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Acetylenic Aquatic Anticancer Agents and Related Compounds†

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Although acetylenes are common as components of terrestrial plants, it is only within the last 30 years that biologically active polyacetylenes having unusual structural features have been reported from aquatic organisms: cyanobacteria, algae, fungi, invertebrates, and other sources. Naturally occurring aquatic acetylenes are of particular interest since many of them display important biological activities and possess antitumor, antibacterial, antimicrobial, antifouling, antifungal, pesticidal, phototoxic, HIV inhibitory, and immuno-suppressive properties. There is no doubt that they are of great interest, especially for the medicinal and/or pharmaceutical industries. This review presents structures and describes cytotoxic and anticancer activities of more than 230 acetylenic metabolites isolated from aquatic organisms. With the computer program PASS some additional biological activities are also predicted, which point toward possible new applications of these compounds. This review emphasizes the role of aquatic acetylenic compounds as an important source of leads for drug discovery.

Keywords: Acetylenic, polyacetylenes, cyanobacteria, aquatic, cytotoxic, anticancer, predicted, activity, metabolites, fatty acids, alcohols, sterols, carotenoids, alkaloids, glycerols, lipids, sesquiterpens, polyethers, acetogenins, sponges, algae, fish.

In the past several decades, natural acetylenic compounds have been isolated from a wide variety of macro- and microalgal species, freshwater and marine cyanobacteria, and other aquatic organisms. Extensive pharmacological screening performed on aquatic species resulted in the discovery of novel antitumor agents [2-4]. The purpose of this review is to summarize antitumor and cytotoxic properties of 236 aquatic acetylenic natural products, belonging to diverse structural classes, including aliphatic and cyclic polyketides, terpenes, steroids, carotenoids and peptides. The species yielding these bioactive compounds comprise a taxonomically diverse group of aquatic organisms [5].

Naturally occurring metabolites possessing an acetylenic unit, as well as polyacetylenes, are of particular interest as many of them display important biological activities, namely antitumor, antibacterial, antimicrobial, antifungal, and others [6-8]. Their structure and biological activities, modes of action, and future prospects are discussed.

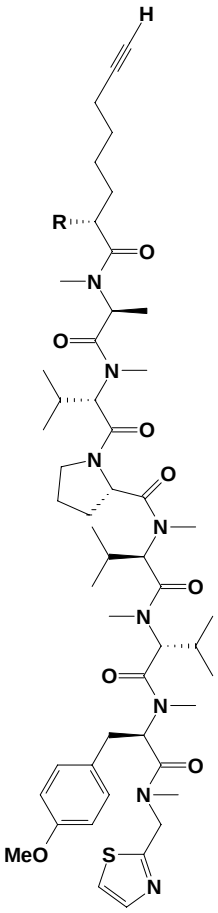
†for part 1 of Acetylenic terrestrial anticancer agents, see Ref. 1

Cyanobacterial Metabolites

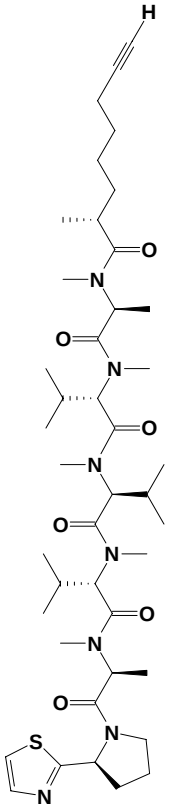
Cyanobacteria (unicellular species of blue-green algae) belong to a diverse group of Gram-negative photosynthetic prokaryotes. They are ubiquitous around the world and found in terrestrial, freshwater, and marine ecosystems. They are able to live in extreme environments such as hot springs and arctic lakes.

Cyanobacteria include nearly 2,000 species, growing as single cells, filaments of cells or through various colonial associations [9-11]. This indicates a high degree of biological adaptation, which has enabled these organisms to thrive and compete effectively in nature. Many of them produce toxic secondary metabolites, in particular nerve and liver toxins as a

form of defense against herbivores. At the same time, some cyanobacterial species represent a source of interesting active metabolites, including acetylenic compounds that possess selective cytotoxicities and which may prove useful for development into commercial drugs [12-15].



1 Apramide A, R = Me
2 Apramide B, R = H

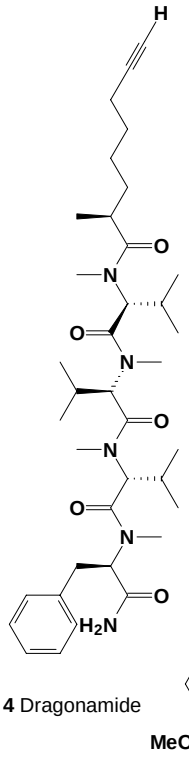


3 Apramide G

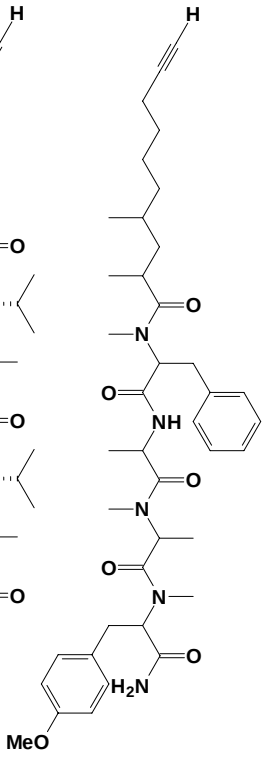
The ubiquitous tropical cyanobacterium *Lyngbya majuscula* is a prolific producer of bioactive metabolites, and approximately 30% of all natural products reported from marine cyanobacteria have been isolated from this species. The plethora of structurally diverse secondary metabolites isolated from *L. majuscula* exhibits a variety of bioactivities including antifeedant, molluscicidal, antiproliferative, and immunosuppressive properties. More than half of the known secondary metabolites of the species are either cyclic or linear lipopeptides, some of them having an acetylenic unit.

The linear lipopeptides named apramides A (**1**), B (**2**), and G (**3**) have been isolated from the cytotoxic fraction of *L. majuscula* collected at Apra Harbor (Guam). Apramide G showed cytotoxic activity, with IC_{50} values of 33 ng/mL and 11 ng/mL against KB and LoVo cells, respectively [16].

Four new metabolites have been isolated from *L. majuscula* collected at Boca del Drago Beach, Bocas del Toro, Panama. These compounds were assigned the trivial names dragonamide (**4**), pseudodysidenin, dysidenamide, and nordysidenin. Dragonamide exhibited cytotoxic activity against P-388, A-549, HT-29, and MEL-28 cells (IC_{50} > 1 μg/mL) [17].



4 Dragonamide

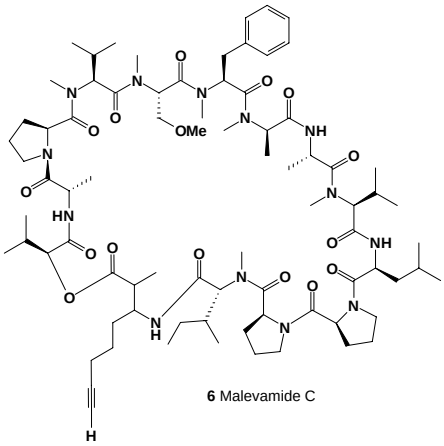


5 Carmabin A

Carmabin A (**5**), a linear lipotetrapeptide, was isolated from the *n*-BuOH extract of *L. majuscul*a. Using the MRC-5 human embryonic lung cell line in the confluent and proliferating states (cytotoxicity assessment assay, Syntex Discovery Research), curacin A and carmabin produced the following IC_{50} values: 6.58 μg/mL (crude extract with curacin A), 0.98 μg/mL (fraction with curacin A), 0.003 μg/mL (pure curacin A); 4.8 μg/mL (crude extract with carmabin), 0.6 μg/mL (fraction with carmabin A), and 0.06 μg/mL (pure carmabin A) [18].

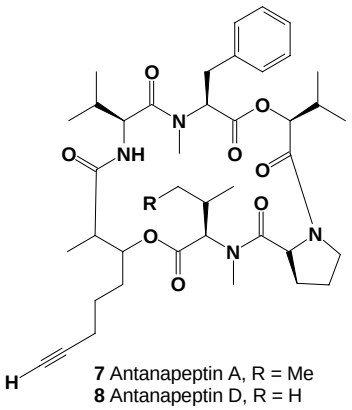
n-Hexane and *n*-butanol extracts of *S. hydnoides* showed cytotoxic activity against HT-29 human colon cancer cells. A new depsipeptide, malevamide C (**6**), was isolated from the cyanobacterium

Symploca laete-viridis, collected near the south shore of Oahu, Hawaii [19]. At a concentration < 2 μg/mL, this compound was found to be active against P-388, A-549, and HT-29 cancer cells. Malevamide contains some unusual amino and hydroxy acids and several methylated and dimethylated residues. Other unusual moieties include 3-amino-2-methylhexanoic acid and 3-amino-2-methyl-7-octynoic acid.



6 Malevamide C

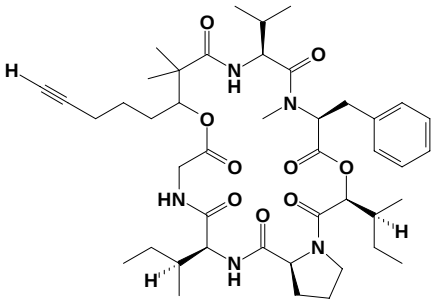
A new series of depsipeptides, antanapeptins A-D, two of them [antanapeptin A (**7**) and D (**8**)] containing acetylenic acid, were isolated from *L. majuscula* of the Antany Mora collection (Madagascar) [20]. Both metabolites showed moderate cytotoxic activity against neuroblastoma-2A cells in mice.



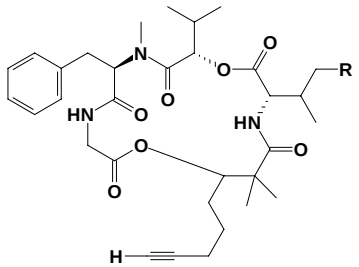
7 Antanapeptin A, R = Me
8 Antanapeptin D, R = H

A new cyclodepsipeptide [named pitipeptolide A (**9**)], isolated from *L. majuscula* collected at Piti Bomb Holes (Guam reefs), an area known for its periodic blue-green algal blooms, appears to be unique in this particular collection of Dr Valerie Paul by the presence of a 2,2-dimethyl-3-hydroxy-7-octynoic acid residue [21]. This compound exhibited weak cytotoxicity against LoVo cancer cells, but possessed moderate antimycobacterial activity and stimulated elastase activity.

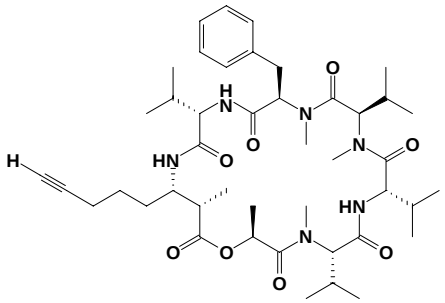
Yanucamides A (**10**) and B (**11**) were isolated from lipid extracts of *L. majuscula* and *Schizothrix* sp. collected at Yanuca Island (Fiji) [22]. Both compounds contain a unique 2,2-dimethyl-3-hydroxy-7-octynoic acid.



9 Pitipeptolide A



10 Yanucamide A, R = H
11 Yanucamide B, R = Me

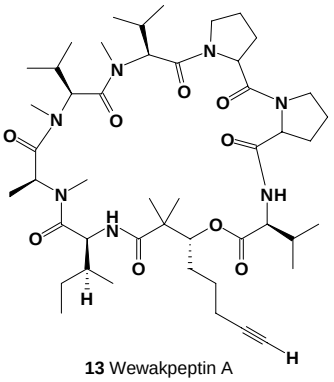


12 Ulongapeptin

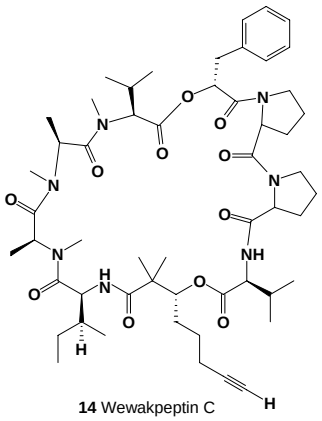
Ulongapeptin (**12**), a cyclic depsipeptide with a β-amino acid, 3-amino-2-methyl-7-octynoic acid, was isolated from a Palauan marine *Lyngbya* sp. The compound was cytotoxic against KB cells with an IC_{50} value of 0.63 μM [23].

Four new depsipeptides have been isolated from the marine cyanobacterium *L. semiplena* collected from Papua New Guinea. The wewakpeptins represent an unusual arrangement of amino and hydroxy acid subunits compared with known peptides of

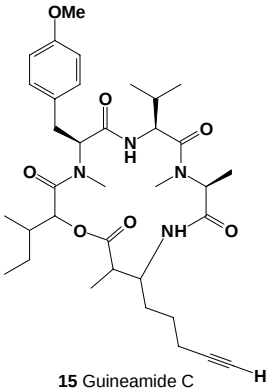
cyanobacterial origin, and possess a bis-ester, a 2,2-dimethyl-3-hydroxy-7-octynoic acid residue.



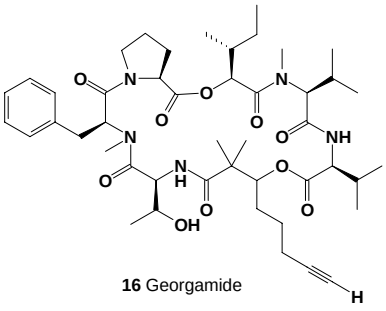
13 Wewakpeptin A



14 Wewakpeptin C



15 Guineamide C



16 Georgamide

Wewakpeptins A (**13**) and C (**14**) were the most cytotoxic among these 4 depsipeptides, with an LC_{50} value of approximately 0.4 μM for both the NCI-H460 human lung tumor and the mouse neuroblastoma-2A cell lines [24].

Guineamide C (**15**) is a novel cyclic depsipeptide isolated and characterized from a Papua New Guinea collection of *L. majuscula* [25]. Guineamide C possesses moderate cytotoxicity to a mouse neuroblastoma-2A cell line with an IC_{50} value of 16 μM. A cyclic depsipeptide, georgamide (**16**), was isolated from a non-identified cyanobacterium (Australia) [20]. Its constituent units were five amino acid residues (L-Thr, L-Pro, L-Val, *N*-Me-L-Val, and *N*-Me-L-Phe), as well as two hydroxy carboxylic acids, 2(*S*)-hydroxy-3(*R*)-methylpentanoic acid and 2,2-dimethyl-3-hydroxy-7-octynoic acid, which are also present in wewakpeptins A and B [26].

Predicted Biological Activities of Metabolites Isolated from Aquatic Organisms

Probable additional biological activities of acetylenic metabolites isolated from aquatic species were evaluated by computer prediction. For this purpose we used the computer program PASS [27-30], which predicts about 2,500 pharmacological effects, mechanisms of action, mutagenicity, carcinogenicity, teratogenicity and embryotoxicity on the basis of structural formulae of compounds. PASS predictions are based on structure-activity relationship (SAR) analysis of the training set consisting of about 60,000 drugs, drug-candidates and lead compounds. The algorithm of PASS prediction is described in detail in several publications [27-30]. Using MOL or SD files as an input for the PASS program, a user may get a list of probable biological activities for any drug-like molecule as an output. An explanation of predicted biological activities for some natural metabolites also was published recently [31].

For each activity, P_a and P_i values are calculated, which can be interpreted either as the probabilities of a molecule belonging to the classes of active and inactive compounds, respectively, or as the probabilities of the first and second kind of errors in prediction. First kind error of prediction reflects the "false-positives", when an inactive compound is predicted to be active. Second kind error of prediction: reflects the "false-negatives", when an active compound is predicted to be inactive.

Interpretation of the predicted results and selection of the most promising compounds are based on flexible criteria, which depend on the purpose of a particular investigation. If the user chooses a rather high value of P_a as a threshold for selection of probable activities, the chance to confirm the predicted

activities by the experiment is also high , but many existing activities will be lost. Typically, there are several dozen biological activities in the predicted biological activity spectra; activity that is predicted with the highest probability is called "focal". Focal biological activities for acetylenic compounds isolated from aquatic organisms are shown below in the Tables No. 1, 2, 4, 5, 7, 8, 10, 12, 13, 14, 16, 17, 19, 20-27, and 29.

Predicted biological activities from acetylenic compounds isolated from aquatic cyanobacteria are presented in Table 1.

Table 1. Predicted biological activities for acetylenic metabolites isolated from cyanobacterial species

No.[a]	Drug-Likeness	Focal Activity Prediction[c]
1	0.975	P_a = 0.733 P_i = 0.040 Amyotrophic lateral sclerosis treatment
2	0.973	0.739 0.037 Amyotrophic lateral sclerosis treatment
3	0.927	0.841 0.006 Amyotrophic lateral sclerosis treatment
4	0.947	0.713 0.010 Nerve growth factor agonist
5	0.915	0.710 0.030 Peptide agonist
6	0.994	0.783 0.006 Tocolytic
7	0.994	0.871 0.007 Antineoplastic (colorectal cancer)
8	0.993	0.853 0.008 Antineoplastic (colorectal cancer)
9	0.993	0.827 0.004 Antibiotic Glycopeptide-like
10	0.993	0.838 0.003 Antibiotic Glycopeptide-like
11	0.993	0.832 0.003 Antibiotic Glycopeptide-like
12	0.993	0.858 0.007 Integrin antagonist
13	0.994	0.824 0.004 Tocolytic
14	0.993	0.789 0.006 Tocolytic
15	0.992	0.800 0.007 General pump inhibitor
16	0.994	0.771 0.004 Antibiotic Glycopeptide-like

[a]Numbering of acetylenic metabolites isolated from aquatic organisms

For instance, if Pa>80% is used as a threshold, about 80% of real activities will be lost; for Pa>70%, the portion of lost activities is 70%, etc. By default, the $P_a > P_i$ value is used as a threshold that provides the mean accuracy of prediction about 90% in leave one out cross-validation (LOO CV). LOO CV procedure is performed for all ~60,000 compounds from the PASS training set, when each compound is sequentially removed from the training set with all associated information about its activities, and prediction is carried out on the basis of the rest part of the training set. The results of prediction are compared with known experimental data, and mean accuracy of prediction is calculated through all compounds and all activities from the training set. The average accuracy of PASS predictions obtained for a heterogeneous evaluation set is almost 90% [29, 31]. PASS also calculates so-called drug likeness according to the method published in ref. 29. More detailed descriptions of PASS and its possibile use to predict biological activity via the Internet are available [30].

