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Marine natural products from zoantharians: bioactivity, biosynthesis, systematics, and ecological roles

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Zoantharians, also improperly known as zoanthids or colonial anemones, are well known by aquarists because of their ease of use in aquaria but also because of their splendid colours. However, high concentrations of the highly toxic palytoxin found in some species of zoantharians maintained in reef aquaria has raised some issues recently, unveiling at the same time a rather unknown chemical diversity hidden in these marine beauties. Herein, we report the structure of the metabolites described in all species of zoantharians up to the end of 2018 and their associated biological activities. As sessile invertebrates, zoantharians harbour a rich diversity of micro-organisms that can play a role in the biosynthesis of these natural products and we detail the current hypotheses on the metabolic pathways leading to the identified ecdysteroids, zoanthoxanthins, zoanthamines, palytoxins and others. Finally, we assess the possible use of these metabolites in the systematics of such a complex group of marine invertebrates and we discuss their possible ecological roles. Altogether, this review brings some insights into the rich chemical diversity of zoantharians and their potential for marine biodiscovery and marine ecology.

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1 Introduction

Long before the scientific explorations of our oceans and the advent of technological instruments such as SCUBA diving, people living in coastal regions had developed an acute sense of observation that often led to the creation of local myths. In remote islands of the Pacific, such as the island of Maui, Hawaii, an old legend described a green moss as "Limu Make O Hana" or the "Seaweed of Death from Hana".[1] The species, later

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identified as the zoantharian *Palythoa toxica*, was indeed found to bioaccumulate one of the most complex and potent toxins called palytoxin.[2] Today, the use of tropical species of zoantharians in reef aquaria is mainly a consequence of the fascinating colours exhibited by these organisms. However, the presence of toxins has become a health concern for aquarists and a better knowledge of their chemical diversity is needed.

Despite the occurrence of bioactive metabolites in relatively common sessile invertebrates of the order Zoantharia, before called Zoanthidea (Cnidaria: Anthozoa: Hexacorallia), this group has largely been overlooked in marine biodiscovery studies mostly focused on sponges or ascidians. Species of this order are nevertheless largely distributed in all the oceans, and they have been described inhabiting very shallow or even intertidal waters until the deep-sea with a large proportion living in close association with cnidarians or sponges. Zoantharians are anthozoan as characterized by an absence of a solid skeleton and their ability of incorporating particles of sand or other material. A search of the natural products classified in the order Zoantharia in the database Marinlit only retrieved 55 metabolites, excluding the first metabolites isolated due to unproper taxonomic description.[3] We therefore believed that a comprehensive inventory of the metabolites isolated from the species of this group or their associated microbiota was necessary. Fatty acid derivatives and peptide/protein derivatives were intentionally excluded as being too borderline with the primary metabolism. The main hypotheses for the biosynthesis of some families of natural products commonly encountered in zoantharians, including ecdysteroids, zoanthoxanthins, zoanthamines and palytoxins, are then detailed. Finally, we discuss the origin of these metabolites, their ecological role and also their possible use as markers for the systematics of this group.

2 Chemical diversity and bioactivities

Zoantharians are divided into two suborders, namely, Brachycnemina and Macrocnemina, displaying a rather diverse chemical diversity. We therefore decided to structure this first part according to this division.

2.1 Suborder Brachycnemina

Four main families of natural products have been described from species of this suborder: sterols, ecdysteroids, palytoxins and zoanthamines.

2.1.1 Sterols

2.1.1.1 Palythoa. The first chemical investigation of a *Palythoa* species was published by Bergmann *et al.* in 1951 from the Caribbean *Palythoa mammilosa* and it was focused on the sterol composition of this species. As the physico-chemical properties of the main sterols showed differences from previously reported sterols, it was named palysterol. Comparison of its optical rotation with the sponge sterol haliclonasterol indicated that palysterol would be a C-24 ethyl sterol, but the authors mentioned it should rather correspond to a mixture.[4] Later, Gupta and Scheuer analysed the sterol fraction of a *Palythoa* sp. from Tahiti and *Palythoa tuberculosa* from the Marshall Islands by gas liquid chromatography. *Palythoa tuberculosa* showed a sterol composition similar to the previously reported palysterol of *P. mammilosa*.[5] The sterols from *P. tuberculosa* were identified as cholesterol (**1**), brassicasterol (**2**), 22,23-dihydrobrassicasterol (**3**), gorgosterol (**4**), campesterol (**5**) and β-sitosterol (**6**) (Fig. 1). Chalinasterol (**7**) was reported as the unique sterol from the Tahitian *Palythoa* sp. The identification of the sterols was deduced from a comparison of the IR and NMR data with those of acetate derivatives and standards. Quinn *et al.* analysed the sterol composition of eight Hawaiian zoantharians, including four species of *Palythoa*, as a preliminary taxonomic assessment.[6] Methylenecholesterol (**7**), also named chalinasterol, was the only sterol found in *P. toxica*, like in *Palythoa* sp. from Tahiti reported by Gupta and Scheuer.[5] The sterol mixture of *P. tuberculosa*, *P. psammophilia* and *P. vestitus* (previously known as *Zoanthus vestitus*) showed a similar composition. The analysis of the sterols of a *Palythoa* sp. collected in Okinawa, Japan, revealed the presence of cholesta-5,22(*E*)-dien-3β-ol (**8**), along with **1**, **2**, **3**, **4**, **7**, and traces of 23,24-dimethylcholesta-5,22(*E*)-dien-3β-ol (**9**) (Fig. 1).[7] The sterol **9**, commonly found in octocorals, is considered an intermediate in the biosynthesis of gorgosterol (**4**). Three species of *Palythoa* were later collected from different sites off the Brazilian coast and studied for their sterol composition.[8] Analysis of the male and sterile colonies of *P. caribaeorum* revealed the presence of the same sterol composition as in *P. mammilosa* reported by Gupta and Scheuer.[5] Analysis of the female or hermaphrodite colonies of *P. caribaeorum* showed a higher concentration of **4** than in male colonies. However, no significant variability with location or sex was evidenced except for one sample CF-72. The sterol mixture of both *Palythoa* sp. from Tahiti and CF-72 showed a high concentration of **2**, and a small amount of **3**. Interestingly, the zooxanthella isolated from CF-72 had the same sterol composition as its host and a double concentration of **4**. The sterol composition of *P. variabilis* showed a sterol composition similar to the one in *P. tuberculosa* as reported by Gupta *et al.*, except for a higher concentration of **3** and lower concentration of **4**. The major sterols of CF-72 were identified as **1**, **2**, **3** and **4**. Sterols **7**, **8** and 24-norcholesta-5,22(*E*)-dien-3β-ol (**10**) were also identified but in lower amounts. In 1986, the sterol composition of *Palythoa dartevellei*, *P. monodi*, *P. senegalensis* and *P. variabilis* collected off the coast of Senegal was reported to be similar to those of *Palythoa* species reported previously.[9] The study of the sterol composition of the zoantharian *Palythoa senegambiensis* by the same group revealed the presence of **1**, **2**, **5** and **6** but also stigmasterol (**11**), fucosterol (**12**) and crinosterol (**13**) (Fig. 1).[10] These sterols were identified by comparison of their retention times in GLC with those of standard sterols. After an absence of work on zoantharian sterols for about 25 years, the chemical study of *P. tuberculosa* from the Red Sea allowed the identification of seven new sterols, oxidized at the C-1 position and named palysterol A–F (**14-19**) and 24-methylenecholest-5-en-1α,3β,11α-triol (**20**) (Fig. 1).[11] Their structures were elucidated based on NMR and MS data. Compound **14** exhibited cytotoxic activity against breast adenocarcinoma (MCF-7) and human

