* (2000) reported the promising antituberculosis activity of three compounds (90-99% inhibition of the growth of *M. tuberculosis*). The first one, litosterol **(17)**, C19-hydroxy steroids first isolated from the Okinawan soft coral *Litophyton virdis* (Iguchi, Saitoh and Yamada, 1989), inhibited 90% of the growth of *M. tuberculosis* $H_{37}Rv$ with an MIC of 3.13 μg/mL. It was reported that the poor solubility of litosterol in the aqueous culture media obscured the assessment of cytotoxic effects.

Heteronemin **(18)**, a scalarane-type sesterterpene primarily isolated from the sponge *Heteronema erecta* (Kazlauskas *et al.*, 1976), induced 99% inhibition with an MIC of 6.25 μg/mL and IC_{50} of 1.3 μg/mL. The high cytotoxicity of this

compound prohibited further biological evaluation; however, chemical modifications of this compound were suggested to produce less toxic and more active derivatives.



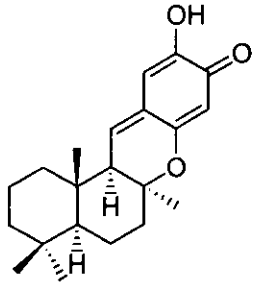
17



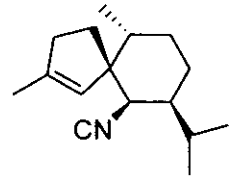
18

The last one, puupehenone **(19)**, was reported to induce 99% inhibition with an MIC of 12.5 μg/mL and IC_{50} of 2.0 μg/mL. The puupehenones are shikimate-sesquiterpene derived metabolites isolated from sponges of the order Verongida and Dictyoceratida from the Hawaiian Island (Nasu *et al.*, 1995).

In a report by Konig, Wright and Franzblau (2000), several compounds were subjected to antituberculosis activity determinations. It was found that the compound with the highest potency was axisonitrile-3 **(20)**, a cyanosesquiterpene isolated from the sponge *Acanthella klethra*. This compound showed antituberculosis activity against *M. tuberculosis* with an MIC of 2.0 μg/mL along with promisingly low cytoxicity (IC_{50} > 20 μg/mL against KB cells).



19

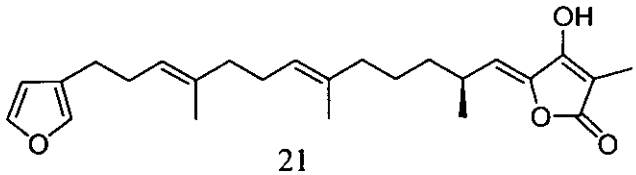


20

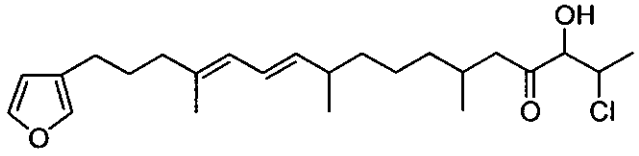
1.3 The sesterterpenoids

The sesterterpenoids arise from geranylfarnesyl diphosphate (GFPP), which is formed by addition of a further isopentenyl diphosphate (IPP) molecule to geranylgeranyl diphosphate (GGPP). With an extensive examples of compounds in this group that are now known, most are nevertheless found principally in fungi and marine organisms, and span relatively few structural types (Dewick, 1997). In fact, the sesterterpenes can be classified into only six main types, including linear, mono-, bi-, tri, tetra-carbocyclic and fungal sesterterpenoids. Some representative examples of each class are shown below.

Marine sponges have been the major sources of a large number of linear sesterterpenoids. Many of these compounds contain a furan ring and a tetronic acid moiety while most of the remainings are the previous group's degradation products. For example, variabilin **(21)** isolated from the sponge *Sarcotragus* sp. (Barrow *et al.*, 1988) and konakhin **(22)** isolated from a Senegalese sponge represents a degradation product of the tetronic acid (N'Diaye *et al.*, 1991).

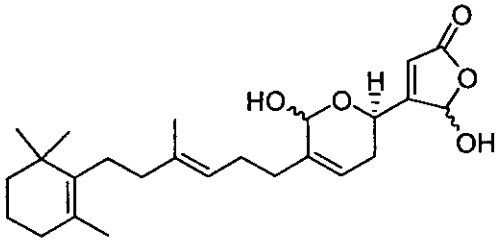


21



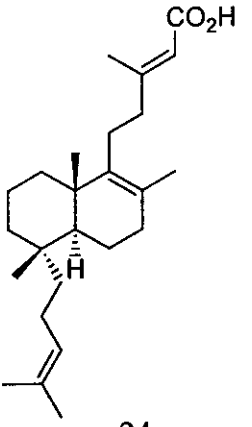
22

Mono-carbocyclic sesterterpenoids are examplified by manoalide **(23)** and its derivatives. Manoalide significantly reduces chemically induced inflammation and was originally found in the sponge *Luffariella variabilis* (de Silva and Scheuer, 1980; Jacobs *et al.*, 1985).

