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Marine Dinoflagellates as source of new bioactive structures

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Abstract

Dinoflagellates are an extremely diverse group of unicellular eukaryotes that represent an important part of marine plankton communities. Amongst other secondary metabolites, these microorganisms are known to produce potent toxins such as the saxitoxins, which can affect marine fauna and ultimately humans who consume seafood in which these toxins have accumulated. The extreme biodiversity of dinoflagellates undoubtedly mirrors an inexhaustible reservoir of compounds, yet the chemodiversity of dinoflagellates is still poorly studied, due in part to constraints linked to the difficulty of isolating and cultivating these organisms. Nevertheless, a number of new dinoflagellate secondary metabolites have been described in recent years. Here we review these new advances in the isolation and characterization of bioactive metabolites from dinoflagellates, some of which might be of therapeutic interest in human health applications.

Keyword: Microalgae, dinoflagellates, chemodiversity, phycotoxins, bioassays.

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1. Introduction

Dinoflagellates (Dinoflagellata, Alveolata) are a lineage of protists that are ecologically important components of marine and freshwater microbial communities. To date, about 2400 species of dinoflagellate belonging to 260 genera have been described [1], making this one of the most diverse protist lineages. Approximately half of dinoflagellate species are photosynthetic, therefore being considered as microalgae. Most photosynthetic dinoflagellates have reddish brown chloroplasts containing chlorophylls a and c2, beta-carotene and a group of unique xanthophylls including peridinin. Some, however, contain golden brown or green chloroplasts originating from independent tertiary endosymbiotic events. Dinoflagellates characteristically possess two dissimilar flagella, one of which (the transverse flagellum) lies in a groove around the cell (the cingulum) and the other that beats posteriorly (longitudinal flagellum). Dinoflagellates have a complex cell covering called an amphiesma composed of a series of membranes, flattened ‘amphiesmal vesicles’ and related structures. In many dinoflagellates these support overlapping cellulose plates to create a sort of armour called the theca (as opposed to ‘athecate’ or ‘naked’ dinoflagellates). Most dinoflagellates have a peculiar form of nucleus, called a dinokaryon, in which the permanently condensed chromosomes are attached to the nuclear membrane. Most dinoflagellates are pelagic, but many benthic forms exist, and some dinoflagellates, such as the genera *Symbiodinium* [2–4], *Pelagodinium* [5], *Brandtodinium* [6] (can form symbiotic relationships with invertebrate animals or other protists). Dinoflagellates are a rich source of bioactive compounds. Many dinoflagellates are well known producers of toxins that can be harmful to humans via syndromes like Paralytic Shellfish Poisoning (PSP) [7–11], Neurotoxic Shellfish Poisoning (NSP) [12–14], Azaspiracid Shellfish Poisoning (AZP) [15,16], Diarrhetic Shellfish Poisoning (DSP) [17] and Ciguatera Fish Poisoning (CFP) [18,19]. These metabolites are generally

detected because of their bioaccumulation through the marine food-chain into marine organisms including fish, crustaceans and mollusks, which are consumed by humans. It is known that these neurotoxins interact with specific neurotransmitter receptors or voltage-sensitive ion channels [20]. Voltage-gated Na^+ , Ca^{2+} , and K^+ ion channels lead to the generation of electrical signals involved in muscle contraction, hormone secretion, sensing of the environment, processing of information in the brain and nervous output from the brain [21]. It is therefore important to obtain information on the molecular structure of these metabolites in order to attempt to develop means to regulate/control their effects. Other dinoflagellate metabolites may have beneficial biological properties, such as amphidinol macrolides that have been shown to exhibit anti-bacterial and anti-fungal activities [22]. The constraints for isolation and characterization of new molecules produced by dinoflagellates remain cumbersome. Most significantly, large-scale cultivation of dinoflagellates to obtain sufficient biomass for purification and characterization of new molecules is relatively complicated because of (i) their relatively low growth rate, and (ii) the difficulty of attaining high cell density in culture. In addition, some heterotrophic or mixotrophic species cannot be cultivated in monoclonal or axenic culture conditions, being dependent on acquisition of eukaryotic or bacterial prey. The extreme biodiversity of dinoflagellates means that they undoubtedly represent a huge potential reservoir of compounds to be discovered and/or molecularly characterized. Interest in this potential source of bioactive compounds is reflected in the recent use of cutting edge technologies for the characterization of chemical structures, leading to a growing number of new secondary metabolites from dinoflagellates having been described. A set of these metabolite structures was selected in this review to highlight these recent advances.

2. Chemical diversity sorted by genus

The present review can be considered as an update of the previous chapter “*Bioactive metabolites from marine dinoflagellates*” in *Comprehensive Natural Products II: Chemistry and Biology* (2010). In light of the vast and diverse chemical reservoir produced by dinoflagellates, this review is not intended to be exhaustive. Rather, we provide here an overview of new advances on the isolation and the structural characterization of metabolites from dinoflagellates that have been shown to exhibit bioactivities of potential interest for applications related to human health (**Table 1**).

Table 1. Selected set of metabolites characterized from dinoflagellates.

Dinoflagellates (Genus)	Metabolites	Chemical class	Biological Activity	Reference
<i>Amphidinium</i>	Amphidinin A (1)	Polyketides	<i>Bacillus subtilis</i> : MIC of 16 µg/mL <i>Aspergillus niger</i> : IC ₅₀ of 4 µg/mL	[23,24]
	Amphidinin C (2)		<i>Staphylococcus aureus</i> : MIC of 32 µg/mL <i>Bacillus subtilis</i> : MIC of 32 µg/mL <i>Aspergillus niger</i> : IC ₅₀ of 12 µg/mL <i>Trichophyton mentagrophytes</i> : IC ₅₀ of 16 µg/mL	[25]
	Amphidinin D (3)		<i>Trichophyton mentagrophytes</i> : IC ₅₀ of 16 µg/mL	[25]
	Amphidinin E (4)		<i>Staphylococcus aureus</i> : MIC of 32 µg/mL <i>Bacillus subtilis</i> : MIC of 32 µg/mL <i>Aspergillus niger</i> : IC ₅₀ of 16 µg/mL <i>Trichophyton mentagrophytes</i> : IC ₅₀ of 16 µg/mL	[25]
	Amphidinin F (5)		<i>Trichophyton mentagrophytes</i> : IC ₅₀ of 16 µg/mL	[25]
	Amphidinin G (7)		<i>Trichophyton mentagrophytes</i> : IC ₅₀ of 8 µg/mL	[23,24]
	Amphidinol 17 (9)		Hemolytic Activity	EC ₅₀ of 4.9 µM in a hemolytic assay [26]
	Amphidinol 18 (10)		Antifungal	<i>Candida albicans</i> : IC ₅₀ of 9 µg/mL [27]
	Amphidinol 19 (11)		-	- [27]
	Amphidinol 20 (12)		Hemolytic Activity	EC ₅₀ of 3 µM in a hemolytic assay [28]
	Amphidinol 21 (13)		-	- [28]
	Amphirionin-2 (14)	Polyketides	Cytotoxicity/Toxic	Caco-2 : IC ₅₀ 0.1 µg/mL; A549 : IC ₅₀ 0.1 µg/mL T/C 120% at dose of 0.5mg/kg (Murine tumor) [29]
	Amphirionin-4 (15)		Cells proliferation activity	950% proliferation propotion of ST-2 cells at 0.1 ng/mL. [30]
	Amphirionin-5 (16)		Cells proliferation activity	282% proliferation propotion of ST-2 cells at 320% proliferation propotion of MC3T3-E1 cells at 10 ng/mL. [31]

Dinoflagellates (Genus)	Metabolites	Chemical class		Biological Activity	Reference
<i>Amphidinium</i>	Iriomoteolides-1a (17)	Macrolides	Cytotoxicity	DG-75: IC ₅₀ of 0.004µg/mL	[32]
	Iriomoteolides-1b (18)			DG-75: IC ₅₀ of 0.9µg/mL	[32]
	Iriomoteolides-1c (19)			DG-75: IC ₅₀ of 0.004µg/mL	[32]
	Iriomoteolides-2 (20)			DG-75: IC ₅₀ of 0.006µg/mL; HeLa: IC ₅₀ of 0.03µM	[33]
	Iriomoteolides-3a (21)			DG-75: IC ₅₀ of 0.08µg/mL	[34]
	Iriomoteolides-3b (22)			DG-75: IC ₅₀ of 0.02µg/mL	[34]
	Iriomoteolides-4 (23)			DG-75: IC ₅₀ of 0.8µg/mL	[35]
	Iriomoteolides-5 (24)			DG-75: IC ₅₀ of 1µg/mL	[35]
	Iriomoteolides-9 (25)			Hela: IC ₅₀ of 15mM	[36]
	Iriomoteolides-10 (26)			DG-75: IC ₅₀ of 1.5 µM; HeLa: IC ₅₀ of 1.5µM MH134: IC ₅₀ of 3.3µM	[37]
	Iriomoteolides-11 (27)			DG-75: IC ₅₀ of 1.5 µM; HeLa: IC ₅₀ of 2µM	[36]
	Iriomoteolides-12 (28)			DG-75: IC ₅₀ of 50µM	[37]
	Amdigenol-A (29)	Polyketides	ND	ND	[38]
	Amdigenol-E (30)		ND	ND	[39]
	Amdigenol-G (31)		ND	ND	[39]
<i>Azadinium</i>	Azaspiracids-33 (33)	Polyether	Cytotoxic	TIB-152: EC ₅₀ of 5.2 nM	[16]
	Azaspiracids-34 (34)			TIB-152: EC ₅₀ of 0.2 nM	[16]
	Azaspiracids-36 (35)			TIB-152: EC ₅₀ of 1.70 nM	[40]
	Azaspiracids-37 (36)			TIB-152: EC ₅₀ of 0.85 nM	[40]
<i>Dinophysis</i>	Acuminolide-A (37)	Polyether	ATPase activity	250% ATPase activity in skeletal muscles at 1µM	[41]