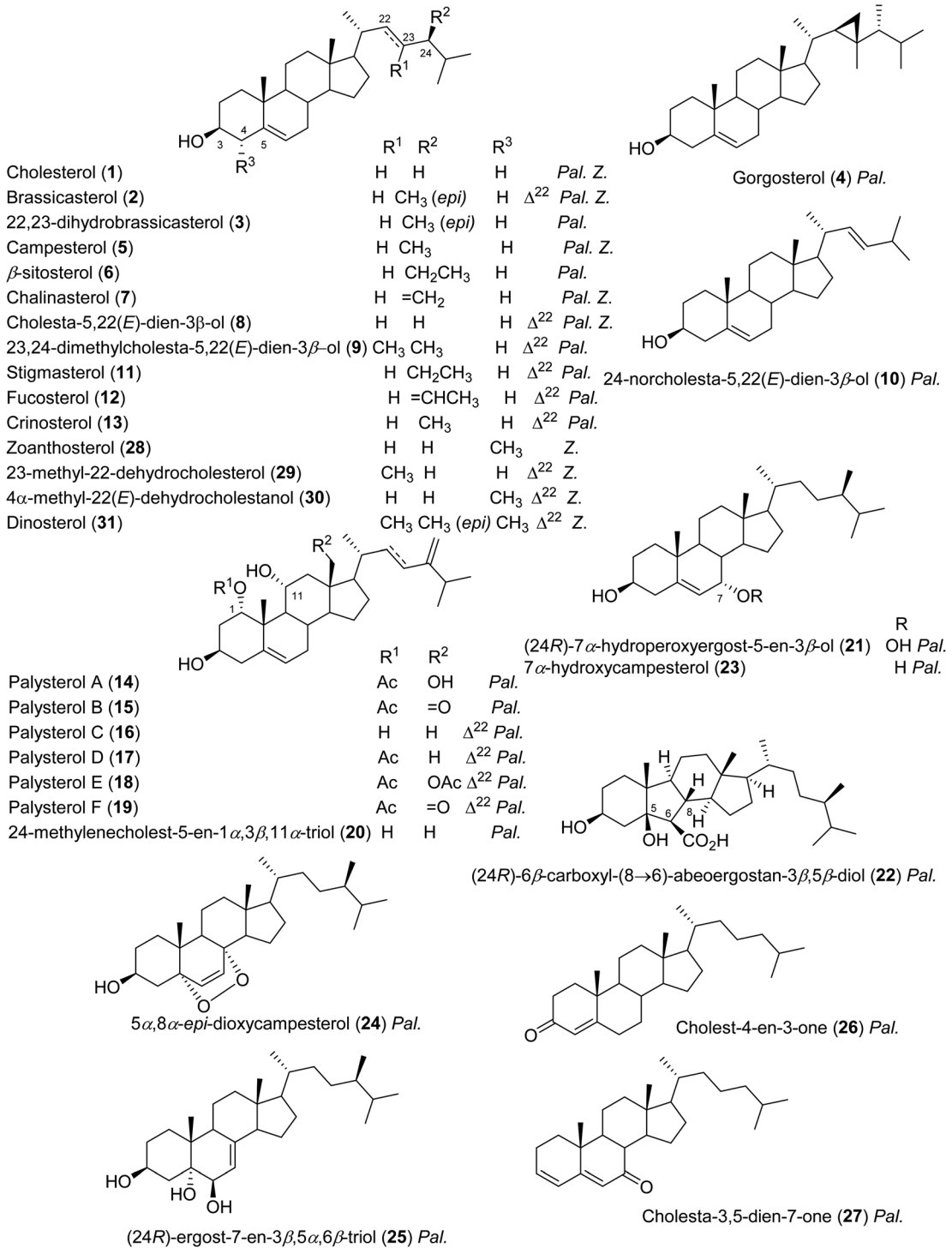


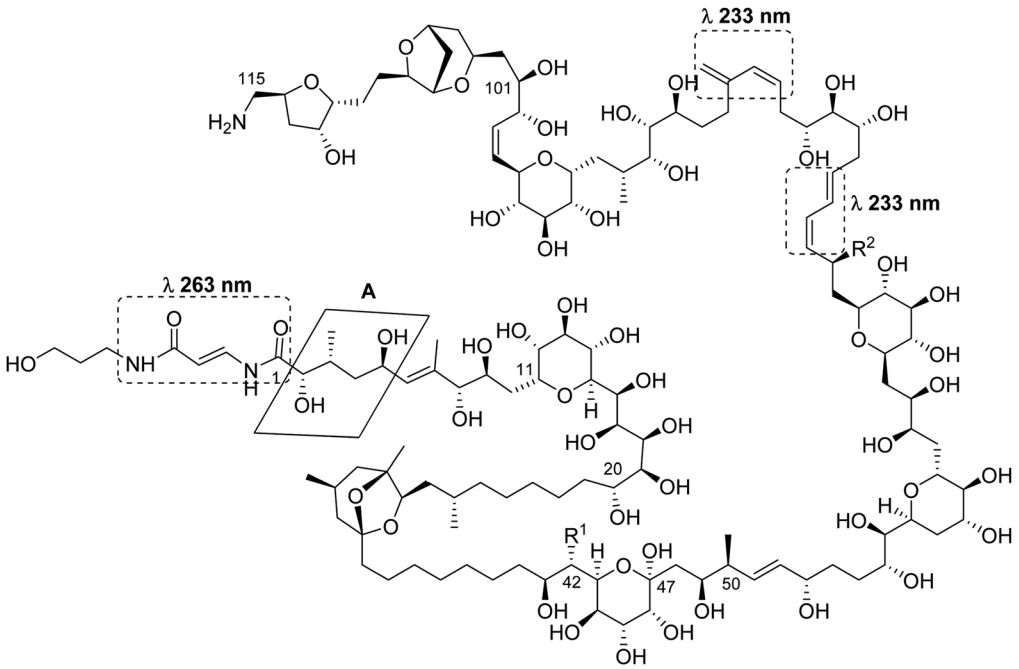
Fig. 1 Structure of sterols from species of the suborder Brachycnemina. From *Palythoa* (Pal.), *Zoanthus* (Z.).

colon adenocarcinoma (HT-29) with IC_{50} values of 170 and 178 μM, respectively, but showed no activity on human cervical carcinoma (HeLa) and the non-tumoral human cell line KMST-6. Compound **19** displayed selective antitumoral activity against MCF-7, HT-29, KMST-6 and HeLa with IC_{50} values of 82, 122, 126 and 128 μM, respectively. Finally, two unusual ergostane-type sterols (24*R*)-7α-hydroperoxy-ergost-5-en-3β-ol (**21**) and 6β-carboxyl-(24*R*)-(8→6)-*abeo*-ergostan-3β,5β-diol (**22**) were identified along with 7α-hydroxycampesterol (**23**), 5α,8α-*epi*-dioxycampesterol (**24**), 24(*R*)-ergost-7-en-3β,5α,6β-triol (**25**), cholest-4-en-3-one (**26**) and cholesta-3,5-dien-7-one (**27**) by GC-MS analysis of the hexane fractions of *P. caribaeorum* and *P. variabilis* collected off the coast of Brazil (Fig. 1).[12] Compound **22** exhibited moderate cytotoxic activity

against the human colorectal tumour cell line (HCT-116) with IC_{50} values of 50 and 4 μM after 24 and 72 h, respectively.

2.1.1.2 Zoanthus. In 1951, Bergmann and co-workers reported chalinasterol (**7**) as the major sterol of *Zoanthus proteus*.[4] The sterol was characterized by its melting point and by comparison with a standard. Cholesterol (**1**), brassicasterol (**2**), campesterol (**5**), and chalinasterol (**7**) were reported from *Zoanthus confertus* (now identified as *Z. pacificus*) collected around Coconut Island, Thailand, by Gupta and Scheuer in 1968 (Fig. 1).[5] A new sterol, zoanthosterol (**28**), was isolated from the Brazilian zoantharian *Zoanthus sociatus* along with **1** and **7**.[13] These sterols were isolated as their acetate derivatives and their structures were deduced from NMR data and by comparison with known analogues. As studies of the sterol composition of dinoflagellates isolated from the Jamaican *Zoanthus sociatus* revealed the presence of several 4α-methyl-5α-stanols, **28** might be produced by the associated algae. Additionally, variation in the sterol composition has been observed since the sterol composition of *Z. sociatus* from Brazil containing mostly sterols with the $\Delta^{24(28)}$ double bond, while those sterols are absent in the associated algae isolated from the Jamaica *Z. sociatus*. Similar variations were reported from the symbionts associated to species of *Palythoa*.[13] Further analysis of the sterol composition of *Zoanthus* sp. from the coast of Brazil led to the identification of sterol **8** together with **1**, **2**, **6** and **7**, identified as their acetate derivatives. Sterols in C_{28} were identified as the major components in species of the genus *Zoanthus*.[14] Finally, the study of the sterol composition of the dinoflagellate isolated from *Zoanthus sociatus* allowed the identification of **8**, 23-methyl-22-dehydrocholesterol (**29**), 4α-methyl-22(*E*)-dehydrocholestanol (**30**) and dinosterol (**31**).[15]

2.1.2 Palytoxins. The first reports of the toxicity exhibited by a *Palythoa* sp. dates back to 1961 when Ciereszko and co-workers experienced the toxicity of *Palythoa caribaeorum* immediately after grinding the dried material through sore throats, chills and fever.[16] Later, the same group reported the high toxicity of *Palythoa mammillosa* and *Palythoa grandis*. Palytoxin (**32**), known as one of the most potent natural and non-proteinaceous toxins for humans, was first isolated by Moore and Sheuer in 1971 from the Hawaiian zoanthid *Palythoa toxica*.[2] This species was previously known by the Hawaiian native population as "limu-make-o-Hana". The authors proposed that palytoxin behaves like a steroidal saponin even though the hydrolysis did not lead to a hydrophilic and a lipophilic part. The lethal dose (LD_{50}) exhibited by palytoxin was measured as 0.1 μg kg^{-1} by intravenous injection and 0.4 μg kg^{-1} by intraperitoneal injection in mice. On the basis of NMR and combustion data, the molecular weight of the toxin was



	A/B	n	R^1	R^2
Palytoxin (**32**)	A	1	H	OH
Homopalytoxin (**33**)	A	2	H	OH
Bishomopalytoxin (**34**)	A	3	H	OH
Neopalytoxin (**35**)	B	1	H	OH
Deoxypalytoxin (**36**)	A	1	H	H
42*S*-hydroxy-50*S*-palytoxin (**37**)	A	1	OH	OH
42*S*-hydroxy-50*R*-palytoxin (**38**)	A	1	OH	OH

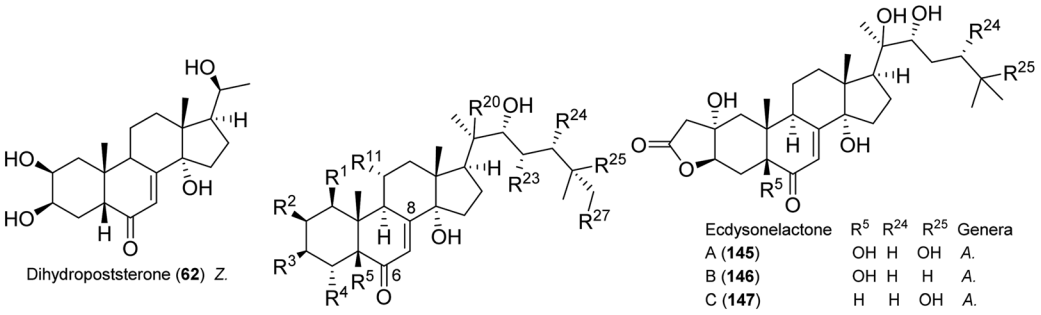
Fig. 2 Structures of palytoxin analogues from species of *Palythoa*.

estimated to be about 3300 Da.[17] It was described as stable in water but a quick decomposition was observed under acidic or alkaline conditions, leading to a reduction of its toxicity.[18] The structure and stereochemistry of palytoxin (**32**) were elucidated a decade after the first report, following intensive work by two independent research groups led by Moore in Hawaii and Hirata in Japan (Fig. 2).[19–22] Hirata's group worked on the structure of palytoxin isolated from *P. tuberculosa* collected in Okinawa, while Moore's group worked on a palytoxin isolated from *P. toxica* collected in Hawaii and Tahiti. The first structural and stereochemical insights into the structure of palytoxin were inferred after the oxidative cleavage of some vicinal diols by $NaIO_4$ followed by a reduction with $NaBH_4$ and acetylation.[21] Slightly different results were first observed with the toxins from Hawaii and Tahiti using the same techniques.[20] The full planar structure of palytoxin, including the absolute configurations of both C-101/C-115 and C-20/C-41 degradation products obtained by X-ray analyses, was first published in 1981 by Hirata's group.[22] Then, the same group deduced the relative configurations of other degradation products by synthesis,[23–25] and this work culminated in the establishment of the 3D structure of the molecule in 1982.[26] Hemiacetal equilibrium at C-47 was suggested to be responsible for the slight differences between compounds of the different species, and the absolute configuration was finally established by Moore's group.[27] Palytoxin (**32**) contains both hydrophilic and lipophilic moieties distributed among 129 aliphatic carbon atoms, 40 secondary alcohols, 64 chiral centres, two amides and 6 olefinic bonds with maximum UV absorbances at λ 233 and 263 nm (Fig. 2).[20,22] The total synthesis of palytoxin was accomplished in 1994 by Kishi's group after several years of attempts.[28–30]