Metabolites of Macro- and Microalgal Species

Extracts of different macrophytes and microphytes have been shown to possess cytotoxic activities [32]. Thirty-nine species of marine algae collected from the coast of China were screened for their antitumor activities against KB, Bel7402 and/or A549 cancer cells. Seven of them were effective against both KB and HT-29 cells: four Rhodophyta (*Symphyocladia latiuscula*, *Rhodomela confervoides*, *Polysiphonia urceolata*, *Gloiopeltis furcata*) and three Phaeophyta (*Leathesia difformis, Punctaria latifolia*, *Scytosiphon lomentaria*). *Ulva pertusa* showed cytotoxicity to both tumor and normal cells. Crude extracts of *Leathesia difformis* showed the most potential selective activity, with an IC_{50} of 12.6 μg/mL against KB cells and 40.6 μg/mL against HT-29 cells. The extracts of *Polysiphonia urceolata* were most active against HT-29 cells (IC_{50} = 26.0 μg/mL), while the extracts from *Symphyocladia latiuscula*, *Rhodomela confervoides* and *Punctaria latifolia* showed cytotoxic activities towards HT-29 and KB cells [32].

The antitumor activity of seaweed extracts from Shandong Province (Qingdao and Weihai) belonging to Rhodophyceae, Chlorophyceae and Phaeophyceae were analyzed for anti-KB and anti-HT-29 activities [33]. Nine species (*Leathesia difformis*, *Polysiphonia urceolata*, *Scytosiphon lomentaria*, *Gloiopeltis furcata*, *Dictyopteris divaricata*, *Punctaria latifolia*, *Symphyocladia latiuscula*, *Rhodomela confervoides*, and *Gracilaria verrucosa*) showed antineoplastic activities. The extracts of *Leathesia difformis* were more toxic against KB cells (LD_{50} = 12.65 μg/mL) than against normal cells (LD_{50} >50 μg/mL). The cytotoxic prominent activities were found in the methanolic extracts of *Polysiphonia urceolata* (LD_{50} = 26.01 μg/mL against human tumor-29). The other algae, *Symphyocladia latiuscula*, *Rhodomela confervoides* and *Punctaria latifolia*, had also shown inhibition of growth of KB cells, and the extracts of *Symphyocladia latiuscula*, and *Rhodomela confervoides* had shown selective activities against HT-29 cells. The ethanol and chloroform extracts of *Polysiphonia urceolata*, the ethanolic extract of

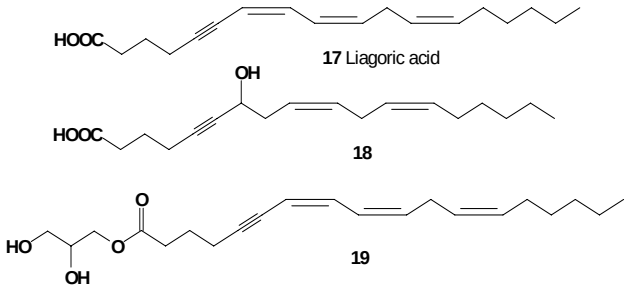
Scytosiphon lomentaria and the *n*-hexane extract of *Dictyopteris divaricata* had strong selective cytotoxic activities; all of the LD_{50} values against KB cells were less than 4.40 μg/mL. The ethanolic extract of *Scytosiphon lomentaria* had strong activity against HT-29 cells (LD_{50} = 1.49 μg/mL). As all the algae grow widely on the Shandong coast and since their extracts inhibited either KB or HT-29 tumor cells with low side effects, they may represent an interesting source of antitumor drugs [33].

The selective cytotoxic activity of extracts from two marine green algae, *Cladophoropsis vaucheriaeformis* and *Halimeda discoidea*, were examined via a dose response assay against mouse leukemia L-1210 cells and normal NIH-3T3 cells [34]. The MeOH extract of *C. vaucheriaeformis* showed selective cytotoxicity to L-1210 cells at concentrations ranging from 50 to 100 μg/mL. In particular, the greatest selectivity for cytotoxic activity was found at the concentration of 50 μg/mL, at which the growth of L-1210 cells, was inhibited completely, whereas that of NIH-3T3 was not affected at all. However, MeOH extracts of the red alga *Laurencia okamurae* and the brown alga *Dictyopteris undulata*, which displayed non-selective cytotoxicity in previous screening tests, did not show similar selective cytotoxicity at any concentration tested. These results indicate that the marine green alga *C. vaucheriaeformis* may contain a unique antitumor substance with selective cytotoxic activity against L-1210 cells [34].

Extracts from 675 species of marine organisms were tested for cytotoxicity toward KB cells (human nasopharyngeal carcinoma) in tissue culture. Sixty species of marine animals and 5 marine algae possessed significant levels of cytotoxicity. A total of 9.6% of the species tested exhibited significant activity. In addition, no significant activity was observed for any species of red alga, which represented >55% of the marine plants collected. No meaningful correlation between organic halogen content and cytotoxic potency was observed [35].
Twenty-two extracts of algae corresponding to eleven species (*Acetabularia acetabulum, Caulerpa prolifera, Codium vermilareae, Enteromorpha intestinalis, Ulva rigida, Corallina elongata, Jania rubens, Pterocladia capillacea, Cystoseira compressa, Dictyopteris membranacea* and *Padina pavonica*) collected from the Tunisian coasts were screened for their potential activities against bacterial strains, an enzyme (PLA2), and tumor cells (KB) [36]. Several extracts showed pertinent activities, among them: *Acetabularia acetabulum* for antifungal activity, *Padina pavonica* for cytotoxicity against KB cells, and *Ulva rigida* as an inhibitor of PLA2 enzyme [36].

An extract of the red marine alga *Liagora farinosa* (Rhodophyta, Nemaliales), collected in the southern part of Brazil, was reported to possess cytotoxic activity [37-39]. Acaricidal activity against *Boophilus micropulus*, of crude ethanol extracts of *L. farinosa*, as well as other marine algae (*Liagora elongata, Laurencia obtusa*, *Padina vickerisiae*, and *Stypopodium lobatum)* was also documented [38]. Pronounced effects of extracts from *Liagora* sp., *Eisenia bicyclis, Sargassum sagamianum, Amphiroa aberrans, Gracilaria verrucosa, Codium fragile, C. intricatum*, and *C. divaricatum* on experimental murine skin rejection models [39] suggest that these algae contain bioactive compounds with immuno-suppressive activity.

The structures of octadec-5-yne-7*Z*,9*Z*,12*Z*-trienoic acid (liagoric acid) (**17**), 7-hydroxy-5-yne-9*Z*,12*Z*-dienoic acid (**18**), and glyceryl octadec-5-yne-7*Z*,9*Z*,12*Z*-trienoate (**19**) isolated from *Liagora farinosa* are presented below.

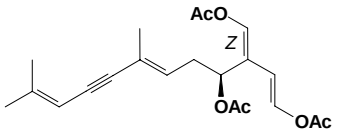


These compounds showed acute toxicity toward *Eupomacentrus leucostictus* from 5 to 8 μg/mL. It was shown that at a concentration of 31 μM, liagoric acid inhibited cyclooxygenase activity [40].

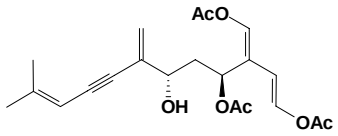
The family Caulerpaceae includes hundreds of multi-shaped species of marine green macroalgae, from grape-like *Caulerpa racemosa*, to feathery *C. sertularioides, C. taxafolia*, and *C. mexicana*, to the solid-bladed *C. prolifera.* Some of them were found to be very invasive and the most devastating representative of this genus, *C. taxifolia*, has the nickname "killer alga". Its aquarium strain was accidentally released in the wastewater from the Oceanographic Museum at Monaco and rapidly

invaded the Mediterranean Sea, the southern Californian and the Australian coasts. *C.taxifolia,* like other species of *Caulerpa*, is coenocytic, representing a gigantic cell and containing millions of nuclei. The ethanolic extract of *C. prolifera* showed antitumor activity against Ehrlich ascites carcinoma *in vitro* [41].

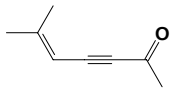
In contrast to other plants that produce a variety of toxins, but in reduced amounts, *C. taxifolia* synthesizes a single major secondary metabolite, caulerpenyne (**20**), in enormous quantities (up to 10% of algal dry mass, depending on season). This sesquiterpene is toxic to herbivores, such as sea urchins and to submarine flora.



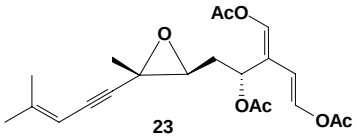
20 Caulerpenyne



21 Caulerpenynol



22 Taxifolione



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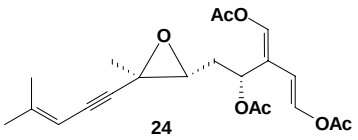


24

Caulerpenyne was found to be cytotoxic to several cell lines. In particular, it induced inhibition of SK-N-SH (neuroblastoma) cell proliferation with an IC_{50} of 10 μmol/mL after either long-time incubation (24 h) or after 2h [42]. In this respect, caulerpenyne was found to be as efficient as anti-tumor drugs oxaliplatinul and paclitaxel. After a short incubation *in vitro*, caulerpenyne blocked polymerization of pure tubulin with an IC_{50} of 21 μM, presumably by inducing its aggregation [42].

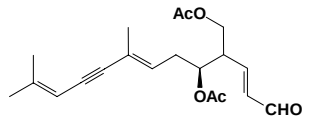
A detailed study of its anti-tumor efficiency and selectivity was performed on 8 cancer cell lines of human origin. It produced growth-inhibitory effects in all cases, with some variability among the cell lines. Cells of colorectal cancer origin were the most sensitive to caulerpenyne, with IC_{50} values from 6.1 to 7.7 μM [43]. As to the major mechanism underlying caulerpenyne cytotoxicity, available data are controversial: both inhibition at the G2/M phase [43] and absence of the effect [42] were reported. On the other hand, the particular action of the compound on the microtubular network assayed both in the *in vitro* tubulin system and on the SK-N-SH cell line [42] could be of great importance in the future search of novel antimitotic agents. Conventional antimitotics either stabilize (Taxol) or depolymerize (*Vinca* alkaloids) microtubules, but do not induce tubulin aggregation.

Tubulin may represent just one of the targets of caulerpenyne. Taking into account an early and strong cytotoxic effect of caulerpenyne, involvement of other anti-proliferative mechanisms was suggested [42]. Indeed, caulerpenyne was reported to block phospholipase A_2 activity and selectively inhibited stimulation of MAPK (mutagen-activated kinase) [cf. 42].

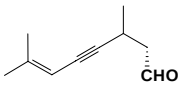
Caulerpenyne is produced in somewhat lower amounts by other Caulerpaceae and has been isolated from several *Caulerpa* species from the Mediterranean Sea (*C. prolifera*), the Pacific Ocean, and the Caribbean Sea (*C. prolifera, C. racemosa, C. lanuginosa*) [44]. Though caulerpenyne represents a major toxic metabolite of *Caulerpa*, its numerous derivatives (**21**-**28**), including furocaulerpin (**29**), an acetylenic sesquiterpenoid possessing a furan ring, could contribute to the cytotoxicity of the species. It should be noted that upon wounding, *C. taxifolia* and other *Caulerpa* species, within seconds, induce transformation of caulerpenyne into highly reactive, and thus potentially more toxic, aldehydes of the oxitoxin family [45,46].

Some of the minor caulerpenyne metabolites (**21**-**24**) were shown to inhibit, *in vitro*, the growth of marine bacteria and marine ciliates (Protozoa) [45]. The toxicity of pure compounds **23**-**28** was also evaluated on three models: mice (lethality), mammalian cells in culture (cytotoxicity), and sea urchin eggs (disturbance of cell proliferation). These caulerpine analogs were found to be more or less toxic, with variations of efficiency depending on the assay [46].

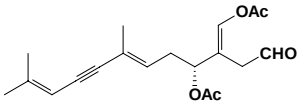
Bioactive furocaulerpin (**29**), an acetylenic sesquiterpenoid possessing a furan ring, was isolated from the marine alga *C. prolifera* [47].



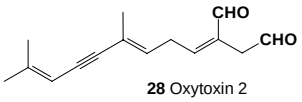
25 Taxifolial A



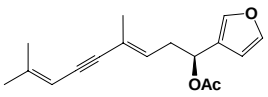
26 Taxifolial D



27 Oxytoxin 1



28 Oxytoxin 2

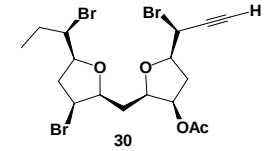


29 Furocaulerpin

Marine red algae of the genus *Laurencia* (order Ceramiales, family Rhodomelaceae) are widely distributed in temperate and tropical waters and in some areas they make up a large component of the algal biomass. They are one of the most prolific producers of secondary metabolites in intertidal habitats. Their crude extracts showed cytotoxic activity against the U937 tumor cell line in the range 0.5 to 40 μg/mL [48a], and strong activity against leishmania *in vitro* [48b].

The chemistry of *Laurencia* species is a very interesting topic of research that never fails to offer the possibility of discovering interesting and novel structures, as well as biologically active metabolites. Species of *Laurencia* have produced more than 200 secondary metabolites which exhibited cytotoxic activity against various cancer cell lines, and/or showed antiviral, antibacterial, antimalarial, antifouling, antifungal, antioxidant and other activities [49-52]. Acetylenic polyethers **30**-**40** and aliphatic oxygenated metabolites **41** and **42** produced by *Laurencia* species have displayed different biological activities (Table 2) [53-56].

The macrocyclic polyketides, polyethers, and acetogenins isolated from marine and/or terrestrial sources have generated substantial interest over the last 30 years in the areas of chemistry, pharmacology, and medicine due to their interesting structures and, more importantly, their activity against numerous cancer cell lines, including those with drug-resistance. Many of them displayed cytotoxic, anticancer and other activities [57-59].



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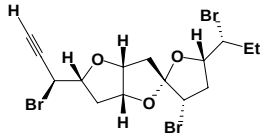

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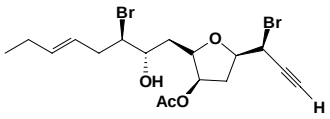

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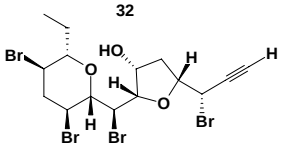

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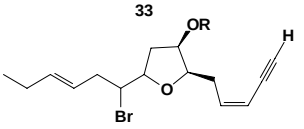
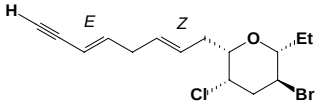
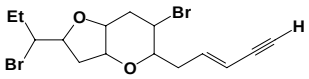


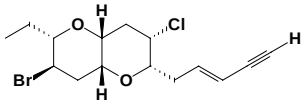
34 *trans*-Kumausyne, R = Ac
35 *cis*-Kumausyne, R = Ac
36 *trans*-Deacetylkumausyne, R = H
37 *cis*-Deacetylkumausyne, R = H



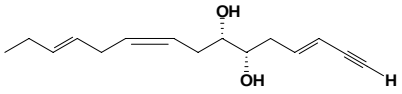
38 Srilankenyne



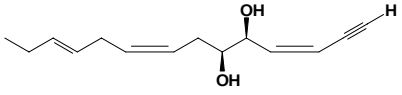
39 Laurobtusin



40 Dactomelyne

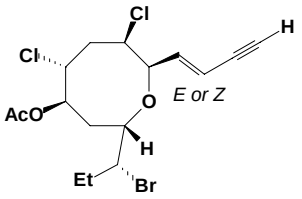


41 *trans*-Laurediol

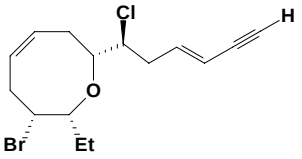


42

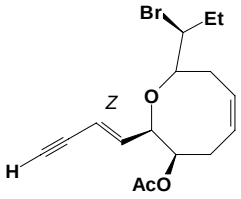
Some bioactive macrocyclic acetogenins (**43**-**59**) isolated from the genus *Laurencia* [60] are shown below, and their predicted activities are shown in Table 2.



43 Laurencienyne, *E*
44 Laurencienyne B, *Z*



45 Laurepinnacin



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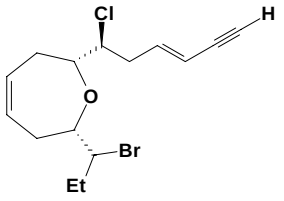
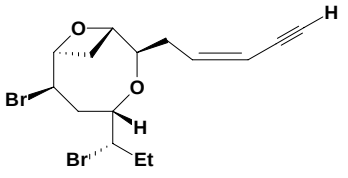
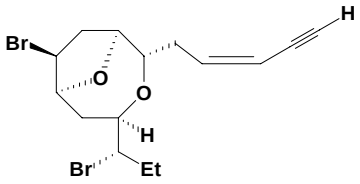


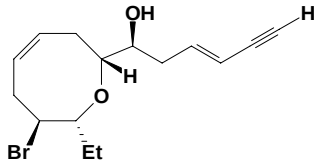
47 Isolaurepinnacin



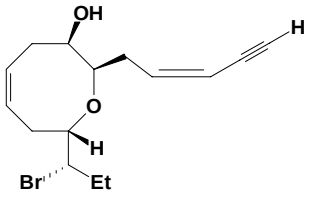
48 Laureatin



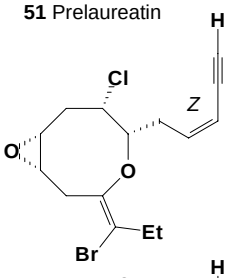
49 Isolaureatin



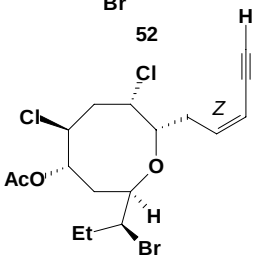
50 Deacetyllaurencin



51 Prelaureatin



52



53 13-Epilaurencienyne

Macro- and microalgae are an important natural source of valuable macromolecules, such as carotenoids (the best known source of the carotenoids is *Spirulina* and other blue green algae), and long-chain polyunsaturated fatty acids, which represent 1 to 5 % of the algal dry matter.

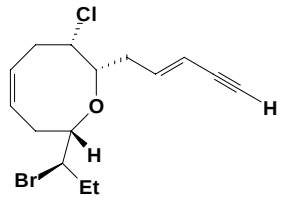
In particular, they contain polyunsaturated "essential" omega-3 and omega-6 fatty acids that play a role in prevention of cardio-vascular diseases, osteoarthritis and diabetes, and in improving immune function.

Besides fatty acids, the unsaponifiable fraction of seaweeds was found to contain carotenoids that demonstrated various biological activities [61,62].

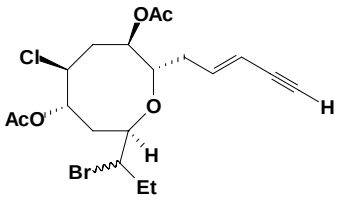
Three carotenoids with an acetylenic unit, named diadinochrome A (**60**) and B (**61**), and diatoxanthin (**62**) were isolated from the freshwater red tide organism *Peridinium bipes* (Dinophyceae). Diadinochrome A was shown to be cytotoxic to HeLa cells, while two other compounds exhibited anti-carcinogenic activity.