Dinoflagellates (Genus)	Metabolites	Chemical class	Biological Activity	Reference
<i>Gambierdiscus</i>	Gambierone (38)	Polyether	Ladder-shaped toxin	A slight cytosolic Ca ²⁺ increase was observed (at 30 nM to SH-SY5Y) [42]
	Gambieroxide (40)	Polyether	ND	ND [43]
<i>Karlodinium</i>	Karlotoxin-1a (42)	Polyketides	Hemolytic Activity	EC ₅₀ of 63 nM in a hemolytic assay [44]
	Karlotoxin-1b (43)			EC ₅₀ of 56 nM in a hemolytic assay [45]
	Karlotoxin-1c (44)			EC ₅₀ of 300 nM in a hemolytic assay [45]
	Karlotoxin-2 (45)			- [45]
	Karlotoxin-3a (46)			EC ₅₀ of 200 nM in a hemolytic assay [45]
	Karlotoxin-3b (47)			EC ₅₀ of 110 nM in a hemolytic assay [45]
	Karlotoxin-3c (48)			EC ₅₀ of 2400 nM in a hemolytic assay [45]
	Karmitoxin (49)	Polyketides	Toxic	RTgill-W1 : LC ₅₀ of 125 ± 1nM Aciarta tonsa (copepod) : LC ₅₀ of 400 ± 100nM [46]
<i>Ostreopsis</i>	Ostreol-A (50)	Polyketides	Toxic	Artemia salina : LD ₅₀ of 0.9 µg/mL. [47]
	Ostreol-B (51)		Cytotoxic	HepG2 : IC ₅₀ of 4.8 µM ; HCT-116 : IC ₅₀ of 0.1 µM Neuro2a : IC ₅₀ of 0.9 µM [48]
<i>Prorocentrum</i>	Prorocentin-1 (52)	Polyketides	Cytotoxic	DLD-1 : IC ₅₀ of 16.7µg/mL RPMI7951 : IC ₅₀ of 83.6µg/mL [49]
	Prorocentin-4 (53)		-	- [50]
	Belizentrin (54)	Macrolides	Neurotoxic	50% reduction in maximum neuronal survival after 24 h EC ₅₀ was estimated at 193 ± 7 nM. [51]
	Limaol (55)	Polyketides	Cytotoxic	HepG2 : IC ₅₀ of 3.7 µM ; HCT-116 : IC ₅₀ of 7.3 µM Neuro2a : IC ₅₀ of 9.6 µM [52]
<i>Vulcanodinium</i>	Pinnatoxin-E (56)	Macrolides	Toxic	LD ₅₀ of 57.0µg/kg (mice by intraperitoneal injection) [53]
	Pinnatoxin-F (57)			LD ₅₀ of 12.7µg/kg (mice by intraperitoneal injection) [53]
	Pinnatoxin-G (58)			LD ₅₀ of 48.0µg/kg (mice by intraperitoneal injection) [53]
	Pinnatoxin-H (59)			LD ₅₀ of 67µg/kg (mice by intraperitoneal injection) [54]

Footnote: Statistics: IC₅₀ half maximal inhibitory concentration, EC₅₀ median effective concentration, MIC minimum inhibitory concentration, T/C Treatment to control (comparative tumor size between treated tumor and control tumor), LD₅₀ median lethal dose. **Cell lines:** A549 lung carcinomatous tissue, Caco-2 human epithelial colorectal adenocarcinoma, DG-75 human B-lymphocyte; HeLa human cervix adenocarcinoma ST-2 bone marrow-derived stroma, MC3T3-E1 osteoblastic, SH-SY5Y Neuroblastoma, RTgill-W1 trout epithelial cell line, HepG2 liver hepatocellular, HCT-116 colorectal carcinoma, Neuro2a mouse neuroblastoma, DLD-1 colorectal adenocarcinoma, RPM1751 Human Malignant Melanoma.

2.1. *Amphidinium*

Amphidinium is a widespread dinoflagellates genus, found in both free-living and benthic states in temperate and tropical marine waters. *Amphidinium* species are often amongst the most abundant dinoflagellates in benthic ecosystems [55]. Over 100 species have been described in this genus [1]. These dinoflagellates are known producers of several families of bioactive macrolides including amphidinolides [56], amphidinols [22,27,57–60], colopsinols [61], luteophanols [62,63] and amdigenols [38,39]. Furthermore, this genus also produces characteristic linear polyketides such as amphidinins [23–25] and amphirionins [29–31].

2.1.1. Amphidinin

Amphidinins are a family of cytotoxic linear polyketides isolated from the marine dinoflagellate *Amphidinium*. Since 2010, six amphidinins have been isolated, the structures of which are illustrated in **Figure 1**. The first amphidinin (**1**) was isolated in 1994 from a symbiotic strain of *Amphidinium sp.* [64]. (**1**) is composed of 17 linear carbons that form a tetrahydrofuran ring. This long backbone is substituted by different chemical groups including 3 hydroxy groups, 2 olefins, 1 methylene and 4 methyls. The absolute configurations of the six stereogenic centers was solved in 2014 [23].

In 2014, four new amphidinins: C-F (**2-5**) were isolated from the same culture of symbiotic *Amphidinium sp.*. The structures of these planar molecules are very similar. (**4-5**) have a hydroxyl on position 6 which is replaced by a ketone group on (**2-3**). Substituents on C₄ introduce also a structural diversity: (**2**) and (**4**) have an hydroxyl group in this position while (**3**) and (**5**) have a ribofuranosyl group [25]. In 2015, another linear polyketide was isolated from the same species: Amphidinin G (**7**). This metabolite is directly related to amphidinin A (**1**). (**7**) was expected to be a precursor of amphidinin A (**1**). In fact, (**1**) is obtained by the formation of tetrahydrofuran ring by the addition of hydroxyl group at C₁₂ to an olefinic carbon at C₉ of (**7**). These molecules are directly associated with another metabolite produced by *Amphidinium*: amphidinolide Q (**6**) [46]. This metabolite corresponds to the cyclization of (**2**) on positions **4-5**.

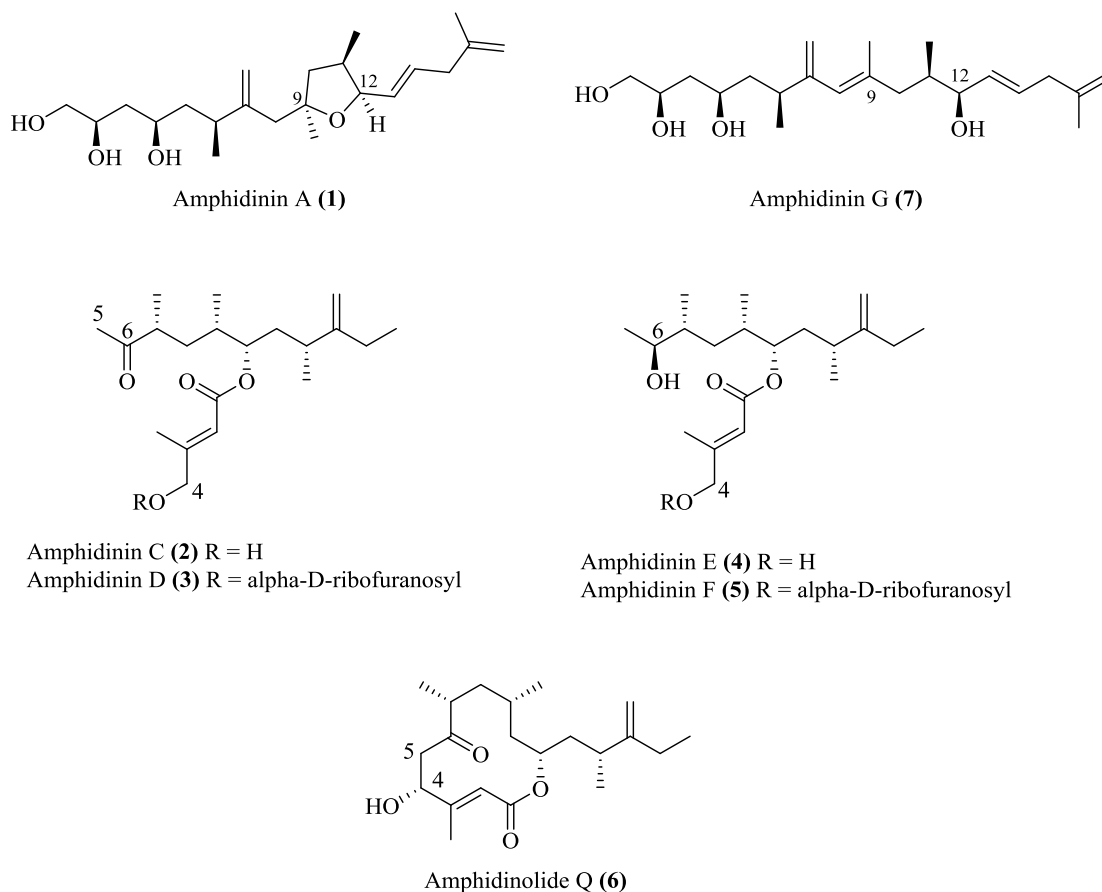


Figure 1. Structures of amphidinins A,C-G and amphidinolide Q (**1-7**).

Amphidinins have been tested for their cytotoxicity, anti-fungal and anti-bacterial activities. Only compound (**5**) showed modest cytotoxicity against murine lymphoma P388 cells (IC_{50} of 5.8 μ g/mL). The majority of compounds were active against the *Trichophyton mentagrophytes* fungus (IC_{50} of 8 μ g/mL (**8**) IC_{50} of 16 μ g/mL (**2-4**) and 32 μ g/mL (**5-6**)). Amphidinin A (**1**), C (**2**) and E (**4**) showed antimicrobial activities against *Bacillus subtilis* (MIC 16 μ g/mL (**1**) and 32 μ g/mL (**2**)(**4**)) and *Aspergillus niger* (IC_{50} of 4 μ g/mL, IC_{50} of 12 μ g/mL, IC_{50} of 16 μ g/mL, respectively) [11]. Only amphidinin C (**2**) and E (**4**) exhibited activity against *Staphylococcus aureus* (MIC 32 μ g/mL for both).