The presence of palytoxin has been reported from several species of *Palythoa*, such as *P. toxica*, *P. tuberculosa*, *P. vestitus*, *P. caribaeorum*, *P.* aff. *margaritae*, *P. canariensis*, *P. heliodiscus* and *P.* aff. *clavata*/*sakurajimensis*.[2,6,17,31–39] The concentration of palytoxin (**32**) in *P.* aff. *clavata*/*sakurajimensis* is one of the highest reported in the literature, with 2.22 mg g^{-1} of wet sample, followed by *P. heliodiscus* reported by Deeds *et al.*, with a concentration of 1 mg g^{-1} wet sample, while deoxypalytoxin (**36**) was found at 3.51 mg g^{-1} wet sample.[37] Even though the presence of the toxin was first thought to be exclusive to the genus *Palythoa*, it was also reported from *Zoanthus solanderi* and *Zoanthus sociatus* from Colombia, *Zoanthus* sp. from Hawaii, and *Zoanthus pulchellus* and *Parazoanthus* sp.[6,40–42] Additionally, this toxin has also been reported from other marine organisms, such as the red alga *Chondria armata* and from various species of xanthid crabs from the Philippines, the warty crab *Eriphia verrucosa*, the trigger fish *Melichtys vidua*, the flathead mullet *Mugil cephalus*, the sea anemone *Radianthus macrodactylus*, the sea urchin *Paracentrotus lividus*, the rock shell *Stramonita haemastoma* and from the marine cyanobacteria *Trichodesmium*.[43–48] The palytoxin analogues homopalytoxin (**33**), bishomopalytoxin (**34**), neopalytoxin (**35**) and deoxypalytoxin (**36**) were reported from *P. tuberculosa* collected in Okinawa (Fig. 2).[17] Two palytoxin epimers, specifically (42*S*)-hydroxy-(50*S*)-palytoxin (**37**) and (42*S*)-hydroxy-(50*R*)-palytoxin (**38**), were isolated from the Hawaiian zoantharians *P. toxica* and *P. tuberculosa*, respectively.[31,49] Compounds **32** and **37** were evaluated against skeletal myotubes, revealing an increase in Ca^{2+} at 6 nM. The increase in Ca^{2+} could have occurred through either an induced activation of voltage calcium channels or by activation of the Na^+/Ca^{2+} exchanger.[31] These compounds also displayed cytotoxic activity against human skin keratinocytes (HaCaT cells), with EC_{50} values of 2.7×10^{-11} M for **32**, 9.3×10^{-10} M for **37** and 1.0×10^{-10} M for **38**.[49] Rossi *et al.* reported a new analogue named palytoxin b from a reference standard purchased from Wako Chemicals.[33] The toxin was only identified through LC-ESI-ToF-MS analysis in the positive mode by comparison of the fragmentation patterns to those of **32**. Compounds **32** and **36** were reported for the first time from the cyanobacteria *Trichodesmium* spp. collected in New Caledonia.[48] These toxins were identified through different methods, including mouse bioassay, neuroblastoma cell-based assay and LC-MS/MS analysis. Fraga *et al.* reported for the first time the presence of **32** and **38** from the Atlantic coral *P. canariensis*.[35] The identification of the toxins was achieved through UPLC-IT-TOF-MS and by comparison of the fragmentation patterns with those of the standard palytoxin. In 2018, the presence of **32** was reported from *P.* aff. *clavata*/*sakurajimensis* maintained in an aquarium.[36] Potent *in vitro* cytotoxicity was exhibited against A549 (lung carcinoma), Hs683 (glioma), U373n (glioma), 9L (gliosarcoma) and B16F10 (melanoma), with IC_{50} values of 0.67, 0.58, 0.56, 0.39 and 0.44 pM, respectively. Palytoxin acts through the Na^+, K^+ ATPase of cell membranes, turning the pump into an ion channel producing a Na^+ influx, K^+ efflux, therefore causing membrane depolarization and leading cells to undergo apoptosis.[38,50,51] Additionally, permeability to Ca^{2+} was observed when palytoxin binds to the Na^+/K^+ pump, and the palytoxin–Ca^{2+} complex was determined through NMR and molecular modelling analysis.[52] Dermal, ocular and respiratory health issues can be caused by direct exposure to some species of zoantharians sold for home aquariums as these species are commonly used for ornamental purposes.[37,53] Palytoxin (**32**) displayed an inhibition of human immunodeficiency virus producing cells (MOLT-4/HIVHTLV-IIIB cells) at 2.0 pg mL^{-1}.[54] Increasing bioactivity was observed when a combination of a TPA-type tumour promoter (teleocidin) was used with a non-TPA type tumour promoter (palytoxin). The increase in bioactivity was suggested to be due to the production of prostaglandin E_2. Additionally, Valverde *et al.* reported the inhibition of human intestinal cell proliferation (Caco-2) by **32** with an IC_{50} value of 0.1 nM.[55] Potent activity against head and neck cancer cell lines was observed for **32**, with LD_{50} values between 1.5 to 3.5 ng mL^{-1}.[56] The compound killed tumour cells in mice, with LD_{50} values between 68 to 83 ng kg^{-1}, and no tumour regression was observed in the control animals.[55]

2.1.3 Ecdysteroids. Highly oxidized steroids featuring a $\Delta^{7,8}$ conjugated ketone at C-6, also named ecdysteroids, have been found in most species of both genera *Palythoa* and *Zoanthus*.

2.1.3.1 Palythoa. The chemical study of an unidentified *Palythoa* sp. from Vietnam allowed the identification of the first members of ecdysteroids: 20-hydroxyecdysone (**39**), 2-*O*-acetyl-20-hydroxyecdysone (**40**) and 3-*O*-acetyl-20-hydroxyecdysone



	R^1	R^2	R^3	R^4	R^5	R^{11}	R^{20}	R^{23}	R^{24}	R^{25}	R^{27}	Genera
20-hydroxyecdysone (**39**)	H	OH	OH	H	H	H	OH	H	H	OH	H	*Pal. Z. Par. S.*
2-*O*-acetyl-20-hydroxyecdysone (**40**)	H	OAc	OH	H	H	H	OH	H	H	OH	H	*Pal. Z. Par.*
3-*O*-acetyl-20-hydroxyecdysone (**41**)	H	OH	OAc	H	H	H	OH	H	H	OH	H	*Pal. Z. Par.*
Palythoalone A (**42**)	H	OH	OH	H	OH	H	OH	H	CH_3	H	OH	*Pal.*
Palythoalone B (**43**)	H	OH	OH	H	OH	H	OH	H	H	H	OH	*Pal.*
Makisterone B (**44**)	H	OH	OH	H	H	H	OH	H	CH_3	H	OH	*Pal.*
Inokosterone (**45**)	H	OH	OH	H	H	H	OH	H	H	H (*R*)	OH	*Pal. Z.*
Palythone A (**46**)	H	H	H	OH	H	H	OH	H	H	OH	H	*Pal.*
3-deoxy-20-hydroxyecdysone (**47**)	H	OH	H	H	H	H	OH	H	H	OH	H	*Pal.*
24-*epi*-makisterone A (**48**)	H	OH	OH	H	H	H	OH	H	CH_3	OH	H	*Pal.*
2-deoxyecdysterone (**49**)	H	H	OH	H	H	H	OH	H	H	OH	H	*Pal. Z.*
α-ecdysone (**50**)	H	OH	OH	H	H	H	H	H	H	OH	H	*Pal. Z.*
Zoanthusterone (**51**)	OH	OH	OH	H	H	H	OH	H	H	H	H	*Z.*
Ponasterone A (**52**)	H	OH	OH	H	H	H	OH	H	H	H	H	*Z.*
Viticosterone E (**53**)	H	OH	OH	H	H	H	OH	H	H	OAc	H	*Z. Par.*
25-*O*-acetylintegristerone A (**54**)	OH	OH	OH	H	H	H	OH	H	H	OAc	H	*Z.*
Ajugasterone C (**55**)	H	OH	OH	H	H	OH	OH	H	H	H	H	*Z. Par. S.*
Dacryhainansterone (**56**)	H	OH	OH	H	H	$\Delta^{10,11}$	OH	H	H	OH	H	*Z.*
Zoanthone A (**57**)	H	OH	OH	H	H	OH	OH	H	H	OAc	H	*Z.*
24(28)-dehydromakisterone A (**58**)	H	OH	OH	H	H	H	OH	H	$\Delta^{24,28}$	OH	H	*Z.*
Pterosterone (**59**)	H	OH	OH	H	H	H	OH	H	OH	H	H	*Z.*
25-*epi*-inokosterone (**60**)	H	OH	OH	H	H	H	OH	H	H	H (*S*)	OH	*Z.*
Turkesterone (**61**)	H	OH	OH	H	H	OH	OH	H	H	OH	H	*Z.*
4-dehydroecdysterone (**143**)	H	OH	OH	$\Delta^{4,5}$		H	OH	H	H	OH	H	*Par.*
Gerardiasterone (**144**)	H	OH	OH	H	H	H	OH	OH	H	OH	H	*S.*

Fig. 3 Structures of ecdysteroids from species of zoantharians. *Palythoa* (*Pal.*), *Zoanthus* (Z.), *Parazoanthus* (*Par.*), *Savalia* (*S.*) and *Antipathozanthus* (*A.*).

(**41**) (Fig. 3).[57] These ecdysteroids were also reported from the Brazilian zoantharians *P. variabilis* and *P. caribaeorum* and from the Taiwanese *P. tuberculosa*.[12,58] Palythoalone A (**42**), palythoalone B (**43**), makisterone B (**44**) and inokosterone (**45**), featuring a hydroxyl group at C-27, were reported from *P. australiae* collected off the coast of Okinawa (Fig. 3).[59]

The new ecdysteroid zoanthusterone (**51**) was isolated along with the known ponasterone A (**52**), viticosterone E (**53**), 25-*O*-acetylintegristerone A (**54**), ajugasterone C (**55**), dacryhainansterone (**56**), **39**, **40**, **45**, **49** and **50** from an unidentified species of *Zoanthus* sp., collected off the coast of Samae-sarn, Thailand.[60] Analysis of the anti-dengue virus activity of the ethanolic extract from *Zoanthus* spp. collected off the coast of Taiwan allowed the identification of a new ecdysteroid zoanthone A (**57**) together with dehydromakisterone A (**58**), pterosterone (**59**), *epi*-inokosterone (**60**), turkesterone (**61**), dihydropoststerone (**62**), **39**, **41**, **45**, **49** and **50**.[61] Compounds **57** and **59** exhibited activity against dengue virus, with EC_{50} values of 19.61 μM and 10.05 μM, respectively.