Extracts of *Peridinium bipes* exerted an inhibitory effect on the growth of *Microcystis aeruginosa* [63-65]. Tsushima and co-workers [64] studied 51 carotenoids, including some with acetylenic unit(s):

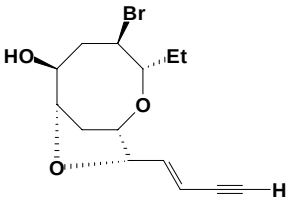
amarouciaxanthin B (sidnyaxanthin), crassostreaxanthin A, diatoxanthin, halocynthiaxanthin, heteroxanthin, mytiloxanthin, mytiloxanthinone, pectenol A and B, and pectenolone. Acetylenic carotenoids showed different level of cytotoxic activity against Raji cells (human neoplasm).



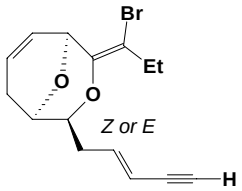
54 13-Epipinnatifidenyne



55 *Z*
56 *E*



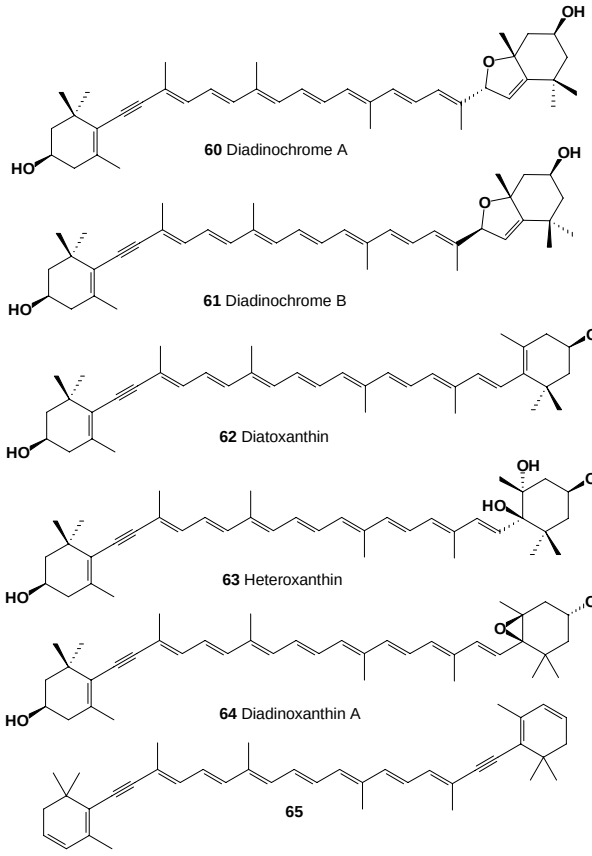
57 Laurefucin



58 *cis*-Chondrin, *Z*
59 *trans*-Chondrin, *E*

Quantitative carotenoid analysis of the microalga *Euglena viridis* revealed the presence of β,β-carotene (5% of total carotenoids), mixed with some β,ε-carotene, the β,ε-carotene derived siphonein (siphonaxanthin 19-dodecenoate, 8%), the allenic neoxanthin (4%), and acetylenic carotenoids >86% [66,67]. Those included the mono-acetylenic diatoxanthin (**62**) (major, 61%), diadinoxanthin (**64**, rearranged to diadinochrome, 12%), heteroxanthin (**63**, 1%), and the diacetylenic 3,4,7,8,3',4',7',8'-octadehydro-β,β-carotene (**65**, 6%).

The significance of the presence of siphonein and diacetylenic carotenoids for algal chemosystematics was briefly discussed. Heteroxanthin was also found in *Euglena gracilis* [66], and Xanthophyceae species [67]. The principal crystallizable xanthophylls of *Tribonema aequale* were diatoxanthin, heteroxanthin, and diadinoxanthin [68].



Carotenoids of two members of the Raphidophyceae (chloromonads), *Gonyostomum semen* and *Vacuolaria virescens*, and of two tentative members of the same class (*Chattonella japonica* and *Fibrocapsa japonica)* were analyzed [69a]. Group I (*G. semen* and *V. virescens*) showed a similar carotenoid pattern, comprised of diadinoxanthin (54-60% of total carotenoids), dinoxanthin (8-17%), β,β-carotene (7%), and heteroxanthin (7%), as well as neoxanthin (*G. semen*, 3%), an epoxidic monoacetate (*G. semen*, 12%), an epoxidic carotenol, possibly 9'-*cis*-diadinoxanthin (*V. virescens*, 8%), an epoxidic diacetate (*V. virescens*, 2%) and vaucheriaxanthin 3,19-diacetate (*V. virescens*, 8%).

Characteristic features common to the carotenoids encountered are a high proportion of epoxidic carotenoids (78-86%), allenic carotenoids (24-82%), acetylated carotenols (18-81%), and acetylenic carotenoids (61-67%; Group I only). The xanthophycean-cultured alga *Pleurochloris meiringensis* contains heteroxanthin, diadinoxanthin

and β-carotene [69b]. Carotenoids extracted from freshwater red tide plankton were shown to include β-carotene (8.1%), peridinin (26.5%), dinochrome A (14.3%), dinochrome B (2.7%), dinoxanthin (1.7%), diadinochrome A (**60**, 2.7%), diatoxanthin (**62**, 6.8%), and 13'-*cis*-7',8'-dihydroneoxanthin-20'-al 3'-β-lactoside (4.7%). Some of the isolated carotenoids were shown to be cytotoxic to mouse tumors [70].

Additional biological activities of acetylenic metabolites isolated from marine micro and macroalgal species are shown in Table 2.

Marine and Freshwater Sponges (Porifera)

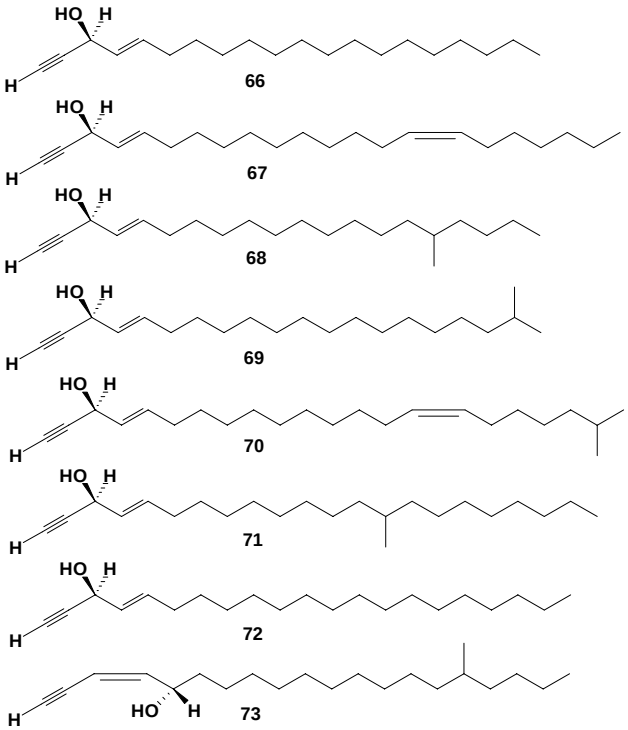
These invertebrates are quite vulnerable and are characterized by a lack of physical defenses. To resist predators and bacteria, to protect themselves from fouling and to compete for space, they developed effective mechanisms of chemical defense by extruding, in particular, very toxic secondary metabolites. In fact, the sponges produce the highest quantity of secondary metabolites compared with all other marine invertebrates [2-6]. Some of the released compounds are of high structural complexity, exhibit unique modes of action, and are active in extremely low doses. No wonder that the sponges are a rich source of biologically active chemical molecules and potentially valuable pharmacological compounds. These porous organisms are known for their ability to serve as harbors for bacteria. Thus many active compounds produced by the sponges are bactericides (more than 200 antibiotics have been isolated from this source), while others could be of bacterial origin. Intensive search for new classes of biologically active molecules led to a discovery of a series of antitumor compounds from marine sponges and the microorganisms associated with them [5,7,10].

More than a hundred polyacetylenic metabolites have been identified in different sponge species [71] and some of these possess pronounced antitumor activities. Antitumor bioassay-guided fractionation of the organic extract of the marine Brown Bowl Sponge (*Cribrochalina vasculum)* resulted in the isolation of several closely related cytotoxic acetylenic alcohols (**66-73**) [72].

Isolated compounds selected from this series showed selective *in vitro* antitumor activity against H-522 non-small cell lung line and IGROV-1 ovarian line.

Table 2. Predicted biological activities for acetylenic metabolites isolated from macro- and microalgal species

No	Drug-Likeness	Focal Activity Prediction
17	0.988	0.927 0.001 Linoleate isomerase inhibitor
18	0.990	0.858 0.007 Antithrombotic
19	0.986	0.882 0.007 Lipid metabolism regulator
20	0.870	0.979 0.001 Carboxypeptidase E inhibitor
21	0.897	0.951 0.001 Carboxypeptidase E inhibitor
22	0.929	0.946 0.002 β-Carotene 15,15'-monooxygenase inhibitor
23	0.780	0.799 0.004 Carboxypeptidase E inhibitor
24	0.780	0.799 0.004 Carboxypeptidase E inhibitor
25	0.879	0.958 0.003 Aminocarboxymuconate-semialdehyde decarboxylase inhibitor
26	0.932	0.959 0.000 Carboxylate reductase inhibitor
27	0.913	0.909 0.021 (-)-(4S)-Limonene synthase inhibitor
28	0.935	0.979 0.001 Carboxypeptidase E inhibitor
29	0.978	0.915 0.002 Carboxypeptidase E inhibitor
30	0.753	0.780 0.000 4-Carboxymethyl-4-methylbutenolide mutase inhibitor
31	0.791	0.910 0.007 Phosphatase inhibitor
32	0.863	0.764 0.012 Antiepileptic
33	0.525	0.806 0.008 Antiepileptic
34,35	0.951	0.719 0.017 Antiepileptic
36,37	0.966	0.759 0.012 Antiepileptic
38	0.921	0.755 0.030 Convulsant
39	0.850	0.774 0.011 Antiepileptic
40	0.901	0.792 0.022 Convulsant
41	0.993	0.833 0.004 Alcohol *O*-acetyltransferase inhibitor
42	0.993	0.809 0.005 Alcohol *O*-acetyltransferase inhibitor
43,44	0.689	0.858 0.001 Interleukin 10 antagonist
45	0.750	0.608 0.002 4-Carboxymethyl-4-methylbutenolide mutase inhibitor
46	0.930	0.701 0.019 *trans*-Cinnamate 4-monooxygenase inhibitor
47	0.511	0.902 0.006 Antiepileptic
48	0.858	0.780 0.000 4-Carboxymethyl-4-methylbutenolide mutase inhibitor
49	0.870	0.714 0.017 Antiepileptic
507	0.986	0.796 0.045 Phosphatase inhibitor
51	0.968	0.721 0.016 Antiepileptic
52	0.916	0.802 0.042 Phosphatase inhibitor
53	0.810	0.843 0.001 Interleukin 10 antagonist
54	0.810	0.723 0.016 Antiepileptic
55	0.917	0.836 0.001 Interleukin 10 antagonist
57	0.991	0.824 0.051 Membrane integrity agonist
58,59	0.989	0.699 0.090 Phosphatase inhibitor
60	0.995	0.850 0.006 Dermatologic
61	0.995	0.850 0.006 Dermatologic
62	0.992	0.960 0.005 Dermatologic
63	0.991	0.882 0.013 β-Adrenergic-receptor kinase inhibitor
64	0.991	0.994 0.002 Antiacne
65	0.912	0.871 0.015 β-Adrenergic-receptor kinase inhibitor



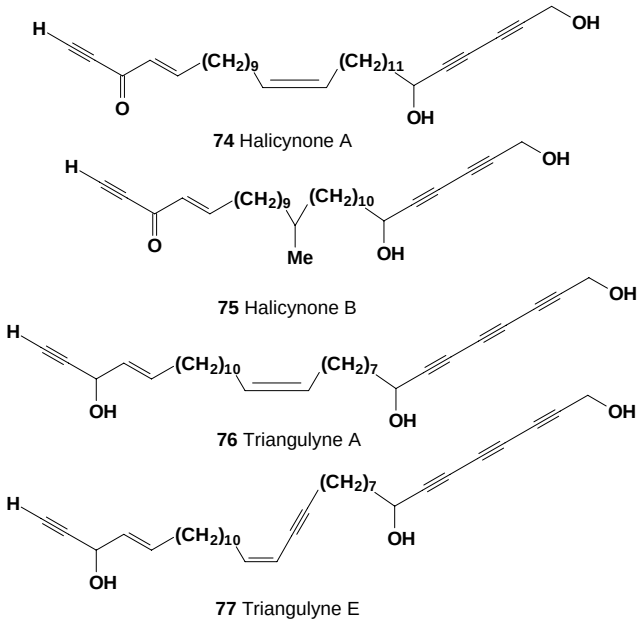
Five acetylenic alcohols (**66,67,69,70** and **72**) with immunosuppressant and antitumor activity were isolated from the sponge *Cribrochalina vasculum* and characterized [73]. The alcohols displayed immunosuppressive activity in mixed lymphocyte reaction and CV-1 cytotoxicity assays (Table 3). Being tested *in vitro* on P388 leukemia cells, and cells from human lung (A549) and colon (HT-29) tumors, these compounds had IC_{50} values that varied from 0.86 to 90 μg/mL.

Table 3. Immunosuppressive activity of acetylenic alcohols isolated from the sponge *Cribrochalina vasculum*

Compound	Dose μg	MLR %[a]	LCV %[b]	Suppression (%)
66	0.1	0	61	100
66	0.01	24	80	76
67 (15*E*)	0.1	0	34	100
67 (15*E*)	0.01	33	67	67
68	0.1	0	42	100
68	0.01	21	78	79
69	0.1	0	49	100
69	0.01	21	82	79
70 (15*E*)	0.1	0	45	100
70 (15*E*)	0.01	20	67	33

[a] Percent of the positive (no drug) MLR control
[b] Percent of the positive (no drug) LCV control

Chromatographic separation of solvent-partitioned fractions of the extract of *Haliclona* sp. yielded two new compounds, halicynones A (**74**) and B (**75**), along with the known compounds triangulyne A (**76**), triangulyne E (**77**) and pellynols A (**78**), B (**79**), C (**80**), D (**81**), and I (**82**) (0.011-0.11% of dry weight). The isolated polyacetylenes were tested for cytotoxicity [74]. Predicted activities for metabolites **66**-**84** are shown in Table 4.



Cultured HCT-116 cells were found to be very sensitive to compounds **78** (IC_{50} 0.026 μg/mL), **79** (0.12 μg/mL), **80** (0.127 μg/mL), **81** (0.103 μg/mL), and **82** (<0.008 μg/mL), while the acetylenic ketones (**74** and **75**) were not effective (IC_{50} >78 μg/mL).

The unusually high cytotoxicities of (**78**-**82**) and the lack of activity of **74** and **75** suggest that not only the relatively rigid, rod-like structure of the molecules, but the presence of the 1-yn-3-ol moiety were required for this type of biological activity. Pellynols A-D, I and F (**83**), having a terminal 1-yn-3-ol, showed strong cytotoxicity against several melanoma and ovarian cancer cell lines (IC_{50} 0.08 - 2.0 μM/mL) [44b,c]. Pellynic acid (**84**) inhibited inosine monophosphate dehydrogenase with an IC_{50} of 1.03 pM/mL.

Acetylenic alcohols, strongylodiols A-D (**85**-**88**), were obtained from the Okinawan marine sponge belonging to the genus *Strongylophora*. Each of these compounds was an enantiomeric mixture in a different ratio and showed cytotoxic activity towards human T lymphocyte leukemia (MOLT-4) cells [75]. Additional activities for compounds **85**-**92** are shown in Table 5.

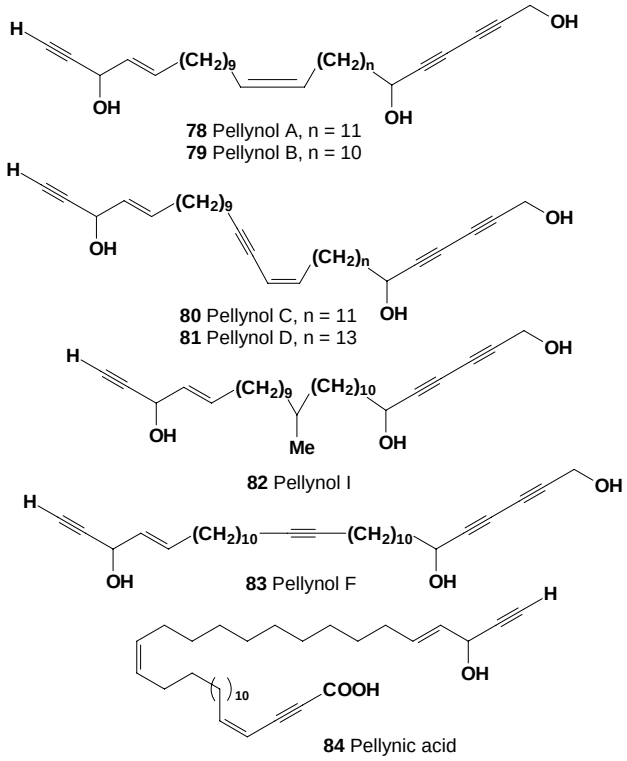
Table 4. Predicted biological activities compounds (**66**-**84**) isolated from sponges

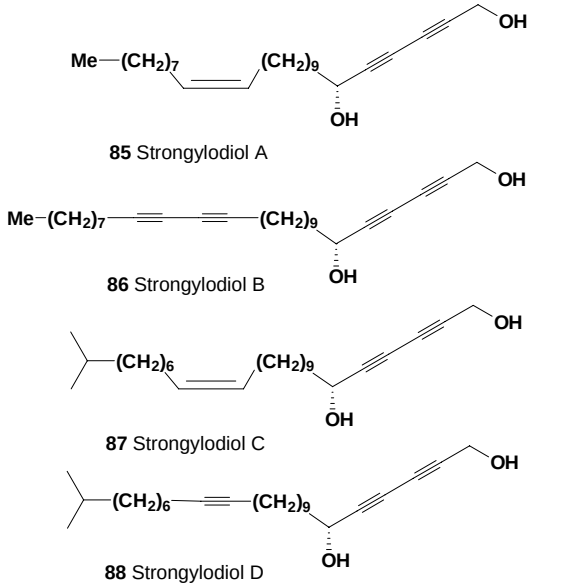
No	Drug-Likeness	Focal Activity Prediction
66	0.991	0.960 0.002 Lactate 2-monooxygenase inhibitor
67	0.991	0.960 0.002 Lactate 2-monooxygenase inhibitor
68	0.991	0.937 0.002 Lactate 2-monooxygenase inhibitor
69	0.991	0.948 0.002 Lactate 2-monooxygenase inhibitor69
70	0.991	0.948 0.002 Lactate 2-monooxygenase inhibitor
71	0.991	0.937 0.002 Lactate 2-monooxygenase inhibitor
72	0.991	0.960 0.002 Lactate 2-monooxygenase inhibitor
73	0.992	0.842 0.009 Pulmonary hypertension treatment
74	0.985	0.839 0.017 Aminocarboxymuconate-semialdehyde decarboxylase inhibitor
75	0.986	0.786 0.003 Alcohol oxidase inhibitor
76	0.990	0.923 0.003 Lactate 2-monooxygenase inhibitor
77	0.992	0.895 0.003 Lactate 2-monooxygenase inhibitor
78	0.990	0.923 0.003 Lactate 2-monooxygenase inhibitor
79	0.990	0.923 0.003 Lactate 2-monooxygenase inhibitor
80	0.992	0.895 0.003 Lactate 2-monooxygenase inhibitor
81	0.992	0.895 0.003 Lactate 2-monooxygenase inhibitor
82	0.990	0.896 0.003 Lactate 2-monooxygenase inhibitor
83	0.991	0.910 0.003 Lactate 2-monooxygenase inhibitor
84	0.976	0.966 0.001 Argininosuccinate lyase inhibitor

Table 5. Predicted biological activities for strongylodiols (A-D), and dideoxypetrosynols (A-D)

No	Drug-Likeness	Focal Activity Prediction
85	0.955	0.832 0.017 Styrene-oxide isomerase inhibitor
86	0.953	0.846 0.015 Styrene-oxide isomerase inhibitor
87	0.969	0.797 0.023 Styrene-oxide isomerase inhibitor
88	0.969	0.810 0.021 Styrene-oxide isomerase inhibitor
89	0.993	0.952 0.002 Lactate 2-monooxygenase inhibitor
90	0.993	0.952 0.002 Lactate 2-monooxygenase inhibitor
91	0.993	0.952 0.002 Lactate 2-monooxygenase inhibitor
92	0.994	0.947 0.002 Lactate 2-monooxygenase inhibitor

Polyacetylenes with cytotoxic activities against human tumor cell lines (A549, SK-OV-3, SK-MEL-2, XF498, and HCT15) have been isolated from the marine sponge *Petrosia* sp. (Table 2), and given the trivial names of dideoxypetrosynols A-D (**89**-**92**) [76]. Compound A (**89**) inhibited DNA replication [77a], and a mechanism of its action on cultured human SK-MEL-2 skin melanoma cells has been suggested [77b].