2.1.2. Amphidinols

Amphidinols form a group of polyhydroxylated and polyunsaturated polyketides very characteristic of dinoflagellates from the genus *Amphidinium*. The first member of the series: AM 1 (**8**) (**Figure 2**) was isolated from *Amphidinium klebsii* in 1991 [57]. These secondary metabolites are constituted by 2 tetrahydropyran rings linked by a short C6 chain [45,65]. Structural modifications occur at the two ends that contain respectively a polyunsaturated

alkyl chain and a long irregular polyhydroxylated chain. In 2017, 21 amphidinols have been described [22,27,57–60]. Only metabolites isolated between 2010-2017 are described here. Amphidinol 17 (AM17; **9**) was isolated from a Bahamas strain of *Amphidinium carterae* [26]. This compound is characterized by the presence of a polyol arm shorter than other amphidinols [26]. Moreover, the polyunsaturated alkyl chain also has one less double bond. Nevertheless, this metabolite did not exhibit an antifungal activity [26].

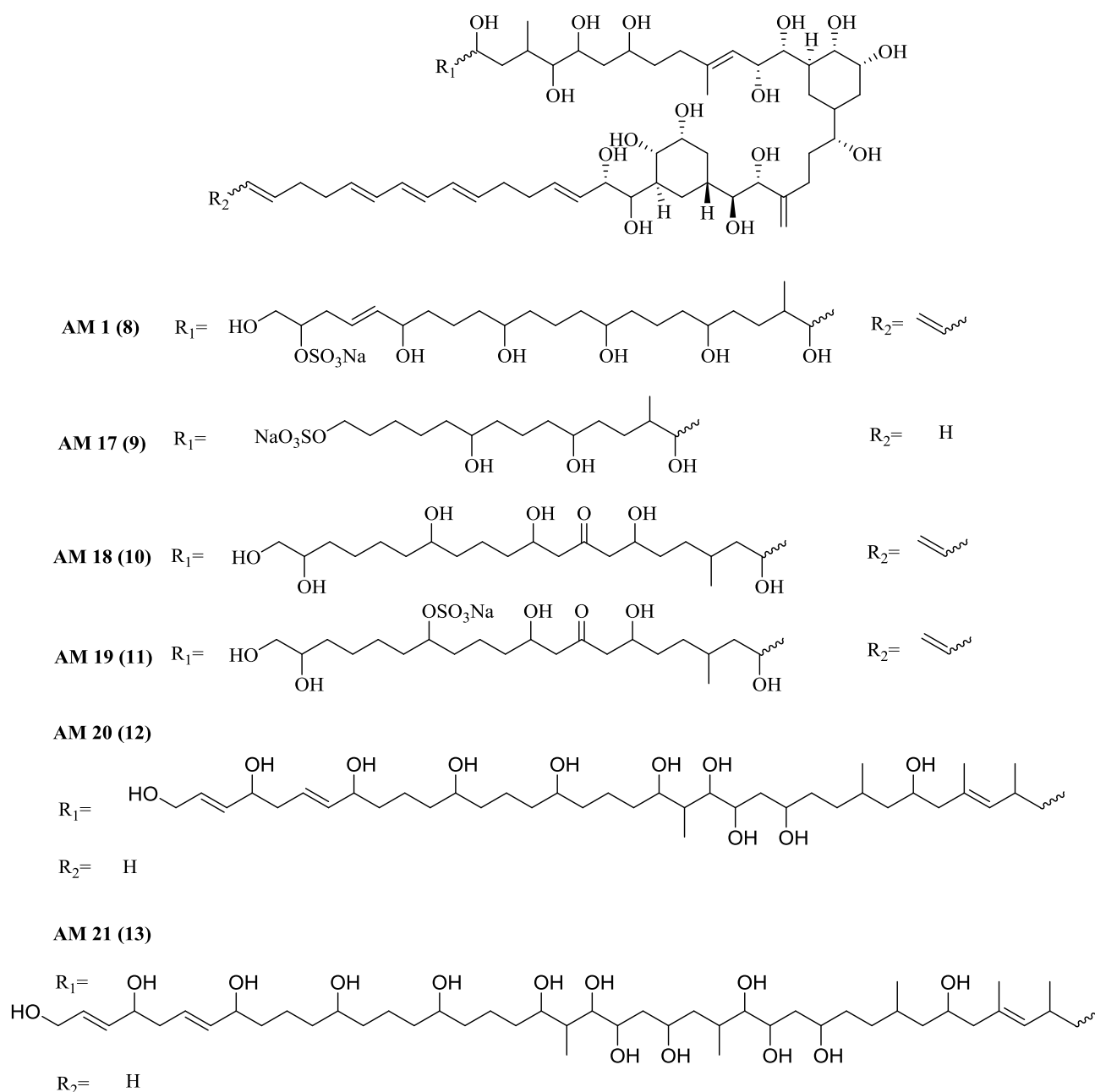


Figure 2. Structures of novel amphidinols 17-21 (**9-13**).

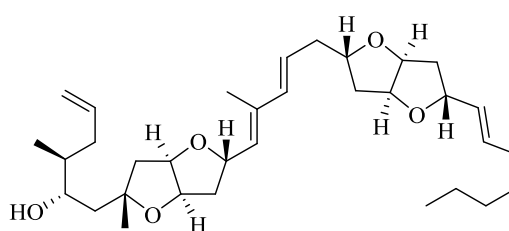
In 2017, a new antifungal amphidinol, AM18 (**10**), and a sulfate analogue, AM 19 (**11**),

were isolated from *Amphidinium carterae* [27]. The main difference from AM17 is on the hydroxyl chain with 15 hydroxyl groups (14 for (**10**)) and 1 ketone group. From a bioactivity point of view, AM18 (**10**) showed moderate antifungal activity against *Candida albicans* (9 $\mu\text{g/mL}$)[27]. The addition of a sulfate group reduces the antifungal activity of the compounds. Two other amphidinols have recently been isolated and characterized: AM20 (**12**) and AM21 (**13**), with the longest polyol chain for compound (**13**) [28]. The dose dependency of hemolysis of AM17 (**9**), AM20 (**12**) and AM21 (**13**) were determined using human red blood cells but only (**9**) and (**12**) were active (EC_{50} of 4.9 μM and 3 μM , respectively) [26,28]. Additionally, AM20 and AM21 were tested for their antifungal activity on *Aspergillus niger* but no activity was found [28].

2.1.3. Amphirionin

2.1.3.1. Amphirionin-2

Amphirionin-2 (**14**) is a polyketide consisting of a linear C30 carbon chain. This is the first *Amphidinium* polyketide possessing a hexahydrofuro[3,2-b]furan moiety [29]. Amphirionin-2 (**14**) showed a modest cytotoxicity against human colon carcinoma (IC_{50} of 0.1 $\mu\text{g/mL}$) and human lung adenocarcinoma (IC_{50} of 0.6 $\mu\text{g/mL}$). The cytotoxicity against human cervix adenocarcinoma Hela cells was also determined, but proved to be low with a 20% growth inhibition at 1 $\mu\text{g/mL}$. In addition (**14**) exhibited activity against an *in vivo* murine tumor (T/C (Treatment to control) 120% at a dose of 0.5 mg/kg). A surface plasmon resonance assay was used to demonstrate an interaction of amphirionin-2 (**14**) with the actin cytoskeleton [29].

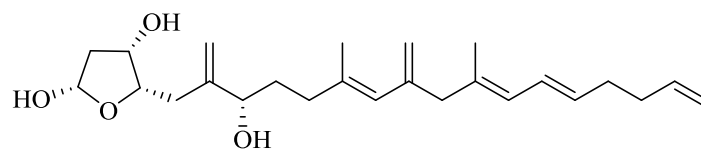


Amphirionin-2 (**14**)

2.1.3.2. Amphirionin-4

A linear polyketide, amphirionin-4 (**15**), was isolated (0.0031% of dry weight) from cultivated cells of *Amphidinium* [30]. This polyketide consists of a linear C22 carbon chain forming a tetrahydrofuran ring. This linear chain is substituted by 3 hydroxyl groups, with 2

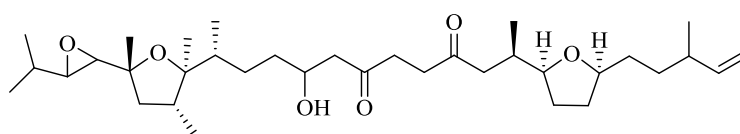
on the cycle. Compound **(15)** exhibited potent proliferation-promoting activity on murine bone marrow stromal ST-2 cells with a value of 950% at a concentration of 0.1 ng/mL [30].



Amphirionin-4 (**15**)

2.1.3.3. Amphirionin-5

Amphirionin-5 (**16**) was isolated from *Amphidinium* in 2014 [31]. This metabolite is a linear C28 polyketide forming 2 tetrahydrofuran rings and containing 2 ketone groups, 1 epoxide and 1 hydroxyl group. Like **(14)** and **(15)**, amphirionin-5 (**16**) promotes cell proliferation: a treatment with 10 ng/mL of **(16)** resulted in a 282% increase in the proliferation of murine bone-marrow derived stromal ST-2 cells and 320% increase in the proliferation of murine osteoblastic MC3T3-E1 cells [30].



Amphirionin-5 (**16**)

2.1.4. Iriomoteolides

Table 2. Lactone ring size and cytotoxicity for iriomoteolides. The various reported IC₅₀ are mentioned in the table.

Iriomoteolides	Lactone size	Cytotoxicity activity		Reference
		Human B-lymphocyte cells	Human adenocarcinoma HeLa cells	
Iriomoteolides-1a	20	0.004 µg/mL		[32]
Iriomoteolides-1b	20	0.9 µg/mL		[32]
Iriomoteolides-1c	20	0.004 µg/mL		[32]
Iriomoteolides-2	23	0.006 µg/mL	0.03 µg/mL	[33]
Iriomoteolides-3a	15	0.08 µg/mL		[34]
Iriomoteolides-3b	15	0.02 µg/mL		[34]
Iriomoteolides-4	16	0.8 µg/mL		[35]
Iriomoteolides-5	20	1 µg/mL		[35]
Iriomoteolides-9	15		15 mM	[36]
Iriomoteolides-10	21	1,5 µM	1,5 µM	[37]
Iriomoteolides-11	19	1,5 µM	2 µM	[36]
Iriomoteolides-12	12	50 µM		[37]

Iriomoteolides are macrolides directly related to amphidinolides that are produced by *Amphidinium* species. They have a variety of backbone skeletons and different size macrolactone rings (**Table 2**). All iriomoteolides contain a vicinal carbon branch, but they differ in the number of hydroxyl groups and methylene units. Most iriomoteolides are potent cytotoxic compounds and with putative antitumor activity (**Table 2**).

