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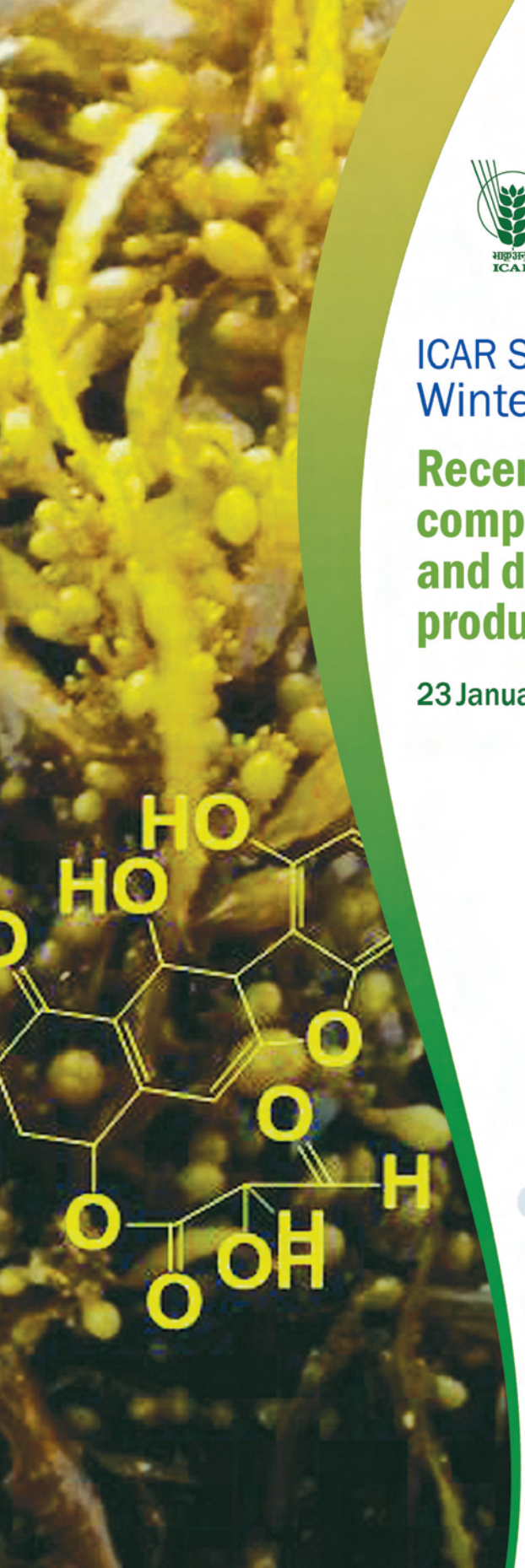
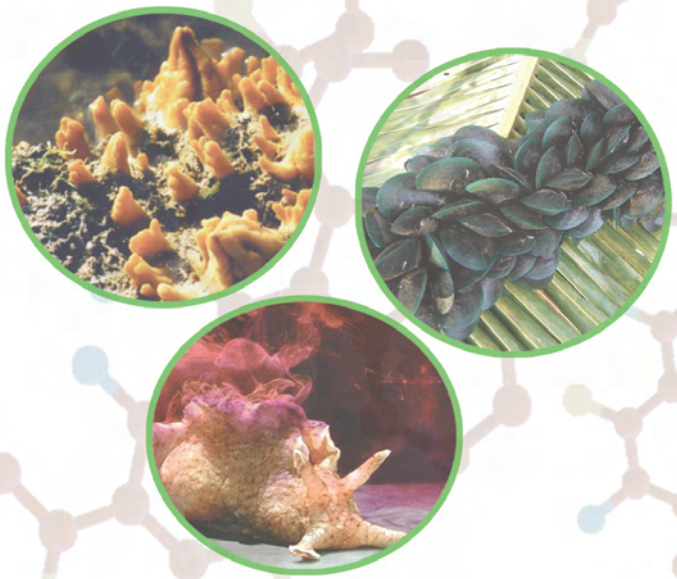


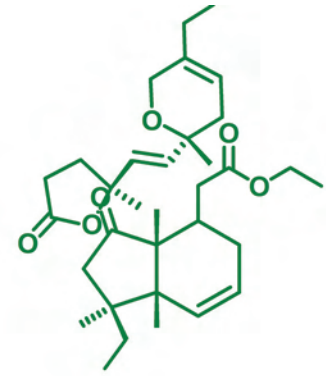
ICAR Sponsored Winter School on

Recent advances in bioactive compounds from marine organisms and development of high value products for health management

23 January to 12 February 2018



Marine Biotechnology Division
ICAR-Central Marine Fisheries Research Institute
Post Box No. 1603, Ernakulam North P.O., Kochi-682 018, Kerala, India



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Course Manual

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Recent advances in bioactive compounds from marine organisms and development of high-value products for health management

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ICAR-Central Marine Fisheries Research Institute,
23 January - 12 February, 2018

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Edited by Foxit Reader

FOREWORD

There has been a growing interest in the marine derived bioactive compounds in the recent years, and the functional foods, enriched with natural ingredients have been proved to provide beneficial action for human health. Marine derived bioactive components and the functional food ingredients demonstrated to possess potential health benefits. High value secondary bioactive metabolites from the marine organisms are attracting attention because of the growing demand for new compounds of 'marine natural' origin, having potential applications in pharmaceutical fields, and concerns about the adverse effects by synthetic drugs and their derivatives. The pioneering R & D works at ICAR-Central Marine Fisheries Research Institute on marine bioprospecting envisaged a systematic approach involving chemical profiling of major species of marine organisms for bioactive pharmacophore leads for activity against various diseases, and a library of molecules with bioactive potential. The research work in this institute developed protocols to prepare various pharmaceutical leads, nutraceuticals/ functional food supplements enriched with lead molecules with different properties against various drug targets for use against various life-threatening diseases.

ICAR-Central Marine Fisheries Research Institute is the pioneering marine research institute in India to work in the frontier area of bioactive molecule discovery from marine organisms as promising therapeutic agents against various diseases, aquatic food product technology, and development of high value products for health management. This prestigious research institute of Indian Council of Agricultural Research is working in the broad national interest of producing high value bioactive leads from the marine organisms, which would provide promising therapeutic agents against various diseases. This institute has developed and commercialized the nutraceutical products Cadalmin™ Green Algal extract (Cadalmin™ GAe) and Antidiabetic extract (Cadalmin™ ADe) as green alternatives to synthetic drugs to combat rheumatic arthritic pains and type-2 diabetes, respectively to a leading biopharmaceutical company in India. The anti-inflammatory nutraceutical Cadalmin™ Green Mussel extract (Cadalmin™ GMe) from Asian green mussel Perna viridis has been commercialized with Amalgam Group of Companies. Cadalmin™ Antihypercholesterolemic extract (Cadalmin™ ACe) has been developed from seaweeds to combat dyslipidemia leading to obesity, and the product was out-licensed to a leading Indian MNC in wellness and obesity management. Antimicrobial therapeutic product from marine bacteria as oral applicant has been developed and the product is in pipeline for commercialization. Seaweed-derived natural template inspired synthetic derivatives as potential pharmacophores were designed and developed. Several nutraceutical and cosmeceutical products from marine organisms are in pipeline, and are being commercialized.

The objective of the National level ICAR Winter School on "Recent advances in bioactive compounds from marine organisms and development of high-value products for health management" is to provide up-to-date information and acquaint the participants with the latest technologies on isolation and characterization of marine natural products of pharmaceutical importance from marine organisms, general and advanced methods of isolation procedures by chromatography, classification of organic compounds and their characterization by advanced spectroscopic experiments. This program further aims to give exposure to the chemical perspectives of marine organisms, primary and secondary bioactive metabolites from fish and marine organisms to develop bioactive compounds and high-value functional food products. Theory and practical classes will be conducted in these areas to provide the participants a hands-on experience.

This ICAR Winter School is organized with the full funding support from ICAR, New Delhi, and the twenty-five participants from various parts of India who are attending this programme were selected after scrutiny of their applications based on their bio-data. They are serving as academicians, such as Professors/Scientists, and in similar posts. The faculties include the knowledgeable scientists and professors from various parts of India and abroad. This training will enable the participants to efficiently carry out their academic programmes, and to plan research on bioactive molecule discovery in their respective laboratories and institutes so that they can formulate the strategies for research.

The Winter School on "Recent advances in bioactive compounds from marine organisms and development of high value products for health management" is very ideal for the current scenario of increasing lifestyle diseases and human health. Understanding the importance of natural products in the health care system of India, ICAR-Central Marine Fisheries Research Institute has reasonably contributed in the various aspects. The Manual released on this occasion covers all aspects of marine natural products prepared by the experts in their respective fields. I congratulate the Course Director of this programme, Dr. Kajal Chakraborty and Head of the Marine Biotechnology Division, Dr. P. Vijayagopal, along with other staff members of Marine Biotechnology Division and Central Marine Fisheries Research Institute for their sincere efforts in bringing out the manual in time, and to arrange the programme in a befitting manner.

A. Gopalakrishnan
Director, ICAR-Central Marine Fisheries Research Institute
Kochi, Kerala

PREFACE

Marine-derived bioactive components and the functional food ingredients with potential health benefits are an emerging area of research. The rich diversity of flora and fauna in the marine and coastal habitats of the Indian subcontinent represent an untapped reservoir of bioactive compounds with valuable pharmaceutical and biomedical use. Considering the underutilization of these groups of marine organisms, exploring bioactive compounds and development of any biologically useful products have benefits as health products. Comprehensive analyses demonstrated that during the last decade the average proportion of bioactive compounds among the new compounds is declining, though there are a large number of marine natural products yet to be explored. This may indicate that the research level of bioactivity is not keeping up with the discovery of new compounds. Thus, the research tools and methods for finding bioactivity need to be improved. The first improvement is about methods of spectral and bioactivity-guided separation and purification of marine-derived secondary metabolites, which combine the discovery of new compounds. These improvements in technology are dependent upon the automation in spectroscopy, which also allows the study of the functions of new compounds extracted from the target marine organisms. Second, for the discovery of new lead compounds and artificial intelligence for drug development evolved to a more mechanistic approach that targets specific molecular lesions. Combined with high-throughput screening through a large number of drug targets, bioactivity research against various life-threatening diseases will be effective in revealing the potentially useful biological properties of marine natural products. Furthermore, the discovery of new bioactive compounds from marine metabolites will form the basis for new drug leads. Thus, the new compounds will absolutely compose an abundant resource for future bioactivity research and drug development. Various medicinal and biomedical products from marine flora and fauna provide a myriad of benefits for human health and multiple life-threatening diseases, and therefore, are the attractive options for the food and pharmaceutical industry. The increasing interest in marine-based functional food ingredients and nutraceutical formulations in the last decade along with increased number of patents filed/granted have appropriately demonstrated the possibilities of bioactive from marine organisms to maintain and improve human health and well-being.