2.1.4 Zoanthamine alkaloids. A very original family of non-aromatic alkaloids called zoanthamines has been isolated mostly from the genus *Zoanthus*. A review of the chemistry and biological activities of these metabolites was published in 2008,[62] following a broader review published earlier on alkaloids from zoantharians.[63]

The first chemical investigation of a toxic *Zoanthus* species started in 1984 by Rao and co-workers when the spray ejected by colonies of an Indian species of *Zoanthus* caused prolonged eye irritation and pain.[64] Even though the zoantharian was first identified as *Zoanthus pacificus*, this species was thought to be endemic to the Caribbean and the authors decided to keep the name *Zoanthus* sp. They were able to isolate and characterize zoanthamine (**63**), the first member of a new family of alkaloids of unknown metabolic pathway characterized by unique features of fused rings culminating in a rare azepane ring (Fig. 4). The structure of **63** was elucidated by mass spectroscopy and NMR, and the relative configuration was deduced from crystallographic and X-ray diffraction analyses. Further chemical studies on the same species allowed the identification of zoanthenamine (**64**) and zoanthamide (**65**).[65]

The structures were elucidated by comparison of the ^1H and ^{13}C NMR data with those of **63**. They both feature a γ-spirolactone ring at C-22. This new lactone ring is probably formed after opening of the unstable hemiaminal at C-10, leading to the

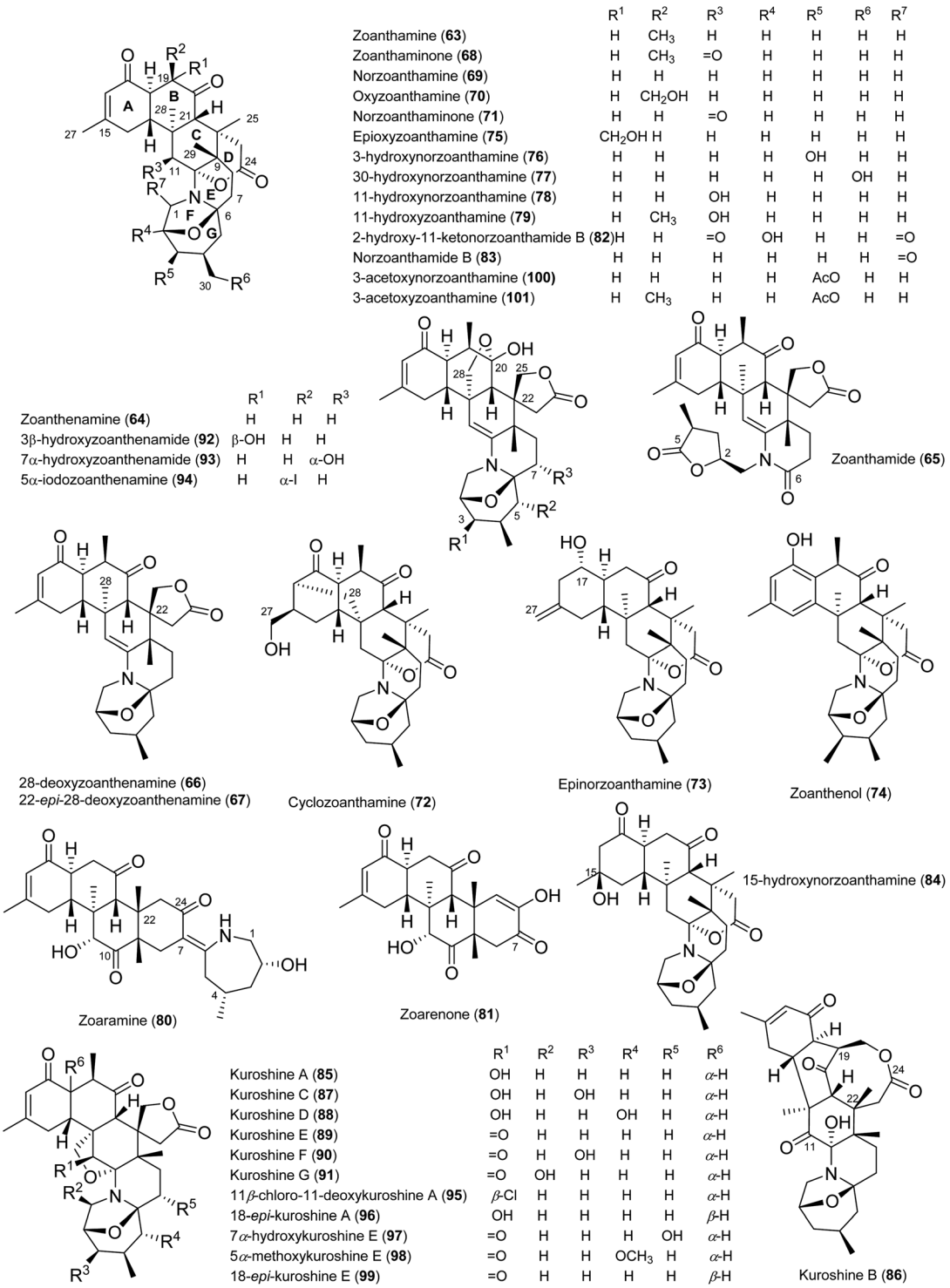


Fig. 4 Structures of zoanthamine alkaloids isolated from species of *Zoanthus*.

corresponding enamine. The spirolactone at C-22 would then result from an esterification between the released carboxylic acid and the primary alcohol formed after oxidation of the methyl C-25. Compound **64** contains an additional hemiacetal at C-20 that could be formed by the addition of a primary alcohol at C-28 onto the ketone at C-20. While this ether ring is absent in **65**, opening of the second hemiaminal at C-6 could lead to a terminal γ-lactone ring and an amide at C-7 after oxidative cleavage of the resulting enamine. Norzoanthamine (**69**), oxyzoanthamine (**70**), norzoanthaminone (**71**), cyclozoanthamine (**72**) and epinorzoanthamine (**73**) were reported from an unidentified species of *Zoanthus* collected off the Ayamaru coast of the Amami Islands, Japan (Fig. 4).[66] Compounds **69**, **71** and **73** are the first zoanthamines that lack the methyl C-

26 at C-19. Zoanthamine (**63**) was proposed to be a precursor of norzoanthamine (**69**) following oxidation of the methyl C-26 and decarboxylation. All five metabolites displayed cytotoxic activity against P388 murine leukemia cells, with IC_{50} values of 24, 7.0, 1.0, 24 and 2.6 μg mL^{-1}, respectively. One of the most promising bioactivities displayed by these alkaloids was against osteoporosis and a review on this potential was published in 2000 by Uemura and co-workers.[67] Compound **69** and its hydrochloride analogue showed the inhibition of Interleukin 6 (IL-6) at 13 and 4.7 μg mL^{-1}, respectively. The structure–activity relationship (SAR) of **69** indicated that the C-15/C-16 double bond and the lactone ring play important roles in the bioactivity of the natural product.[68] The potent anti-osteoporotic effect of norzoanthamine hydrochloride was confirmed through an *in vivo* assay on ovariectomized mice and by theoretical studies through molecular dynamics and docking investigations of zoanthamine analogues as matrix metalloproteinases-1 inhibitors.[69,70] Additionally, Kinugawa *et al.* reported that **69** increases the production of collagen-hydroxyapatite composite, one of the major solid components of bone tissue, by a non-specific binding to the polyvalent binding sites of collagen.[71] Therefore, the anti-osteoporosis mode of action was suggested to be due to the collagen-norzoanthamine supramolecular association preventing collagen from undergoing proteolytic cleavage. The protective function of **69** was further confirmed by Genji *et al.* They studied the distribution of norzoanthamine in *Zoanthus* sp., revealing a high concentration of the alkaloid in the epidermal tissue of the organism.[72] Tachibana and co-workers synthesized the bisaminal unit of norzoanthamine, which exhibited similar collagen protection as the parent compound. The absence of the D ring led to the hydrolysis of the bisaminal, inducing a decrease in the protective activity.[73] In 1998, epioxyzoanthamine (**74**), an isomer of **70** at C-19, was isolated from a *Zoanthus* sp. collected around the Canary Islands.[74] An unusual deuterium exchange was observed in the presence of D_2O for the methylene signal at C-11 in **69** and **74**, as previously reported for **65**. The deuterium exchange could originate from an equilibrium between the hemiaminal and the enamine form in aqueous solution. Later, zoanthenol (**75**), the first zoanthamine with an aromatized A ring, 3-hydroxynorzoanthamine (**76**), 30-hydroxynorzoanthamine (**77**), 11-hydroxynorzoanthamine (**78**) and 11-hydroxyzoanthamine (**79**) were reported from another *Zoanthus* sp. (Fig. 4).[75] No deuterium exchange at C-11 was observed for **78** and **79**, suggesting that the first step in the enamine formation would be the elimination of H-11β. During a study on the activity against human platelet aggregation, a strong inhibition of collagen, thrombin and arachidonic acid-induced aggregation was observed for compounds **69** and **78**, both at 0.3, 0.5 and 1 mM. The inhibitory effect caused by the zoanthamine analogues was suspected to be induced by the stimulation of the calcium input into the cell, the activation of protein kinase C or the reduction of intracellular cyclic adenosine monophosphate levels. Additionally, compounds **76** and **77** selectively inhibited platelet aggregation induced by collagen at 0.3 and 1 mM, while **75** was effective at 0.125, 0.5 and 1 mM.[76] Hirai *et al.* synthesized the ABC-ring and CDEFG-ring of **75** to study its SAR. The presence of the hydrochloride in the CEFG-ring was suggested to be the active pharmacophore to inhibit IL-6 production.[77] Furthermore, a pro-aggregation effect was observed for **68** at 1 mM and for **70** between 0.125 and 1.5 mM. Interestingly, the bioactivity was enhanced in the presence of hydroxyl groups at C-3, C-11 or C-30 and by the addition of a double bond at C-10/C-11 of the norzoanthamine skeleton.[76] Two structurally different zoanthamine analogues, specifically zoaramine (**80**) and zoarenone (**81**), were isolated from an unidentified species of *Zoanthus* sp. collected off the coast of Punta del Hidalgo, Tenerife (Fig. 4).[78] Compound **80** was characterized by the absence of the N/C-10 bond and the presence of a C-7/C-24 bond. The relative configuration was assigned by ROESY experiment and the coupling constant values and confirmed through Gauge-Independent Atomic Orbital (GIAO) NMR calculations using density functional theory (DFT) analysis and the probabilistic DP4. It was reported that zoarenone (**81**) is closely related to **80** but the azepane ring has been lost. Additionally, the same research group reported three oxidized zoanthamine analogues: 2-hydroxy-11-ketonorzoanthamide B (**82**), norzoanthamide B (**83**) and 15-hydroxynorzoanthamine (**84**) from a *Zoanthus* sp. (Fig. 4).[79] Kuroshine A (**85**) and kuroshine B (**86**) were then reported from the Taiwanese zoantharian *Z. kuroshio*.[80] Compound **85** is characterized by the presence of an ether bridge between C-10 and C-28, while **86** contains an eight membered lactone ring between C-24 to C-26. These compounds did not show any activity against human platelet aggregation, inflammation or cytotoxicity. Further studies on *Z. Kuroshio* led to the identification of kuroshines C-G (**87-91**), 3β-hydroxyzoanthenamide (**92**) and 7α-hydroxyzoanthenamide (**93**) (Fig. 4).[81] Kuroshines C-G contain the same ring system as **85**, while **92** and **93** share the same ether bridge as zoanthenamine (**64**). These compounds were evaluated for their anti-inflammatory, antiviral, antimicrobial, antiosteoporotic and cytotoxic activity. Only compound **89** displayed weak toxicity against melanoma cell line B16, with an IC_{50} value of 120 μM. Two halogenated zoanthamine analogues, namely 5α-iodozoanthenamine (**94**) and 11β-chloro-11-deoxykuroshine A (**95**), were isolated from *Z. kuroshio* along with 18-*epi*-kuroshine A (**96**), 7α-hydroxykuroshine E (**97**), 5α-methoxykuroshine E (**98**) and 18-*epi*-kuroshine E (**99**).[82] Compounds **96** and **99** were the first zoanthamines with a *cis* A/B junction. The anti-inflammatory assay revealed a significant activity of **94** at 10 μM. Finally, the first chemical study of *Z. cf. pulchellus* collected off the coast of mainland Ecuador led to the isolation of 3-acetoxynorzoanthamine (**100**) and 3-acetoxyzoanthamine (**101**) together with the known compounds **63**, **69** and **76**.[83] These compounds were evaluated for their antioxidant and anti-inflammatory activity in the microglia BV-2 cell line. Compounds **69** and **101** displayed dose-dependent activity on reactive oxygen species (ROS), while the other compounds exhibited significant inhibitory activities on ROS and nitric oxide generation (NO).