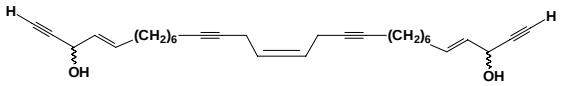
It is worthy of mention that dideoxypetrosynols B (**90**), C **(91)**, and D (**92**) revealed, depending on the

test used, cytotoxic activites one order of magnitude higher than those found for doxorubicine (Table 6).
Lembehyne A (**93**), a novel long chain polyacetylene, was isolated from the Indonesian marine sponge, *Haliclona* sp. [78,79]. Lembehyne A induced bipolar neuritogenesis of Neuro-2A cells at 1 μg/mL.

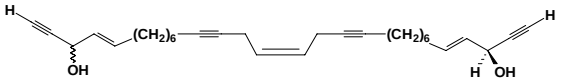
Table 6. *In vitro* cytotoxicites (ED_{50}, μg/mL) of dideoxypetrosynols against human solid tumors

Compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
89	1.43	0.02	0.01	0.16	0.17
90	1.98	0.21	0.11	1.83	1.56
91	12.41	1.83	1.27	1.83	1.87
92	5.78	0.02	0.02	3.02	1.94
Doxorubicin	0.09	0.16	0.11	0.13	1.02

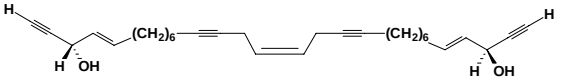
A549, human lung cancer; SK-OV-3, human ovarian cancer; SK-MEL-2, human skin cancer; XF498, human CNS cancer; HCT15, human colon cancer



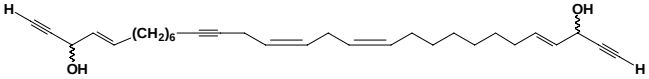
89 Dideoxypetrosynol A



90 Dideoxypetrosynol B



91 Dideoxypetrosynol D



92 Dideoxypetrosynol C

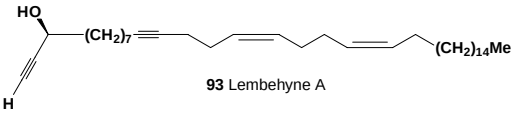
Acetylcholinesterase activity of Neuro-2A was also increased by treatment with **93**. Furthermore, the cell cycle of Neuro-2A cells was found to be specifically blocked by **93** at the G1 phase. Lembehynes B (**94**) and C (**95**), which possess different types of long carbon-chain parts compared with that of lembehyne A, also exhibited neuritogenic activity against a neuroblastoma cell line, Neuro-2A. This indicates the importance of a particular stereochemistry (presence of a hydroxyl group at C-3) of lembehynes for the revealed activity [47].

The sponge *Reniera fulva* from Algeciras Bay, Spain contains, in particular, fulvinol (**96**), that was shown to be cytotoxic to P-388 mouse lymphoma, A-549 human lung carcinoma, HT-29 human colon carcinoma, and MEL-28 human melanoma, ED_{50} 1 μg/mL) [80].

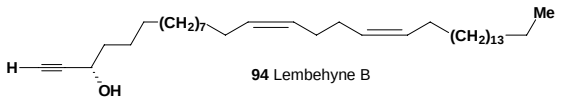
Adociacetylenes A-D (**97**-**100**) were isolated as new polyacetylenes from the Okinawan marine sponge *Adocia* sp. [81]. Adociacetylenes A, C, and D exhibited inhibitory activity in the *in vitro* endothelial cell-neutrophil leukocyte adhesion assay. All acetylenes were highly cytotoxic to P388, A-549, HT-29, and MEL-28 melanoma cells.

Table 7. Predicted biological activities for compounds (**93**-**104**) isolated from sponges

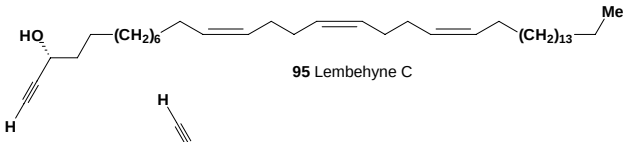
No	Drug-Likeness	Focal Activity Prediction
93	0.986	0.945 0.002 Lactate 2-monooxygenase inhibitor
94	0.981	0.954 0.002 Lactate 2-monooxygenase inhibitor
95	0.981	0.954 0.002 Lactate 2-monooxygenase inhibitor
96	0.992	0.965 0.001 Lactate 2-monooxygenase inhibitor
97	0.994	0.935 0.002 Lactate 2-monooxygenase inhibitor
98	0.992	0.926 0.003 Lactate 2-monooxygenase inhibitor
99	0.992	0.919 0.003 Lactate 2-monooxygenase inhibitor
100	0.993	0.940 0.002 Lactate 2-monooxygenase inhibitor
101	0.993	0.952 0.002 Lactate 2-monooxygenase inhibitor
102	0.991	0.947 0.001 Argininosuccinate lyase inhibitor
103	0.989	0.930 0.003 Lactate 2-monooxygenase inhibitor
104	0.992	0.965 0.001 Lactate 2-monooxygenase inhibitor



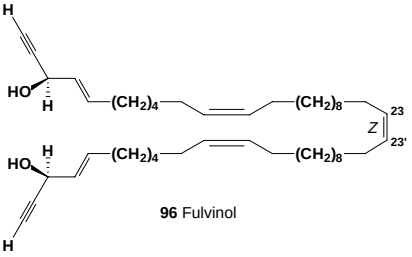
93 Lembehyne A



94 Lembehyne B



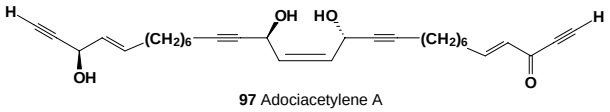
95 Lembehyne C



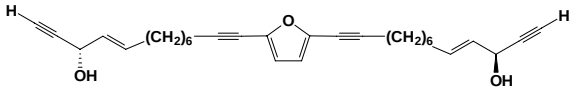
96 Fulvinol

Two acetylenic compounds, petrosynol (**101**), and petrosolic acid (**102**) from the Red Sea sponge *Petrosia* sp. inhibited reverse transcriptase of human immunodeficiency virus [82].

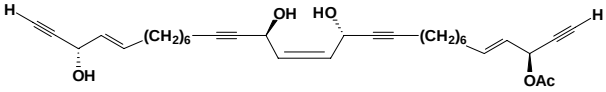
The sponge *Strongylophora durissima* yielded two new acetylenic derivatives, durissimols A and B (**103**), and duryne (**104**) [83]. Among them, durissimol B and duryne showed potent cytotoxicity against human gastric tumor (NUGC) cells.



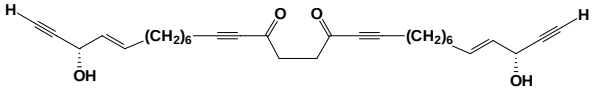
97 Adociacetylene A



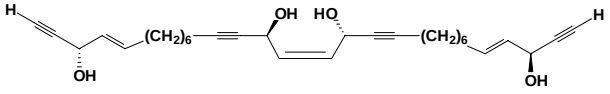
98 Adociacetylene B



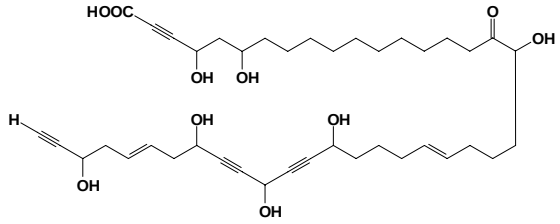
99 Adociacetylene C



100 Adociacetylene D

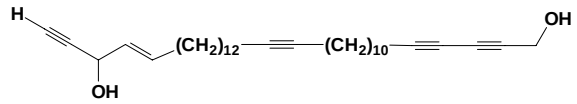


101 Petrosynol

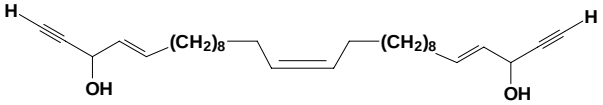


102 Petrosolic acid

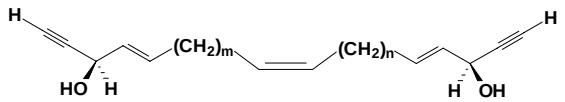
Duryne, a cytotoxic metabolite that inhibits the growth of both mouse and human tumor cell lines *in vitro* was previously isolated from the marine sponge *Cribrochalina dura* [83b,c]. Additional activities for **93-104** are shown in Table 7.



103 Durissimol B



104 Duryne



105 Vasculyne, m + n = 31

A new C43 acetylenic alcohol, vasculyne (**105**), was isolated by cytotoxicity-guided fractionation of the Caribbean sponge *Cribrochalina vasculum* [84]. Vasculyne (**105**) yielded average $G1_{50}$, TGI, and LC_{50} values of 0.2, 0.7, and 6.7 μg/mL, respectively, and exhibited modest differential cytotoxicity toward the melanoma and colon tumor cell-line subpanels when tested against the NCI's 60-cell antitumor screening panel. Structurally, **105** is closely related to the C_{30} compound duryne, previously isolated from the Caribbean sponge *C. dura.*

Osirisynes A-F (**106-111**, respectively), highly oxygenated C_{47} polyacetylenes, have been isolated from the sponge *Haliclona osiris* collected from Guam [85]. These compounds are characterized by the presence of a diacetylenic carbinol and an α-acetylenic carboxylic acid. Osirisynes A-F exhibited moderate cytotoxicity against a human leukemia cell-line (KS62), with LC_{50} values of 25, 48, 52, 25, 20, and 22 μM for **106-111**, respectively. In addition, **108**, **110**, and **111** inhibited Na^+/K^+-ATPase and reverse transcriptase at concentrations of l μg/mL. Additional activities for **106-111** are shown in Table 8.

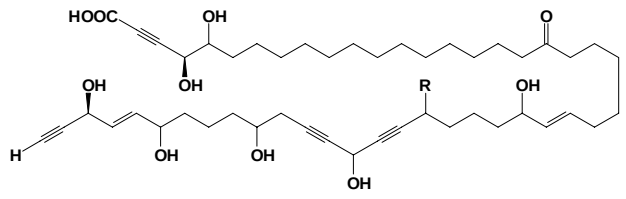
A novel acetylenic compound, taurospongin A (**112**), was isolated from the Okinawan marine sponge *Hippospongia* sp. [86], and amino acid analysis of the hydrolysis products of **112** showed the presence of taurine. Two fragments, a trihydroxylamide and an unsaturated fatty acid methyl ester, were obtained by methanolysis to elucidate the lengths of the methylene chains. Metabolite **112** showed inhibitory activity against c-erbB-2 kinase (IC_{50} 28 μg/mL).

Table 8. Predicted biological activities for osirisynes (A-F)

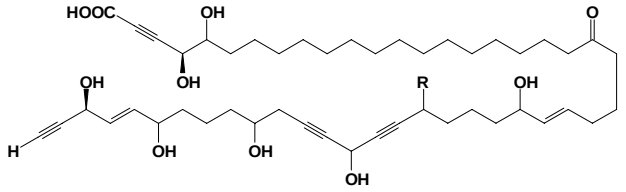
No	Drug-Likeness	Focal Activity Prediction
106	0.993	0.938 0.002 Argininosuccinate lyase inhibitor
107	0.994	0.938 0.002 Argininosuccinate lyase inhibitor
108	0.993	0.938 0.002 Argininosuccinate lyase inhibitor
109	0.994	0.938 0.002 Argininosuccinate lyase inhibitor
110	0.994	0.950 0.001 Argininosuccinate lyase inhibitor
111	0.994	0.934 0.002 Argininosuccinate lyase inhibitor

Callyspongamide A (**113**), a cytotoxic polyacetylenic amide, has been isolated from the marine sponge *Callyspongia fistularis* collected in the Red Sea.

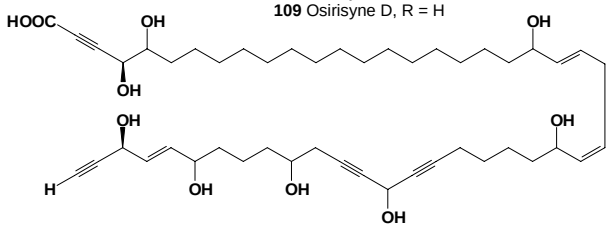
Callyspongamide A is an amide derivative of a C_{17}-polyacetylenic acid and phenethylamine. It showed a moderate cytotoxicity against HeLa cells with an IC_{50} value of 4.1 μg/mL [87].



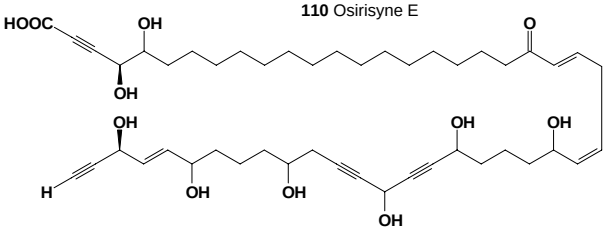
106 Osirisyne A, R = OH
107 Osirisyne B, R = H



108 Osirisyne C, R = OH
109 Osirisyne D, R = H



110 Osirisyne E



111 Osirisyne F

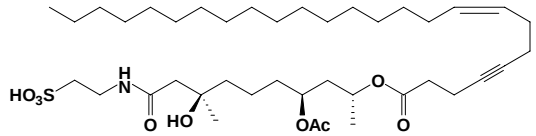
Investigation of the organic extract of a Red Sea sponge, *Callyspongia* sp., resulted in the isolation and identification of three new C22-polyacetylenic alcohols, callyspongenols A-C (**114-116**), together with dehydroisophonochalynol (**117**) [88].

Table 9. Cytotoxicity of compounds (**114-117**) against P388 and Hela Cells (IC_{50}: μg/mL)

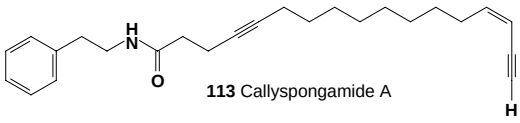
Compound	**114**	**115**	**115**	**117**	adriamycin[a]
P388	2.2	10.0	2.2	2.2	0.04
HeLa	4.5	10.0	3.9	5.1	0.066

[a] Positive cytotoxicity control.

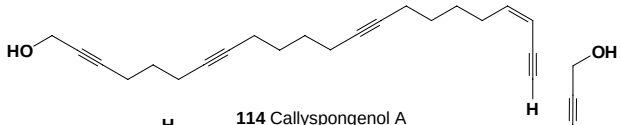
All compounds showed moderate cytotoxicity against P388 and HeLa cells. The cytotoxicity of compounds **114-117** against HeLa and P388 cells is presented in Table 9. Previously, dehydroisophonochalynol (**117**) was isolated from the sponge *Siphonochalina* sp. [88b].



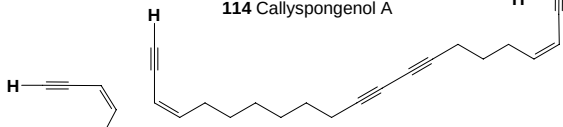
112 Taurospongin A



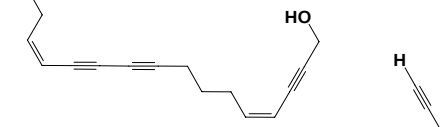
113 Callyspongamide A



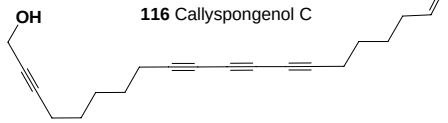
114 Callyspongenol A



115 Callyspongenol B



116 Callyspongenol C



117 Siphonochalynol

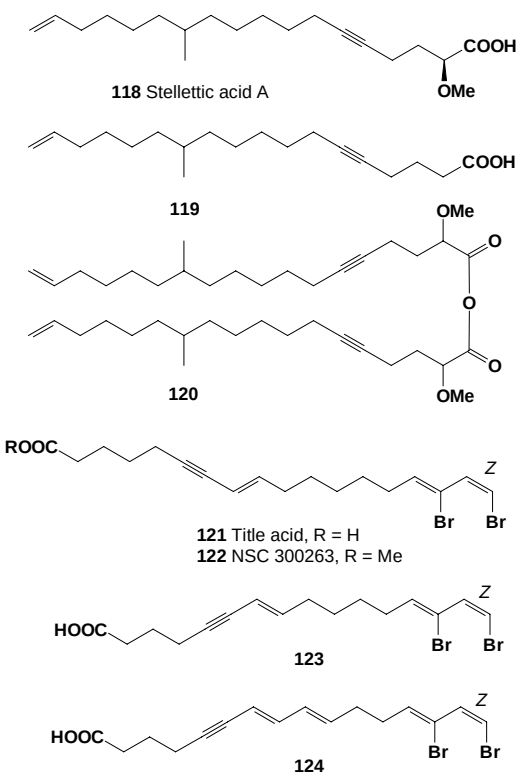
Three new acetylenic metabolites (**118-120**) were isolated from the sponge *Stelletta* sp. collected from Gagu-Do, Korea [89]. These compounds exhibited no significant antimicrobial activity and displayed only weak cytotoxicity against the human leukemia cell-line K562 with LC_{50} values of 43.5, 51.3, and 62.5 μg/mL for **118-120**, respectively. Predicted activities for **112-124** are shown in Table 10.