The present ICAR Winter School on "Recent advances in bioactive compounds from marine organisms and development of high-value products for health management" is designed to acquaint the participants with the advances in marine bioactive compounds with emphasis on the latest technologies on isolation and characterization of marine natural products of pharmaceutical importance. The course is planned in such a way that it covers both theoretical and practical aspect of recent advances in bioactive compounds from marine organisms. This programme will strengthen the knowledge of participants with regard to

the general and advanced methods of isolation procedures by chromatography, and their characterization by advanced spectroscopic experiments aspects.

I wish to thank the Education Division of Indian Council of Agricultural Research for giving us an opportunity to organize this ICAR Winter School. We are grateful to Dr. A. Gopalakrishnan, Director, ICAR-Central Marine Fisheries Research Institute, for his guidance, continuous interest in the course and providing all necessary facilities. I am highly obliged to Dr. P. Vijayagopal, Head, Marine Biotechnology Division for his guidance and support for the programme. All the scientists of Marine Biotechnology Division, technical staff, supporting staff and research scholars supported us in organizing the ICAR Winter School. I recall with gratitude the marvellous effort and help in preparing this manual by Minju Joy, Research Scholar of Marine Biotechnology Division. I take this opportunity to thank all the faculty members who have devoted their valuable time and contributed material for the preparation of the manual. I am confident that the Course Manual would aid the participants to enhance their knowledge and competence in the area of marine bioactive compounds and their applications for the development of high-value products for health management.

Kajal Chakraborty
Course Director

January, 2018

CONTENTS

CHAPTER

04

CLASSIFICATION OF MARINE NATURAL PRODUCTS - CHEMISTRY AND BIOACTIVITY

Kajal Chakraborty, Soumya Salas, Minju Joy, Prima Francis, Subhajit Dhara, Tima Antony

Bioprospecting Section of Marine Biotechnology Division, ICAR-Central Marine Fisheries Research Institute, Kochi

Enthusiasm of the scientific community towards the discovery of new drug leads from marine habitats is unquenchable. Marine natural products have been recognized as a source of highly desirable chemodiversity to support drug discovery and pharmacology applications by the diverse chemical features and the wide range of biological activities possessed by them. According to the latest review by Blunt et a,l., 1378 new compounds were isolated from marine organisms in 2014 compared with 332 in 1984 (Blunt et al., 2016; Faulkner, 1994). Marine flora and fauna play a momentous role as a foundation of new molecular entity. Over 5 million species of the world in about 30 different phyla reside in the oceans. Because of the diversities of marine organism and habitats, marine natural products enfold a wide variety of chemical classes, including Terpenes, Shikimates, Polyketides, Acetogenins, Peptides, and Alkaloids of varying structures and multitude of compounds of mixed biosynthesis. These natural products are secondary metabolites and enhance survival fitness and may serve as chemical weapons used against bacteria, fungi, viruses and small or large animals. A good number of the natural products of interest to the pharmaceutical industry are secondary metabolites and several such compounds, derived from marine organisms, have been in clinical trials as experimental drugs. Over the past 50 years, numerous novel compounds have been isolated from marine flora and fauna having biological activities such as antibacterial, antiviral, antitumor, antiparasitic, anticoagulants, antimicrobial, anti-inflammatory and cardiovascular compounds.

BIODIVERSITY OF MARINE ENVIRONMENT - A SOURCE OF CHEMICAL DIVERSITY

Marine environment is a natural habitat for a broad variety of living organisms having different physiology and capacity to adapt their environment. Out of over 33 animal phyla known today, a total of 32 phyla are embodied in the marine environment out of which 15 varieties are exclusively present in the marine environment (Margulis and Schwartz, 1998). The marine environment provides different biosynthetic conditions to organisms living in it. Such genetic diversity renders chemical diversity which is promising for new drug development. Highly stressed oceanic conditions, including a combination of light and O2, was reported to trigger oxidative stress in marine species, including molluscs and seaweeds, generating the formation of ROS and other strong oxidizing agents (Dykens et al., 1992). However, absence of oxidative damage in structural components, their stability to quench toxic ROS suggest that their cells are equipped with a powerful anti-inflammatory and

antioxidant system (Jiménez-Escrig et al., 2001). Seaweed species collected from the coasts of Quintana Roo and Yucatan showed antioxidant and anti-inflammatory activities, suggesting that tropical macroalgae develop an effective antioxidant defense system due to strong UV radiation in tropical environment.

CHEMISTRY OF MARINE NATURAL PRODUCTS

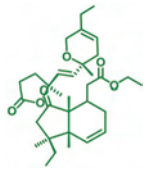
Marine natural products chemistry has passed through several phases of development. The collection of materials from deep seas has been made trouble-free by scuba diving. Many potent compounds have been provided in pure form by effective methods of isolation. Many intricate structural and stereochemical problems have been solved by advanced instrumentation methods such as nuclear magnetic resonance, mass spectrometric techniques and X-ray diffraction. The present section is a minor effort to throw light into readers regarding bioactive marine natural products. There is no inappropriate claim that a comprehensive coverage of all bioactive compounds has been made. Nevertheless efforts have been taken for not excluding any of the major class of bioactive compounds.

BIOACTIVE SECONDARY METABOLITES OF MARINE ORGANISMS

BIOACTIVE MARINE STEROLS

Sterols, also known as steroid alcohols, are a subgroup of steroids, which are ubiquitously found in marine invertebrates, plants, and fungi. Sterols from marine sources have been reported to possess diverse bioactivities, including antiproliferative, cytotoxic, anti-fouling, and farnesoid X-activated receptor antagonistic activities (Ioannou et al., 2009; Shin et al., 2012). 5a,8a-Epidioxysterols have been reported to possess growth inhibiting activity against human cancer cells from lung denocarcinoma, gastric carcinoma, promyelocytic leukemia, colon adenocarcinoma, myelogenous leukemia, nasopharyngeal carcinoma, breast adenocarcinoma, liver adenocarcinoma, and acute monocytic leukemia (Sheu et al., 2000; Gauvin et al., 2000; Takei et al., 2005; Pan et al., 2006). 5a,8a-epidioxysteroids (4.1a) with a double bond between C-24 and C-28 (a terminal vinyl group) isolated from a marine sponge of the genus Monanchora offer potential leads for the discovery of new antipancreatic cancer agents.

There has been a report of isolation of a phytosterol 24-propylidene-cholest-5-en-3–ol (4.1b) (24-branched Ä^5 sterols) from extract of red seaweed *L. papillosa* as antibacterial agent (Kavita et al., 2014). The sterols from marine algae are reported to be non-toxic and have the ability to reduce blood cholesterol level. They are also reported to reduce the tendency to form a fatty liver and excessive fat deposition in the heart (Heinemann et al., 1991). Sterols with unusual structural features such as 5,6-epoxy-(22E,24R)-3,11-dihydroxy-9,11-secoergosta-7-en-9-one (4.1c) was isolated from Gorgonian corals belonging to the genus *Pinnigorgia* which displayed inhibitory effects on the generation of superoxide anions



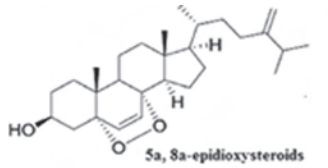
and the release of elastase by human neutrophils.

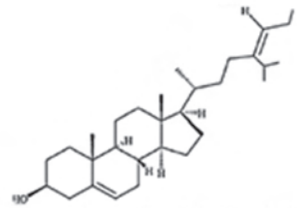
Asterosaponins are sterol derivatives of echinoderm and reported to have hemolytic, antineoplastic, cytotoxic, antitumor, antibacterial, antiviral antifungal and anti-inflammatory activities. (Leung and Stefano, 1983; Leung and Stefano,1984). The novel neuritogenic steroid glycosides Linckosides A and B (4.1d) were isolated from the Okinawan starfish *Linckia Laevigata*. It was reported that a novel sterol, which isolated from marine sponge *Actinomadura* sp. SBMs009, showed the translocation inhibition of NF-kB at the half maximal inhibitory concentration (IC_{50}) of 71μM. The inhibition of NF-kB translocation suggested that Bendigole F (4.1e) has potential anti-inflammation activity (Simmons et al., 2011).

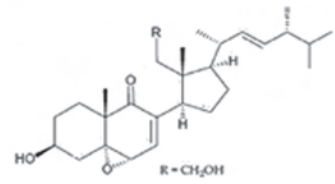
Sterol glycoside Carijoside A (4.1f) which isolated from octocorals belonging to genus *Carijoa* (=Telesto), had shown the anti-inflammatory activity, significant inhibition of superoxide anion generation (IC_{50}=1.8 μg/ml), and elastase release (IC_{50}=6.8 μg/ml) by human neutrophils (Liu et al., 2010). 3-Hydroxy-26-norcampest-5-en-25-oic acid (4.1g) from *Euryspongian* sp. was reported that it has effect on COX pathway and thus could be a potential anti-inflammatory natural compound derived from marine sponge (Mandeaua et al., 2005). It has been reported that isolated sterol from sponge *Dysidea arenaria*, 9a,11a-epoxycholest-7-ene-3b,5a,6a,19-tetrol-6-acetate (ECTA) (4.1h), showed the enhancement in antifungal activity when combined with fluconazole, one common antifungal drug. Fluconazole alone showed IC_{50}=300 μMon drug resistance *Candida albicans*; however, the IC_{50} of fluconazole is decreased to 8.5μM when combined with 3.8 mM of ECTA (Jacob et al., 2003). Sterols from the Red Sea marine sponge *Lamellodysidea herbacea*, cholesta-8,24-dien-3b,5a,6a-triol and cholesta-8(14),24-dien-3b,5a,6a-triol, also showed the antifungal activity against *Candida tropicalis* (Sauleau and Bourguet-Kondracki, 2005). The sulfated sterol ibisterol (4.1i) was isolated from the deep water Caribbean sponge *Topsentia sp*. that was investigated as anti-HIV agent (McKee et al., 1993). Sun et al., (1991) reported that weinbersterol disulfates A (4.1j) was isolated from the sponge *Petrosia weinbergi*, which is active *in vitro* against HIV. Clathsterol (4.1k) isolated from Red Sea sponge *Clathria* sp. inhibit HIV-1 reverse transcriptase activity at a concentration of 10 μM (Rudi et al., 2001). Studies on sterols isolated from marine sponge have shown that ibisterol sulfate from *Topsentia* sp., halistanol sulfate (4.1l) from *Halichondria* cf. moorei, and 26-methylhalistanol sulfate and 25-demethylhalistanol sulfate from *Pseudaxinyssa digitata* showed essentially complete protection against the cytopathic effects of HIV-1 infection at half maximal effective concentration (EC_{50}) of 13, 6, 3, and 6 mM, respectively. Two sterols 5a,6a-epoxy-24R-ethylcholest-8(14)-en-3b,7a-diol and 5a,6a-epoxy-24R-ethylcholest-8-en-3b,7a-diol purified from marine sponge *Polymastia tenax* showed antiproliferative activity toward lung (A549), colon (HT-29 and H-116), mice endothelial (MS-1), and human prostate carcinoma (PC-3) cell lines (Santafe´ et al., 2002). Orostanal (4.1m), purified sterol from marine sponge *Stellatta*