2.1.5 2-Aminoimidazole alkaloids. Some original aromatic alkaloids containing 2 or more 2-aminoimidazole rings are commonly found in zoantharians.

2.1.5.1 Palythoa. During a survey of fluorescent aromatic alkaloids in different zoantharians, Cariello *et al.* reported the presence of zoanthoxanthin (**102**), parazoanthoxanthin D (**103**), parazoanthoxanthin F (**104**), epizoanthoxanthin B (**105**), palyzoanthoxanthin A–C (**106-108**) pseudozoanthoxanthin (**109**), homopseudozoanthoxanthin (**110**) and dimethylpseudozoanthoxanthin (**111**) in small amounts from a *Palythoa* sp. collected in Indonesia, *P. mammilosa* in the Caribbean and *P. tuberculosa* in the Marshall Islands (Fig. 5).[84] They feature a fully aromatized tricyclic bisguanidine core.

2.1.5.2 Zoanthus. Zoanthus sociatus from the Caribbean and *Z.* aff. *pacificus* were also screened for their content of the pigment zoanthoxanthins.[84] The composition was different and for the *Zoanthus* species they found zoanthoxanthin (**102**), parazoanthoxanthin A (**112**), parazoanthoxanthin B (**113**), parazoanthoxanthin D (**103**), parazoanthoxanthin G (**114**), palyzoanthoxanthin A (**106**), epizoanthoxanthin A (**115**), pseudozoanthoxanthin (**109**), 3-norpseudozoanthoxanthin (**116**), and homopseudozoanthoxanthin (**110**) (Fig. 5).

2.1.6 Miscellaneous

2.1.6.1 Palythoa. In 1978, the water-soluble amino acid mycosporine-Gly (**117**) was isolated from *Palythoa tuberculosa* collected in Japan and converted into the stable methyl ester for its characterization by spectroscopy (Fig. 6).[85] An ultraviolet protection function was proposed in the organism because of its high UV absorbance at 310 nm. Palythine (**118**), palythinol (**119**) and palythene (**120**) were isolated from the same species and these compounds showed high UV absorption at 310, 332 and 360 nm, respectively.[86,87] Additional studies on UV-absorbing compounds from *P. tuberculosa* led to the isolation of the pyrazine derivatives palythazine (**121**) and isopalythazine (**122**), characterized by their spectroscopic data and chemical synthesis (Fig. 6).[88] In 1982, two glycerol ethers named chimyl alcohol (**123**) and batyl alcohol (**124**) were identified along with a mixture of other glycerol derivatives from the ligroin fraction of the species *P. liscia* (Fig. 6).[89]

Two prostaglandins PGA_2 (**125**) and PGB_2 (**126**) were isolated in 2006 from *P. kochii* collected off the coast of Okinawa, Japan.[90] The structures were established based on their NMR data and by comparison to those of the commercial PGA_2. Compound **125** displayed cytotoxic activity and neurite-extension inhibition (NGF-PC12 cell), with IC_{50} values of 70 and 20 μM, respectively, and antitubulin polymerization activity at 100 μM. The PGA_2 bioactivity of **125** was comparable to that of paclitaxel (Taxol), but **126** was found to be inactive. The chemical study of the Brazilian zoantharian *Protopalythoa variabilis* allowed the identification, of two lipidic α-aminoacids for the first time from natural sources.[91,92] The mixture of the two

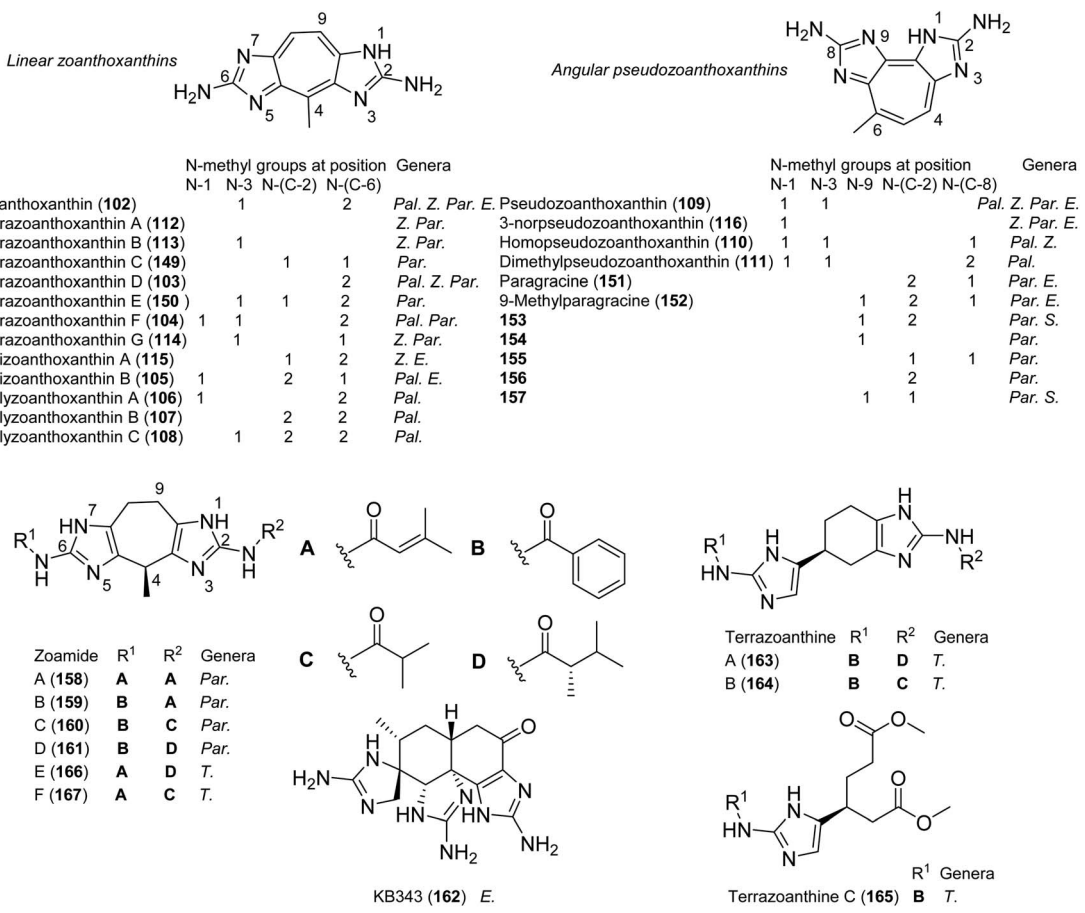


Fig. 5 2-Aminoimidazole alkaloids from zoantharians. *Palythoa* (*Pal.*), *Zoanthus* (Z.), *Parazoanthus* (*Par.*), *Savalia* (*S.*), *Epizoanthus* (*E.*), *Terrazoanthus* (*T.*).