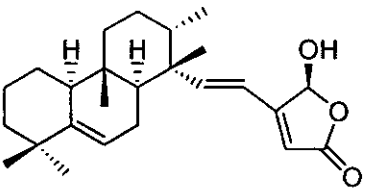


23

Dysideapalaunic acid **(24)** and aplysolide A **(25)** are, respectively, examples of bi- and tri-carbocylic sesterterpenoids. Dysideapalaunic acid was isolated from the sponge *Dysidea* sp., and showed the inhibition toward aldose reductase (Hagiwara and Uda, 1991). Aplysolide A is hydroxy-butenolides obtained from a sponge *Aplysinopsis* sp. (Crews, Jimenez and Neil-Johnson, 1991).



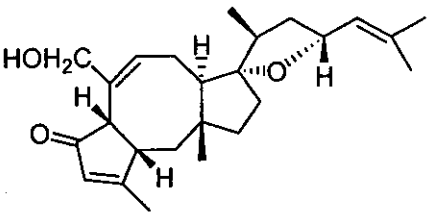
24



25

The scalaranes, which belongs to the tetracarbocyclic type, are the most common sesterterpenoids and most extensively studied compounds. The details regarding their chemistry and bioactivities will be discussed in the next section of this chapter.

The last group, the fungal sesterterpenoids, are mainly produced by plant fungal pathogens of the genus *Drechslera*. Ophiobolin A **(26)**, for example, is a phytotoxic metabolite isolated from *Drechslera sorghicola*, which is a pathogen on sorghum and Johnson grass (Sugawara *et al.*, 1988).



26

1.3.1 Scalarane-type sesterterpenoids

Scalarane-type sesterterpenes are found widely distributed in several marine sponge species, especially those from the family *Dictyoceratida* (Hanson, 1992). Certain members of this group can also be found in nudibranches, which assoicate with the sponges containing these compounds. The scalarane skeleton is shown in Figure 2.

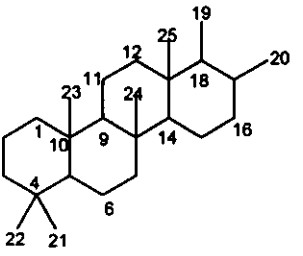


Figure 2 Scalaranes skeleton

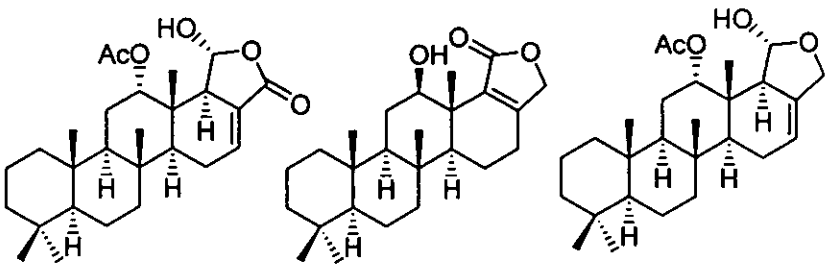
To date, there are up to more than 100 naturally occuring scalarane-type sesterterpenes reported, which can be classified into two categories, furanoscalaranes and non-furanoscalaranes.

1.3.1.1 Furanoscalaranes

Constructing the major category, most scalarane-type sesterterpenes reported to date are belonging to the furanoscalaranes. The main skeleton of the members in this class possesses a tetracarbocyclic ring fused with an extended furan moiety onto C17-C18 of ring D. The oxidation state, as well as the joining positions, of the furan residue are varied, from a simple hydrofuran, to aromatic

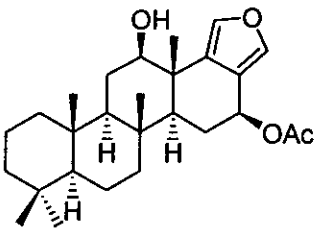
and oxygenated furans. Some selected prototypes of the furanoscalaranes are examplified below.

The oxygenating degree in the lactone moiety varies from hydroxy lactone, as seen in scalarin **(27)** from the sponge *Cacospongia scalaris* (Fattorusso *et al.*, 1972), to simple lactone, as seen in scalarolide **(28)** from the sponge *Spongia idia* (Walker, Thompson and Faulkner, 1980) and lactol as seen in heteronemin **(18)** from the sponge *Heteronema erecta* (Kazlauskas *et al.*, 1976) and deoxoscalarin **(29)** from the sponge *Spongia officinalis* (Cimino *et al.*, 1977). Normally, the oxygenating position is found at either C-19 or C-20.