2.1.4.1. Iriomoteolide-1a, 1b and 1c

Iriomoteolides-1a, b and c (**17-19**) are 20-membered macrolides isolated from *Amphidinium sp.*. Iriomoteolide-1a (**17**) has a six-membered hemiacetal ring, a methylene group and 4 hydroxyl groups [32]. It has a hydroxyl group at C₉ and a ketone at C₁₃ conjugated with an E-double bond. Iriomoteolide-1c (**19**) is considered a homologue of (**17**) with a 4-hydroxy-3-methylpentyl side chain instead of a 3-hydroxy-2-methylbutyl side chain [32]. Iriomoteolide-1a, b and c (**17-19**) exhibited potent cytotoxicity against human B-lymphocytes with a value of IC₅₀ of 0.004 µg/mL for (**17**) and (**19**).

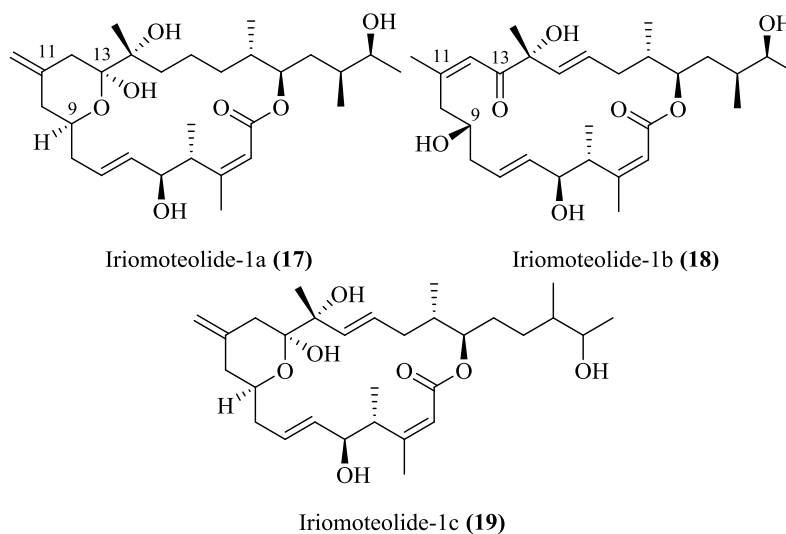
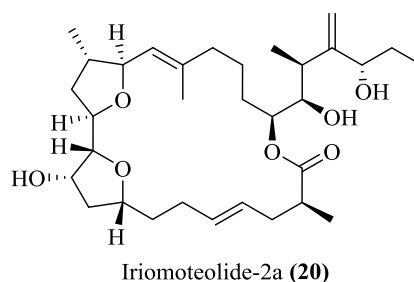


Figure 3. Structures of iriomoteolides-1a, 1b and 1c. (**17-19**).

However, the IC₅₀ value (0.9 µg/mL) of iriomoteolide-1b (**18**) against human B lymphocytes was weak compared to (**17**) and (**19**) [32]. This result suggests that the six-membered hemiacetal ring (C₉–C₁₃) and/or the methylene unit (C₁₁) in iriomoteolide-1a (**17**) is important for cytotoxic activity.

2.1.4.2. Iriomoteolide-2a

Iriomoteolide-2a (**20**) is the first reported natural product possessing a 23-membered macrolactone ring consisting of a polyketide chain with 2 tetrahydrofuran rings [29]. (**20**) exhibited potent cytotoxicity against human B-lymphocyte DG-75 cells (IC_{50} of 0.006 $\mu\text{g/mL}$) and human cervix adenocarcinoma HeLa cells (IC_{50} of 0.03 $\mu\text{g/mL}$) [29]. Moreover, this compound exhibited activity against murine tumor *in vivo* (T/C 132%) at a dose of 0.2 mg/kg [29].



2.1.4.3. Iriomoteolide-3a et 3b

Iriomoteolide-3a (**21**) and its 7,8-*oisopropylidene* derivative (**22**) (**Figure 4**) are 15-membered macrolides with an allyl epoxide, 3 hydroxyl groups and 2 methyl groups [34]. Although two classes of 15-membered macrolides (amphidinolides J and O) have been isolated from the symbiotic dinoflagellate *Amphidinium*, the carbon chain length and oxygen-substituted positions for (**21**) are quite different from those of these known 15-membered macrolides [34,66]. A cell-based screening on cytotoxicity and antiviral activities showed that iriomoteolide-3a (**21**) and iriomoteolide-3b (**22**) are potently cytotoxic against human B-lymphocyte DG-75 cells (IC_{50} of 0.08 and 0.02 $\mu\text{g/mL}$, respectively) and Raji cells (IC_{50} of 0.05 and 0.02 $\mu\text{g/mL}$, respectively) [34].

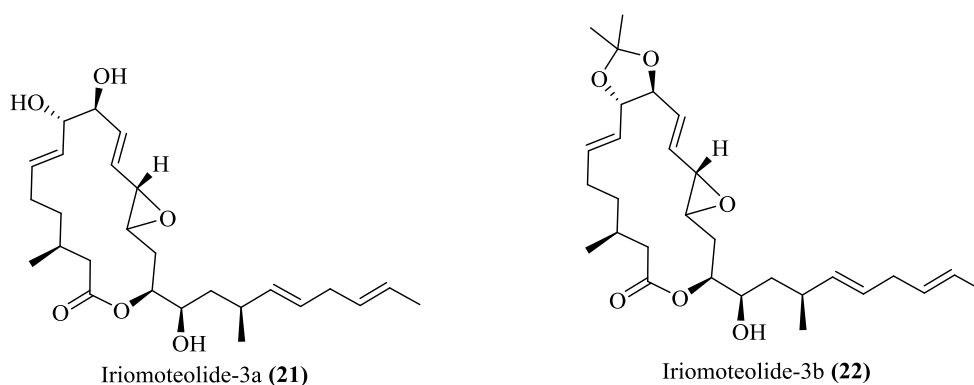
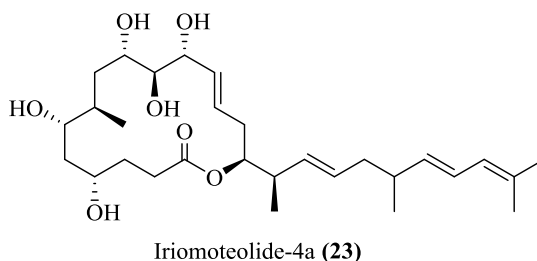


Figure 4. Structures of iriomoteolides-3a and b. (**21-22**).

2.1.4.4. Iriomoteolide-4a

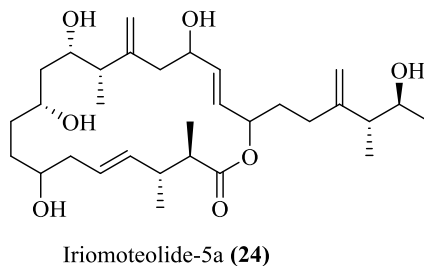
Iriomoteolide-4a (**23**) is a 16-membered macrolide possessing 5 hydroxyl groups and substituted by an isoprene chain on the side chain. This metabolite is the second *Amphidinium* macrolide with a 16-membered macrocyclic ring [35].



Iriomoteolide-4a (**23**) was tested *in vitro* for the capacity to inhibit the growth of tumor cells: (**23**) showed a moderate toxicity against human B-lymphocyte DG-75 cells with an IC_{50} of 0.8 $\mu\text{g/mL}$ [35].

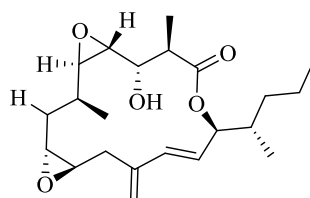
2.1.4.5. Iriomoteolide-5a

Iriomoteolide-5a (**24**) is a 20-membered macrolide with a unique carbon skeleton associated with five hydroxyl groups [35]. Although two classes of 20-membered macrolides (amphidinolides A [67] and U [68]) had been isolated from the symbiotic dinoflagellate *Amphidinium*, the carbon chain length and C1-branches of (**24**) are different from those of these macrolides. Iriomoteolide-5a (**24**) exhibited moderate toxicity against human B-lymphocyte DG-75 cells with an IC_{50} of 1 $\mu\text{g/mL}$ [35].



2.1.4.6. Iriomoteolide-9a

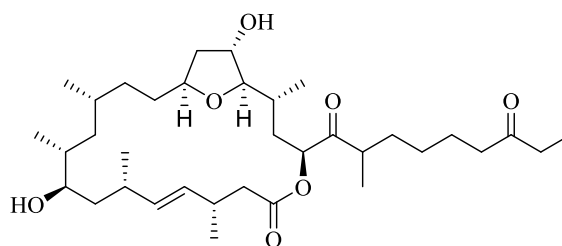
Iriomoteolide-9a (**25**) is another macrolide isolated from *Amphidinium* in 2017. It is a 15-membered macrolide with 2 epoxy rings, 4 methyl groups, and 1 hydroxyl group [36]. Several 15-membered macrolides had previously been isolated such as amphidinolides O and P [66] and iriomoteolide-3a [34]. This molecule is only slightly cytotoxic against human cervix adenocarcinoma HeLa cells with an IC_{50} of 15 μ M [36].