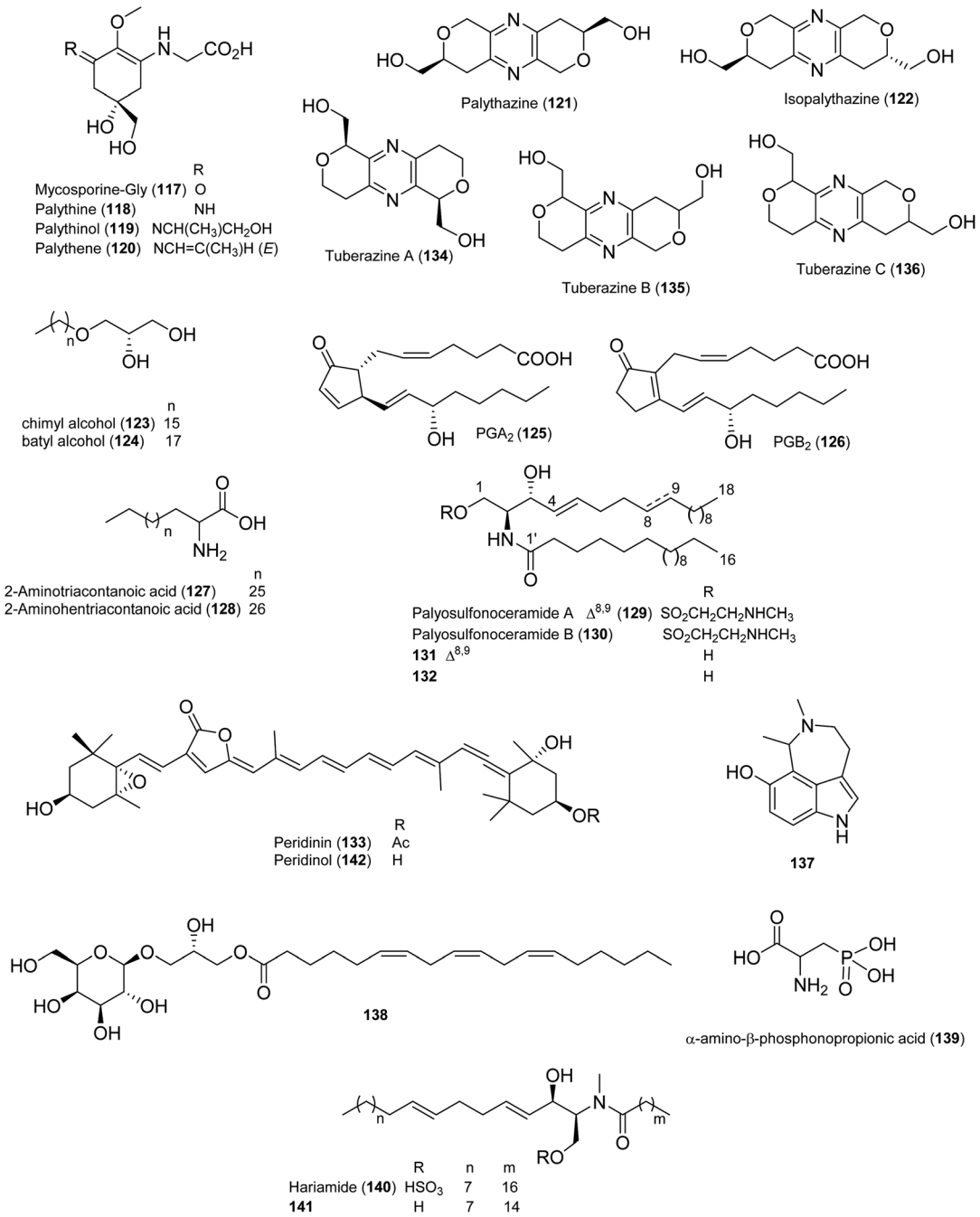


Fig. 6 Structures of other metabolites isolated from *Palythoa* and *Zoanthus* species.

amino acids **127** and **128** exhibited potent cytotoxicity against human colon cancer (HCT-8), melanoma (MDA-MB-435), CNS glioblastoma (SF-295) and leukemia (HL-60), with IC_{50} values of 0.13, 0.05, 0.07 and 0.1 μg mL^{-1}, respectively (Fig. 6).

In 2012, two unprecedented sulfonoceramides named palysulfonoceramide A (**129**) and palysulfonoceramide B (**130**) along with the known ceramides **131** and **132** were isolated from *Palythoa caribaeorum* and *Palythoa variabilis* collected off the coast of Brazil.[93] Remarkably, sulfonylated groups on ceramides have only been obtained through synthesis before. These ceramides were tested against the human colon adenocarcinoma (HCT-116) cell line with no significant bioactivity. The DCM fraction exhibited high toxicity in the *Artemia* lethality test with an LC_{50} value of 52.10 μg mL^{-1}. All the fractions showed low haemolytic and no antimicrobial activity at 100 μg mL^{-1}.[94]

The anti-dengue apocarotenoid pigment peridinin (**133**) was isolated from the Vietnamese *Palythoa mutuki* (Fig. 6).[95] The pigment exhibited potent antiviral activity against all serotypes of DENV 1–4, with EC_{50} values of 7.62, 4.50, 5.84 and 6.51 μM, respectively. Additionally, **133** showed an inhibition of DENV protease activity with an EC_{50} value of 8.50 μM, suggesting it

could be a potential anti-dengue virus candidate. Some pyrazine derivatives named tuberazines A–C (**134-136**) were isolated along with **137**, tyramine, *N*-methylserotonin, phenethylamine, isobutylamine, isoamylamine, **138**, **40** and **41** from the Taiwanese zoantharian *Palythoa tuberculosa* (Fig. 6).[58] The absolute configuration of **134** was assigned through ECD analysis, and compound **136** exhibited anti-lymphangiogeneic activity in human lymphatic endothelial cells (LECs), with an IC_{50} value of 33 μg mL^{-1}.

The variability of the metabolomic profiles using the MS/MS fragmentation patterns and global molecular networking analysis (GNPS) was assessed on two species of zoantharians *Palythoa caribaeorum* and *P. variabilis* collected from different locations off the coast of Brazil.[96] This new approach deepened our knowledge on the metabolome of zoantharians. The study revealed the presence of minor components, such as mycosporine-like amino acids, ecdysteroids, phosphatidylcholine derivatives, indole diterpenes, sulphonoceramides and zoanthamine alkaloids, through annotation by HRMS/MS.

2.1.6.2 Zoanthus. The α-amino-β-phosphonopropionic acid (**139**) was identified from an aqueous–ethanolic extract of the Caribbean *Zoanthus sociatus* by Kittredge and Hughes in 1964 (Fig. 6).[97] The identification of the amino acid was based on comparison with the synthetic labelled analogue by paper chromatography, paper electrophoresis and ion-exchange chromatography. In 1997, hariamide (**140**), a sulphated sphingolipid, was isolated together with the ceramide *N*-palmitoyl-D-(+)-erythrooctadecasphinga-4(*E*)-8(*E*)-dienine (**141**) from an unidentified species of *Zoanthus* sp. collected off the Indian coast (Fig. 6).[98] The anti-spasmodic C_{37} carotenoid pigment peridinol (**142**) was isolated from a *Zoanthus* sp. collected off the coast of Goa, India.[99]

2.2 The suborder Macrocnemina

This suborder contains more genera than the suborder Brachycnemina, and five of them have been chemically studied: *Savalia*, *Parazoanthus*, *Epizoanthus*, *Terrazoanthus* and *Antipathozoanthus*. Two families of natural products have been commonly identified in species of these genera, namely ecdysteroids and zoanthoxanthins, but halogenated tyrosine derivatives have also been identified.

2.2.1 Ecdysteroids

2.2.1.1 Parazoanthus. In 1979, Fedorov *et al.* were the first to report the presence of 20-hydroxyecdysone derivatives **39–41** from a *Palythoa* sp., but also from a *Parazoanthus* sp. collected off the coast of Vietnam (Fig. 3).[57] The structures were deduced from their NMR and MS data and by comparison with those of previously reported analogues. The new 4-dehydroecdysterone (**143**) was isolated from an Australian *Parazoanthus* sp., together with the known 20-hydroxyecdysone (**39**) and ajugasterone C (**55**) (Fig. 3).[100,101] The relative configuration of **143** was assigned through coupling constants analysis and nOe correlations. An *in vivo* feeding assay to test the protective role of ecdysteroids present in marine invertebrates was performed in the study. No protective role was observed through treatment with up to 1% of ecdysteroid. This finding was further confirmed by a palatability study carried out by Fenical and co-workers. No antifungal activity was found in the tested metabolites. Recently during a deep chemical investigation of two morphotypes of *P. axinellae* in the Mediterranean Sea, the four ecdysteroids **39**, **40**, **41** and **53** were identified.[102]

2.2.1.2 Savalia. In 1982, 20-hydroxyecdysone (**39**) was isolated as the major component of the Mediterranean zoantharian *Gerardia savaglia* (now named *Savalia savaglia*) (Fig. 3).[103] The structure was deduced from NMR analyses and by comparison with the commercially available ecdysterone. Consequently, gerardiasterone (**144**) and then ajugasterone C (**55**) were isolated from *S. savaglia* by Pietra's group.[104,105] This was the first ecdysteroid reported with four hydroxyl groups on the side chain, including the new OH at C-23. Tsubuki and co-workers synthesized gerardiasterone *via* a diastereoselective synthesis and confirmed the absolute configuration of the side chain to be 20*R*, 22*R* and 23*S*.[106,107]

2.2.1.3 Antipathozoanthus. Only one chemical study has been published on a species of the genus *Antipathozoanthus*. Ecdysonelactones A–D (**145-148**) were reported from the Tropical Eastern Pacific zoantharian *A. hickmani*, collected at the marine protected area El Pelado, Ecuador (Fig. 3). Ecdysonelactones are characterized by the presence of a γ-lactone ring fused at the C-2/C-3 bond of the ecdysteroid ring A. The structures were inferred from their NMR and HRESIMS data. The relative configurations were assigned through NOESY correlations, coupling constants analysis and by comparison of the NMR data to those of 4-dehydroecdysterone. These compounds did not display antimicrobial nor cytotoxic activity.[108]

2.2.2 2-Aminoimidazole alkaloids

2.2.2.1 Parazoanthus. The first alkaloids isolated from a zoantharian are characterized by an unusual heteroaromatic system cyclohepta[1,2-*d*:4,5-*d*′]diimidazole or cyclohepta[1,2-*d*:3,4-*d*′]diimidazole. These alkaloids can be classified depending on their skeleton type: zoanthoxanthin (linear system) or pseudozoanthoxanthin (angular system). Within the zoanthoxanthin type, they are differentiated into *para* and *epi* zoanthoxanthins depending on the methylation pattern on the imidazole ring. The fluorescent pigment zoanthoxanthin (**102**) was first isolated in 1973 from the Mediterranean zoantharian *Parazoanthus* cfr. *axinellae* (Fig. 5).[109,110] The structure of this compound featuring a new heterocyclic system was determined by spectroscopy data, chemical derivatization and X-ray crystallography of its chloro derivative. Compound **102** displayed a non-competitive inhibition of succinic oxidase activity of beef heart mitochondria, with an IC_{50} value of 5.7×10^{-4} M.[111] Further chemical studies on the same species led to the isolation of parazoanthoxanthines A–D (**112**, **113**, **149**, **103**) (Fig. 5). Only compounds **112** and **103** were fully characterized, by comparison of their spectroscopic data with those of **102**. The structures of **113** and **149** could not be fully elucidated due to the small amount of material isolated.[112] Compound **112** inhibited DNA synthesis at 2.7×10^{-4} M, and an electrostatic binding of **112** to the double-stranded DNA was suggested as its mechanism of action.[113] Compound **112** also showed anticholinesterase activity with inhibition constant values (Ki) of 19 and 26 μM and inhibition of the nicotinic acetylcholine