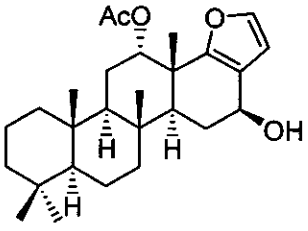


27 28 29

The non-oxygenated furano type, although found less frequently, was also reported. The prototype of such group include scalarafuran **(30)**, from the sponge *Spongia idia* (Walker, Thompson and Faulkner, 1980), of which the extended furan-subunit is the fully aromatized. Also rare were rearranged furanoscalaranes, in which the furan moiety is otherwise attached on its *b*-face, suggesting an oxidative cleavage-recyclization biosynthetic scheme. The example of such furanoscalarane is furoscalarol **(31)** from the sponge *Cacospongia mollior* (Cimino *et al.*, 1978).



30

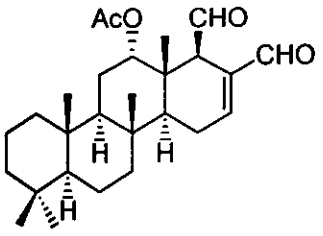


31

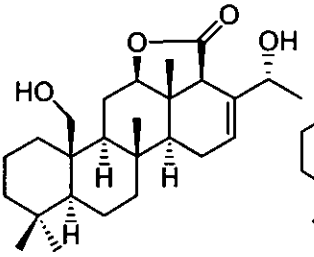
1.3.1.2 Non-furanoscalaranes

The members of this group belong to the tetracarbocyclic scalaranes with no furan residue on ring D. Most often, the functional group variation is found substituted at C-12, C-16, C-19 and C-20. The prototype of this group is scalaradial **(32),** which was first isolated from the sponge *Cocospongia mollior* (Cimino *et al.*, 1974), and sednolide **(33)** from the nudibrance *Chromodoris sedna* (Hochlowski and Faulkner, 1983).

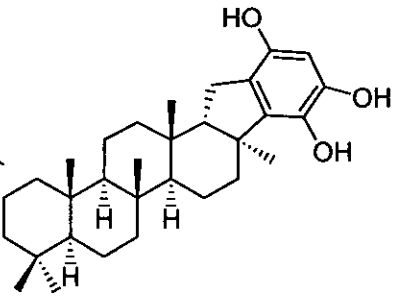
Additionally, there are some other members that incorporate structural subunit from other biosynthetic pathway. These include disidein **(34)**, pentacyclic scalaranes combined with a hydroxyhydroquinone ring, isolated from the sponge *Disidea pellscens* (Cimino *et al.*, 1975). The hydroquinone residue of **34** is clearly demonstrating the involvement of triketide intermediate during the biosynthetic pathway.



32



33



34

The activities and biological sources of all members in scalarane-type sesterterpenes reported to date are summarized in Table 2.

Table 2 Biological sources and activities of scalarane-type sesterterpenoids

Compounds	Sources	Activities	References
1. Furanoscalaranes			
1.1 furanone-type			
Scalarin	*Cacospongia scalaris* (sponge)	N/A	Fattorusso *et al.*, 1972
12-Epi-scalarin	*Spongia nitens* (sponge)	N/A	Cimino *et al.*, 1977
Scalarolide	*Spongia idia* (sponge)	N/A	Walker *et al.*, 1980
23-Hydroxy-20-methylscalarolide	*Chromodoris sedna* (nudibranch)	N/A	Hochlowski and Faulkner, 1983
Phyllofolactone A	*Phyllospongia foliascens* (sponge)	N/A	Zeng *et al.*, 1991

Table 2 (cont.)

Compounds	Sources	Activities	References
Phyllofolactone B	*Phyllospongia foliascens* (sponge)	N/A	Zeng *et al.*, 1991
Phyllofolactone B acetate	*Carteriospongia foliascens* (sponge)	N/A	Barron *et al.*, 1991
Phyllactone A	*Phyllospongia foliascens* (sponge)	Cytotoxic (IC_{50} 20 μg/mL against KB)	Fu *et al.*, 1992
Phyllactone B	*Phyllospongia foliascens* (sponge)	Cytotoxic (IC_{50} 20 μg/mL against KB)	Fu *et al.*, 1992
Phyllactone C	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1992
Phyllactone D	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1992
Phyllactone E	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1992
Phyllactone F	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1993
Phyllactone G	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1993
12-*O*-Deacetyl scalarin	*Hyrtios* sp. (sponge)	Nerve growth factor synthesis-stimulating (concentration 30-100 μg/mL)	Doi *et al.*, 1993

Table 2 (cont.)