Iriomoteolide-9a (**25**)

2.1.4.7. Iriomoteolide-10a

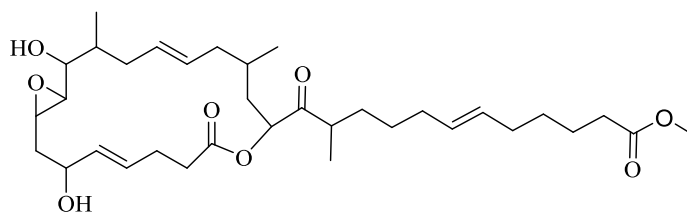
Iriomoteolide-10a (**26**) is the first 21-membered macrolide containing 1 tetrahydrofuran ring, 2 hydroxyl groups, 6 methyl groups and 2 ketone groups on the C1-carbon chain [37]. Iriomoteolide-10a (**26**) exhibited cytotoxic activity against human cervix adenocarcinoma HeLa (IC_{50} of 1.5 μ M), human B-lymphocyte DG-75 cells (IC_{50} of 1.5 μ M), and murine hepatocellular carcinoma MH134 cells (IC_{50} of 3.3 μ M) [37].



Iriomoteolide-10a (**26**)

2.1.4.8. Iriomoteolide-11a

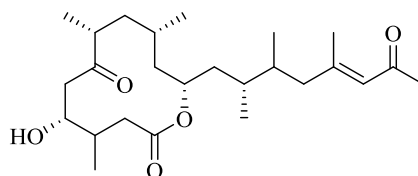
Iriomoteolide-11a (**27**) is a unique 19-membered macrolide possessing a methyl carboxylic group and an exo-ketone on side chains in addition to 1 epoxy ring, 3 methyl groups, 2 hydroxyl groups [36]. Compound (**27**) exhibited cytotoxic activity against human cervix adenocarcinoma HeLa cells with an IC_{50} of 2 μ M. Moreover, experiments using SPR revealed that compounds (**25**) and (**27**) are able to interact with actin [36].



Iriomoteolide-11a (**27**)

2.1.4.9. Iriomoteolide-12a

Iriomoteolide-12a (**28**) is one of the smaller macrolides produced by *Amphidinium* with a 12-membered lactone ring [37] possessing 2 ketone groups, 1 hydroxyl group and 6 methyl groups. This metabolite can be directly associated to amphidinolide Q macrolides. Compound (**28**) showed moderate cytotoxicity against DG-75 cells (IC_{50} of $50\mu M$) [37].



Iriomoteolide-12a (**28**)

2.1.5. Amdigenol

The polyol compound amdigenol A (**29**), isolated from the dinoflagellate *Amphidinium* sp., consists of a C98-linear carbon backbone likely formed linearly by 2 amphidinol analogs [38]. In 2014, two other polyols were isolated from *Amphidinium* sp.. Amdigenol G (**31**) is a typical amphidinol analog with 2 linear carbon side chains connected to the core portion. Its structure is similar to that of luteophanol-A [69], but an oxymethine and a methylene (C43 and C47) position are switched [39]. In amdigenol E [39], the carbon chain is inserted between the 2 tetrahydropyrans, also possessing 2 epoxide groups. Amdigenols A (**29**), E (**30**), and G (**31**) inhibited an increase of the intracellular Ca^{2+} concentration in differentiated IMR-32 neuroblastoma cells. The effect on Ca^{2+} concentration was observed in the presence of amdigenols A, E, and G at $10\mu M$ for (**29**) and $40\mu M$ for (**30**) and (**31**) [38,39]. The amdigenols were tested for their antimicrobial activity against

Streptomyces aureus, *Escherichia coli* and *Pseudomonas aeruginosa*, but did not show inhibition [38,39].

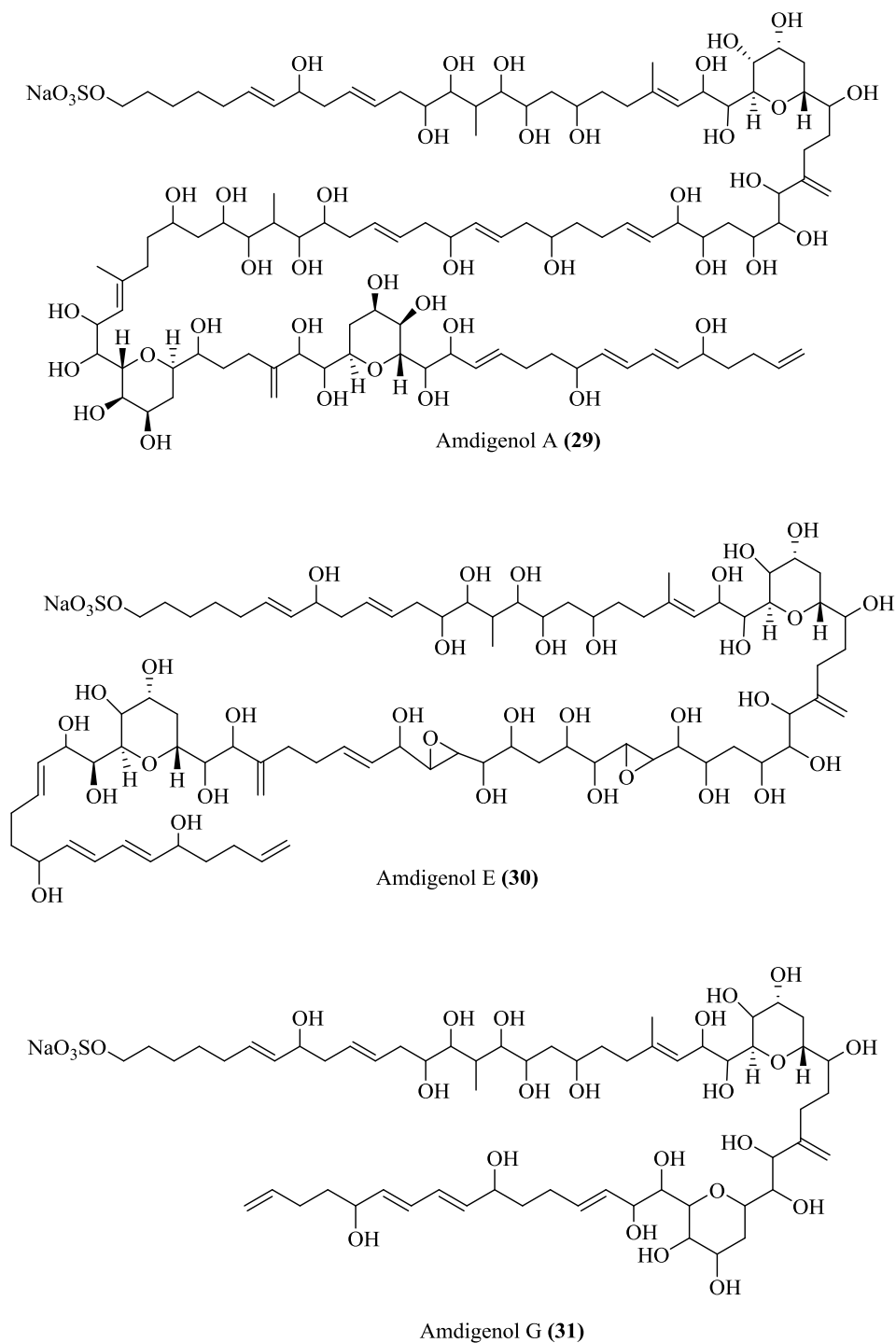


Figure 6. Structures of amdigenol A (**29**), E (**30**) and G (**31**).

2.2. Azadinium

2.2.1. Azapiracids

Azaspiracids (AZA) are a family of marine lipophilic polyether metabolites having 2 spiro-ring assemblies, a cyclic amine and a carboxylic acid functionality at one terminus [48]. They were first detected in 1995 following human illness following ingestion of contaminated mussels (*Mytilus edulis*) from Ireland. In 2009, a novel dinoflagellate species, *Azadinium spinosum*, was identified and described as the source of AZA production [15]. Several AZA-related structures were subsequently isolated from other *Azadinium* species: *A. poporum*, *A. dexteroporum*, and *A. spinosum* [16]. However, the genus *Azadinium* is not the only producer of Azaspiracids. Recently, the *Amphidomataceae* lineage was also shown to produce Azaspiracids. Specifically, *Amphidoma languida* has been found to produce a novel toxin belonging to the azaspiracids and implicated in a molluscan contamination event in the southern Atlantic coast [70].