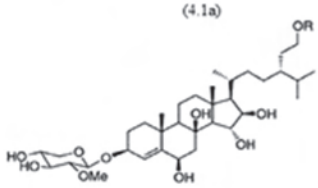
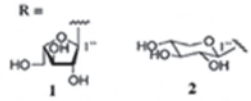
hiwasaensis inhibited proliferation in human leukemia (HL-60) cell line at IC_{50} 1.7 mM (Miyamoto et al., 2001).

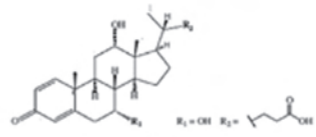
Lobophytosterol from soft coral *Lobophytum laevigatum* showed cytotoxicity effect on A549 and HL-60 with IC_{50} values of 4.5 and 5. mM, respectively. The new compounds 5b,6b-

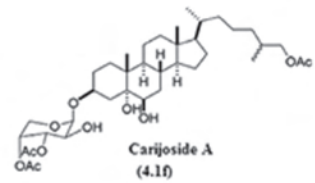

5a, 8a-epidioxysteroids
(4.1a)

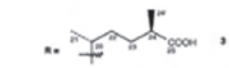
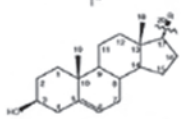

24-propylidene cholest-5-en-3-ol
(4.1b)

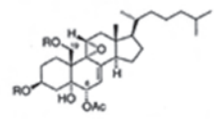

5,6-epoxy-(22E,24R)-3,11-dihydroxy-9,11-secoergosta-7-en-9-one (4.1c)

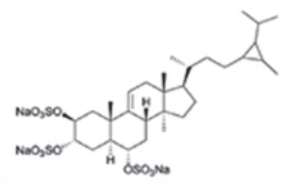


Linckoside A (1), B (2) (4.1d)

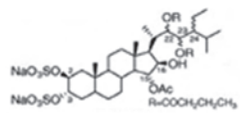

Bengidole F
(4.1e)

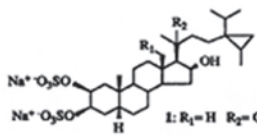

Carijoside A
(4.1f)

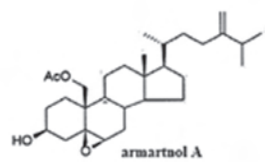


3-Hydroxy-26-norcampest-5-en-25-oic acid
(4.1g)

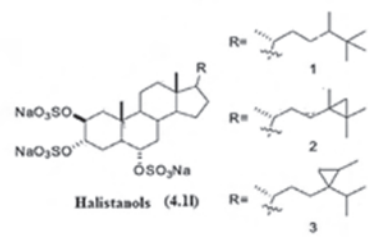

(ECTA) (4.1h)

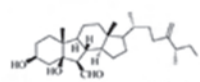

Ibisterol (4.1i)

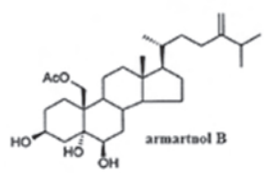

Clathsterol (4.1k)

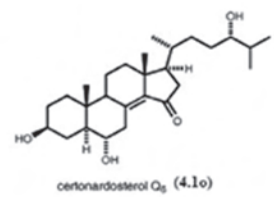

weinbersterol disulfates A(1) and B(2) (4.1j)

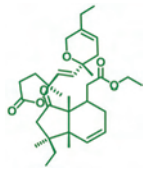

armartnol A


Halistanols (4.1l)


Orostanal (4.1m)


armartnol B
(4.1n)


certonardosterol G_5 (4.1o)



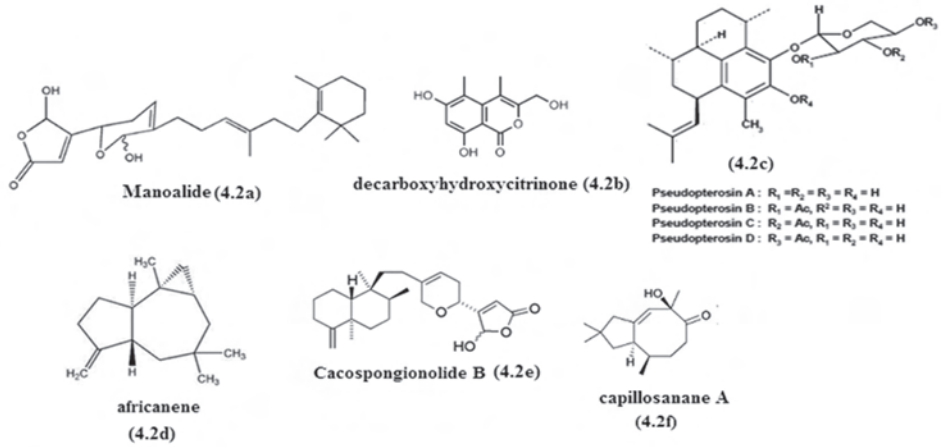
epoxyergost-24(28)-ene-3b,7b-diol, together with the known compound ergost-24(28)-ene-3a,5b,6b-triol, exhibited strong and selective cytotoxicity against the HT-29 cell line with an ED_{50}=0.1 µg/ml, and an ED_{50}=0.25 µg/ml for ergost-24(28)-ene-3b,5a,6b,7b-tetrol (Rueda et al., 2001). Purified armartnol A (4.1n) from soft coral *Nephthea armata* exhibited cytotoxicity against A549, HT-29, and P-388 (mouse lymphocytic leukemia) cell lines with IC_{50} value of 7.6, 6.5, and 6.1 mM, respectively. Moreover, armartnol B (4.1n) showed cytotoxicity against P-388 and HT-29 cells with IC_{50} values of 3.2 and 3.1 mM, respectively (El-Gamal et al., 2004). Wang et al., (2004) reported that certonardosterol Q6 (4.1o) from starfish *Certonardoa semiregularis* exhibited cytotoxicity effect on A549, SK-OV-3 (human ovarian cancer), SK-MEL-2 (human skin cancer), and HCT 15 cell line at ED_{50} values of 0.43, 0.22, 0.17, and 0.48 mg/ml, respectively. On the whole, sterols from marine resources have potential in development of anticancer agents.

BIOACTIVE TERPENOIDS

These compounds are derived from a five-carbon isoprene structure, and depending on the combination of units, can be subdivided into biogenetic classes such as monoterpenes, sesquiterpenes, diterpenes, sesterpenes, triterpenes (steroids), and tertraterpenes (carotenoids). The green algae of the genera *Halimeda, Penicillus* and *Udotea* are found to contain highly active but unstable sesquiterpenoids and diterpenoids. Some of these diterpenoids exhibit cytotoxic and antimicrobial activities (Paul, 1984; Tillekeratne, 1984). Manoalide (4.2a) is sesquiterpenoid isolated from the Indo-Pacific sponge *Luffariella variabilis*. Manoalide which contains an á,â-unsaturated ã-lactone function, had antiinflammatory activity and was found to be an inhibitor of phospholipaseA2 (Freita and Jacob,1984). Tsukada et al., (2011) isolated the decarboxyhydroxycitrinone (4.2b), a terpene, from the marine fungus *Arthrinium sacchari*, with antiangiogenic activity. The pseudopterosins (4.2c) are tricyclic diterpene glycosides isolated from the Caribbean Sea whip (gorgonian) *Pseudopterogorgia elisabethae* (Gorgoniidae). They are potent anti-inflammatory and analgesic agents and appear to inhibit eicosanoid biosynthesis by inhibition of both PLA2 and 5-lipoxygenase. The pseudopterosins have been licensed to a small pharmaceutical firm, OsteoArthritis Sciences Inc., for medical use as potential anti-inflammatory drugs. However, the pseudopterosins extract has found its way to the marketplace. It is used as an additive to prevent irritation caused by exposure to the sun or the chemicals in the Estée Lauder cosmetic skin care product, Resilience.

Sesquiterpene africanene (4.2d) isolated from the soft coral *Sinularia leptoclados* resulted in a more potent reduction of paw volume than that produced by 100 mg/kg body weight of ibuprofen, in carrageenan-induced rat edema assay. A novel sesterterpene Cacospongionolide B (4.2 e) isolated from the sponge *Fasciospongia cavernosa* was found to be an inhibitor of human synovial phospholipase A2. It irreversibly inhibited both secretory

PLA2 in vitro and group II secretory PLA2 *in vivo*. Sesquiterpenes capillosanane A (4.2f) isolated from *Sinularia capillosa* (Sanya Bay, Hainan Province, China) (Cheng et al., 2013) exhibited antifouling activity against *B. amphitrite*.