Compounds	Sources	Activities	References
16-*O*-Deacetyl-16-episcalarol butenolide	*Hyrtios erecta* (sponge)	Cytotoxic (IC_{50} 0.4 μg/mL against P-388)	Ryu *et al.*, 1996
12-*O*-Deacetyl-16-*O*-deacetyl-16-epi scalarolbutenolide	*Hyrtios erecta* (sponge)	Cytotoxic (IC_{50} 0.4 μg/mL against P-388)	Ryu *et al.*, 1996
12-Deacetoxy-21-acetoxyscalarin	*Hyrtios erecta* (sponge)	Cytotoxic (IC_{50} 0.4 μg/mL against P-388)	Ryu *et al.*, 1996
12-Epi-acetylscalarolide	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 1-2 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
19-Deoxyscalarin	*Cacospongia scalaris* (sponge)	N/A	Rueda *et al.*, 1997
12-Deacetyl-12-epi-19-deoxyscalarin	*Hyrtios erecta* (sponge)	Cytotoxic (ED_{50} 2.9 μg/mL against P-388)	Pettit *et al.*, 1998
Sesterstatin 1	*Hyrtios erecta* (sponge)	Cytotoxic (ED_{50} 0.46 μg/mL against P-388)	Pettit *et al.*, 1998
Sesterstatin 2	*Hyrtios erecta* (sponge)	Cytotoxic (ED_{50} 4.2 μg/mL against P-388)	Pettit *et al.*, 1998
Sesterstatin 3	*Hyrtios erecta* (sponge)	Cytotoxic (ED_{50} 4.3 μg/mL against P-388)	Pettit *et al.*, 1998

Table 2 (cont.)

Compounds	Sources	Activities	References
12-*O*-Acetyl-16-*O*-deacety-12,16-epi scalarolbutenolide	*Chromodoris inornata* (nudibranch)	Cytotoxic (IC_{50} 2.4 μg/mL against L1210)	Miyamoto *et al.*, 1999
Phyllofolactones C	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1999
Phyllofolactones D	*Phyllospongia foliascens* (sponge)	N/A	Fu *et al.*, 1999
Hyrtiolide	*Hyrtios erecta* (sponge)	N/A	Miyaoka *et al.*, 2000
16-Hydroxy scalarolide	*Hyrtios erecta* (sponge)	N/A	Miyaoka *et al.*, 2000
Phyllofolactones H	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
Phyllofolactones I	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
Phyllofolactones J	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
Phyllofolactones K	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
3-Acetylsesterstatin 1	*Hyrtios erecta* (sponge)	N/A	Youssef *et al.*, 2002
19-Acetylsesterstatin 3	*Hyrtios erecta* (sponge)	N/A	Youssef *et al.*, 2002
1.2 furanol-type			
Deoxoscalarin	*Spongia officinalis* (sponge)	N/A	Cimino *et al.*, 1977

Table 2 (cont.)

Compounds	Sources	Activities	References
12-Epi-deoxoscalarin	*Spongia nitens* (sponge)	N/A	Cimino *et al.*, 1977
Heteronemin	*Heteronema erecta* (sponge)	Cytotoxic (IC_{50} 1.2 μg/mL against KB); Antituberculosis (MIC 6.25 μg/mL against *M. tuberculosis* ($H_{37}Rv$)	Kazlauskas *et al.*, 1976; Doi *et al.*, 1993; El *sayed et al.*, 2000
Scalardysin A	*Dysidea herbacea* (sponge)	N/A	Kashman and Zviely, 1979
Scalardysin B	*Dysidea herbacea* (sponge)	N/A	Kashman and Zviely, 1979
12-Deacetyl-20-methyl-12-epideoxoscalarin	*Chromodoris sedna* (nudibranch)	N/A	Hochlowski and Faulkner, 1983
23-Hydroxy-20-methyldeoxoscalarin	*Chromodoris sedna* (nudibranch)	N/A	Hochlowski and Faulkner, 1983
12-α-Acetoxy-19,20-epoxy-20-hydroxy-20,22-dimethyl scalarane	*Carteriospongia foliascens* (sponge)	Ichthyotoxic (LD_{50} 40 mg/L against *Lebistes reticulatus*)	Braekman *et al.*, 1985
Heteronemin acetate	*Hyrtios erecta* (sponge)	N/A	Crews and Bescansa, 1986
12-Epi-heteronemin acetate	*Hyrtios erecta* (sponge)	Cytotoxic (IC_{50} 2.7 μg/mL against KB)	Crews and Bescansa, 1986; Doi *et al.*, 1993

Table 2 (cont.)