The title acid **121** and its methyl ester 33B were isolated from the sponge *Xestospongia muta*. The EDs of **121** for 50% inhibition *in vivo* PS and L1210 cell culture evaluations were 24 and 34 μg/mL, respectively, and the corresponding doses of **122**were 29 and 34 μg/mL [90a]. Similar brominated fatty acids **123** and **124** were isolated from an Indonesian sponge, *Oceanapia* sp. [90b]. Their common structural feature is a (13*E*, 15*Z*)-14,16-dibromodiene terminus. Both compounds are unstable oils. The mixture exhibits mild cytotoxicity towards KB cells.

Table 10. Predicted biological activities for compounds (**112-124**)

No	Drug-Likeness	Focal Activity Prediction
112	0.936	0.895 0.007 Squalene-hopene cyclase inhibitor
113	0.904	0.752 0.018 Insulinotropin agonist
114	0.965	0.861 0.002 Alcohol oxidase inhibitor
115	0.980	0.848 0.002 Alcohol oxidase inhibitor
116	0.980	0.848 0.002 Alcohol oxidase inhibitor
117	0.971	0.854 0.002 Alcohol oxidase inhibitor
118	0.957	0.903 0.007 Phosphoenolpyruvate-protein phosphotransferase inhibitor
119	0.962	0.910 0.021 (-)-(4S)-limonene synthase inhibitor
120	0.904	0.856 0.042 (-)-(4S)-limonene synthase inhibitor
121	0.949	0.756 0.004 Phosphoenolpyruvate carboxykinase (GTP) inhibitor
122	0.905	0.682 0.008 Skin diseases treatment
123	0.949	0.756 0.004 Phosphoenolpyruvate carboxykinase (GTP) inhibitor
124	0.956	0.732 0.006 Skin diseases treatment

A methanol-soluble extract of the frozen marine sponge *Petrosia* sp. showed significant activity in the brine shrimp larvae lethality bioassay (LD_{50} 30 μg/mL) [91], and cytotoxic activities against a panel of human solid-tumor cells.



A number of very long-chain C_{46} polyacetylenic alcohols named petrocortynes (**125-130**) have been identified from marine sponge *Petrosia* sp. These compounds showed different cytotoxic activities human tumor cells (Table 11) [91]; for predicted activities see Table 12.

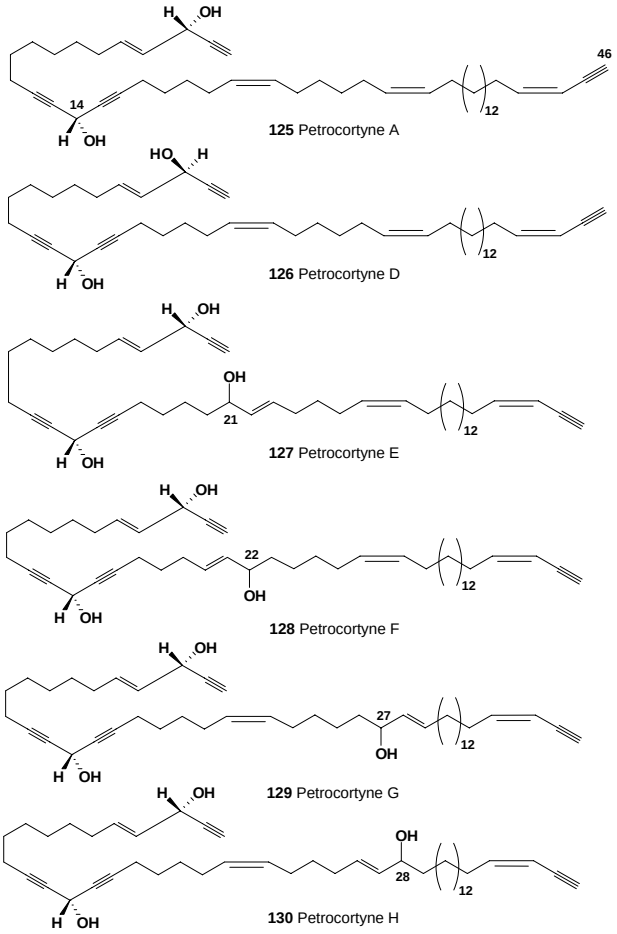


Table 11. Cytotoxic activity petrocortynes (ED_{50} μg/mL) isolated from sponge *Petrosia* sp.

Comp.	A549	SK-OV3	SL-MEL2	XF489	HCT15
125	1.1	0.6	1.1	1.7	10.
126	1.6	0.5	0.9	1.7	1.0
127	1.7	2.2	1.9	>3	3.7
128	1.3	0.1	0.1	0.6	0.8
129	>3	>3	>3	>3	>3
130	1.4	0.1	0.2	1.2	1.2
DOX*	0.1	0.2	0.2	0.2	0.9

*DOX, doxorubicin
A549; human lung carcinoma; SK-OV-3; human ovarian cancer; SK-MEL-2; human skin cancer; XF498; human CNS cancer; HCT15; human colon cancer

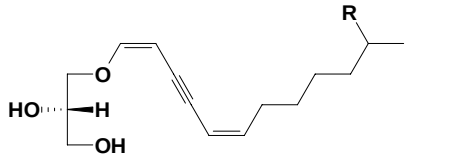
Acetylenic enol ethers of glycerols, including bioactive compounds **131-136,** have been isolated from a sponge of the genus *Petrosia*. Compounds **131** and **133** exhibited weak cytotoxicity against a human leukemia cell-line (K-562) [92]. Bioactivities of glyceryl enol ether compounds (**131, 132** and **133**), of

the yne-diene series, exhibited weak cytotoxicity against the human leukemia cell-line K-562 (LC_{50} 9.2, 57, 29 μg/mL, for **131, 132** and **133**, respectively, while **134-136**, possessing the yne-ene group, were less active (LC_{50} > 100 μg/mL); for predicted activities see Table 13.

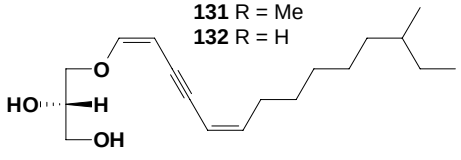
Table 12. Predicted biological activities for petrocortynes isolated from sponge *Petrosia* sp.

No	Drug-Likeness	Focal Activity Prediction
125	0.995	0.942 0.002 Lactate 2-monooxygenase inhibitor
126	0.995	0.942 0.002 Lactate 2-monooxygenase inhibitor
127	0.995	0.931 0.003 Lactate 2-monooxygenase inhibitor
128	0.995	0.931 0.003 Lactate 2-monooxygenase inhibitor
129	0.995	0.931 0.003 Lactate 2-monooxygenase inhibitor
130	0.995	0.931 0.003 Lactate 2-monooxygenase inhibitor

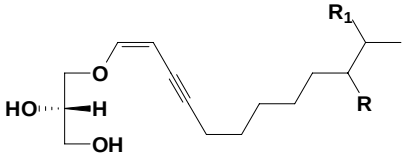
The marine sponge, *Prianos osiros* from Pohnpei, gave a new cytotoxic acetylenic carotenoid, 3,3',5,19'-tetrahydroxy-7',8'-didehydro-γ,ε-carotene-8-one (**137**) [93a], which was cytotoxic toward cultured human colon tumor cells, HCT 116 (IC_{50} 4.38 μg/mL). Two new carotenoids, the neoplasm inhibitors, 19-hexanoyloxymytiloxanthin (**138**) and 19-butanoyloxymytiloxanthin (**139**), have been isolated from the marine sponge *Phakellia stelliderma* collected in Okinawa.



131 R = Me
132 R = H



133



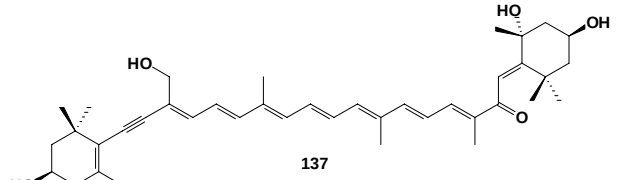
134 R = R_1 = H
135 R = Me, R_1 = H
136 R = H, R_1 = Me

Both compounds showed mild cytotoxic activity against P388 mouse leukemia cells [93b].

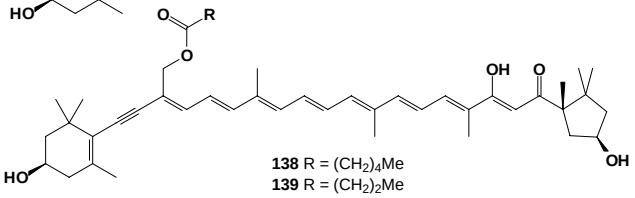
Table 13. Predicted biological activities for acetylenic enol ethers of glycerols

No	Drug-Likeness	Focal Activity Prediction
131	0.941	0.883 0.007 CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase inhibitor
132	0.917	0.901 0.006 CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase inhibitor
133	0.953	0.870 0.007 CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase inhibitor
134	0.880	0.907 0.005 CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase inhibitor
135	0.935	0.877 0.007 CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase inhibitor
136	0.917	0.889 0.006 CDP-diacylglycerol-glycerol-3-phosphate 3-phosphatidyltransferase inhibitor

Many different carotenoids have been isolated from marine (orders Poecilosclerida and Axinellida) and freshwater (*Spongilla fragilis*) sponges [94-96], including the acetylenic carotenoids tedaniaxanthin (*Tedania digitata*, *Microciona prolifera*), alloxanthin (*Microciona prolifera*), 3,3',4,4',7,7',8,8'-octadehydro-β,β-carotene (*Polymastia granulosa*), suberixanthin (*Suberites massa*), and the series of sulfated carotenoids, bastaxanthin(*Ianthella basta)*, crocoxanthin (*Microciona prolifera, Tedania digitata*), isotedaniaxanthin (*Tedania digitata*), and 7,8-didehydro-φ,χ-carotene (*Reniera japonica*). The biological activity of the isolated acetylenic carotenoids has not been reported [97-101]. Structures of the isolated sponge acetylenic carotenoids are presented in a review [94].



137

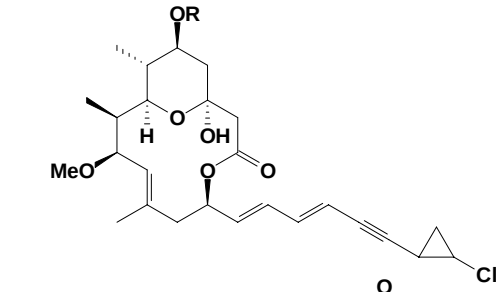


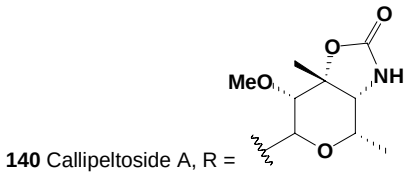
138 R = $(CH_2)_4Me$
139 R = $(CH_2)_2Me$

Callipeltoside A (**140**), the first member of a novel class of marine glycoside macrolides, was isolated from the lithistid sponge *Callipelta* sp. by Minale and co-workers in 1996 [102a]. Preliminary biological assays indicated that this marine natural product exhibited cytotoxic activity against NSCLC-N6 human bronchopulmonary non-small-cell lung carcinoma and P388 cell lines. Callipeltoside A (**140**), B (**141**) and C (**142**) are moderately cytotoxic against NSCLC-N6 cells with IC_{50} values of 10.0, 15.1, and 30.0 μg/mL, respectively [102b].

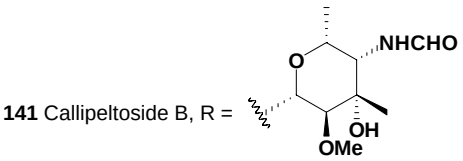
Table 14. Predicted activities for acetylenic carotenoids (**137**-**139**)

No	Drug-Likeness	Focal Activity Prediction
137	0.993	0.859 0.018 β-Adrenergic-receptor kinase inhibitor
138	0.967	0.970 0.002 Integrin antagonist
139	0.967	0.971 0.002 Integrin antagonist

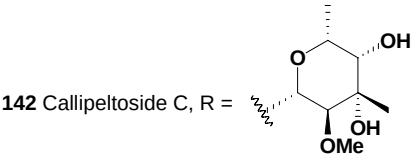




140 Callipeltoside A, R =



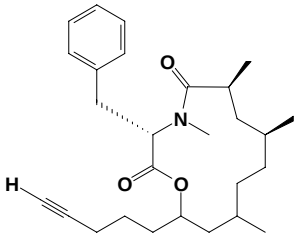
141 Callipeltoside B, R =



142 Callipeltoside C, R =

A novel macrolide, spongidepsin (**143**) has been isolated from the Vanuatu marine sponge *Spongia* sp. [103]. The structure of **143** contains 9-hydroxy-2,4,7-trimethyltetradeca-14-ynoic acid and *N*-methylphenylalanine residues joined in a 13-membered ring. Spongidepsin showed cytotoxic activity against J774.A1, WEHI-164 and HEK-293 cancer cell lines, with an IC_{50} value in the sub-micromolar range (see Table 15). Predicted activities are in Table 16.

New acetylenic sterols, gelliusterol A (**144**, 26,27-bisnorcholest-5-en-23-yn-3β,7α-diol), its corresponding 7-ketone, gelliusterol B (**145**, 26,27-bisnorcholest-5-en-23-yn-3β-ol-7-one), gelliusterols C (**147**, cholest-5-en-23-yn-3β,7-one) and D (**148**, cholest-5-en-23-yn-3β,25-diol-7-one) were isolated from an unidentified species of sponge, *Gellius* sp. [104,105].



143 Spongidepsin

Table 15. *In vitro* antiproliferative activity of spongidepsin

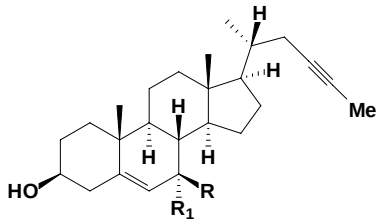
Cell lines	Spongidepsin IC_{50} (μM)	6-Mercaptopurine IC_{50} (μM)
J774.a1	0.56	0.003
HEK-293	0.66	0.007
WEHI-164	0.42	0.017

Steroids containing an atypical acetylenic unit as a component of the side chain have been obtained from extracts of the sponge *Calyx nacaaensis*, where 26,27-bisnorcholest-5-en-23-yn-3β-ol (**146**) and cholest-5-en-23-yn-3β-ol (**149**) were minor components.

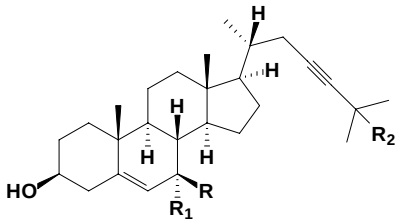
Table 16. Predicted activities for callipeltosides (A-C) and spongidepsin

No	Drug-Likeness	Focal Activity Prediction
140	0.993	0.829 0.030 Phosphatase inhibitor
141	0.994	0.856 0.007 Antineoplastic
142	0.995	0.860 0.007 Antineoplastic
143	0.991	0.790 0.014 Antineoplastic (colorectal cancer)

Biological evaluation of gelliusterols A (**144**), B (**145**), and C (**147**) was performed on cancer cell lines P-388, HT-29, A-549, DU-145, and MEL-28. Gelliusterols A and B exhibited moderate activity, with IC_{50} values greater than 1 μg/mL. An activity of 0.5 μg/mL was observed with gelliusterol C against HT-29, while the other cell lines gave IC_{50} values above 1 μg/mL. The quantity of gelliusterol D was insufficient for biological testing [104].

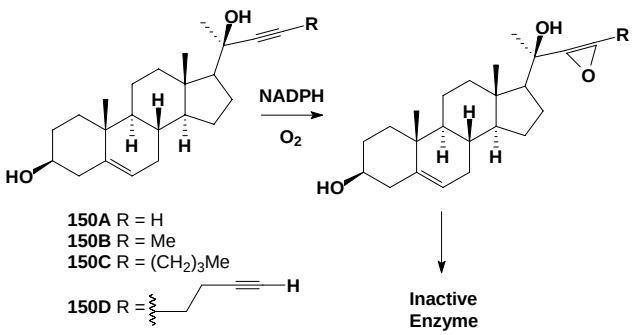


144 Gelliusterol A, R = H, R_1 = OH
145 Gelliusterol B, R,R_1 = O
146 R = R_1 = H



147 Gelliusterol C, R, R_1 = O, R_2 = H
148 Gelliusterol D, R,R_1 = O, R_2 = OH
149 R = R_1 = R_2 = H

Several synthesized acetylenic steroids (**150A-D**) having the same structure, except for the side chains, are excellent inhibitors of P-450scc, although they appear to inactivate the enzyme in a manner distinct from the action of acetylenes on the microsomal enzyme [105]. Incubation of (**150B-D**) with P-450scc in the presence of electron donors and oxygen led to a time-dependent absorbance decrease in the Soret region. This absorbance decrease was found to be dependent on the presence of adrenodoxin, adrenodoxin reductase, NADPH, and oxygen. The proposed mechanism of P-450scc inhibition is shown in Figure 1. Predicted activities for these steroids are shown in Table 17.



150A R = H
150B R = Me
150C R = $(CH_2)_3$Me
150D R =

Fig.1. Steroid derivatives with acetylenic side chains as substrates of P-450scc which would generate a reactive species in the active site, thus leading to suicide inhibition of the enzyme

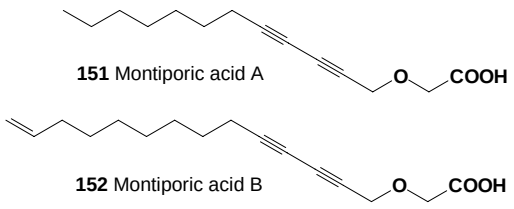
Soft Corals and Other Coelenterates

The old phylum Coelenterata, now included in the phylum *Cnidaria*, contains the corals, jellyfish, hydras, and sea anemones. Soft corals can be found worldwide in tropical environments, and they can contain symbiotic dinoflagellate algae (Zooxanthellae) that provide the coral with food in return for a safe environment. Most corals feed on zooplankton in the water. The defense of soft corals is based mainly on their stinging cells. At the same time, some fish and mollusks are attracted by the fleshy body of the soft corals.