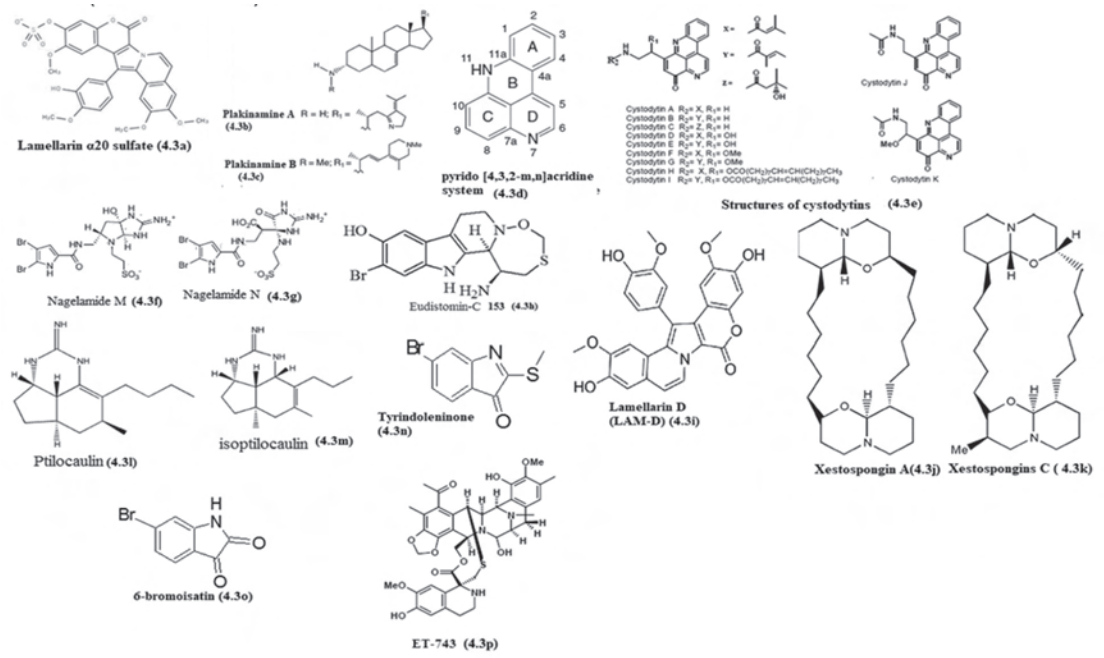
BIOACTIVE MARINE ALKALOID

Three alkaloids having an unusual decahydroquinoline skeleton and showing significant and selective antiplasmodial and antitrypanosomal activity were obtained from a new tunicate species of the genus *Didemnum* (Wright et al., 2002) These bioactive alkaloids may serve as lead structure for the development of new antimalarial drugs. Lamellarin á20 sulfate (4.3a), an ascidian alkaloid inhibited HIV-1 integrase of virus in cell culture (Reddy et al., 1999). Two steroidal alkaloids, plakinamine A (4.3b) and plakinamine B (4.3c) which could act as antimicrobial metabolites by inhibiting the growth of *Staphylococcus aureus* and *Candida albicans* were obtained from *Plakina* spp. of marine sponges (Roser and Faulkner, 1984). The compounds Pyridoacridines (4.3d) are alkaloids that have a common tetracyclic heteroaromatic parent-pyrido [4,3,2-m,n]acridine system. The yellow tunicate *Cystodytes dellechiajei* from Okinawa has yielded nine cytotoxic tetracyclic pyridoacridine alkaloids named cystodytins (4.3e) (Kobayashi et al., 1991).

Two new bromopyrrole alkaloids, nagelamides M (4.3f) and N(4.3g), which displayed antimicrobial activity have been isolated from an Okinawan marine sponge Agelas species (Kubota et al., 2008). The ascidian *Eudistoma olivaceum* is an extraordinary rich source of tryptophan derived alkaloids. Several alkaloids named eudistomins having a â-carboline system and significant antiviral activity had been isolated from this source (Rinehart, 1987). Significant antiviral activity was observed for a series of indole alkaloids, Eudistomin-C 153 (4.3h) from Tunicate *Eudistoma olivaceum* being most effective against Human Simplex

Virus. An alkaloid Lamellarin D (LAM-D) (4.3i) initially isolated from a prosobranch mollusk of the genus Lamellaria, exhibits cytotoxicity against many different tumors.

The Xestospongins (4.3j and 4.3k) (from Australian sponge *Xestospongia exigua*) (Orabi et al., 2002) represent a new class of macrocyclic alkaloid incorporating two 1-oxaquinolizidine rings and were reported as vasodilative compounds which induce relaxation of blood vessel *in vivo*. Ptilocaulin (4.3l) and isoptilocaulin (4.3m) isolated from *Ptilocaulistaff* and *P. spiculifer* (Cafieri et al., 1998) exhibit high order of antimicrobial activity against Grampositive and Gram-negative bacteria, and also inhibit cell growth against L 1210 leukemia cells. Tyrindoleninone (4.3n) and 6-bromoisatin (4.3o) are indole derivatives from marine mollusk *Dicathais orbita* that induces apoptosis in female reproductive cancer cell lines ovary, granulosa, and choriocarcinoma (OVCAR-3, KGN, Jar), respectively (Edward et al., 2012). Ecteinascidin-743 or ET-743 (4.3p) is a tetrahydroisoquinoline alkaloid derived from the colonial tunicate *Ecteinascidia turbinata*, a sea squirt that lives in clusters in the Caribbean and Mediterranean seas. Early on, the compound demonstrated very potent activity against a broad spectrum of tumor types in animal models (Reinhart, 2000).



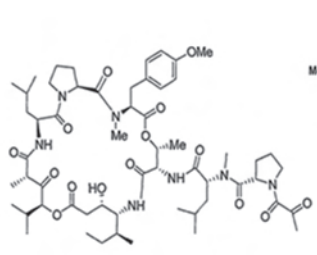
BIOACTIVE MARINE PEPTIDES

Aplidine (4.4a), a marine depsipeptide from Mediterranean tunicate *Aplidium albicans* was in phase-II clinical trial (Earbo et al., 2002). Aplidine in human Molt-4 leukaemia cells

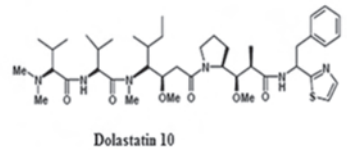
was found to be cytotoxic at nanomolar concentrations.

Dolastatin 10 (4.4b) is a linear peptide isolated from the sea hare *Dollabella auricularia* from the Indian Ocean and is a well known antitumour agent with ED50 = 0.046 ng/ml against P 388 cells. Dolastatin 15, a potent antineoplastic peptide from the mollusk *Dolabella auriculata* was undergoing clinical trials in Europe and North America (Hu et al., 1999)

Vitilevuamide (4.4c) a bicyclic peptide was isolated from marine ascidians *Didemnum cuculiferum* and *Polysyncranton lithostrotum*. The peptide was cytotoxic in several human tumor cell lines with IC50 values ranging from 6 to 311 nM. *Haliclona* sp. a marine sponge yielded **kendarimide A (4.4d)**, a novel peptide which reversed glycoprotein mediated multidrug resistance in tumor cells. Two new cyclic depsipeptide derivatives, Kahalalides R and S, together with two known congeners, **Kahalalides F (4.4e)** and D, were isolated from the Indian sacoglossan mollusk *Elysia grandifolia* (marine gastropod mollusks). The new derivative Kahalalide R was found to exert comparable or even higher cytotoxicity than the

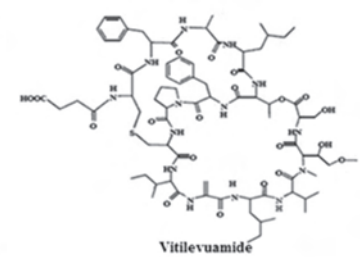


Aplidine (4.4a)

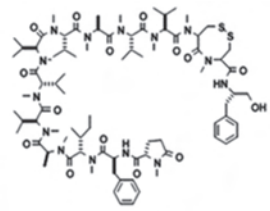


Dolastatin 10

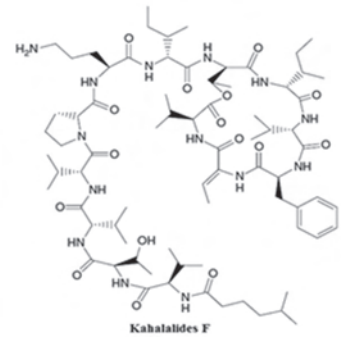
(4.4b)



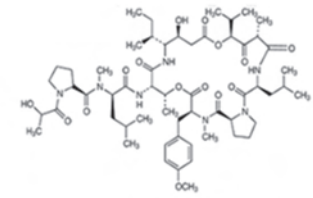
Vitilevuamide
(4.4c)



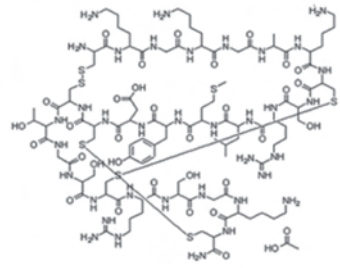
kendarimide A (4.4d)



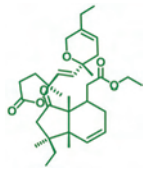
Kahalalides F



. Didemnin B (4.4f)



Ziconitide (4.4g)



potential drug candidate kahalalide F toward the MCF7 human mammary carcinoma cell line.

Didemnin B (4.4f) is one of a number of related depsipeptides isolated from the Caribbean tunicate *Tridemnum solidum* (Didemnidae). It was later found to display antineoplastic, antiviral and subsequently immunosuppresive activities (Rinehart 1988). A close relative of didemnin B- dehydrodidemnin B, isolated from a Mediterranean tunicate *Aplidium albicans*, is currently in Phase II studies in the United States and Europe, to determine its anticancer properties.

Ziconitide (4.4g) is a 25 aminoacid peptide from the venom of a predatory snail *Conus magnus*. It acts by binding to and inhibiting presynaptic calcium channels, thereby preventing neurotransmitter release. It is licensed by Elan Pharmaceuticals under the name Prialt.

POLYSACCHARIDES

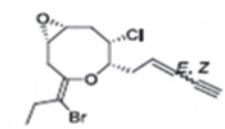
Sulfated polymannuroguluronate (SPMG), a marine sulfated polysaccharide entered in phase II clinical trial in China as the first AIDS drug (Miao et al., 2004). A new polysaccharide exhibiting anti-HIV activity and made up of galactan sulfate was isolated from the marine clam *Meretrix petechialis* (Amornrut et al., 1999). The antiviral activity observed in green algae *Codium elongatum* and the two species of *Hypnea* was attributed to the polysaccharides (Kamat et al., 1992). Carrageenans from *Chondrus crispus* is known to be antiviral. The activity has been reported to be due to the sulphated galactose unit of the phycocolloid. Algal polysaccharides such as laminarin, fucoidan, and algin, which lack sulfated galactose unit exhibit no antiviral activity.