receptors.[114,115] The screening of zoanthoxanthin alkaloids in zoantharians led to the detection of other parazoanthoxanthin derivatives E (**150**), F (**104**) and G (**114**) in *P. axinellae* (Fig. 5).[84] The histamine-like activity of the methanolic fractions of the Japanese zoantharia *Parazoanthus gracilis* allowed the isolation of the yellowish-green fluorescent compound paragracine (**151**).[116] Its structure was established based on its chemical reactivity, spectroscopy data and by X-ray crystallography analysis of its dehydrobromide trihydrate derivative. Compound **151** showed a papaverine-like activity and a frequency-dependent blocking action of the sodium channel.[117] In addition to **151**, six paragracine-related alkaloids, namely 9-methylparagracine (**152**), **153**, pseudozoanthoxanthin (**109**), **154**, **155**, and **156**, were subsequently isolated from the Japanese *P. gracilis* (Fig. 5).[118] A papaverine-like activity was exhibited by these compounds. Earlier, and during the chemical study of a Hawaiian zoantharian *Parazoanthus* sp. collected at a depth of 350 m, the pseudozoanthoxanthin analogue **153** was also isolated by Scheuer and co-workers.[119] However, a following publication by the same group admitted an uncertainty in the taxonomy of the species in that it could also belong to the genus *Savalia*.[120] Further analysis of the chemical content of this species allowed the characterization of **153** and another pseudozoanthoxanthin derivative **157**. The inhibition of acetylcholinesterase was reported from an ethanolic extract of *P. axinellae* from the Northern Adriatic Sea, leading to the isolation of the known **109**.[121] This fluorescent compound displayed similar activity *in vivo* as the extract in mice and crabs, with an inhibition constant of 4 μM. The components of the extract were also cholinergic agonists on the nicotinic and muscarine receptors. The *in vivo* results on the rat phrenic nerve-diaphragm suggested a possible binding of the compounds to the acetylcholine receptor. Finally, a recent study of the same species allowed the identification of **116** and **151**.[102]

For the first time, non-fully aromatic zoamides A–D (**158-161**) were reported in 1997 from an unidentified dark species of *Parazoanthus* sp. from the Philippines (Fig. 5).[122] They feature an unprecedented acylation on both terminal primary amines.

2.2.2.2 Epizoanthus. The first metabolites reported from a species of the genus *Epizoanthus* were four zoanthoxanthin derivatives, namely epizoanthoxanthin A (**115**), epizoanthoxanthin B (**105**), pseudozoanthoxanthin (**109**) and 3-norpseudozoanthoxanthin (**116**), isolated from the Mediterranean *Epizoanthus arenaceus* collected in the Bay of Naples.[123] The structures of the compounds were established by comparison with those of zoanthoxanthin derivatives previously reported from *Parazoanthus axinellae*. In 1993, 9-methylparagracine (**152**) was isolated together with paragracine (**151**) and **102** from an undescribed species of *Epizoanthus* collected in the Namena Islands, Fiji.[124] Compound **152** displayed cytotoxic activity against two human colon adenocarcinoma (HCT8 and HT29), human lung carcinoma (A549) and mouse lymphocytic leukaemia (P-388), with IC_{50} values of 1.61, 0.82, 2.38 and 1.77 μg mL^{-1}, respectively. Recently, a bioactive-guided chemical study of the extract of *Epizoanthus illoricatus* collected in Palau allowed the isolation of KB343 (**162**), an unusual cyclic tris-2-aminoimidazole alkaloid.[125] Its relative configuration was deduced from NOESY correlations, while its absolute configuration was established through electronic circular dichroism (ECD) analysis. Compound **162** exhibited moderate toxicity activity against the murine leukaemia cell line (L1210), human tumor cell line (HeLa) and model neuronal cell line derived from human bone marrow (SH-SY5Y), with IC_{50} values between 2 and 5 μM.

2.2.2.3 Terrazoanthus. A new family of 2-aminoimidazole alkaloids named terrazoanthines A–C (**163–165**) were isolated from the zoantharian *Terrazoanthus patagonichus* (formerly known as *T. onoi*) collected off the coast of mainland Ecuador (Fig. 5).[126] This is the unique report on the chemical diversity of a species of the genus *Terrazoanthus*. The alkaloids **163** and **164** feature a novel skeleton with a 2-aminoimidazole ring fused to a cyclohexene and they still contain the two 2-aminoimidazoles of zoanthoxanthins. The acylation on both amines is similar to the one observed for zoamides.

Two additional non-fully aromatized bisguanidine derivatives, namely zoamide E–F (**166–167**), were later found in a sister species *Terrazoanthus* cf. *patagonichus* collected in the same area of the Tropical Eastern Pacific.[127] They differ from the other zoamides by the acyls substituted on both primary amines, and the absolute configuration at the central methyl was proposed based on ECD calculations.

2.2.3 Halogenated tyrosine derivatives

2.2.3.1 Parazoanthus. A new class of halogenated tyrosine alkaloids named parazoanthines A–E (**168-172**) were reported from the Mediterranean zoanthid *Parazoanthus axinellae* (Fig. 7).[128] They feature a rare 3,5-disubstituted hydantoin skeleton. Even if the antitumoral and antimalarial assays did not evidence significant activity for these compounds, the Microtox® bioassay revealed that **170** displayed the highest toxicity, with a significant EC_{50} value of 1.64 μM. Later, additional parazoanthines F–J (**173–177**) were identified by MS/MS analysis of the crude extract of *P. axinellae*.[129,130] The structures were deduced by comparison of the fragmentation patterns to those of previously reported analogues and by MS/MS spectra anticipation of the predicted compounds. The absolute configuration was assigned using an online UPLC-ECD system.

Two simple brominated and iodinated tyramine derivatives **178** and **179** were finally identified from *P. darwini* collected off the coast of Ecuador (Fig. 7).[127]

2.2.3.2 Antipathozoanthus. Four halogenated dipeptides named valdiviamides A–D (**180–183**) were isolated from *A. hickmani* off the coast of Ecuador (Fig. 7).[127] These compounds feature bromine and iodine atoms in the *ortho* position of the phenol ring of a common tyrosine amino acid. The relative and absolute configurations were assigned by nOe correlations, comparison of the theoretical and experimental ECD data and by DP4 calculations based on ^{13}C NMR chemical shifts. Compound **181** exhibited moderate cytotoxic activity against the hepatocellular carcinoma cell line (HepG2), with an IC_{50} value of 7.8 μM.

3 Biosynthetic considerations

3.1 Ecdysteroids

As seen in part 2, ecdysteroids represent a family of oxidized steroids broadly distributed among the two-suborders and

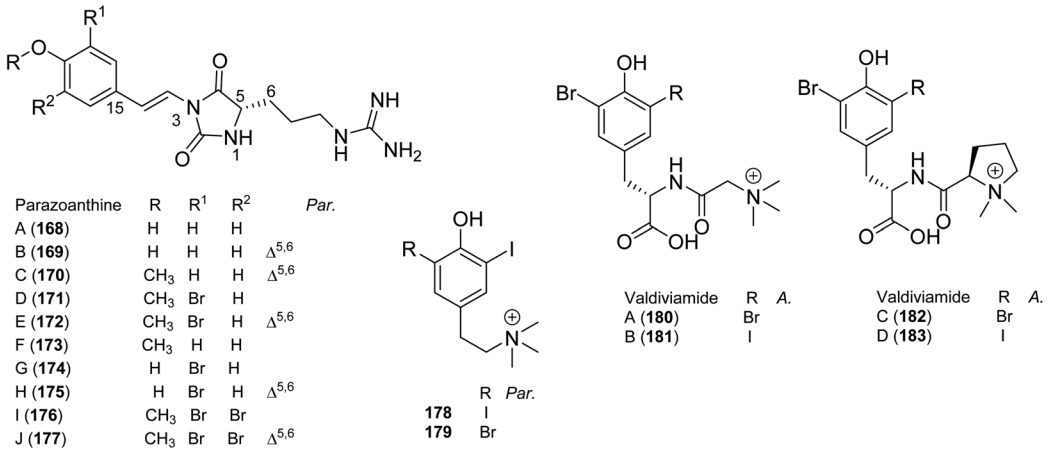


Fig. 7 Halogenated tyrosine derivatives isolated from the genera *Parazoanthus* (Par.) and *Antipathozoanthus* (A.).

genera of zoantharians. They are characterized by the presence of a $\Delta^{7,8}$ conjugated ketone at C-6 of ring B, and they also incorporate several hydroxyl groups at the C-2, C-3, C-14 and C-22 positions among others. Ecdysteroids have been recognized as pivotal hormones of the Ecdysozoa group, a superphylum and monophyletic clade of animals composed of 8 terrestrial and marine phyla.[131] Ecdysozoans include animals that grow by ecdysis, moulting their exoskeleton, with crustaceans examples of marine species of this clade. Ecdysteroids were early on recognized as moulting hormones for many species of this clade even though their presence was later evidenced in some plants too, therefore suggesting alternative ecological roles.[132] In 1982, the group of Pietra was the first to report an ecdysteroid from a zoantharian, the Mediterranean *Savalia savaglia* (Fig. 3).[103–105] Additional ecdysteroids were then found in some species of *Palythoa* and *Parazoanthus* off the coast of Vietnam in South-East Asia by Fedorov's group.[57] These discoveries revealed a broad presence of this family of natural products in most if not all the zoantharians, as proposed in a recent study on diverse species collected off the coast of mainland Ecuador in South Amer*ica.*[133] To the best of our knowledge, no biosynthetic studies have been undertaken on zoantharians for this particular family of natural products, so we can only report the results obtained on insects or plants presuming that they share the same metabolic pathways.[134] Two model animals allowed a better understanding of the metabolic pathways involved: the insect *Drosophila melanogaster* and the nematode *Caenorhabditis elegans*. Like for most of the ecdysozoans, insects utilize cholesterol and/or plant sterols as precursors of ecdysteroids. However, as they lack an enzyme for sterol biosynthesis, most ecdysozoans must intake cholesterol and/or other sterols from their diet. The biosynthesis of these hormones has been mostly studied to discover pesticides acting on the different steps of the metabolic pathways of pest insects. The first well-characterized step in the biosynthesis of all ecdysteroids is a dehydrogenation step at the C-7 position of cholesterol, leading to a diene in cycle B (Fig. 8).