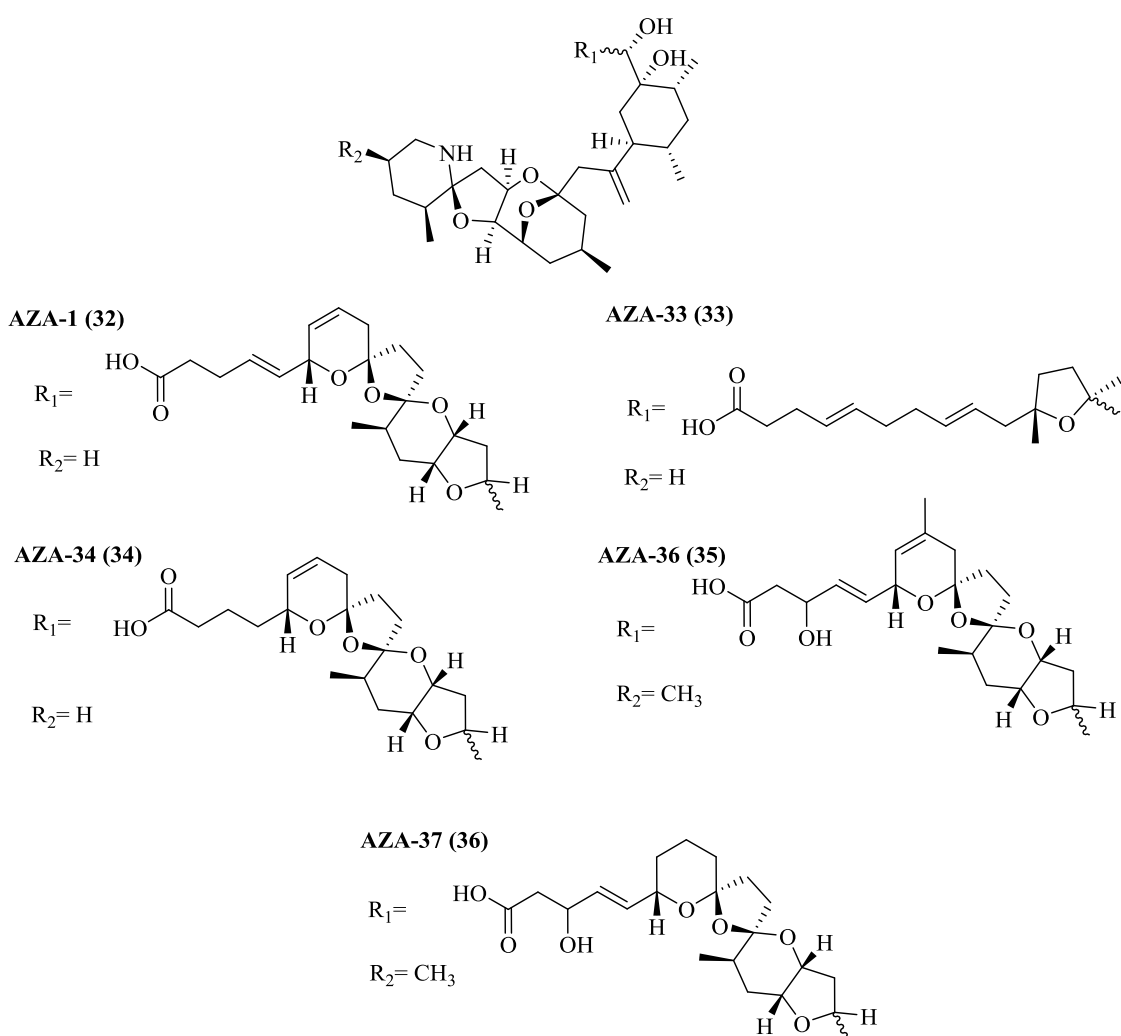


Figure 7. Structures of novel azaspiracids (AZA1, AZA 33, 34, 36, 37) (36-41).

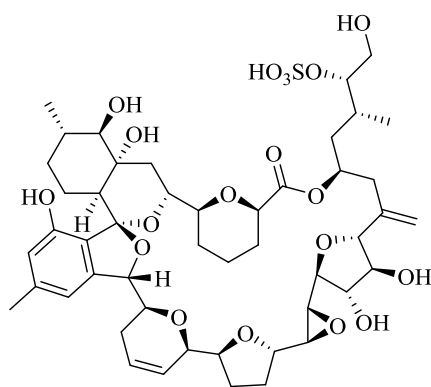
In 2015, three new azaspiracids were isolated from *Azadinium spinosum* (AZA-33 (**33**), AZA-34 (**34**) and AZA-35). These compounds differ significantly at the carboxyl end of the molecule from known AZA analogues. The cytotoxicity of two analogues was tested on Jurkat T-lymphocyte cells, revealing that AZA-33 (**33**) and AZA-34 (**34**) are less and more toxic respectively compared to AZA-1 (**32**) [16]. New structures from *Azadinium poporum* were identified to be 3-hydroxy-8-methyl-39-demethyl-azaspiracid-1 (AZA-36 (**35**)) and 3-hydroxy-7,8-dihydro-39-demethyl-azaspiracid-1 (AZA-37 (**36**)). Compound (**35**) was found to be less toxic to T-lymphocyte cells (EC_{50} of 1.70 nM) compared to (**36**) (EC_{50} of 0.85 nM) and (**31**) (EC_{50} of 0.28 nM) [16]. From a structure-activity point of view, the addition of a hydroxyl function or the addition of a methyl on the heterocycle would decrease the activity, whereas decrease of the aliphatic chain length would increase the bioactivity.

2.3. *Dinophysis*

Over 50 species have been described in the genus *Dinophysis* [1], some of which are well-known harmful dinoflagellates. *Dinophysis* has received special attention because it can seriously impact the bivalve culturing industry when DSP toxins that it produces, such as okadaic acid (OA) [17], dinophysistoxin (DTX) [71,72] and pectenotoxin (PTX) [72], are found in commercialized mussels. These compounds have already been well-characterized and described in the literature, but a new macrolide, Acuminolide [41] was described from *Dinophysis* in 2014.

2.3.1. Acuminolide

Acuminolide-A (**37**) is a new 33-membered polyether macrolide isolated from the dinoflagellate *Dinophysis acuminata*. This compound is composed of 2 tetrahydrofurans, 2 dihydropyrans, 1 dihydroisobenzofuran, 1 octahydroisochromene, and 1 epoxide [41]. This compound did not show any cytotoxicity against four cancer cell lines [41].



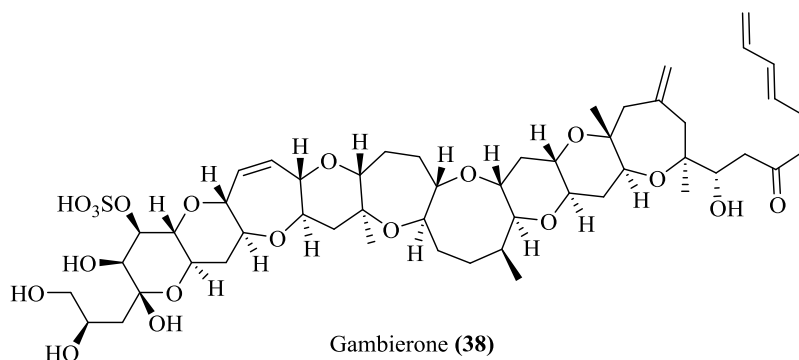
Acuminolide-A (**37**)

2.4. *Gambierdiscus*

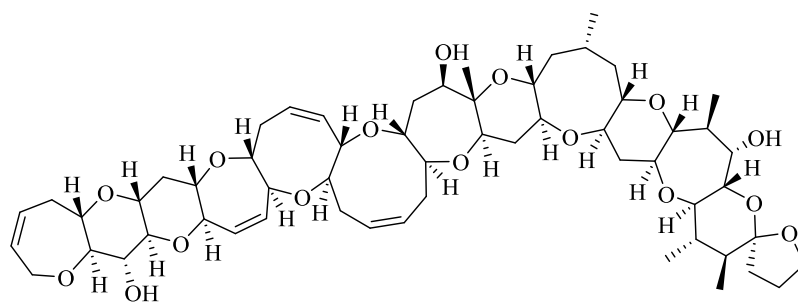
Eleven species have been described in the genus *Gambierdiscus* [1] which is one of the most extensively studied dinoflagellate genera. These dinoflagellates are well-known producers of hazardous metabolites such as ciguatoxin [73], gambieric acid [74], and maitotoxin [75–78]. Since 2010, two novel families of compounds have been described from these dinoflagellates: gambierone [42] and gambieroxide [43].

2.4.1. Gambierone

Isolated from *Gambierdiscus belizeanus*, gambierone (**38**) is a new macrolide described in 2017 [42]. The structure of this compound features an unprecedented polyether skeleton and an unusual right-hand side chain. Its relative configuration was fully determined by interpretation of several NMR analyses. The succession of cycles is very different from that of ciguatoxin, but compound (**38**) has a molecular formula and biological activity similar to those of ciguatoxin-3C (**39**) [79].



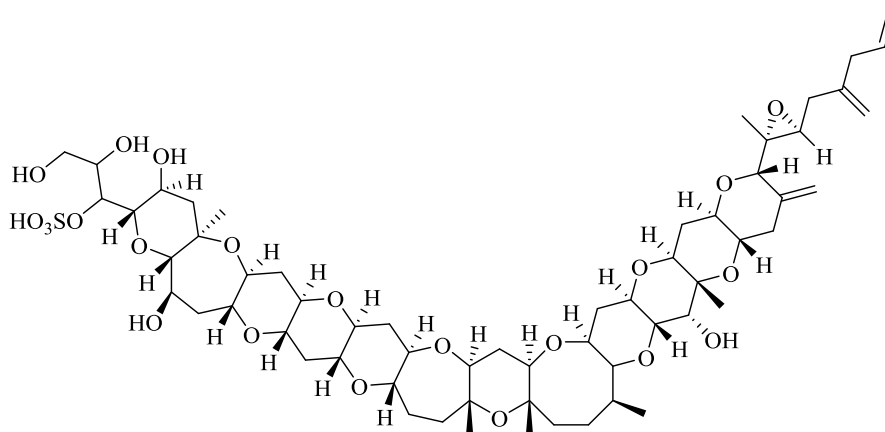
Gambierone (**38**)



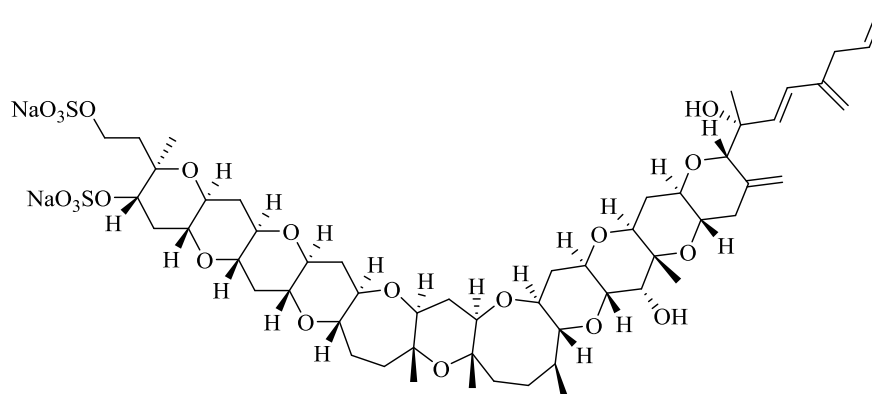
Ciguatoxin-3C (**39**)

2.4.2. Gambieroxide

A novel epoxy polyether compound named gambieroxide (**40**) was isolated from the benthic dinoflagellate *Gambierdiscus toxicus*. It is intriguing that the chemical structure of (**40**) is similar to that of yessotoxin (YTX) (**41**) produced by the dinoflagellate *Protoceratium reticulatum* [43], which is phylogenetically distant from *Gambierdiscus*. The bioactivity of this metabolite has not been studied, but from a structural point of view it is likely to show similar biological properties to YTX (**41**) [43].