BIOACTIVE ACETOGENINS

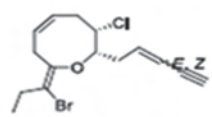
Acetogenins are a large cluster of nonterpenoid molecules that derive in the polyketide pathway (Dembitsky et al., 2003). They are relatively widespread in certain plant families, particularly Annonaceae, and are renowned for their biological activities. Acetogenins derived from annonaceous are larger molecules (C_{35} or C_{37}) bearing ether groups (Liaw et al., 2010) and in seaweeds, acetogenins are frequently halogenated, and are generally thought to originate from a common C_{15} precursor (Wang et al., 2013). Acetogenins are recognized as chemotaxonomic markers for marine algae (Kubanek, 2010). In spite of a few linear bioactive compounds, most algal C_{15} acetogenins are cyclic ether metabolites with different ring sizes and a conjugated enyne (C= C-C a" CH) or bromoallene (C= C=CHBr) terminus. Earlier alkyne groups were considered to be rare in the nature, but now, it is well identified that compounds with the acetylenic group are also common in seaweeds such as *Petrosia* sp ranging from C_{44} to C_{47} (Minto and Blacklock, 2008). Acetogenins are recognized as chemotaxonomic markers for red algae belonging to the family Rhodomelaceae, in particular,

to the genus *Laurencia* (Stout and Kubanek, 2010). The most common biological activities described for acetogenins include antibacterial activity towards different microorganisms.

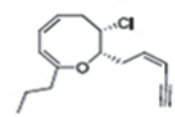
The first reported acetogenins from *Laurencia venusta* venustin A (4.6a) and B (4.6b) (Suzuki and Kurosawa, 1980) which were renamed as 3-E-epoxyvenustin and 3-E-venustin (Suzuki et al., 1983a), respectively. A related metabolite 3-Z-epoxyvenustin (4.6c), major component comprising 10% of the extract, and also 3-Z-venustin (4.6d) and 3-Z-venustinene (4.6e) were reported in Japan for a sample collected at Moura, near Asamushi, Aomori Prefecture(Suzuki et al., 1983c) Kaul et al., (2011) reported that the metabolites (4.6b)-(4.6e) prolonged sleep-time in mice induced by pentobarbitone. 3-Z-dactylyne an acetogenin of the hydropyran subclass, isolated from a *Laurencia* species from Japan (Suzuki et al., 1999) presented pronounced activity in increasing blood levels of pentobarbital in a dose-dependent manner. It increased both the half-life and the duration of action of the drug, possibly due to the inhibition of metabolic elimination of pentobarbital caused by 3-Z-dactylyne itself, or perhaps by a metabolite (Kaul and Kulkarni, 1978). Palaniveloo and Vairappan (2014) collected samples of *A.dactylomela* from different islands of Malaysia, and isolated 12-Z-lembyne A (4.6f), which was also reported for a non-identified *Laurencia* sp.



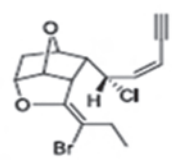
venustin A (4.6a) E
Venustin B (4.6b) Z



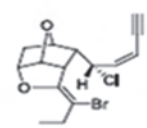
Venustin B (4.6b) E
3-Z-venustin (4.6d) Z



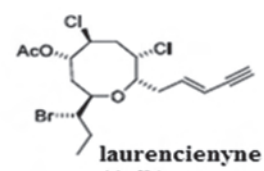
3-Z-venustinene
(4.6e)



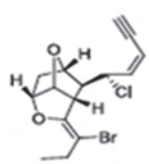
12-Z-lembyne A
(4.6f)



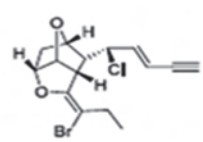
12-E-lembyne A
(4.6g)



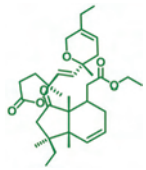
laurencienyne
(4.6h)



12-Z-cis-maneonene D
(4.6i)



12-E-cis-maneonene E
(4.6j)



from Malaysia (Vairappan et al., 2001a) and 12-E-lembyne A (4.6 g) which was also isolated from *L. mariannensis* from Japan (Vairappan et al., 2001b). Both presented prominent antibacterial activity against marine bacteria (Vairappan et al., 2001a, b). The isomer 3-Z-chlorofucin isolated from *L. elata* (Dias and Urban, 2011) showed antibacterial activity against *Chromobacterium violaceum* (Suzuki et al., 2001). Eight-membered cyclic ether acetogenins laurencienyne (4.6h), were reported from algae collected in Sicily, was active against *Bacillus subtilis* and *Escherichia coli* (Caccamese et al., 1980). *Laurencia obtusa* collected in the Saudi Arabia Red Sea Coastal Jeddah also afforded acetogenins from the maneonene class: 12-Z-cis-maneonene D (4.6i), 12-E-cis-maneonene E (4.6j), and 12-Z-trans-maneonene C(4.6k) compounds (4.6i) and (4.6j) inhibited apoptosis of blood neutrophils, suggesting that they may be involved in regulation of programmed death in the initiation and propagation of inflammatory responses (Ayyad et al., 2011).

BIOACTIVE POLYKETIDES

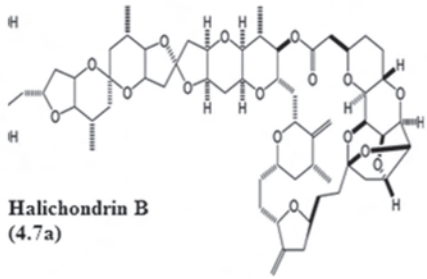
Marine polyketides are a class of natural compounds containing multiple â-hydroxyketone or â-hydroxyaldehyde (-$H_2C(=O)CH_2CH(OH)CH_2C(=O)$-) functional groups that present remarkable biological properties. Due to the flexibility of their biosynthetic production mechanism, these compounds exhibit remarkable diversity, in terms of structural complexity. The marine polyketide Halichondrin B (4.7a) was isolated for the first time from the sponge *Halichondria okadai* in 1986 along with other halichondrins and norhalichondrins (Hirata et al., 1986; Aicher et al., 1992). Halichondrins display low nanomolar cytotoxic activity; however, insufficient supply posed a problem for their further development.

Eribulin (4.7b) is an approved anticancer drug which is a synthetic derivative from Japanese marine sponge *Halichondria okadai*. It acts by interfering with the microtubular growth ultimately leading to apoptosis after prolonged mitotic blockage. Bryostatins (4.7c) are a family of polyketide compounds that shows remarkable in vitro and in vivo anticancer activity with no significant side effects (Hale et al. 2002). The first members of the bryostatin family were isolated by Pettit and co-workers in 1968 from the invertebrate bryozoans *Bugula neritina* and *Amathiaconvulata*. The didemnin family of polyketides was isolated from the Caribbean tunicate *Tridid*emmum *solidum* in 1981 (Rinehart et al., 1981). Didemnin B (4.7d) was the most potent analog of this series of compounds and entered clinical trials as an anticancer agent. Nevertheless, this compound showed cardiac and neuromuscular toxicities and the clinical process was put to halt. The family congener aplidine (4.7e) (plitidepsin) was later isolated from the Mediterranean tunicate *Aplidinium albicans* (Urdiales et al., 1996) which displayed a similar pharmacological profile but less toxicity, and replaced didenmin B in multiple phase I and phase II trials as an anticancer agent (Lee et al., 2012). Salinosporamide A (4.7f) (marizomib; NPI-0052) is ã-lactam-â-lactone isolated by Fenical,

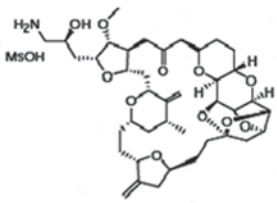
Jensen and co-workers from the bacteria *Salinosporatropica*, which are found in marine sediment collected in the Bahamas (Feling et al., 2003). It is a novel, potent proteasome inhibitor. It induces apoptosis by a caspase-8 dependent mechanism in multiple myeloma and leukemia cells. The spongistatins are a family of marine polyketide macrolides with highly complex structures and interesting anticancer properties. Among this family of natural products, Spongistatin 1 (4.7g) is the most interesting macrocycle because of its extraordinary biological activity. Spongistatin 1 was isolated by Pettit and co-workers from sponges *Spirastrella spinispirulifera* and *Hyrtios* (Pettit et al., 1993; Schwarzenberg et al., 2013). (+)-Discodermolide (4.7h) was isolated by Gunasekera and co-workers from the Bahamian deep water sponge *Discodermia dissolute* (Gunasekhara et al., 1990). The first biological activity investigation of (+)-Discodermolide was related to its strong immunosuppressive effect (De souza, 2004). It was later also recognized as a cytotoxic agent. (+)-Discodermolide is an antimitotic agent which produces disruption of cellular division by microtubule stabilisation. (+)-Discodermolide is often compared to Taxol® (Paclitaxel), a widely used drug in chemotherapy. Both drugs present a similar tubulin-binding mechanism; however, (+)-discodermolide has been described to be more potent and to have higher affinity than paclitaxel. The sponge *Lithoplocamialithistoides* from Madagascar was the source of two new polyketide compounds: PM060184 (4.7i) and its chloride derivative PM050489 (4.7j) isolated by PharmaMar. Currently PM060184 is undergoing phase I clinical trials in late stage cancer Patients (Pera et al., 2013).

Marine polyketides in the clinical pipeline.

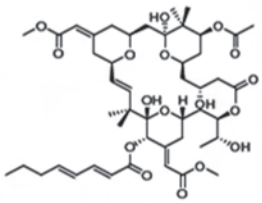
Compound	Structure	Target	Clinical status	Disease Area	Company
Eribulin	Macrocyclic PK	Microtubules	Approved (2010)	Cancer	Eisai Co., Ltd.
Bryostatin Institute	Macrocyclic PK	Protein kinase C	Phase I-II	Alzheimer	National Cancer
Aplidine	Macrocyclic mixed PK-NRP	Protein Kinases	Phase I-III	Cancer	PharmaMar S.A.
PM060184	Linear PK	Microtubules	Phase I	Cancer	PharmaMar S.A.
Salinosporamide A	γ-lactam-β-lactone mixed PK-NRP	20S proteasome	Phase I	Cancer	Triphase A.C.
Spongistatin	Macrocyclic PK	Tubulin	Preclinical	Cancer	National Cancer Institute
Discodermolide	Linear PK	Microtubules	Discontinued	Cancer	Novartis A.G.