Compounds	Sources	Activities	References
Deoxoscalarin acetate	*Spongia officinalis* (sponge)	N/A	De Giulio *et al.*, 1989
(-)-12-Epi-deoxoscalarin	*Spongia officinalis* (sponge)	N/A	De Giulio *et al.*, 1989
24-Acetoxy-12-deacetyl-12-epi-deoxoscalarin	*Hyatella intestinalis* (sponge)	N/A	Karuso *et al.*, 1989
12-Epi-heteronemin	*Hyrtios erecta* (sponge)	N/A	Bourguet-Kondracki *et al.*, 1994
12-Epi-deoxoscalarin-3-one	*Chromodoris inornata* (nudibranch)	Cytotoxic (IC_{50} 6.6 μg/mL against L1210)	Miyamoto *et al.*, 1999
Deoxoscalarin-3-one	*Chromodoris inornata* (nudibranch)	Cytotoxic (IC_{50} 0.95 μg/mL against L1210)	Miyamoto *et al.*, 1999
21-Acetoxydeoxo scalarin	*Chromodoris inornata* (nudibranch)	Cytotoxic (IC_{50} 0.35 μg/mL against L1210)	Miyamoto *et al.*, 1999
21-Hydroxydeoxo scalarin	*Chromodoris inornata* (nudibranch)	Cytotoxic (IC_{50} 4.1 μg/mL against L1210)	Miyamoto *et al.*, 1999
12-Deacetoxy-12-oxodeoxoscalarin	*Glossodoris atromarginata* (nudibranch)	Cytotoxic (25% of mortality against human thyroid carcinoma	Fontana *et al.*, 1999
12-Deacetyl-12-epi-deoxoscalarin	*Glossodoris atromarginata* (nudibranch)	N/A	Fontana *et al.*, 1999

Table 2 (cont.)

Compounds	Sources	Activities	References
12-Deacetyl-23-acetoxy-20-methyl 12-epi-deoxoscalarin	*Glossodoris sedna* (nudibranch)	N/A	Fontana *et al.*, 2000
1.3 non-oxygenated furan-type			
Furoscalarol	*Cacospongia mollior* (sponge)	N/A	Cimino *et al.*, 1978
Scalarafuran	*Spongia idia* (sponge)	Cytotoxic (IC_{50} 7.2 μg/mL against KB)	Walker *et al.*, 1980; Doi *et al.*, 1993
16-Deacetyl-12-epi-scalafuran acetate	*Spongia officinalis* (sponge)	N/A	De Giulio *et al.*, 1989
Isoscalarafuran A	*Spongia hispida* (sponge)	N/A	Davis and Capon, 1993
Isoscalarafuran B	*Spongia hispida* (sponge)	N/A	Davis and Capon, 1993
12-*O*-Deacetyl furoscalarol	*Hyrtios* sp. (sponge)	Nerve growth factor synthesis-stimulating (concentration 30-100 μg/mL)	Doi *et al.*, 1993
16-Acetyl furoscalarol	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 2.5-10 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
12-*O*-Desacetyl furoscalar-16-one	*Cacospongia sp* (sponge).	N/A	Cambie *et al.*, 1998
Salmahyrtisol B	*Hyrtios erecta* (sponge)	N/A	Youssef *et al.*, 2002

Table 2 (cont.)

Compounds	Sources	Activities	References
2. Non-furanoscalaranes			
Scalaradial	*Cocospongia mollior* (sponge)	Brine shrimp lethality (LD_{50} 0.18 μg/mL); Fish antifeedant (MIC 60 μg/cm^2 against *Carassius carassius*); Inhibited PLA_2 (IC_{50} 0.6 μM)	Cimino *et al.*, 1974; De Rosa *et al.*, 1994; Fontana *et al.*, 2000
Disidein	*Disidea pellscens* (sponge)	N/A	Cimino *et al.*, 1975
12-Epi-scalaradial	*Spongia nitens* (sponge)	N/A	Cimino *et al.*, 1979
12,18-Diepi-scalaradial	*Spongia nitens* (sponge)	N/A	Cimino *et al.*, 1979
Scalarherbacin A	*Dysidea herbacea* (sponge)	N/A	Kashman and Zviely, 1979
Scalarherbacin B	*Dysidea herbacea* (sponge)	N/A	Kashman and Zviely, 1979
Scalarherbacin A acetate	*Dysidea herbacea* (sponge)	N/A	Kashman and Zviely, 1979
Scalarherbacin B acetate	*Dysidea herbacea* (sponge)	N/A	Kashman and Zviely, 1979
12-Deacetyl-12,18-diepi-scalaradial	*Spongia idia* (sponge)	N/A	Walker *et al.*, 1980

Table 2 (cont.)