Gambieroxide (**40**)



Yessotoxin (**41**)

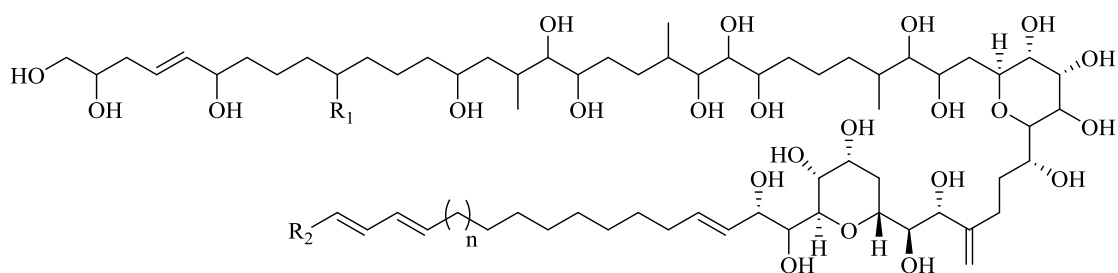
2.5. Karlodinium

The genus *Karlodinium*, which only contains 12 described species [1,80], is known to produce karlotoxins [45] and karmitoxins [46] which act as anti-grazing or allelopathic compounds.

2.5.1. Karlotoxin

The karlotoxins (**Figure 8**) (**42-48**) represent a class of metabolites that are structurally close to the amphidinols [44,45] produced by *Amphidinium*, having three distinct regions: (i) a polyol arm with variable hydroxylation and methylation, (ii) a hinge region containing 2 ether rings, (iii) and a lipophilic arm that often includes conjugated trienes in amphidinols [22,27,57,58]. Note here that the terminal diene is chlorinated in several karlotoxins [45]. Three types of karlotoxins, type-1 (**42-44**), type-2 (**45**) and type-3 (**46-48**), have been described, differing in the length of the lipophilic arm. Several analogues also exist, with a sulfate group replacing a hydroxyl group or variable terminal chlorination of the lipophilic chain [45] (**Figure 8**).

Various biological activities have been reported for these metabolites, including hemolytic properties [45]. The length of the lipophilic arm appears to modulate their hemolytic activity. Sulfated compounds have lower activity, whereas the presence of a chloric group on the C-terminal increases the hemolytic activity.

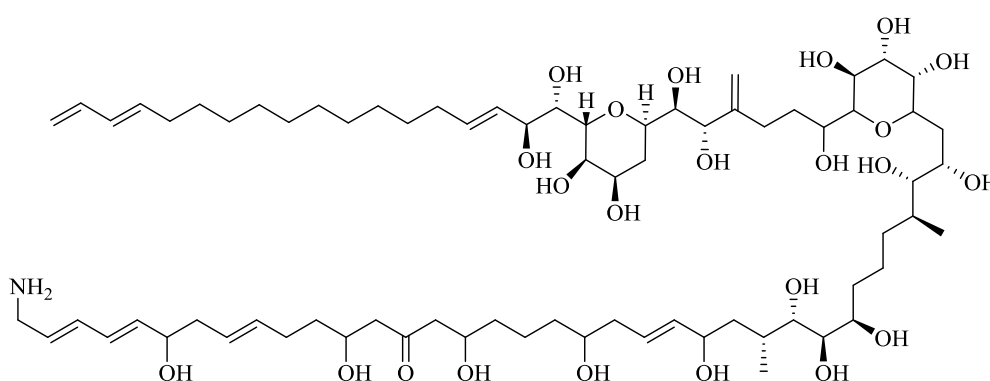


Name	n	R ₁	R ₂	Hemolytic Activity EC ₅₀ (nM)
KmTx 1a (42)	3	OH	H	63
KmTx 1b (43)	3	OH	Cl	56
KmTx 1c (44)	3	OSO ₃ H	H	300
KmTx 2 (45)	1	OH	Cl	<u>NB</u>
KmTx 3a (46)	2	OH	H	200
KmTx 3b (47)	2	OH	Cl	110
KmTx 3c (48)	2	OSO ₃ H	H	2400

Figure 8. Structures of karlotoxins (KmTxs).

2.5.2. Karmitoxin

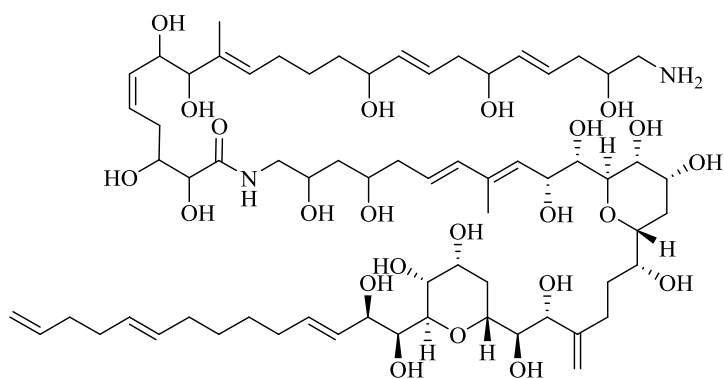
Karmitoxin (**49**) is a macrolide isolated in 2016 from the dinoflagellate *Karlodinium armiger*. Its structure is related to those of the amphidinols and karlotoxins. Karmitoxin (**49**) differs by having a longer aliphatic chain with a primary amine at the end [46]. Compound (**49**) exhibited cytotoxic activity against RTgill-W1 cells from the fish *Oncorhynchus mykiss* (LC₅₀ of 125 nM) and toxicity to copepods has also been observed (LC₅₀ of 400 nM) [46].



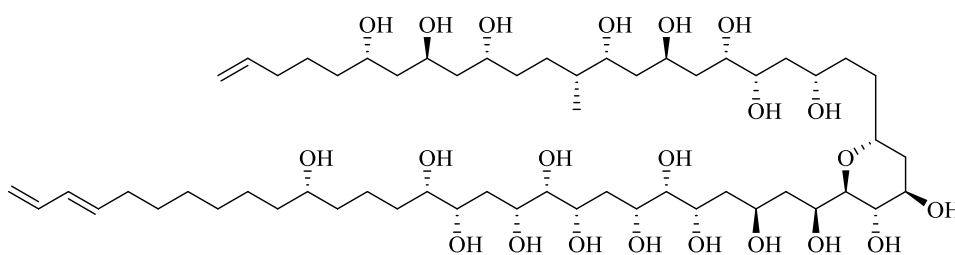
Karmitoxin (**49**)

2.6. *Ostreopsis*

Ostreopsis species are epiphytic benthic dinoflagellates, and some species are harmful to marine organisms (and to humans). Many studies have reported that these organisms produce the potent biotoxins palytoxin [81] and ovatoxin [82]. In 2013, 2 macrolides, specifically ostreols, were isolated and described from *Ostreopsis ovata* [47,48]. These new polyhydroxyl chain compounds are called Ostreol A (**50**) and B (**51**).



Ostreol A (**50**)



Ostreol B (**51**)

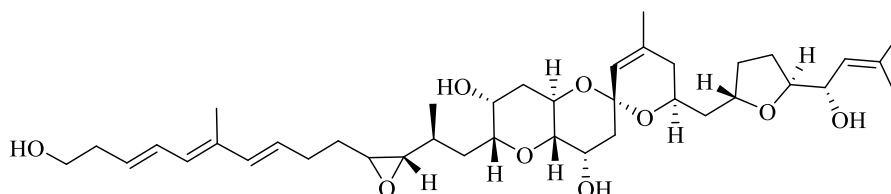
The former has 3 linear polyhydroxyls separated by 2 tetrahydropyran like amphidinols or karlotoxins. The specificity is the presence of a primary amino group, an amide bond and a double bond. Ostreol A (**50**) was determined to have *in vitro* cytotoxicity against *Artemia salina* with a LD₅₀ value of 0.9 µg/mL [47]. Ostreol B (**51**) is structurally different from Ostreol A (**50**) which contains only a hydroxyl function at number 21 and only 1 tetrahydropyran [48]. Ostreol B was shown to exhibit moderate cytotoxicity against hepatocarcinoma HepG2 (IC₅₀ of 4.8 µM), neuroblastoma Neuro-2a (IC₅₀ of 0.1 µM) and colon cancer HCT-116 (IC₅₀ of 0.9 µM) cell lines [48].

2.7. *Prorocentrum*

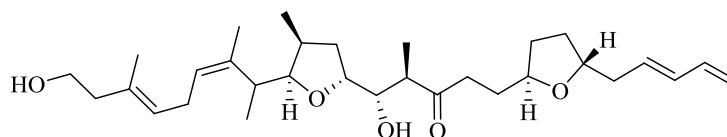
Prorocentrum is well-known for producing DSP toxins. Apart from these compounds, skeletally diverse bioactive compounds have also been isolated from cultured *Prorocentrum* species, including prorocentrolide [83], hoffmanniolide [84], prorocentin [49, 50], formosalides A and B [85], belizentrin [51], belizeanolide [86] and limaol [52].

2.7.1. Prorocentin

Prorocentins 1 and 4 (**52-53**) are linear polyketides produced by *Prorocentrum* sp. and *Prorocentrum lima*, respectively [50]. Prorocentin-1 (**52**) exhibited a moderate cytotoxic activity against the human colon adenocarcinoma cell line DLD-1 ($IC_{50} = 16.7 \mu\text{g/mL}$) and the human malignant melanoma cell line RPMI-7951 ($IC_{50} = 83.6 \mu\text{g/mL}$) [49]. By contrast, prorocentin-4 (**53**) was not cytotoxic when tested on the same cell lines.