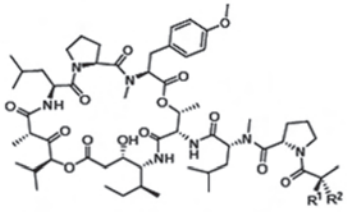
Halichondrin B
(4.7a)



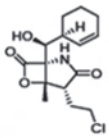
Eribulin (4.7b)



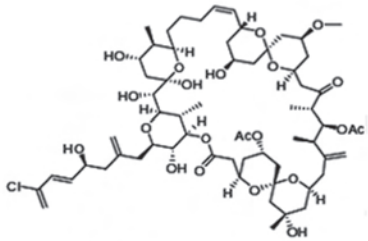
Bryostatins (4.7c)



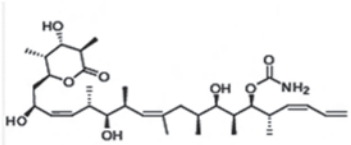
Didemnin B R1=H R2=OH (4.7d)
aplidine R1,R2=O (4.7e)



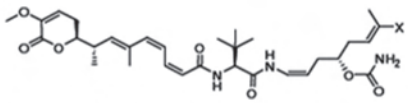
Salinosporamide
A (4.7f)



Spongistatin 1 (4.7g)



Discodermolide (4.7h)



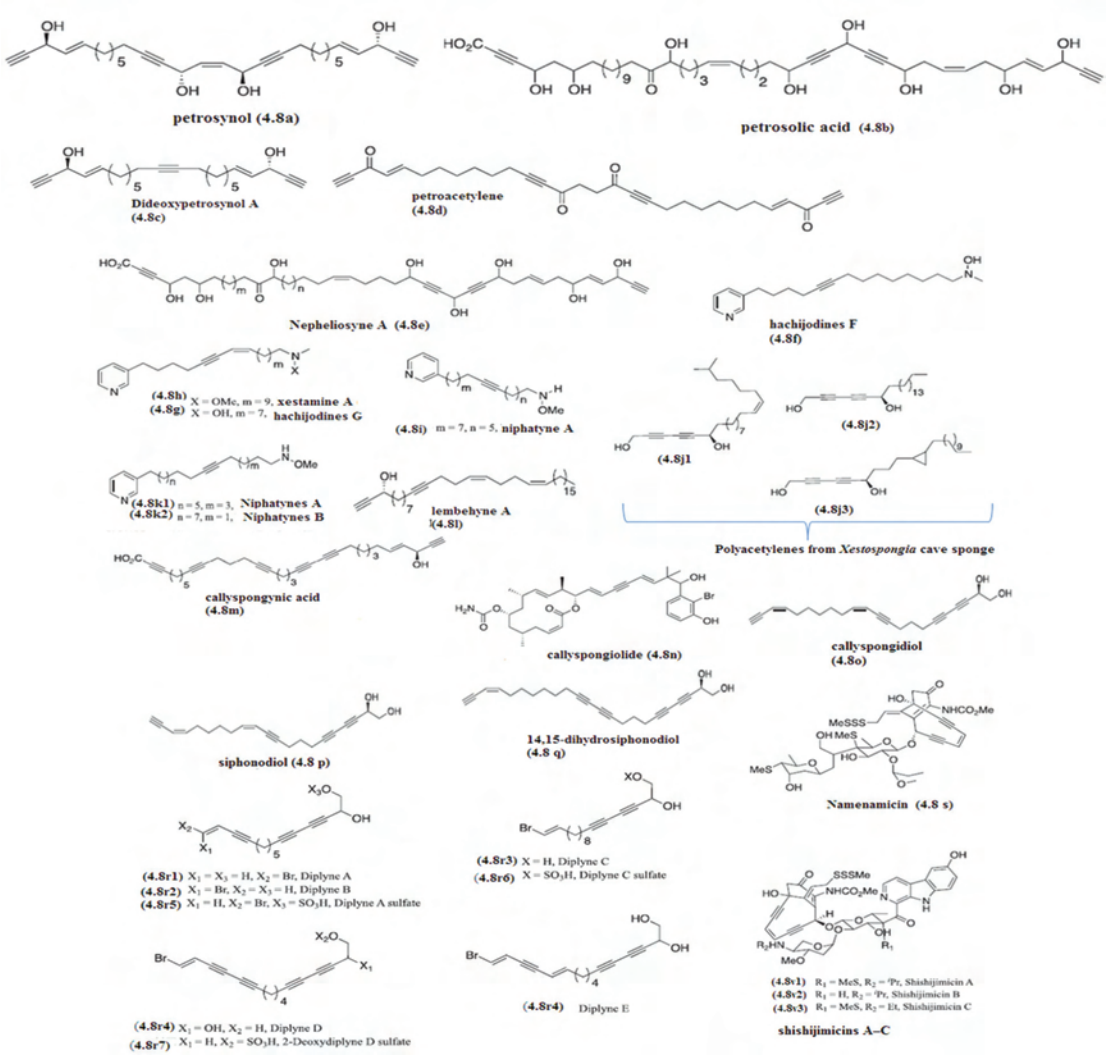
PM060184 X=H (4.7i)
PM050489 X=Cl (4.7j)

Polyacetylenes

Acetylene compounds or "polyacetylenes" are a general name for a substantial class of natural products,2 of which all contain one or more carbon"carbon triple bond functionalities in their molecules. Several polyacetylenes exhibiting significant selective cytotoxicity against human tumor cell lines were isolated from the marine sponge *Petrosia* sp. (Kim et al., 2002). Marine organisms are a great source of polyacetylenes. Among marine organisms, sponges are the main source of polyacetylenic compounds. Several polyacetylenes exhibiting significant selective cytotoxicity against human tumor cell lines were isolated from the marine sponge *Petrosia* sp. (Kim et al., 2002). The symmetric metabolite petrosynol (4.8a) and petrosolic acid (4.8b) were isolated from a Red Sea *Petrosia sp*. by Isaac et al., (1993). Petrosolic acid is notable because C44 carboxylic acids are relatively rare in natural products chemistry. Petrosynol inhibited the cell division of sea urchin eggs and petrosolic acid inhibited DNA polymerase activity of HIV reverse transcriptase (Isaac et al., 1993). Dideoxypetrosynol A

(4.8c), obtained from the marine sponge *Petrosia* sp., shows significant selective cytotoxic activity against several human cancer cell lines (Park et al., 2007). The petroacetylene (4.8d), a C30 linear polyacetylene isolated from the Japanese marine sponge *Petrosia solida*, inhibited blastulation of starfish embryos at a concentration of 3.1 ìg/mL or greater (Ohta et al., 2013). Nepheliosyne A (4.8e) is the first polyacetylene isolated from Xestospongia sp. by Kobayashi et al., (1994) is one of the longest marine acetylenic acids with a C47 chain length (Fig. 13). Nepheliosyne A exhibited a weak in vitro cytotoxicity against L-1210 lymphoma and human epidermal carcinoma KB cells (IC50 > 20 ìg/mL) (Kobayashi et al., 1994). The acetylenic 3-alkylpyridines hachijodines F (4.8f) and G (4.8g), xestamine A (4.8h), and niphatyne A (4.8i) were found in *Xestospongia* and *Amphimedon* sponges (Fig. 15) (Sakemi et al., 1990; Quinoa and Crews, 1987; Tsukamota et al., 2000). They bear long alkyl chains terminated by N-methoxy-N-methylamino or N-methylhydroxylamino substituents. The hachijodines were found to have cytotoxicity against P388 murine leukemia cells (1.0 ìg/mL). Micronesian *Xestospongia* cave sponge was the source of three polyacety-lenes (4.8j1-4.8j3) with low ìM activity against *Pseudomonas aeruginosa* (Ankisetty, 2012). Niphatynes A and B (4.8k1, 4.8k2) were the first metabolites with the 3-alkylpyridine skeleton isolated from marine sponges. Niphatyne A exhibited a cytotoxic activity against leukemic cells P388 (IC_{50}=0.5 ìg/mL) (Quinoa and Crews, 1987) (Fig. 17). The linear C36 diacetylenic alcohol, lembehyne A (4.8l), was isolated from an Indonesian *Haliclona* sp. (Aoki et al., 2001). Lembehyne A induced neurite outgrowth of rat pheochromocytoma PC12 cells at a minimal concentration of 2 mg/mL and induced neuritogenesis in mouse neuroblastoma Neuro 2A cells at 0.1 mg/mL. Isolated from *Callyspongia truncata*, callyspongynic acid (4.8m) contains the á,â-acetylenic acid and the 4-en-1-yn-3-ol termini. Callyspongynic acid is a specific inhibitor of á-glycosidase (IC50 0.25 µg/mL) (Fig. 23). A macrolide, callyspongiolide (4.8n), was isolated from the marine sponge *Cal-lyspongia* sp. collected in Indonesia (Pham et al., 2014).

Callyspongiolide showed strong cytotoxicity against human Jurkat J16 T and Ramos B lymphocytes, with IC_{50} values of 70 and 60 nM, respectively, after 48 h of treatment. The EtOAc extract of the sponge *Callyspongia* sp. showed significant activ-ity against human promyelocytic leukemia cells (HL-60). Further bioassay-guided fractionation of the EtOAc extract led to the isolation of three polyacetylene metab-olites: callyspongidiol (4.8o) (28A) (Umeyama et al., 2010), siphonodiol (4.8 p) and 14,15-dihydrosiphono-diol (4.8 q) (Masao et al., 2008). They exhibited antiproliferative activity against HL-60 with IC_{50} values of 6.5, 2.8, and 6.5 µg/mL, respectively. HIV-1 integrase inhibition assay used in the bioguided purification of *Diplastrella* spp. extracts allowed the identification of diplynes A-E (4.8r1-4.8r4) , jointly purified with diplyne A 1-sulfate (4.8r5) , diplyne C 1-sulfate (4.8r6), and deoxydiplyne D sulphate (4.8r7); only sulfated compounds were bioactive (Lerch et al., 2003).