Compounds	Sources	Activities	References
12-Deacetyl scalaradial	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 0.58 μg/mL against L-1210)	Yasuda and Tada, 1981
Foliaspongin	*Phyllospongia foliascens* (sponge)	Antiinflammatory (18.1% inhibition at concentration 10 μg/disk utilizing chrio-allantoic membrane of chick embryo	Kikuchi *et al.*, 1983
Sednolide	*Chromodoris sedna* (nudibranch)	N/A	Hochlowski and Faulkner, 1983
Sednolide-23-acetate	*Chromodoris sedna* (nudibranch)	N/A	Hochlowski and Faulkner, 1983
Hyrtial	*Hyrtios erecta* (sponge)	Antiinflamatory (43% decrease in ear weight of PMA induced inflammation at concentration 50 μg/mL)	Crews *et al.*, 1985; Crews and Bescansa, 1986
12-α,16-β-Diacetoxy-20,22-dimethyl-20-oxo-19-norscalarane	*Carteriospongia foliascens* (sponge)	Ichthyotoxic (LD_{50} 20 mg/L against *Lebistes reticulatus*)	Braekman *et al.*, 1985

Table 2 (cont.)

Compounds	Sources	Activities	References
12-α-Acetoxy-16-β-hydroxy-20,22-dimethyl-20-oxoscalar-19-al	*Carteriospongia foliascens* (sponge)	Ichthyotoxic (LD_{50} 5 mg/L against *Lebistes reticulatus*)	Braekman *et al.*, 1985
12-Deacetyl-12-epi-scalaradial	*Hyrtios erecta* (sponge)	N/A	Crews and Bescansa, 1986
12-Deacetyl-18-epi-12-oxoscalaradial	*Chromodoris youngbleuthi* (nudibranch)	N/A	Terem and Scheuer, 1986
Triacetyl disidein	*Disidea pallescens* (sponge)	N/A	Cimino *et al.*, 1987
6′-Cl-disidein	*Disidea pallescens* (sponge)	N/A	Cimino *et al.*, 1987
6′-Br-disidein	*Disidea pallescens* (sponge)	N/A	Cimino *et al.*, 1987
Phyllofoliaspongin	*Phyllospongia foliascens* (sponge)	Cytotoxic (84% inhibitory at 5 μg/ml against P-388); Antithrombocyte (IC_{50} 2.35 μg/ml against adenosine diphosphate)	Kitagawa *et al.*, 1989
Dehydrofoliaspongin	*Phyllospongia foliascens* (sponge)	N/A	Kitagawa *et al.*, 1989
Phyllofenone A	*Phyllospongia foliascens* (sponge)	N/A	Zeng *et al.*, 1991

Table 2 (cont.)

Compounds	Sources	Activities	References
Phyllofenone B	*Phyllospongia foliascens* (sponge)	Cytotoxic (IC_{50} 5 μg/mL against P-388)	Zeng *et al.*, 1991
12-Deacetoxy scalaradial	*Cacospongia mollior* (sponge)	Fish antifeedant (MIC 30 $\mu g/cm^2$ against *Carassius carassius*); Brine shrimp lethality (LD_{50} 0.77 μg/mL)	De Rosa *et al.*, 1994
18-Epi-scalaradial	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 0.2-0.5 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
19-Dihydro scalaradial	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 2-2.5 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
Norscalaral A	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50}1-2 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
Norscalaral B	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 2 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997

Table 2 (cont.)

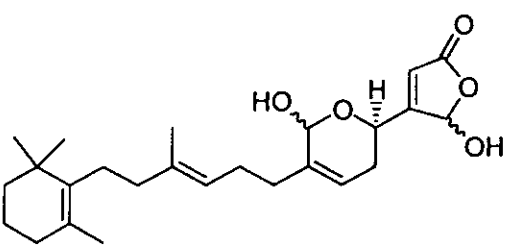
Compounds	Sources	Activities	References
Norscalaral C	*Cacospongia scalaris* (sponge)	Cytotoxic (ED_{50} 1.2-2.5 μg/mL against P-388, Schabel, A-549, HT-29 and MEL-28)	Rueda *et al.*, 1997
25,26-Bishomo-scalarane	*Cacospongia scalaris* (sponge)	N/A	De Rosa *et al.*, 1998
12-Deacetyl-Δ^{17}-hyrtial	*Hyrtios erectus* (sponge)	Antiproliferative (IC_{50} 2.82 μg/mL against KB)	Miyaoka *et al.*, 2000
Honu'enone	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
Phyllofenone C	*Strepsichordaia aliena* (sponge)	N/A	Jimenez *et al.*, 2000
12-Deacetyl-23-acetoxy-20-methyl-12-epi-scalaradial	*Glossodoris sedna* (nudibranch)	Ichthyotoxic (0.1 ppm against *Gambusia affinis*); Inhibited PLA_2 (IC_{50} 18 μM)	Fontana *et al.*, 2000