Table 17. Predicted activities for several natural and synthesized steroids

No	Drug-Likeness	Focal Activity Prediction
144	0.990	0.891 0.012 Prostaglandin-E2 9-reductase inhibitor
145	0.975	0.896 0.010 Prostaglandin-E2 9-reductase inhibitor
146	0.985	0.905 0.008 Prostaglandin-E2 9-reductase inhibitor
147	0.960	0.892 0.011 Prostaglandin-E2 9-reductase inhibitor
148	0.961	0.882 0.014 Prostaglandin-E2 9-reductase inhibitor
149	0.972	0.901 0.009 Prostaglandin-E2 9-reductase inhibitor
150A	0.983	0.865 0.018 Prostaglandin-E2 9-reductase inhibitor
150B	0.990	0.882 0.014 Prostaglandin-E2 9-reductase inhibitor
150C	0.985	0.882 0.014 Prostaglandin-E2 9-reductase inhibitor
150D	0.980	0.847 0.022 Prostaglandin-E2 9-reductase inhibitor

Thus they release poisonous secondary metabolites (mostly terpenes) to deter potential predators, as well as to protect themselves from algal and bacterial colonization. In addition, the soft corals are territorial and will defend their territory by releasing toxic compounds inhibiting the growth of neighboring animals and algae [106-108]. Some coral's secondary metabolites showed anti-bacterial, anti-fungal, cytotoxic and anticancer activities.



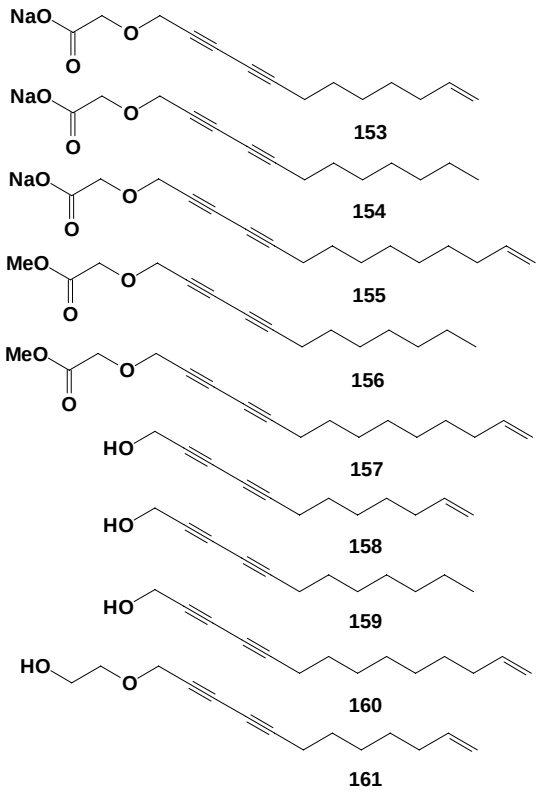
151 Montiporic acid A

152 Montiporic acid B

The genus *Montipora* is very rich in acetylenic compounds and many of them were shown to be cytotoxic and/or to possess antifungal and antibacterial properties. Two polyacetylene carboxylic acids, montiporic acids A (**151**) and B (**152**), have been isolated from the eggs of the scleractinian coral *M. digitata* [109]. They exhibited antimicrobial activity against *Escherichia coli* and

cytotoxicity towards P-388 murine leukemia cells. Montiporic acids A and B were not only antibacterial against *Escherichia coli*, but also cytotoxic against P-388 murine leukemia cells, with IC_{50} values of 5 and 12 μg/mL, respectively.

Coral metabolites **153-167** and two known diacetylenes (**151**,**152**) have been isolated from the methanolic extract of the stony coral *Montipora* sp. [110]. The compounds exhibited significant cytotoxicity against a small panel of human solid tumor cell lines (see Table 18). Compounds **153-161** share a 2,4-diyne moiety suggesting a common biosynthetic precursor. Compounds **162-166** are similar to **153-161** in having a diyne group, but the position is different.



2,4-Diynes are encountered frequently in corals, and this may raise a question of the origin of **162-166**. The isolated compounds have been tested for cytotoxicity against a small panel of human cancer cell lines (Table 18), and most were found to be cytotoxic.

Compound **162** showed significant cytotoxicity against human skin cancer and human ovarian cancer cell lines. In general, diacetylenes with the α-hydroxy ketone functionality (**162-164**) were found to be more active. The *trans*-isomer (**165**) was more active than the *cis*-isomer (**166**), as in the case of montiporyne A-D. Montiporyne A (**167**), an analog of **165**, showed significant cytotoxicity towards human solid tumor cell lines. Montiporyne A (**167**) showed significant cell cycle inhibition in the HCT116 cell.

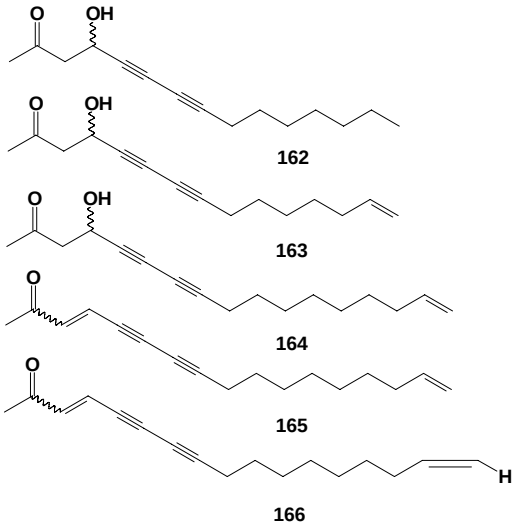


Table 18. Cytotoxic activities (ED_{50} μg/mL) of compounds (**153-166**) against human solid tumor cells

Compd	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
153	>30	>30	>30	>30	>30
154	6.31	7.50	7.97	7.72	8.30
155	6.26	4.88	4.68	4.96	4.47
156	>30	20.52	>30	>30	25.61
157	>30	>30	>30	>30	>30
158	13.78	9.79	9.56	10.78	12.93
159	5.48	4.63	4.45	5.59	5.90
160	3.90	3.23	3.94	5.26	3.32
161	22.73	17.94	25.08	16.88	24.05
162	4.17	1.81	1.40	3.70	3.73
163	4.97	3.85	3.74	3.87	3.42
164	4.91	3.34	3.52	4.45	4.18
165	6.39	3.52	4.21	5.50	4.56
166	>30	5.23	4.61	29.16	11.30

Key to cell lines used: A549 = human lung cancer; SK-OV-3 = human ovarian cancer; SK-MEL-2 = human skin cancer; XF498 = human CNS cancer; HCT15 = human colon cancer

Six acetylenic compounds, montiporyne A-F (**167-172**, respectively), with cytotoxic activities against human solid tumor cell lines SK-OV-3, SK-MEL-2, XF498, and HCT15, have been isolated from the stony coral *Montipora* sp. (Table 19) [111].

Polyacetylenes (**173-176**), found in three species of the hermatypic corals, *Montipora* sp., *M. mollis*, and *Pectinia lactuca*, represent metabolites of coelenterates, which exhibit ichthyotoxicity and inhibit the growth of some bacteria, fungi, and fish

[112]. Predicted activities of the compounds isolated from corals are shown in Table 20.

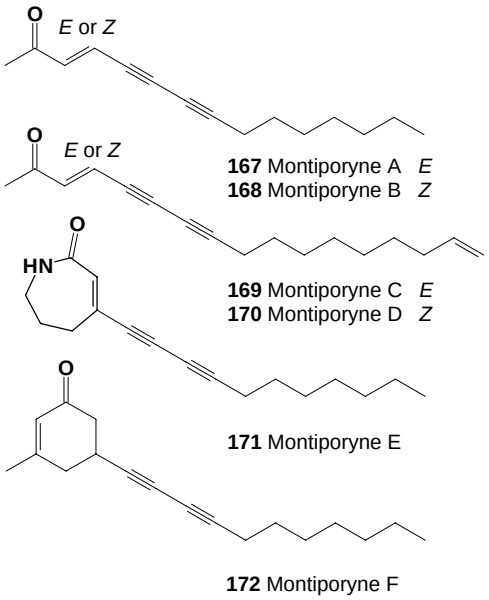


Table 19. *In vitro* cytotoxicities (ED_{50}, μg/mL) of montiporynes against human solid tumor cells

Comp.	A549	SK-OV3	SL-MEL2	XF489	HCT15
167	>50	3.2	1.4	1.9	3.7
168	>50	2.5	1.5	3.2	5.2
169	>50	25.9	42.6	>50	>50
170	>50	45.1	43.1	>50	>50
171	>50	>50	>50	>50	>50
172	>50	29.2	35.7	31.3	45.1
CIS*	0.6	0.9	0.7	0.6	0.6

CIS*, cisplatin; A549: human lung cancer; SK-OV-3: human ovarian cancer; SK-MEL-2: human skin cancer; XF498: human CNS cancer; HCT15: human colon cancer. Compounds were assayed in two separate batches.

During separation of the astaxanthin-proteins of the mantle tissue of jellyfish-like *Velella velella* (phylum Cnidaria, class Hydrozoa), three unusual acetylenic carotenoids (**177-179**) were isolated [113], and their predicted biological activities are shown in Table 21.

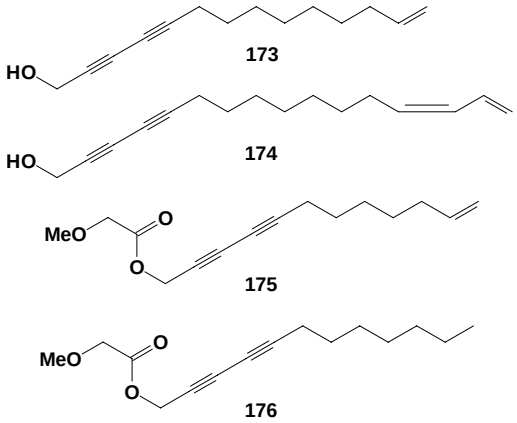
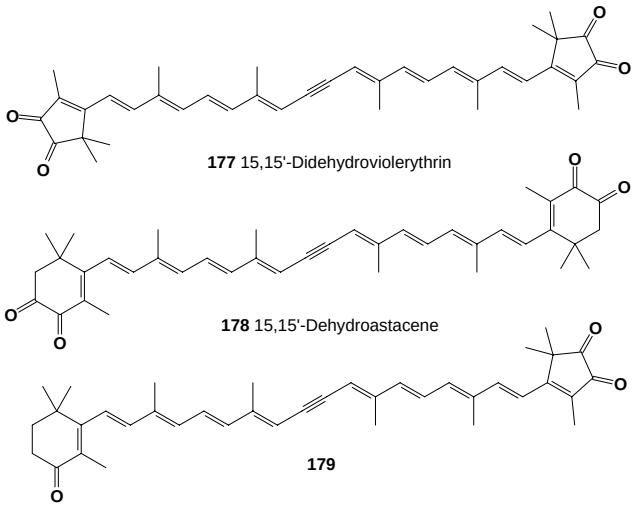


Table 20. Predicted activities for compounds (**151-176**) isolated from soft corals

No	Drug-Likeness	Focal Activity Prediction
151	0.869	0.948 0.005 Sarcosine oxidase inhibitor
152	0.891	0.914 0.008 Sarcosine oxidase inhibitor
153	0.891	0.914 0.008 Sarcosine oxidase inhibitor
154	0.869	0.948 0.005 Sarcosine oxidase inhibitor
155	0.891	0.914 0.008 Sarcosine oxidase inhibitor
156	0.717	0.908 0.008 Sarcosine oxidase inhibitor
157	0.776	0.899 0.025 (-)-(4S)-Limonene synthase inhibitor
158	0.895	0.940 0.012 (-)-(4S)-Limonene synthase inhibitor
159	0.865	0.849 0.002 Alcohol oxidase inhibitor
160	0.895	0.940 0.012 (-)-(4S)-Limonene synthase inhibitor
161	0.843	0.922 0.017 (-)-(4S)-Limonene synthase inhibitor
162	0.958	0.868 0.007 *trans*-Pentaprenyl-transtransferase inhibitor
163	0.954	0.897 0.008 Phosphoenolpyruvate-protein phosphotransferase inhibitor
164	0.954	0.897 0.008 Phosphoenolpyruvate-protein phosphotransferase inhibitor
165	0.951	0.919 0.018 (-)-(4S)-Limonene synthase inhibitor
166	0.951	0.919 0.018 (-)-(4S)-Limonene synthase inhibitor
167, 168	0.947	0.904 0.007 Aminocarboxymuconate-semialdehyde decarboxylase inhibitor
169, 170	0.951	0.919 0.018 (-)-(4S)-limonene synthase inhibitor
171	0.852	0.752 0.008 Anti-inflammatory
172	0.970	0.832 0.052 Antiseborrheic
173	0.895	0.940 0.012 (-)-(4S)-Limonene synthase inhibitor
174	0.942	0.926 0.007 β-Adrenergic-receptor kinase inhibitor
175	0.584	0.993 0.001 (-)-(4S)-Limonene synthase inhibitor
176	0.478	0.992 0.001 (-)-(4S)-Limonene synthase inhibitor

Table 21. Predicted activities for acetylenic carotenoids

No	Drug-Likeness	Focal Activity Prediction
177	0.993	0.955 0.002 β-Carotene 15,15'-monooxygenase inhibitor
178	0.990	0.949 0.002 β-Carotene 15,15'-monooxygenase inhibitor
179	0.993	0.944 0.002 β-Carotene 15,15'-monooxygenase inhibitor

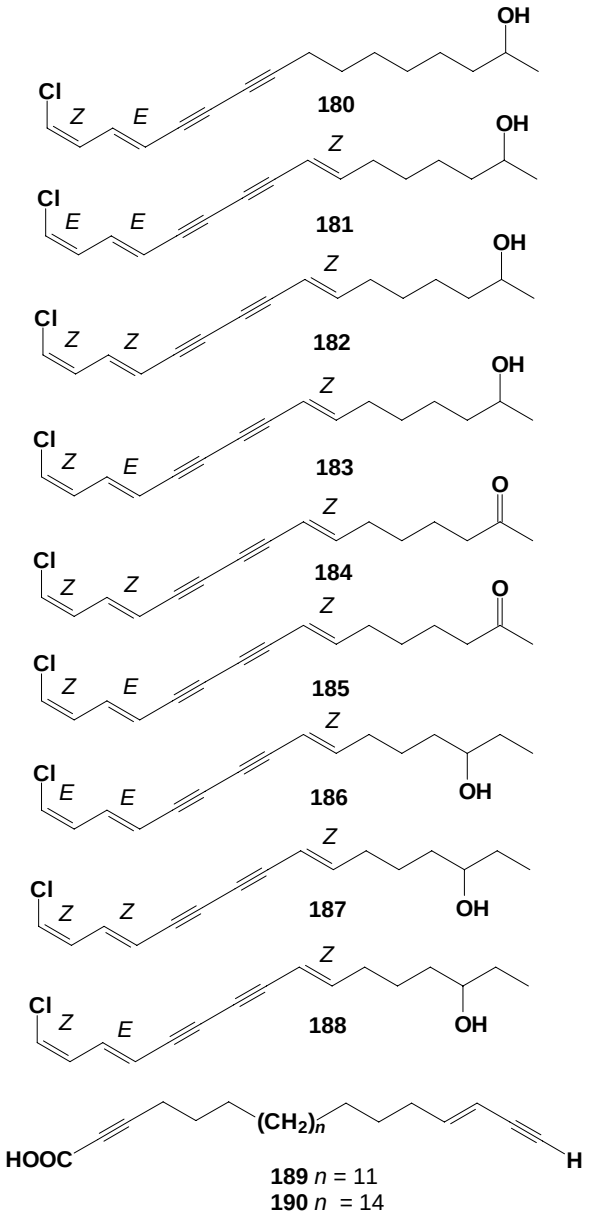


Marine and Freshwater Mollusks

The mollusks (= molluscs) are the large and diverse phylum Mollusca, which includes eight living classes: Caudofoveata (deep-sea wormlike creatures), Aplacophora (solenogasters, deep-sea wormlike creatures), Polyplacophora (chitons), Monoplacophora (deep-sea limpet-like creatures), Scaphopoda (tusk shells), Cephalopoda (squids, octopuses, nautilus, and cuttlefish), Bivalvia (clams, oysters, scallops, and mussels), and Gastropoda (nudibranchs, snails and slugs, limpets, and sea hares) [114]. Only the last two classes (Bivalvia and Gastropoda), have been well studied and are of great interest from the pharmaceutical point of view. These two mollusc classes produced many biologically active compounds, including acetylenic metabolites, some of which showed cytotoxic, anticancer, and other activities [115].

The ethanol extract of the mucous secretion from the opisthobranch mollusk *Oxynoe olivacea* was examined and found to contain two novel ichthyotoxic metabolites, oxytoxin 1 (**27**) and 2 (**28**) [116a]. The structures of the two compounds are closely related to the metabolites previously isolated from the alga *Caulerpa prolifera*. The activity of the most stable compound was studied to investigate the possibility of a further biological role for these metabolites, which represent an uncommon example of bioactive molecules produced *in vivo* from a dietary precursor. Another acetylenic bioactive metabolite, caulerpenyne (**20**), was isolated from the mesogastropod *Littorina irrorata*, which used the macrophytes *Caulerpa prolifera* and *Cymopolia barbata* for its diet [116b].

The nudibranch *Diaulula sandiegensis* produced nine bioactive chloroacetylenes (**180**-**188**) as chemical defensive agents [117a]. Both the nudibranchs *Dendrodoris grandiflora* and *D. limbata* contain two acetylenic acids (**189** and **190**) [117b]. Predicted biological activities for compounds (**180**-**190**) are shown in Table 22.



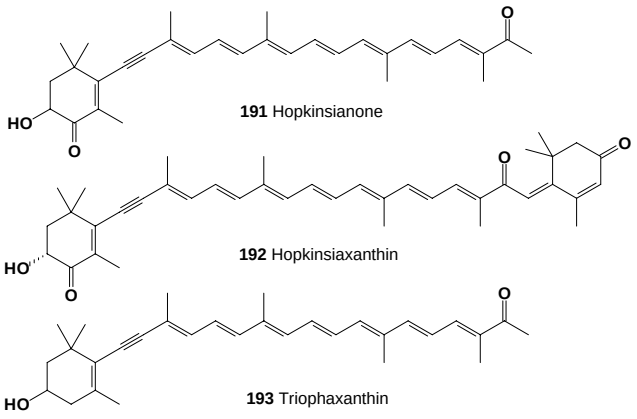
Two pigments, hopkinsianone (**191**) and hopkinsiaxanthin (**192**), were found in the marine mollusk, *Hopkinsia rosacea*, which gives the animal its striking rose-pink color [118]. The pigments isolated were identical to those of its food organism, the bryozoan *Eurystomella bilabiata*. The nudibranchs, *Anisodoris nobilis*, *Dendrodoris fulva*, and *Doriopsilla albopunctata*, contained unusually

high percentages of carotenes in their integumental carotenoids. The main pigment of *Triopha carpenteri* (triophaxanthin **193**), identified as an acetylenic apocarotenoid, was also found in the food carotenoids, as were all of the other fractions isolated from this nudibranch. Astaxanthin, the only carotenoid found in *Flabellinopsis iodinea*, was isolated in three different forms: free, esterified, and conjugated to a protein [118].