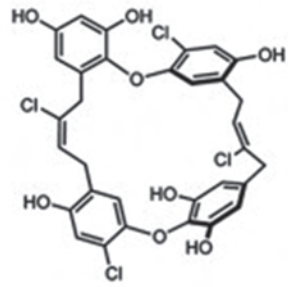


Namenamicin (4.8 u) (McDonald et al.,1996) and shishijimicins A-C (4.8v1-4.8v3) (Oku et al., 2003) showed interesting cytotoxic properties have been isolated from the ascid-ians *Polysyncraton lithostrotum* and *Didemnum proliferum*, respectively.

SHIKIMATES

Shikimates are metabolite derivatives from the shikimate pathway, produced by bacteria, fungi, plants, and some protozoaroans for the biosynthesis of some aromatic amino acids, such as L-phenylalanine, L-tyrosine, and L-tryptophan. This pathway is not present in animals, and the shikimate-derived aromatic ami-no acids have been obtained through diet.

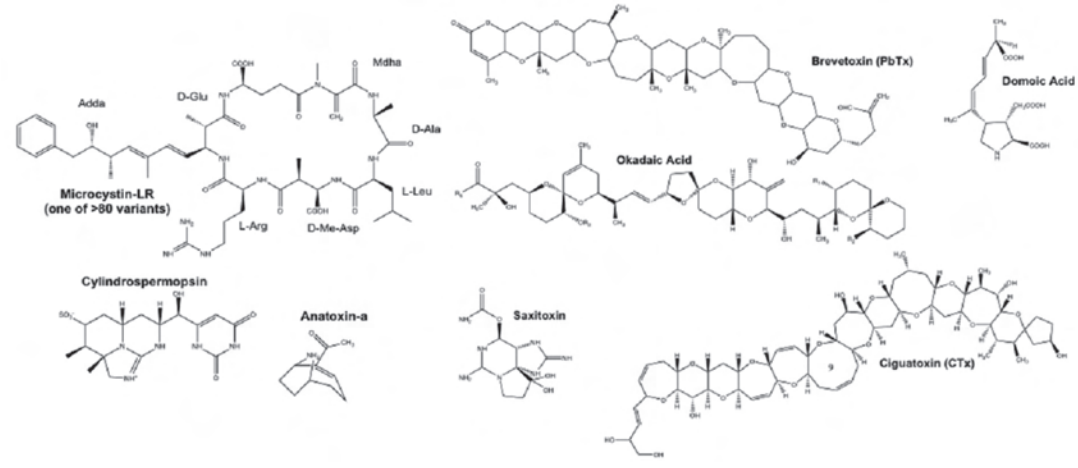
Chrysophaentin A was isolated by Plaza et al., (2010) from the marine chrysophyte alga *Chrysophaeum taylori* and tested against several multiresistant bacteria, including methicilin-resistant *Staphylococcus aureus*, multidrug resistant *Staphylococcus aureus*, and vancomycin-resistant *Enteroccocus faecium*.

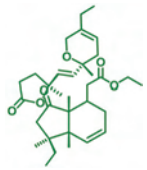


chrysophaentin A

BIOACTIVE MARINE TOXINS

The chemistry of the toxins is imperative to devise proper counter measures, such as detection, determination and therapeutic methods. It is important to understand their mechanism of action at molecular level. Many of the toxins have been found to be useful tools for probing biological or pharmacological phenomena, such as use of tetrodotoxin in sodium channel studies,1 and okadaic acid in protein phosphatase studies (Bialojan and Takai, 1988). A team from the University of Melbourne has extracted the conotoxin from a cone-shell snail which not only inhibits pain as being 10,000 times more powerful than morphine, but also accelerates the recovery of injured nerves (Holmes, 2002). Aplysiatoxins and oscillatoxins isolated from blue-green algae *Schizothrix calcicola* and *Oscillatoria nigroviridis* possess antileukaemic activity but their high toxicity precludes their medicinal use. Anatoxin-a, an exogenic toxin of blue-green alga *Anabaena flosaquae* (Stjerulof et al., 1989) is one of the most potent nicotinic receptor agonist. Saxitoxin isolated from algae *Gonyaulax catenella* blocks nerve conduction by specifically interfering with the initial increase in sodium permeability of the membrane. The symptoms caused by the toxin include peripheral paralysis. The saxitoxin compound isolated from algae have biomedical application, with the neurotropic effect of saxitoxin use for important drugs formation. Domoic acid also produced from algae have different pharmaceutical uses.





Ciguatoxin and maitotoxin both groups of toxins are produced by the epiphytic dinoflagellate *Gambierdiscus toxicus*.

CONCLUSION

Marine natural products demonstrate compelling bioactivities and outstanding pharmacological properties that make them well suitable for medical use. However their complexities makes their supply difficult, along with their structural and stereochemical assignment, which further decelerates their development as drug leads. Nowadays, the main counter to overcome supply issues are either aquaculture of the producing organism or the application of synthetic or semi synthetic methodologies to produce them in large enough scale quantities. On the other hand, genetic engineering is starting to offer an alternative for the production of certain metabolites. Aquaculture of marine invertebrates can be used as supply route by finding the appropriate aquaculture conditions. This is the most straightforward way to obtain gram quantities of a drug candidate in a short period of time, but the fact that production is highly dependent on the external conditions and that this can alter the production of some metabolites should be considered. Moreover, aquaculture usually has an important environmental cost, as farms may have to be located in oceans and the host.

SUGGESTED READINGS

Aguilera, J., Bischof, K., Karsten, U., Hanelt, D., Wiencke, C. 2002. Seasonal variation in ecophysiological patterns in macroalgae from an Arctic fjord. II. Pigment accumulation and biochemical defense systems against light stress. Marine Biology, 140,1087-1095.

Aicher TD, Buszek KR, Fang FG, Forsyth CJ, Jung SH, Kishi Y. 1992. Total synthesis of halichondrin B and norhalichondrin B. Journal of the American Chemical Society, 114, 3162-3164.

Amornrut, C., Toida, T., Imanari, T., Woo ER, Park, H., Linhardt, R., Wu SJ., Kim YS. 1999. Carbohydrate Research, 321, 121.

Ankisetty, S., Slattery, M. (2012). Antibacterial Secondary Metabolites from the Cave Sponge Xestospongia sp. Marine Drugs, 10, 1037-1043.

Aoki, S., Matsui, K., Takata, T., Hong, W., Kobayashi, M. 2001. Lembehyne A, a spongean polyacetylene, induces neuronal differentiation in neuroblastoma cell. Biochemical and Biophysical Research Communications, 289, 558-563.

Ayyad SEN, Angawy, R., Alarif WM, Saqer EA, Badria FA. 2014. Journal of Biosciences, 69 (3/4), 117-123.

Bialojan, C., Takai, A. 1998. Bendigoles D-F, bioactive sterols from the marine sponge-derived Actinomadura sp. Biochemical Journal, 256- 283.

Blunt JW, Copp BR, Keyzers RA, Munro MHG, Prinsep MR. 2016. Marine natural products. Natural Product Reports, 33(3), 382-431.

Caccamese, S., Azzolina, R., Duesler EN, Paul IC, Rinehart KL. 1980. Laurencienyne, a new acetylenic cyclic ether from the marine red alga Laurencia obtusa.Tetrahedron Letters, 21, 2299-2302.

Cafieri, F., Fattorusso, E., Taglialatela-Scafati. 1998. Journal of Natural Products, 61, 122.

De Freitas JC., Jacobs RS. 1984**.** Federation of American Societies for Experimental Biology, 43, 374.

De Souza MV. (+)-discodermolide: a marine natural product against cancer. 2004. Scientific World Journal, 4, 415-436.

Dykens JA, Shick JM, Benoit, C., Buettner GR, Winston GW. 1992. Oxygen radical production in the sea anemone Anthopleura elegantissima and its endosymbiotic algae. Journal of Experimental Biology, 168, 219-241.

Earbo, E., Bassano, L., Di Liberti, G., Muradore, J., Chiorino, G., Ubezio, P., Vignati, S., Codegoni, A., Desiderio MA, Faircloth, B. 2002. British Journal of Cancer, 86, 1510.

Edwards, V., Benkendorff, K., Young,F. 2012. Marine Compounds Selectively Induce Apoptosis in Female Reproductive Cancer Cells but Not in Primary-Derived Human Reproductive Granulosa Cells. Marine Drugs, 10, 64-83.

El-Gamal AAH, Wang SK, Dai CF, Duh CY. 2004. New nardosinanes and 19-oxygenated ergosterols from the soft coral Nephthea armata collected in Taiwan. Journal of Natural Products, 67, 1455-1458.

Faulkne DJ. 1984. Marine natural products: metabolites of marine invertebrates. Natural Product Reports, 1(6), 551-598.

Feling RH, Buchanan GO, Mincer TJ, Kauffman CA, Jensen PR, Fenical W. 2003. Salinosporamide A: a highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus salinospora. Angewandte Chemie International Edition, 42, 355-357.

Gauvin, A., Smadja, J., Aknin, M., Faure, R., Gaydou EM. 2000. Isolation of bioactive 5a,8a-epidioxy sterols from the marine sponge Luffariella cf. variabilis. Canadian Journal of Chemistry, 78, 986-992.

Gunasekera SP, Gunasekera M., Longley RE, Pierce F., Schulte GK. 1990. Discodermolide: A New Bioactive Polyhydroxylated Lactone from the Marine Sponge Discodermia dissoluta. Journal of Organic Chemistry, 55, 4912-4915.

Heinemann T., Kullak-Ublick GA, Pietruck B., Von Bergmann K. 1991. Mechanisms of actionof plant sterols on inhibition of cholesterol absorption. European Journal of Clinical Pharmacology, 40, 59-63.

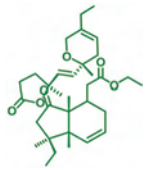
Hirata Y., Uemura D. 1986. Halichondrins - antitumor polyether macrolides from a marine sponge. Pure and Applied Chemistry, 58, 701-710.

Hu MK, Huang W.S. 1999. Peptide Research, 54, 460.

Ioannou, E., Abdel-Razik, M., Zervou, D., Christofidis, X., Alexi, C., Vagias MN, Alexis, Roussis. 2009. 5a,8a-Epidioxysterols from the gorgonian Eunicella cavolini and the ascidian Trididemnum inarmatum: Isolation and evaluation of their antiproliferative activity. Steroids, 74, 73-80.