Note: N/A = not available

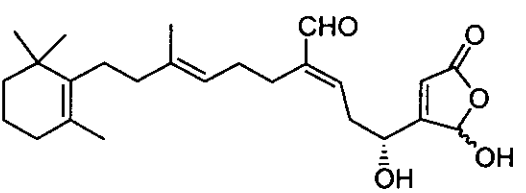
1.3.2 Manoalide-type sesterterpenoids

The members in this class are monocarbocyclic sesterterpenoids normally containing butenolide end. Most of them have been reported from the sponge *Luffariella variabilis*. Their bioactivities are mostly antiinflammatory. Substituted position on butenolide moiety can be used to classify this group into two major types, including β-substituted- and γ-substituted-butenolide-type sesterterpenes.

Manoalide **(23)** is the prototype of alkylated trimethyl- cyclohexenyl with β-substituted-α,β-unsaturated-γ-hydroxybutenolide moiety. The compound was first isolated from a sponge *Luffariella variabilis* (de Silva and Scheuer, 1980) and was reported to reduce chemically induced inflammation. Seco-manoalide **(35)** possesses the same structural type but lack of cyclized α,β-unsaturated δ-lactol moiety and was isolated from the same sponge species (de Silva and Scheuer, 1981).

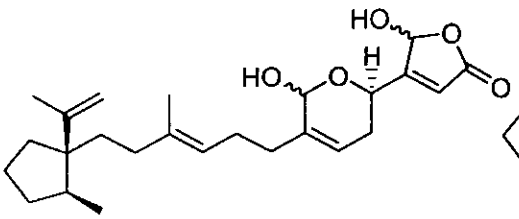


23

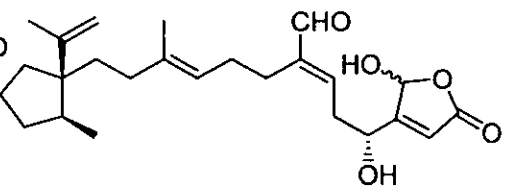


35

Luffariellins A **(36)** and B **(37)** are geometric isomers of **23** and **25**, respectively, and were also isolated from the same sponge species (Kernan and Faulkner, 1987). These compounds possess alkylated cyclopentanyl with β-substituted-α,β-unsaturated-γ-hydroxybutenolide moiety.

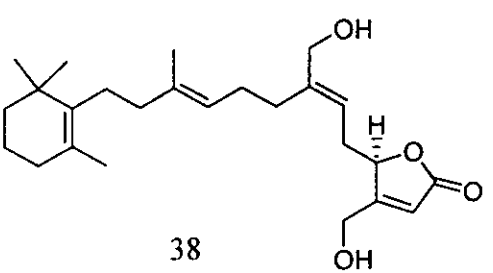


36

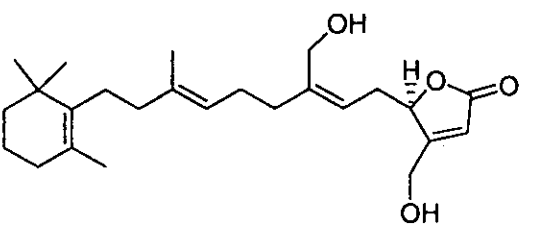


37

The γ-substituted-butenolide-type sesterterpenes are examplified by *E*- and *Z*-neomanoalide (**38** and **39**, respectively). These compounds also possess the alkylated trimethylcyclohexenyl group with β,γ-disubstituted-α,β-unsaturated-butenolide moiety.



38



39

The activities and biological sources of all members are summarized in Table 3.

Table 3 Biological sources and activities of manoalide-type sesterterpenoids

Compounds	Sources	Activities	References
1. β-substituted-butenolide-type			
Manoalide	*Luffariella varabilis*	Antiinflam-matory (inactivated directly PLA_2); Analgesic; Prevent paralytic action of β-bungaro-toxin on the rat phrenic nerve-hemidiaphragm preparation; Cytotoxic (IC_{50} 0.022 and 0.26 μg/mL against L1210 and KB, respectively)	de Silva and Scheuer, 1980; de Freitas *et al.*, 1984; Jacobs *et al.*, 1985; Kobayashi *et al.*, 1994
Seco-manoalide	*Luffariella varabilis*	Antiinflam-matory, inhibit aldose reductase (82% inhibition with MIC 2×10^{-6} M)	de Silva and Scheuer, 1981; Katsumura *et al.*, 1987
Luffariellin A	*Luffariella varabilis*	Antiinflam-matory (IC_{50} 5.6×10^{-8} M against bee venom PLA_2)	Kernan and Faulkner, 1987
Luffariellin B	*Luffariella varabilis*	Antiinflam-matory (IC_{50} 6.2×10^{-8} M against bee venom PLA_2)	Kernan and Faulkner, 1987

Table 3 (cont.)