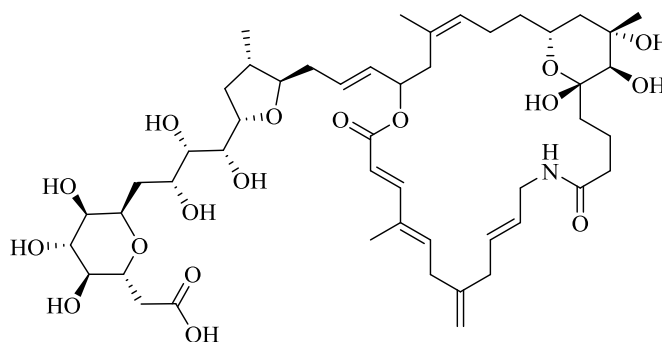
Prorocentin 1 (**52**)



Prorocentin 4 (**53**)

2.7.2. Belizentrin

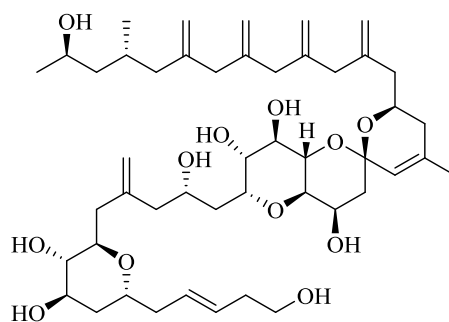
Belizentrin (**54**) is a 25-membered polyketide-derived macrocycle isolated from the marine dinoflagellate *Prorocentrum belizeanum* [51]. This macrolide is composed of an unsaturated 25-membered cycle containing 2 hydroxyls. This ring is substituted by a linear chain with 6 hydroxyls and 1 carboxylic acid moiety at the end of the chain. Several biological experiments have been performed on belizentrin (**54**), revealing toxicity against several neublastoma cell lines (EC_{50} approximately 200 nM) [51].



Belizentrin (**54**)

2.7.3. Limaol

Limaol (**55**) is a linear polyketide isolated from the benthic marine dinoflagellate *Prorocentrum lima*. Limaol (**55**) is constituted by a long aliphatic chain with 4 tetrahydropyran and 8 hydroxyl groups. From a biological point of view, limaol (**55**) exhibited moderate cytotoxicity against three tumoral cell lines: HepG2 (hepatocellular carcinoma), HCT-116 (colon adenocarcinoma), and Neuro2a (neuroblastoma) with EC₅₀s of 3.7, 7.3 and 9.6 μ M, respectively [52].



Limaol (**55**)

2.8. *Vulcanodinium*

The dinoflagellate genus *Vulcanodinium*, first described in 2010, is found in many locations around the world, including Australia, Japan, China, France and Hawaii [53,87]. *Vulcanodinium* is a producer of a potent neurotoxin, pinnatoxin [53,54], and of the macrolide, portimine [88].

2.8.1. Pinnatoxin

In 1995, a potent neurotoxin called Pinnatoxin, a polycyclic ether with a spiroimine function, was isolated from an Okinawan bivalve [89]. Since this discovery, several Pinnatoxin analogues have been isolated and characterized [90]. It was only in 2010 that *Vulcanodinium rugosum* was recognized as a producer of pinnatoxin E (**56**), F (**57**) and G (**58**) [53]. In 2014, a new Pinnatoxin H (**59**) was isolated from *V. rugosum*. (**59**) shares close structural similarities with other pinnatoxins, having an identical carbon skeleton but harboring different substituents [54]. The LD₅₀ value of (**59**) in mice by intraperitoneal injection was 67 μ g/kg and by gavage was 163 μ g/kg [54], similar to the values for other

pinnatoxin compounds.

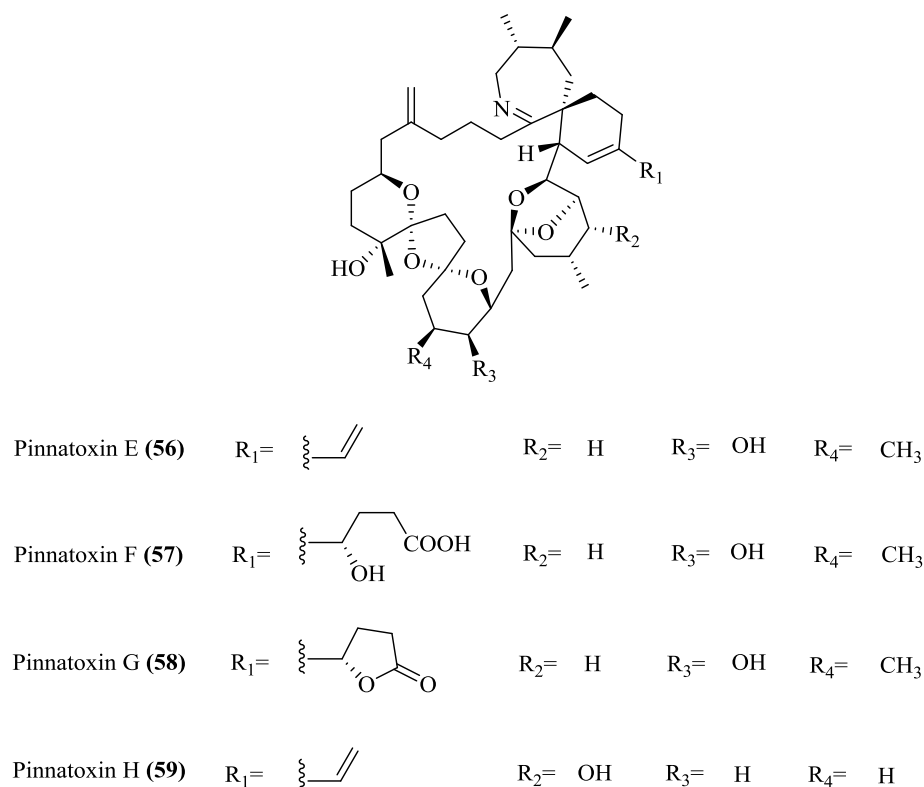
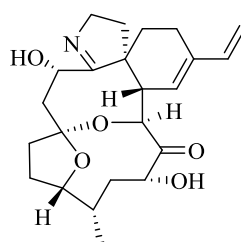


Figure 9. Structures of Pinnatoxins from *Vulcanodinium rugosum*

2.8.2. Portimine

A five-membered cyclic imine called portimine (**60**) was isolated from *Vulcanodinium rugosum* [88]. This compound shares some similarities with pinnatoxins: a spiro-linked cyclic imine and a polycyclic ether backbone. Compared to pinnatoxins, portimine (**60**) exhibited relatively low toxicity on mice, but higher toxicity on P388 mouse leukemia cells (EC₅₀ of 2.7 nM) by inducing cell death in an apoptotic manner [88].



Portimine (**60**)

3. Concluding remarks

Dinoflagellates have developed unique biosynthetic pathways leading to the production of sophisticated and specific metabolites, many of which are likely involved in protection mechanisms in the highly competitive environment in which the cells live. The set of bioactive molecules so far known to be produced by dinoflagellates includes various polyketide compounds such as amphidinols, lutheophanol and ostreols, as well as macrolides including acuminolide and iriomoteolides. It is noteworthy that these molecules have been isolated from a very broad diversity of species, including members of most of the main dinoflagellate families: Gymnodiniales (*Amphidinium*, *Karlodinium*), Peridinales (*Vulcanodinium*), Gonyaulacales (*Gambierdiscus*, *Ostropsis*), Prorocentrales (*Prorocentrum*) and Dinophysiales (*Dinophysis*). Phylogenetic trends in metabolite structure may exist (as would appear to be the case for example for the structural similarity between amphidinols and karlotoxins which are both produced by members of the Gymnodiniales), but much more data on other dinoflagellate species would be required to assess whether these trends are robust (which might lead to development of a certain degree of predictive capability). It should be noted that a huge number of metabolites have been found firstly into macro-organisms as fishes, sponges and crustaceans derived of the bioaccumulation or molecular transfer pro-symbiotic with microorganisms. It is very likely that other marines compounds described is initially derived from dinoflagellates. The interest will be to determine the real producer of bioactive metabolites to be controlled or promoted. However, the discovery and characterization of new metabolites from dinoflagellates is the result of a long process including: (i) isolation of culture strains and their characterization; (ii) scale-up from laboratory scale cultivation (from mL up to 100L) to production scale (up to several m³); (iii) extraction, fractionation and structural elucidation of metabolites; (iv) screening of the purified compounds using disease-relevant assays. Despite some of the recently discovered natural products from dinoflagellates being too toxic, the majority have potential therapeutic value as anti-cancer, anti-fungal and/or anti-bacterial agents. The road from identification of an interesting bioactivity to marketable product is long and restrictive, involving for example intensive medicinal chemistry studies in order to improve the efficiency and selectivity of products, but there are numerous examples of successful development of natural products, such as the multi-kinase inhibitor Midostaurin (Rydapt, Novartis Pharmaceuticals, Inc.), approved in 2017 in the EU and USA for the treatment of FMS-like tyrosine kinase 3 (FLT3)

mutation-positive acute myeloid leukemia [92]. Midostaurin is an indolocarbazole and a direct derivative of staurosporin, a natural alkaloid purified from the bacterium *Streptomyces staurosporeus* (first described in 1977). This natural product, considered as a “pan-kinase” inhibitor (high- affinity inhibitor of nearly all mammalian protein kinases), and its derivatives were under investigation for decades before reaching the market for cancer treatment in combination notably with the marine natural product, cytarabin [91]. With current technological advances, notably in bioinformatics, mass spectrometry, and microfluidics for miniaturization of high-throughput screening assays, a growing number of metabolites from microorganisms will be isolated and characterized, and in this context we hope that the present review highlights the potential potency of dinoflagellate metabolites for human therapeutics. Specific new cultivation techniques will be needed as a key step towards further exploring this potential, and further efforts should be made to influence the induction of metabolite synthesis (via the use of specific culture media and/or co-cultures with several organisms for example) in order to reveal minor compounds. Pharmaceutical companies need access to new chemical diversity. We believe that the development of dinoflagellates as producers of high value chemicals will pave the way for promising new therapeutic applications.

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Abbreviations

AM: Amphidinol

AMP: Amphidinolide

ASP: Amnesic shellfish poisoning

AZA: Azaspiracid
CTX: Ciguatoxins
CFP: Ciguatera fish poisoning
DSP: Diarrheic shellfish poisoning
EC₅₀: Half maximal effective concentration
GI₅₀: Half maximal inhibition of cell proliferation
GTX: Gonyautoxins
IC₅₀: Half maximal inhibitory concentration
KmTx: Karlotoxin
LD₅₀: Median Lethal Dose
MTX: Maitotoxin
NSP: Neurotoxic shellfish poisoning
OA: Okadaic acid
PTX: Palytoxin
PSP: Paralytic shellfish poisoning
STX: Saxitoxin
YTX: Yessotoxin

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