Jacob MR, Hossain CF, Mohammed KA, Smillie TJ, Clark AM, Walker LA, Jime´nez-Escrig, A., Jime´nez-Jime´nez, I., Pulido, R., Saura-Calixto, F. 2001. Antioxidant activity of fresh and processed edible seaweeds. Journal of the Science of Food and Agriculture, 81, 530-534.

Kamat SY, Wahidulla, S., D'Souza, L., Naik CG, Ambiye, V., Bhakuni DS, Goel AK, Garg HS, Srimal RC. 1992. Botanica Marina, 35, 161.



Kavitaa, K., Singha VK, Jhaa, B. 2014. 24-Branched 5 sterols from Laurencia papillosa red seaweed withantibacterial activity against human pathogenic bacteria. Microbiological Research, 169, 301- 306.

Kim DK, Lee MY, Lee HS, Lee DS, Lee JR, Lee BJ, Jung JH. 2002. Cancer Letters, 185, 95.

Kobayashi, J., Naitoh, K., Ishida, K., Shigemori, H., Ishibashi, M. 1994. Journal of Natural Products, 57, 1300-1303.

Kobayashi, J., Tsuda, M., Tanabe, A., Ishibashi, M., Cheng JF, Yamamura, S., Sasaki, T. 1991. Journal of Natural Products, 54, 1634.

Lee, J., Currano JN, Carroll PJ, Joullié MM. 2012. Didemnins, tamandarins and related natural products. Natural Product Reports, 29, 404-424.

Lerch ML, Harper MK, Faulkner DJ. 2003. Journal of Natural Products, 66, 667-670.

Leung, M., Stefano GB. 1983. Life Sciences, 33, 77.

Leung, M., Stefano GB. 1984. Proceedings of the National Academy of Sciences, 81, 955.

Mandeaua, A., Debitus, C., Aries MF, David, B. 2005. Isolation and absolute configuration of new bioactive marine steroids from Euryspongian sp. Steroids, 70, 873-878.

Masao, T., Akemi, U., Noboru, S., Toshihiro, H. 2008. Immunopharmacology and Immunotoxicology, 30 (2), 425-435.

McDonald LA, Capson TL, Krishnamurthy, G., Ding WD, Ellestad GA, Bernan VS, Maiese WM, Lassota, P., Discafani, C., Kramer RA, Ireland CM. 1996. Journal of the American Chemical Society, 118, 10898-10899.

McKee TC, Cardellina JH, Tischler, M., Snader KM, Boyd MR. 1993. Ibisterol sulfate, a novel HIV-inhibitory sulfated sterol from the deep water sponge Topsentia sp. Tetrahedron Lett. 34, 389-392.

Miao, B., Geng, M., Li, J., Li, F., Chen, H., Guan, H., Ding. 2004. Journal of Biochemical and Pharmacological Research, 68, 641.

Miyamoto, T., Kodama, K., Aramaki, Y., Higuchia, R., Van Soest RWM. 2001. Orostanal, a novel abeo-sterol inducing apoptosis in leukemia cell from a marine sponge, Stelletta hiwasaensis. Tetrahedron Letters, 42, 6349-6351.

Ohta, S., Ogawa, T., Ohta, E., Ikeuchi, T., Kamemura, K., Ikegami, S. 2013. Natural Product Research, 27(20), 1842-1847.

Orabi KY, El Sayed KA, Hamann MT, Dunbar DC, Al-Said MS, Higa,T., Kelly, M. 2002. Journal of Natural Products, 65, 1782.

Pan MH, Huang YT, Chang CI, Ho CT, Pan BS. 2006. Apoptotic-inducing epidioxysterols identified in hard clam (Meretrix lusoria). Food Chemistry, 102, 788-795.

Park, C., Jung JH, Kim ND, Choi YH. 2007. International Journal of Oncology, 30 (1), 291-298.

Paul VJ, Fenical, W. 1984**.** Tetrahedron, 40, 3053.

Pera, B., Barasoain, I., Pantazopoulou, A., Canales, A., Matesanz, R., Rodriguez-salarichs, J. 2013. New Interfacial Microtubule Inhibitors of Marine Origin, PM050489/ PM060184, with Potent Antitumor Activity and a Distinct Mechanism. Journal of Chemical Biology, 8, 2084-2094.

Prinsep MR, Yao B, Nicholson BK, Gordon DP. 2004. The pterocellins, bioactive alkaloids from the marine bryozoan Pterocella vesiculosa. Phytochemistry Reviews, 3, 325-331.

Qi, J., Ojika, M., Sakagami, Y. 2002. Bioorganic & Medicinal Chemistry Letters, 10, 1961.

Quinoa, E., Crews, P. 1987. Tetrahedron Letters, 28 (22), 2467-2468.

Reddy MV, Rao MR, Rhodes, D., Hansen MS, Rubins, K., Bushman FD, Venkateswarlu, Y., Faulkner DJ. 1999, Journal of Medicinal Chemistry, 42, 1901.

Rinehart KL, Gloer JB, Cook JC, Mizsak SA, Scahill TA. 1981. Structures of the didemnins, antiviral and cytotoxic depsipeptides from a Caribbean tunicate. Journal of the American Chemical Society, 103, 1857-1859.

Rinehart KL, Kobayashi, J., Harbour GC, Gilmore, J., Mascal, M., Holt TG, Shield LS, Lafargue, F. 1987. Journal of the American Chemical Society, 109, 1378

Rinehart KL, Kishore, V., Bible KC, Sakai, R., Sullins DW, Li KM. 1988. Didemnins and Tunichlorin: Novel Natural Products from the Marine Tunicate Trididemnum solidum. Journal of Natural Products, 51, 1-21.

Rinehart KL. 2000. Antitumor Compounds from Tunicates. Medicinal Research Reviews, 20, 1-27.

Rosser RM, Faulkner DJ. 1984. Journal of Organic Chemistry, 49, 5157.

Rudi, A., Yosief, T., Loya, S., Hizi, A., Schleyer, M., Kashman, Y. 2001. Clathsterol, a novel anti-HIV-1 RT sulfated sterol from the sponge Clathria species. Journal of Natural Products, 64, 1451-1453.

Rueda, A., Zubý´a, E., Ortega MJ, Salva, J. 2001. Structure and cytotoxicity of new polyhydroxylated sterols from the Caribbean gorgonian Plexaurella grisea. Steroids, 66, 897-904.

Sakemi, S., Totton LE, Sun HH. 1990. Journal of Natural Products, 53, 995-999.

Sheu JH, Chang KC, Duh CY. 2000. A cytotoxic 5a,8a epidioxysterol from a soft coral Sinularia species. Journal of Natural Products, 63, 149-151.

Shin KJ, Chin, D., Hahn, J., Lee, H., Hwang DH, Won, J., Ham, H., Choi ES, Kang, H., Kim MK, Ju SJ, Nam, Kang, H. 2012. Sterols from a soft coral, Dendronephthya gigantean as farnesoid X-activated receptor antagonists. Steroids, 77, 355-359.

Simmons, L., Kaufmann, K., Garcia, R., Schwa¨r, G., Huch, V., Mu¨ ller, R. 2011. Sterols from the Colombian marine sponge Polymastia tenax. Journal of Natural Products, 65, 1161-1164.

Stjerulof, P., Trogen, L., Aderson, A., 1989. Acta Chem. Scand., Ser. B., 43, 917.

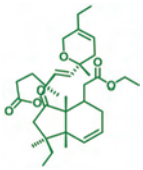
Stout EP, Kubanek, J., 2010. Marine macroalgal natural products. In: Moore, B.,Crews, P. (Eds.), Comprehensive Natural Products. II: Chemistry and Biology.Elsevier, Kidlington, 41-65.

Sun HH, Gross SS, Gunasekera, M., Koehn FE. 1991. Weinbersterol disulfates A and B, antiviral steroid sulfates from the sponge Petrosia weinbergi. Tetrahedron, 47, 1185-1190.

Suzuki, T., Koizumi, K., Suzuki, M., Kurosawa, E. 1983a. Kumausallene, a new bro-moallene from the marine red alga Laurencia nipponica Yamada. Chemistry Letters, 12, 1639-1642.

Takaaki Kubota, Atsushi Araki, Junji Ito, Yuzuru Mikami, Jane Fromont, Jun'ichi Kobayashi. 2008. Nagelamides M and N, new bromopyrrole alkaloids from sponge Agelas species. Tetrahedron, 64, 10810-10813.

Takei, T., Yoshida, M., Ohnishi-Kameyama, M. Kobori. M. 2005. Ergosterol peroxide, an apoptosis-inducing component isolated from Sarcodon aspratus (Berk.) S. Ito. Bioscience, Biotechnology, and Biochemistry, 69, 212-215.



Tsukada, M., Fukai, M., Miki, K., Shiraishi, T., Suzuki, T., Nishio, K., Sugita, T., Ishino, M., Kinoshita, K., Takahashi, K., Shiro, M., Koyama, K. 2011. Journal of Natural Products, 74, 1645-1649.

Tsukamoto, S., Takahashi, M., Matsunaga, S., Fusetani, N., Van Soest RWM. 2000. Journal of Natural Products, 63, 682-684.

Umeyama, A., Matsuoka, N., Mine, R., Nakata, A., Arimoto, E., Matsui, M., Shoji, N., Arihara, S., Takei, M., Hashimoto, T. 2010. Journal of Natural Medicines, 64, 93-97.

Wang WH, Jang HJ, Hong JK, Lee CO, Im KS, Bae SJ, Jung JH. 2004. Additional cytotoxic sterols and saponins from the starfish Certonardoa semiregularis. Journal of Natural Products, 67, 1654-1660.

Wright AD, Goclik, E., Konig GM, Kaminsky, R. 2002. Journal of Medicinal Chemistry, 45, 3067.

Yu-Chia Chang, Tsong-Long Hwang , Chih-Hua Chao, Ping-Jyun Sung. 2017. New Marine Sterols from a Gorgonian Pinnigorgia sp. Molecules, 22, 393.

Inauguration of winter school 2018 by Padma Bhushan Dr. Manju Sharma

Photo with Dr. K. Gopakumar, Formerly DDG ICAR (Fy)

Field visit to India Sea Foods

Field visit to BOS Naturals

Field visit to Accelerated Freeze Drying Co. Ltd

Photo with Dr. Meledath Govindan

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