Compounds	Sources	Activities	References
Manoalide 25-acetate	*Thorectandra excavatus*	N/A	Cambie and Craw,1988
Thorectolide 25-acetate	*Thorectandra excavatus*	N/A	Cambie and Craw,1988
Luffariellolide	*Fascaplysinopsis* sp.	N/A	Roll *et al.*, 1988
Dehydro luffariellolide diacid	*Fascaplysinopsis reticulata*	N/A	Jimenez *et al.*, 1991
Luffariolide A	*Luffariella* sp.	Cytotoxic (IC_{50} 1.1 μg/mL against L1210)	Tsuda *et al.*, 1992
Luffariolide B	*Luffariella* sp.	Cytotoxic (IC_{50} 1.3 μg/mL against L1210)	Tsuda *et al.*, 1992
Luffariolide D	*Luffariella* sp.	Cytotoxic (IC_{50} 4.2 μg/mL against L1210)	Tsuda *et al.*, 1992
Luffariolide E	*Luffariella* sp.	Cytotoxic (IC_{50} 1.2 μg/mL against L1210)	Tsuda *et al.*, 1992
2. γ-substituted-butenolide-type			
E-Neomanoalide	*Luffariella varabilis*	Cytotoxic (IC_{50} 9.8 μg/mL against L1210)	de Silva and Scheuer, 1981; Tsuda *et al.*, 1992

Table 3 (cont.)

Compounds	Sources	Activities	References
Z-Neomanoalide	*Luffariella varabilis*	Cytotoxic (IC_{50} 5.6 μg/mL against L1210)	de Silva and Scheuer, 1981; Tsuda *et al.*, 1992
Luffariolide C	*Luffariella* sp.	Cytotoxic (IC_{50} 7.8 μg/mL against L1210)	Tsuda *et al.*, 1992
Z-2,3-Dihydro neomanoalide	*Luffariella* sp.	Antibacterial (MIC 1 and 5 μg/mL against *Escherichia coli* and *Bacillus subtilis*, respectively	Konig *et al.*, 1992
Z-24-Acetoxy-2,3-dihydro neomanoalide	*Luffariella* sp.	Antibacterial (MIC 3 and 11 μg/mL against *B. subtilis and Micrococcus luteus*, respectively	Konig *et al.*, 1992
Z-24-Acetoxy neomanoalide	*Luffariella* sp.	Antibacterial (MIC 8 and 2 μg/mL against *B. subtilis* and *M. luteus*, respectively	Konig *et al.*, 1992
E-Neomanoalide - 24-al	*Luffariella* sp.	Antibacterial (MIC 4 μg/mL against *B. subtilis* and *M. luteus*	Konig *et al.*, 1992

Table 3 (cont.)

Compounds	Sources	Activities	References
Luffarin-P	*Luffariella geometrica*	N/A	Butler and Capon, 1992

Note: N/A = not available

1.4 The Genus *Brachiaster*

The identification of sponge species is not easy even for experts and requires special technique. Sponges are taxonomically classified by means of skeleton structures (spicule and spongin), external characteristics (shape, size, color, texture, mucous production, smell) and biochemical, reproductive and ecological characteristics. They are taxonomically classified into four classes; Demospongiae, Hexactinellida, Calcarea and Sclerospongiae (Bergquist, 1978; Hooper, 2000). Approximately 95% of sponges are in the class Demonspongiae, which the genus *Brachiaster* belongs to (Hopper, 2000).

The taxa of this Genus is as followed; Phylum Porifera, Class Demospongiae, Order Choristida, Family Pachastrellidae and Genus *Brachiaster*.

Hooper (2000) described the characteristic of the Family Pachastrellidae as following;

> ...Encrusting, massive and plate-shaped growth forms, with ostia and oscules on opposite sides; megascleres calthrops, short-shafted triaenes, and oxeas; microscleres streptasters of various types (metasters, spirasters and amphiasters), but never euasters; desmas common in some genera ('lithistid' or 'sublithistid' grades of construction). Seventeen genera are included for this family...

To our knowledge, there are no reports regarding chemical constituents from the sponges of the genus *Brachiaster*.