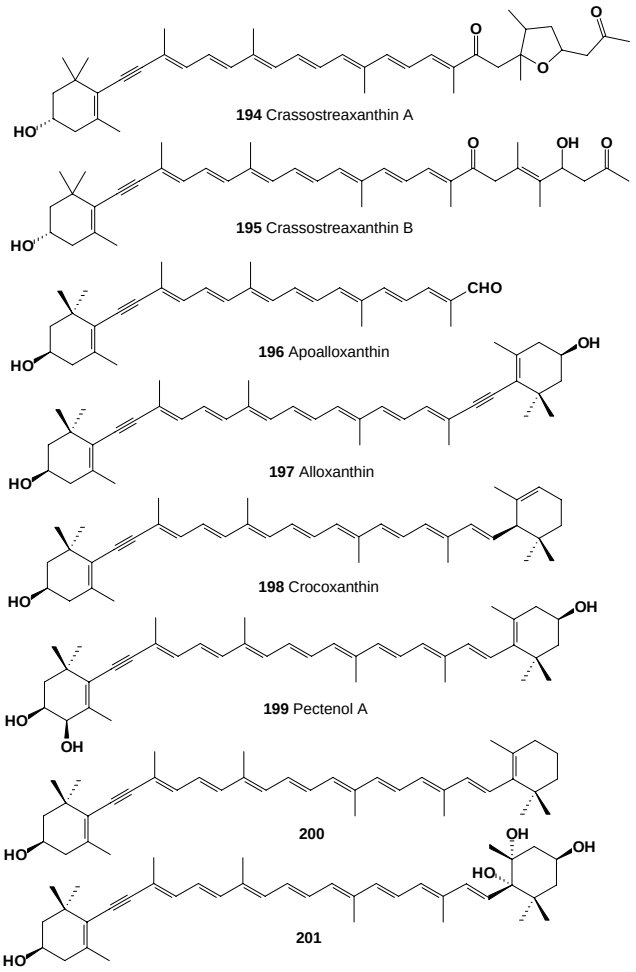
Table 22. Predicted activities for metabolites (**180**-**190**) isolated from mollusk species

No	Drug-Likeness	Focal Activity Prediction
180	0.944	0.815 0.006 Skin irritation, inactive
181-183	0.952	0.824 0.006 Skin irritation, inactive
184, 185	0.775	0.882 0.018 Mucomembranous protector
186-188	0.946	0.746 0.008 Skin irritation, inactive
189	0.958	0.975 0.001 Argininosuccinate lyase inhibitor
190	0.958	0.975 0.001 Argininosuccinate lyase inhibitor

Cytotoxic carotenoids, named crassostreaxanthins A (**194**), and B (**195**), and apoalloxanthinal (**196**), were isolated from the oyster *Crassostrea gigas* (Ostreidae), and apocarotenoid (**200**) from the marine shellfish *Mytilus coruscus* [119]. Predicted biological activites are see in Table 23.



Acetylenic carotenoids (**62, 63, 197, 198, 199**, 7,8-didehydro-β-cryptoxanthin **200**), and 6-epi-heteroxanthin (**201**) have been isolated from three species of corbicula clams, *Corbicula japonica*, *C. sandai*, and *Corbicula* sp. (Chinese freshwater corbicula clam) [120]. Total carotenoid content of the muscle of *Peronidia venulosa* and *Corbicula fluminea*, and of the gonad of *Atrina pinnata* and *Chlamys farreri* ranged from 2.51 to 6.83 mg per cent, values that are relatively higher than those of other shellfishes. Carotenoids of *Crassostrea gigas* and *Corbicula fluminea* were also studied. The antimutagenic effect of the carotenoids isolated from shellfish and tunicata against 2-amino-3-methylimidazol [4,5-f]quinoline (IQ) for *S. typhimurium* TA 98 was proportional to the amounts (20, 50 and 100 μg/plate) used. The growth of HeLa cells by β-carotene, cynthiaxanthin, astaxanthin and halocynthiaxanthin, NCI-H87 cells by β-carotene, astaxanthin, cynthiaxanthin, and halocynthiaxanthin, HT-29 cells by β-carotene, cynthiaxanthin, mytiloxanthin and halocynthia-xanthin, and MG-63 cells by β-carotene, cynthiaxanthin, astaxanthin, cantha-xanthin and halocynthiaxanthin were significantly reduced [121].



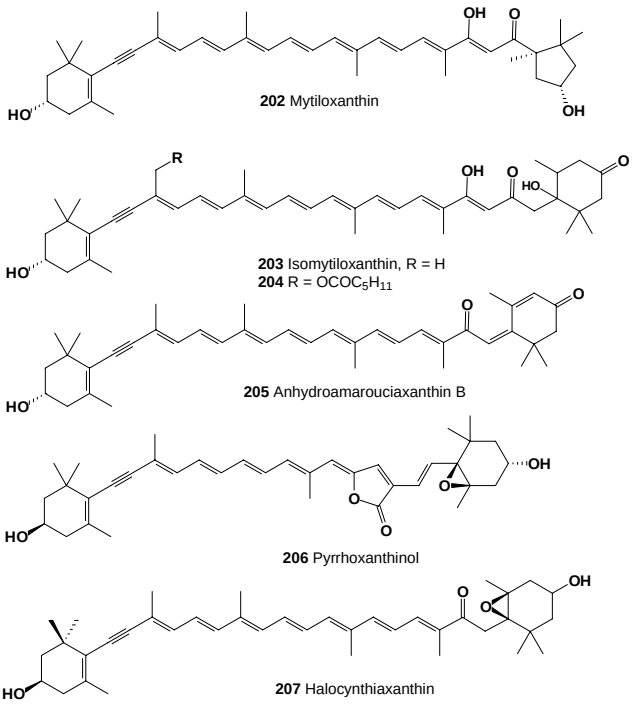
Several cytotoxic acetylenic carotenoids (**62**, **63**, **197**, **199**), isomytiloxanthin (**203**), 19'-(hexanoyloxy)-isomytiloxanthin (**204**), hydroamarouciaxanthin B (**205**), pyrrhoxanthinol (**206**), and halocynthiaxanthin (**207**) have been identified from muscle of *Mytilus edulis* [122,123]. Predicted biological activities of

several acetylenic carotenoids are shown in Tables 23 and 24.

Table 23. Predicted activities for acetylenic carotenoids (**191**-**201**)

No	Drug-Likeness	Focal Activity Prediction
191	0.980	0.830 0.031 Mucomembranous protector
192	0.982	0.833 0.024 β-Adrenergic-receptor kinase inhibitor
193	0.982	0.874 0.014 β-Adrenergic - receptor kinase inhibitor
194	0.991	0.843 0.007 Apoptosis agonist
195	0.984	0.856 0.018 β-Adrenergic - receptor kinase inhibitor
196	0.983	0.877 0.014 β-Adrenergic - receptor kinase inhibitor
197	0.990	0.908 0.008 β-Adrenergic - receptor kinase inhibitor
198	0.991	0.886 0.012 β-Adrenergic - receptor kinase inhibitor
199	0.993	0.932 0.006 β-Adrenergic - receptor kinase inhibitor
200	0.992	0.972 0.004 Dermatologic
201	0.991	0.882 0.013 β-Adrenergic - receptor kinase inhibitor

In addition, the cytotoxic carotenoids mytiloxanthinone (**208**), pectenol A and B (**209**), and pectenolone (**210**) have also been isolated from shellfish [122,123].



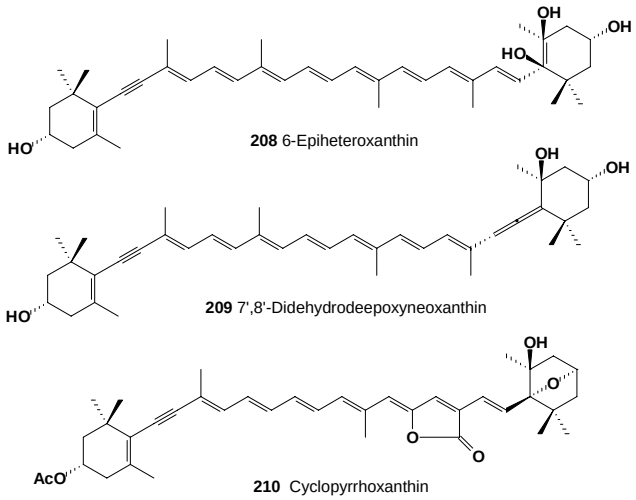
Diatoxanthin, alloxanthin, and pectinolone from *Pectene maximus* [124a] and *Patinopectene yessoensis* [124b], pectinols A and B from *Mytilus coruscus* [125a], crassostreaxanthins A and B from *Crassostrea gigas* [125b], and a series of carotenoids with a 5,6-dihydro-β-end group from *Fushinus perplexus* [126a] have been reported as the principal carotenoids in marine shellfish.

Table 24. Predicted activities for acetylenic carotenoids (**202**-**210**)

No	Drug-Likeness	Focal Activity Prediction
202	0.976	0.981 0.001 Integrin antagonist
203	0.985	0.759 0.043 β-Adrenergic-receptor kinase inhibitor
204	0.977	0.791 0.082 Antiseborrheic
205	0.984	0.875 0.014 β-Adrenergic-receptor kinase inhibitor
206	0.973	0.883 0.005 Antipsoriatic
207	0.989	0.962 0.005 Dermatologic
208	0.991	0.882 0.013 β-Adrenergic -receptor kinase inhibitor
209	0.993	0.839 0.022 β-Adrenergic -receptor kinase inhibitor
210	0.949	0.800 0.006 Antipsoriatic

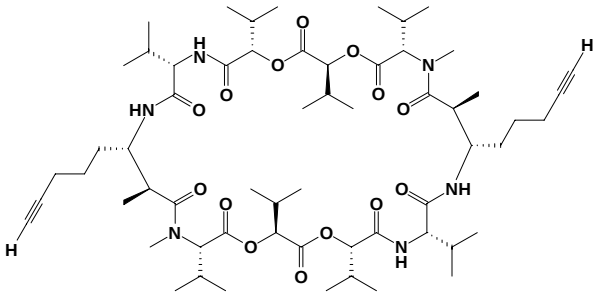
Carotenoids in eight species of freshwater and sea mollusks from Russia were investigated [126b]. Alloxanthin, mytiloxanthin, isomytiloxanthin, halocynthiaxanthin ether from *Modiolus modiolus*, and *Crenomytilus grayanus*; alloxanthin, and mytiloxanthin from *Mytilus galloprovincialis*; alloxanthin, mytiloxanthin, isomytiloxanthin, halocynthiaxanthin ether, and pectenolon from *Mizuhopecten yessoensis* have been isolated [126b].

Three new acetylenic carotenoids, 6-epiheteroxanthin (**208**), 7',8'-didehydrodeepoxy-neoxanthin (**209**), and cyclopyrrhoxanthin (**210**), were isolated from *Corbicula japonica* (Shijimi in Japanese) [127].

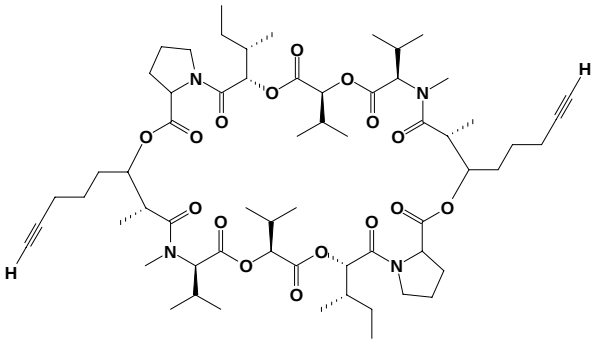


Marine depsipeptides are natural bio-oligomers composed of hydroxy and amino acids linked by

amide and ester bonds, and many of them showed very promising biological activities, including anticancer, antibacterial, antiviral, antifungal, anti-inflammatory, anti-clotting and anti-antherogenic properties. Depsipeptides have shown the greatest therapeutic potential as anticancer agents [128a].

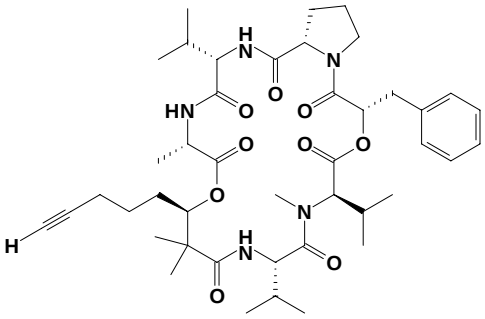


211 Onchidin

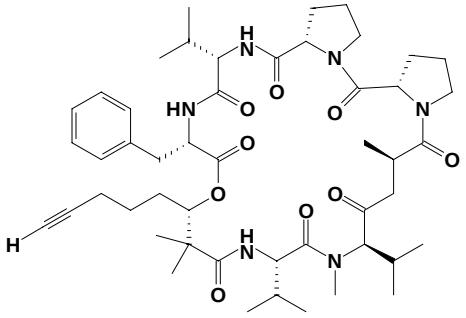


212 Onchidin B

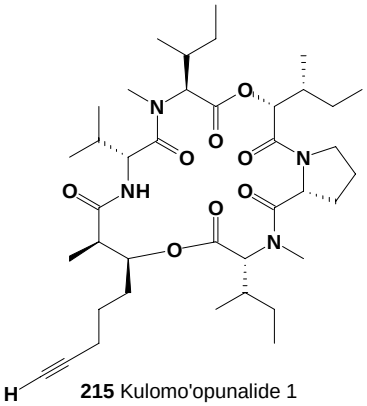
The depsipeptides onchidin (**211**), and onchidin B (**212**) were isolated from the pulmonate mollusk *Onchidium* sp. Onchidin contains the β-amino acid, 3-amino-2-methyl-7-octynoic acid, and onchidin B, the β-hydroxy acid, 3-hydroxy-2-methyl-7-octynoic acid. The onchidins are known to be cytotoxic, but no details were given in regard to this activity [128b,c]. Kulolide (**213**), a cyclic depsipeptide, was isolated from a cephalaspidean mollusk, *Philinopsis speciosa* [129]. Kulolide is made up of five amino acid residues, one each of L-Ala, L-Pro, and N-Me-D-Val and two of L-Val, and two carboxylic acids, L-3-phenyllactic acid and the unprecedented (*R*)-3-hydroxy-2,2-dimethyl-7-octynoic acid. Kulolide was active against L-1210 leukemia cells and P388 murine leukemia cells, with IC_{50} values of 0.7 and 2.1 μg/mL, respectively. Kulolide caused morphological changes of rat 3Y1 fibroblast cells at a concentration of 50 μM. In addition to five new depsipeptides related to kulolide-1 (**213**), further examination of the mollusk *Philinopsis speciosa* has yielded a linear peptide, pupukeamide, and an unprecedented macrolide, tolytoxin-23-acetate. The chemical makeup suggested that the compounds originate from cyanobacteria, which are transmitted *via* herbivorous mollusks to *P. speciosa* [129]. Combined extracts [(EtOH and $CHCl_3$/MeOH (1:1)] of the mollusk *Philinopsis speciosa* yielded kulolide-1 (**213**), kulolide-2, kulolide-3, kulokainalide-1 (**214**), kulomo'opunalide-1 (**215**), kulomo'opunalide-2 (**216**), and tolytoxin 23-acetate.



213 Kulolide



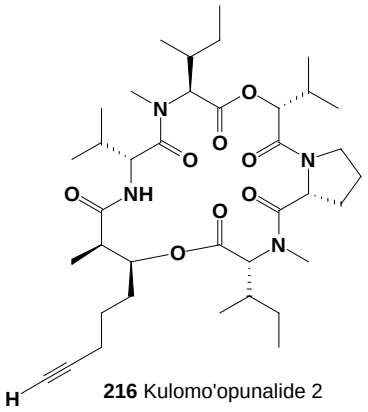
214 Kulokainalide 1



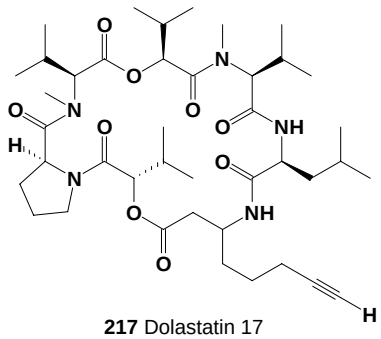
215 Kulomo'opunalide 1

Kulolide-1 (**213**) caused morphological changes to rat fibroblast cells at a concentration of 50 μM. Less than 0.1% contamination with tolytoxin might account for this activity. Peptides **214-216** showed only moderate cytotoxicity against P388 cells, and additional activities shown in Table 25.

The unusual cyclodepsipeptide dolastatin 17 (**217**) was isolated from the Papua New Guinea sea hare *Dolabella auricularia* (Gastropoda, Orthogastropoda, Aplysiidae) and found to contain an acetylenic β-amino acid, designated dolayne. Dolastatin 17 exhibited significant human cancer cell growth inhibitory activity (GI_{50} 0.45-0.74 μg/mL range) [130].



216 Kulomo'opunalide 2



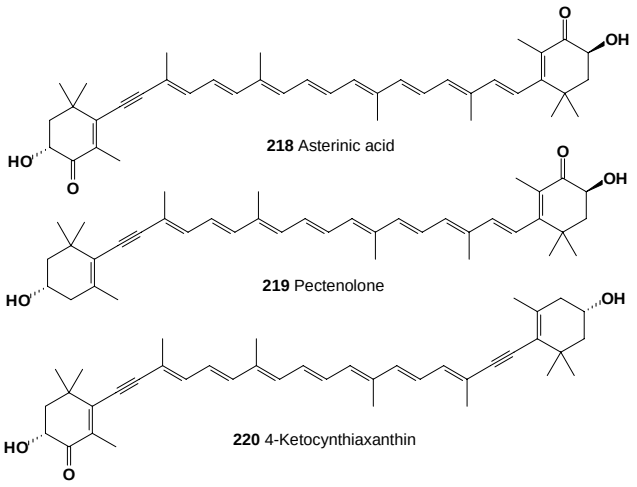
217 Dolastatin 17

Table 25. Predicted activities for depsipeptides (**211**-**217**) isolated from mollusks

No	Drug-Likeness	Focal Activity Prediction
211	0.994	0.800 0.004 Antibiotic Glycopeptide-like
212	0.994	0.766 0.009 Transplant rejection treatment
213	0.993	0.751 0.011 Transplant rejection treatment
214	0.992	0.762 0.007 Tocolytic
215	0.994	0.784 0.008 Transplant rejection treatment
216	0.994	0.815 0.012 Antineoplastic (colorectal cancer)
217	0.994	0.812 0.015 Integrin antagonist

Starfish and Other Echinoderms

Echinoderms ("spiny skin" in Greek, including starfish, brittle stars, crinoids, sea urchins, and sea cucumbers) are radially symmetrical invertebrates that are only found in the sea. Most echinoderms live on the bottom of the ocean floor [131]. More than 6,500 species have been recorded in the phylum Echinodermata. A variety of biologically active substances have been isolated from the echinoderms: carotenoids, ether lipids, glycolipids, saponins, naphthoquinones, porphyrins, and others; some of the isolated metabolites contain the acetylenic unit [132]. Several substances unique to echinoderms have also been reported, some of which showed high potential as new medicaments.



218 Asterinic acid

219 Pectenolone

220 4-Ketocynthiaxanthin

From the calyx and arms of *Lamprometra klunzingeri* (family Mariametridae, class Crinoidea, Echinodermata), collected in the Red Sea, the cytotoxic carotenoids diadinochrome (**60**), alloxanthin (**197**), cynthiaxanthin, pectenoxanthin, and asterinic acid (**218**) have been isolated [133].

Asterinic acid (**218**) was found in Echinoderms from the Adriatic Sea: *Coscinasterias tenuispina, Marthasterias glacialis, Paracentrotus lividus,* and *Sphaerechinus granularis* [134]. The cytotoxic acetylenic carotenoids diatoxanthin, and alloxanthin (other names: cynthiaxanthin and pectenoxanthin) were present in the gonads of Australian and Japanese species of the echinoids *Heliocidaris erythrogramma* and *H. tuberculata* [135a], in the sea urchin *Pseudocentrotus depressus* [135b], in *Peronella japonica* [136a], in seven species of sea-urchins, belonging to the orders Cidaroida, Echinothurioida, Diadematoida, and Arbacioida, as well as pectenolone (**219**) and 4-ketocynthiaxanthin (**220**) [136b].

Asterinic acid was isolated from *Asterias rubens*, *Acanthaster planci*, *Coscinastrias acutispina*, *Leiaster leachii*, *Asterias amurensis*, *Ophidiaster ophidianus*, *Asterina panceri*, *Asteropecten*

aurantiacus, and *Marthasterias glacialis* [137-139]. Mytiloxanthin was found in *Ophiocomina nigra* [91a], and derivatives of the cytotoxic acetylenic carotenoids, (3S,4S,3'S,5'R)-4-hydroxy-mytiloxanthin, (3S,4S,3'S,4'S)-4,4'-dihydroxydiatoxanthin, (3S, 4S,3'S,4'S)-4,4'-dihydroxy-alloxanthin, (3S,3'S,4'S)-4-keto-4'-hydroxydiatoxanthin, and (3S,3'S,4'S)-4-keto-4'-hydroxyalloxanthine were isolated from the starfish, *Asterina pectinifera* and *Asterias amurensis* [140].

Table 26. Predicted activities for acetylenic carotenoids (**218**-**224**) isolated from echinoderms

No	Drug-Likeness	Focal Activity Prediction
218	0.990	0.911 0.008 β-Adrenergic-receptor kinase inhibitor
219	0.991	0.918 0.007 β-Adrenergic-receptor kinase inhibitor
220	0.990	0.874 0.014 β-Adrenergic-receptor kinase inhibitor
221	0.988	0.980 0.001 Integrin antagonist
222	0.992	0.904 0.009 Beta-adrenergic-receptor kinase inhibitor
223	0.992	0.844 0.021 β-Adrenergic-receptor kinase inhibitor
224	0.993	0.929 0.006 β-Adrenergic-receptor kinase inhibitor

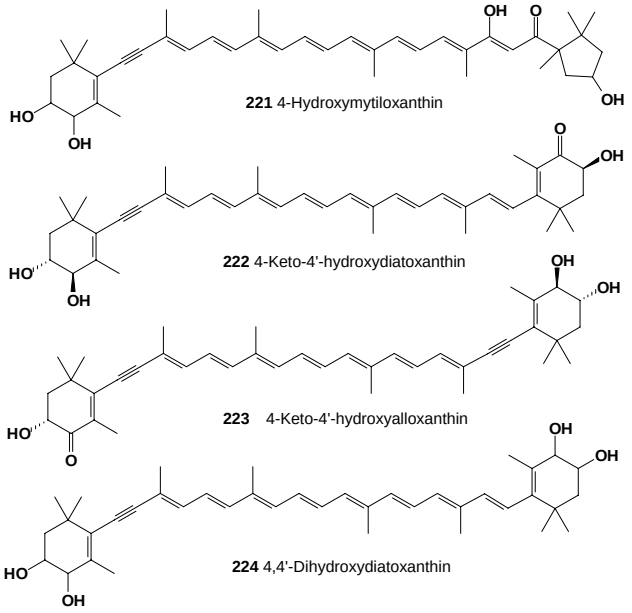
Carotenoid content of the seven species of sea cucumber (*Stichopus japonicus*, *Holothuria leucospilota*, *H. moebi*, and *H. pervicax* of the order Aspidochirotida, *Cucumaria japonica*, *C. echinata*, and *Pentacta australis* of the order Dendrochirotida) was reported [141]. β-Carotene, β-echinenone, canthaxanthin, phoenicoxanthin, and astaxanthin were common in all the sea cucumbers examined. Alloxanthin, diatoxanthin, and pectenolone were isolated as minor carotenoids. The bluish violet pigment in the dorsal skin of *Asterias rubens* was isolated as an amorphous powder [142].

Asteric acid (**221**) and four derivatives of alloxanthin, diatoxanthin, and mytiloxanthin (**224**-**227**) were isolated and their structures elucidated. More detailed information on the carotenoids and other bioactive compounds was also reported [143]. Predicted biological activities of these acetylenic carotenoids are shown in Table 26.

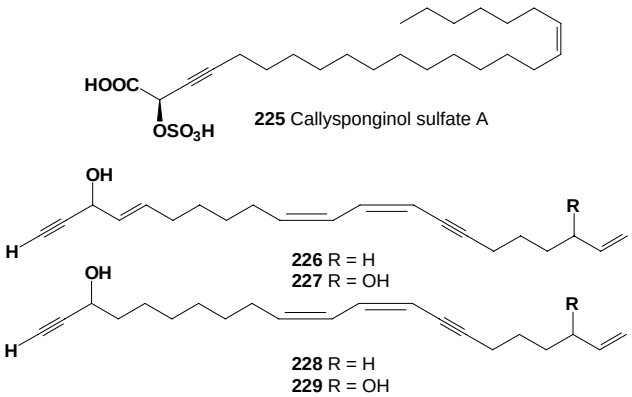
Tunicates (or Sea Squirts)

Tunicates (Urochordates) are small, box-like filter-feeding animals that live either alone or in colonies cemented to the sea floor. Many species of tunicata produce bioactive compounds [144].

Callysponginol sulfate A (**225**), a sulfated C24 acetylenic fatty acid from the marine sponge *Callyspongia truncata*, is a membrane type 1 matrix metalloproteinase (MT1-MMP) inhibitor with an IC_{50} value of 15.0 μg/mL [145a]. and sodium 1-(12-hydroxy)octadecanyl sulfate was isolated from a marine tunicate as a matrix metalloproteinase 2 (MMP2) inhibitor [145b]. This compound inhibited MMP2 with an IC_{50} value of 9.0 μg/mL.



Four novel straight-chain polyacetylenic alcohols (**226**-**229**) were isolated from a marine ascidian (Phyllum Chordata, subphyllum Urochordata) collected off Vigo, along the Atlantic coast of northwestern Spain [146]. Predicted activities of these acetylenic alcohols are shown in Table 27.

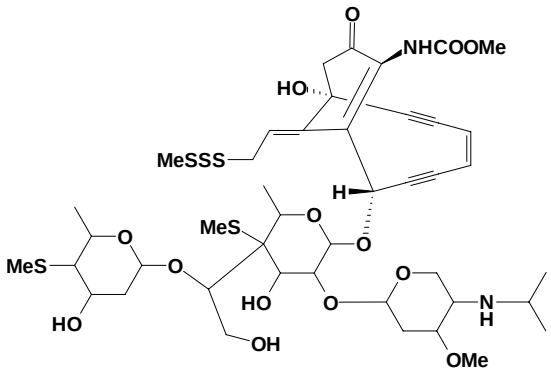


The carotenoids of the muscles and tunic of the tunicates *Halocynthia aurantium*, *H. roretzi*, *Styela clava*, and *Styela plicata*, were isolated and identified [147].

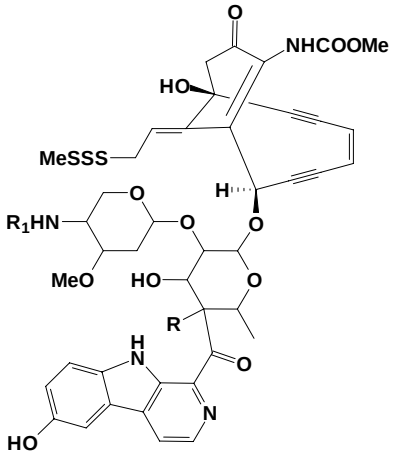
Table 27. Predicted activities for acetylenic alcohols (**225-229**) isolated from tunicates

No	Drug-Likeness	Focal Activity Prediction
225	0.979	0.971 0.003 Benzoate-CoA ligase inhibitor
226	0.993	0.918 0.003 Lactate 2-monooxygenase inhibitor
227	0.993	0.964 0.003 Phosphoenolpyruvate-protein phosphotransferase inhibitor
228	0.991	0.918 0.003 Lactate 2-monooxygenase inhibitor
229	0.992	0.966 0.003 Phosphoenolpyruvate-protein phosphotransferase inhibitor

Antimutagenic activities of the carotenoids towards *S. typhimurium* TA 98 and cytotoxic activity for cancer cell lines were detected. Total carotenoid contents in the muscle of tunicata ranged from 18.65 to 2.39 mg per 100 g fresh meat.



230 Namenamicin



231 Shishijimicin A, R = SMe, R_1 = iPr
232 Shishijimicin B, R = H, R_1 = iPr
233 Shishijimicins C, R = SMe, R_1 = Et

The highest amount of total carotenoid was found in the muscle of *Halocynthia aurantium*, followed by *Styela clava*, *H. roretzi* and *S. plicata*, in that order. The major carotenoids of *H. roretzi*, *H. aurantium*, S. *plicata*, and *S. clava* were cynthiaxanthin (25.1-42.2%), halocynthiaxanthin (9.7-26.3%), diatoxanthin (8.0-18.7%) and β-carotene (7.7-21.7%). Diadinochrome, mytiloxanthin, diatoxanthin, alloxanthin, pectenolone, and halocynthiaxanthin were isolated from *Halocynthia roretzi* [147]. Halocynthiaxanthin (**207**), with little side effects at 2 μg/mL inhibited growth of HeLa, COLO32ODM, HGC-27, PANC-I, and GOTO cells, *in vitro* [148a]. Halocyanthiaxanthin (5 μg/mL), from the sea squirt *Halocynthia roretzi*, caused complete suppression of human neuroblastoma GOTO cell proliferation, reducing the growth rate by 88.8% compared with the control. Furthermore, halocyanthiaxanthin also inhibited the growth of other human malignant tumor cells. Thus halocyanthiaxanthin seems to be a promising antineoplastic agent [148b].

Table 28. Cytotoxicity of namenamicin and shishijimicins (IC_{50}, pg/mL)

Cell line	**230**	**231**	**232**	**233**
2Y1	13.0	2.0	3.1	4.8
HeLA	34.0	1.8	3.3	6.3
P-388	3.3	0.47	2.0	1.7

Extracts of the colonial marine ascidian, *Polysyncraton lithostrotum*, collected from Namenalala Island, Fiji Islands, showed induction of the SOS repair response in a Biochemical Induction Assay (BIA) and potent cytotoxicity against a panel of human tumor cell lines. Bioassay guided fractionation of the extract, following BIA activity, yielded namenamicin (**230**), a new enediyne antitumor antibiotic. DNA cleavage experiments showed that namenamicin cleaves DNA less specifically than calicheamicin [149]. The MeOH and EtOH extracts of the tunicate, following reverse-phase HPLC, afforded shishijimicins A (**231**), B (**232**), and C (**233**), together with the known namenamicin (Tables 28 and 29).

Table 29. Predicted activities for namenamicin and shishijimicins (A-C)

No	Drug-Likeness	Focal Activity Prediction
230	0.992	0.977 0.005 Antineoplastic
231	0.992	0.954 0.006 Antineoplastic
232	0.994	0.951 0.006 Antineoplastic
233	0.992	0.954 0.006 Antineoplastic

Crustacea

Crustacea is the only group of arthropods that is primarily marine, though there are also many fresh water species. There are some semi-terrestrial species, but these are not, in general, well adapted for

life on land [150]. Carotenoids are widespread in many aquatic species, as well as in crustacea. They are unable to synthesize carotenoids *de novo*, and rely upon the diet as a source of these compounds. Over recent years, there has been considerable interest in dietary carotenoids with respect to their potential in alleviating age-related diseases in humans. Dietary carotenoids are the sole biological precursors of retinoids in crustaceans. Retinoids play a prominent role in many developmental processes, including embryonic development and differentiation of various cell types [150,151].

Alloxanthin was found in some crustacean species: *Eupagurus prideauxii*, *Calanus finmarchicus*, *Emerita analoga*, *Parribacus antarcticus*, *Scyllarides squamosus*, and *Paralithodes brevipes* [152-153]. Diatoxanthin was present in six species of Black Sea crustaceans: crabs (*Eriphia spinifrons* and *Portunus holsatus*), shrimp (*Crangon crangon* and *Leander adspersus*), and isopods (*Idothea algirica* and *I. chelipes*) [154]. Many other non-acetylenic carotenoids were detected in different crustacean species, and their metabolism has also been reported [154-158].

Acetylenic Carotenoids of Marine and Freshwater Fish

Acetylenic metabolites, including alcohols, acids, sterols, and other compounds have not been found in fish. The group of carotenoids found in fish is known as xanthophylls, the major ones of which are astaxanthin, canthaxanthin, and their derivatives. The dominant carotenoids are astaxanthin, which is common to red fish, lutein, common to freshwater species, tunaxanthin common to Scombrina, Carangina and Percina fish, and a few other carotenoids common to some groups of fish.

Astaxanthin is the primary source of pigmentation in crustaceans and ornamental fish. Normally carotenoids are obtained via the natural diet of organisms in the wild, however, in aquaculture, carotenoids have to be specifically added to their artificial diet. In addition to the dominant ones, the fish usually contain other carotenoids, for example, acetylenic, in smaller amounts, the proportion of which often differs between samples, possibly due to their physiological and/or dietal condition [159-161]. Major cytotoxic and other acetylenic carotenoids isolated and identified from marine and freshwater fish are shown in Table 30. Predicted biological activities of alloxanthin and diatoxanthin isolated from fish species are shown in Tables 2 and 23.

Table 30. Distribution of major cytotoxic and other acetylenic carotenoids in marine and freshwater fish

Latin Name	Carotenoid	Ref.
Diodon holocanthus *Mugil cephalus* *Trachurus japonicus* *Spheroides niphobles*	alloxanthin diatoxanthin	[162a]
Gnathopogon *Hemibarbus barbus* *Pseudogobio esocinus* *Sarcocheilichthys variegatus*	alloxanthin diatoxanthin	[162b]
Chaenogobius isaza *Cottus pollus* *Cottus reinii*	alloxanthin diatoxanthin	[163a]
Hypomesus japonicus *Salangichthys microdon*	alloxanthin diatoxanthin	[163b]
Cololabis saira *Exocoetus volitans* *Gasterosteus aculeatus* *microcephalus* *Oryzias latipes* *Prognichthys agoo* *Pungitius sinensis*	alloxanthin diatoxanthin	[164a]
Ischikauia steenackeri *Moroco steindachneri* *Opsariichthys uncirostris* *Tribolodon hakonesis* *Zacco platypus* *Zacco temmincki*	alloxanthin diatoxanthin	[164b]
Tilapia nilotica	alloxanthin diatoxanthin	[165a]
Oncorhynchus masou *macrostomus* *Oncorhynchus masou masou* *Salmo gairdneri* *Salmo trutta* *Salvelinus fontinalis* *Salvelinus leucomaenis* *Salvelinus namaycush*	alloxanthin diatoxanthin	[165b]
Channa maculata *Cichlasoma citrinellum* *Cichlasoma elisalium* *Lepomis macrochirus* *Serrasalmo nattereri* *Serrasalmus nattereri*	alloxanthin diatoxanthin	[166]
Pagrus major	alloxanthin diatoxanthin	[167]
Coryphaena hippurus *Pacific mackerel* *Prognichthys agoo* *Seriola quinqueradiata*	alloxanthin diatoxanthin	[168]
Crucian carp *Sarocheilichthys variegatus* *Carassius gibelio langsdorfi*	alloxanthin asterinic acid diatoxanthin 4,4'-diketo- cynthiaxanthin	[169]
Siniperca scherzeri	alloxanthin, diatoxanthin	[170]
Salvelinus malma malma	alloxanthin diatoxanthin	[171]
Coreoperca herzi *Siniperca scherzeri*	alloxanthin diatoxanthin	[172]
Plecoglossus altivelis	alloxanthin diatoxanthin	[173]
Rhodeus uyekii	alloxanthin diatoxanthin	[174]
Acheilognathus koreensis	alloxanthin diatoxanthin	[175]
Rhinogobius brunneus	alloxanthin diatoxanthin	[176]

Concluding Remarks

Intensive searches for new classes of pharmacologically potent agents produced by cyanobacteria, micro- and macroalgae, and marine and freshwater invertebrates have resulted in the discovery of dozens of compounds possessing high cytotoxic activities. However, only a limited number of them have been tested in pre-clinical and clinical trials.

One of the reasons is a limited supply of the active ingredients from the natural sources. However, the pre-clinical and clinical development of many marine-derived natural products into pharmaceuticals is often hampered by a limited supply from the natural source. Total synthesis is of vital importance in these situations, allowing for the production of useful quantities of the target compound for further biological evaluation.

Organic compounds that contain acetylenic unit(s) that are produced by diverse cyanobacteria, micro- and macroalgae, marine and freshwater invertebrates, have stimulated interdisciplinary studies by chemists and biologists. Many of the compounds exhibit biological activity relevant to human physiology and disease states. The extraordinary diversity of marine life and global occurrence of marine natural products asks some fundamental questions: how are they distributed and what are their natural functions? How do marine natural products serve their hosts and influence the life histories of individual species and even large-scale marine community structures? Studies in biodiversity and chemical ecology reveal roles of natural products and organizing principles, not only at the species level, but also within the context of marine environmental dynamics that shape communities ranging from tropical reefs to the Antarctic benthos.

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