No enzyme was characterized in a "black box" converting this intermediate into a compound named diketol. The following steps leading to 20-hydroxyecdysone have now been well established. As in most insects, 20-hydroxyecdysone (**39**) seems a key ecdysteroid for zoantharians and this metabolite can therefore be considered as a precursor of the other isolated analogues. Acetylation at the *O*-C-2 and *O*-C-3 positions is also quite common and indeed, the acetylated products **40** and **41** were found in 3 genera of zoantharians. Oxidation at C-5, as found in palythoalones **42** and **43**, can be explained through a Michael addition on the Δ^4-diketol, but also by oxidation at the alpha position of the ketone at C-6. The rare presence of a methyl at C-24 reveals the possibility to extend the metabolism to other sterols in C28, like ergostane derivatives (and not campestane) in order to respect the absolute configuration at C-24. While hydroxylation is very common at C-25, some other oxidations have been observed at the C-1, C-4 positions, but also at C-11, C-23, C-24 or C-27. The formation of the γ-lactone ring for the recently discovered and original ecdysonelactones (**145-148**) could stem from an addition of the nucleophilic enolate of the acyl at *O*-C-3 on a ketone present at C-2.[108] Overall, the biosynthetic pathways leading to ecdysteroids in zoantharians still remain to be elucidated and their analogy with insect ecdysteroid pathways to be fully assessed.

3.2 2-Aminoimidazole alkaloids

The first bis(2-aminoimidazole) alkaloids isolated from a zoantharian were members of the fully aromatic zoanthoxanthin family (Fig. 5). Here also, the first identification of zoanthoxanthin (**102**) was performed in Italy by Prota's group from the Mediterranean zoantharian *Parazoanthus axinellae* and reported in 1973.[109,110] Some analogues were later described with a varied substitution pattern of the methyl on the 6 nitrogens. From another Mediterranean species *Epizoanthus arenaceus*, the same research group identified the second family of fully aromatic bisguanidine alkaloids called pseudozoanthoxantin (**109**).[123] While the three cycles of zoanthoxanthins are arranged linearly those of pseudozoanthoxanthins are placed in an

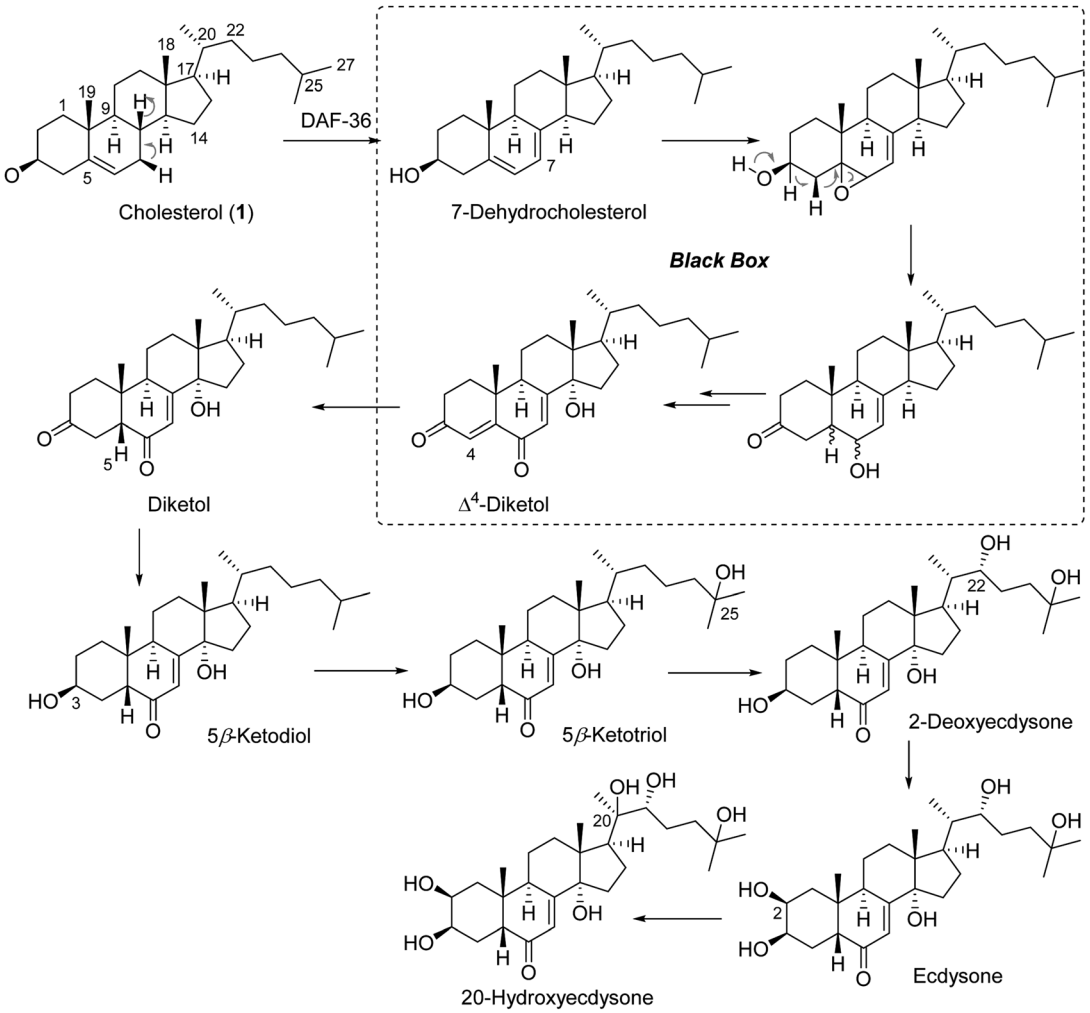


Fig. 8 Metabolic pathway of ecdysteroids in the fly *Drosophila melanogaster*.

angular arrangement. Even if a third isomeric form would be defined where the methyl at C-6 is located at C-4 on the other side of the seven membered-ring, they would represent tautomers of the pseudozoanthoxantins.[124]

The first non-fully aromatic congeners of this family were the zoamides **159–162** reported in 1997 from a *Parazoanthus* collected in the Philippines.[122] These compounds maintain both terminal 2-aminoimidazoles, but two contiguous carbons of the seven membered-ring are saturated and the terminal primary amines are both acylated. Later in 2017, terrazoanthines were described from *Terrazoanthus patagonichus*, in which, for the first time, the seven membered-ring was replaced by a cyclohexene.[126] Finally, a unique tris(2-aminoimidazole) alkaloid named KB343 (**163**) was recently reported from the zoantharian *Epizoanthus illoricatus* from Palau.[125] As commonly observed for marine invertebrates, no experimental data are available on the biosynthesis of these alkaloids and therefore the hypotheses are based on some structural analyses of the different derivatives but also through some synthetic work showing the feasibility of the proposed transformations.

These polyguanidine alkaloids seem to be intertwined around a unified metabolic pathway featuring arginine as the provider of a unit in C_5N_3. Cariello *et al.* were the first to propose 2 molecules of arginine as precursors of zoanthoxanthins.[112] The hypothesis was later supported by total syntheses starting from this precursor by Büchi's[135,136] and Horne's[137,138] groups. As the first steps, a succession of decarboxylation, oxidation and condensation processes transform arginine into the key and highly reactive 4-vinyl-2-aminoimidazole. Similar transformations were already proposed for pyrrole 2-aminoimidazoles present in some sponges starting from lysine (Fig. 9).[139] A [6 + 4] cycloaddition between two molecules of 4-vinyl-2-aminoimidazole could either lead to the linear zoanthoxantins or the angular pseudozoanthoxanthins after aromatization of the three rings. Alternatively, a rare [4 + 2] cycloaddition between the same units would afford the terrazoanthine skeleton found in *Terrazoanthus*. Interestingly, zoamides and terrazoanthines are the only non-fully aromatic alkaloids in this family, and, at the same time, they are the only compounds that are acylated instead of being methylated. It seems that an extended aromaticity would favour methylation

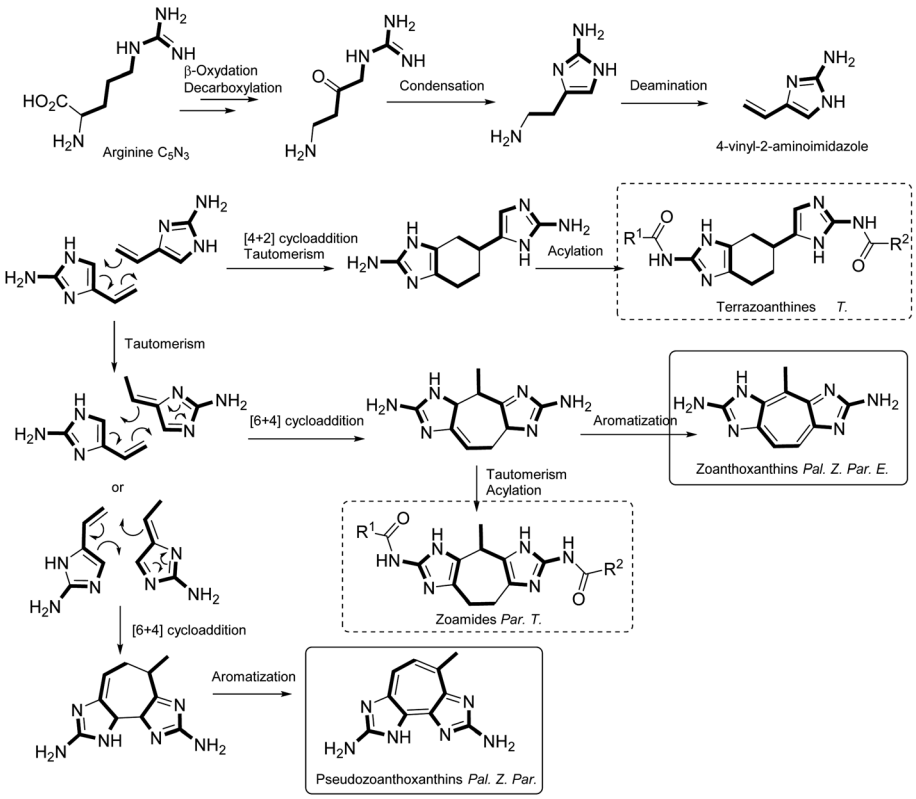


Fig. 9 Biosynthetic hypotheses for 2-aminoimidazole alkaloids isolated from zoantharians.

when a decrease in the aromaticity would render the primary amines more reactive, therefore enabling acylation processes, especially in *Terrazoanthus* species.

3.3 Zoanthamines

Aside from the ecdysteroids and 2-aminoimidazole alkaloids largely distributed among zoantharians of different genera, some metabolites are more restricted to a genus. The first example is the family of non-aromatic alkaloids called zoanthamines, which are mostly found in species of the genus *Zoanthus*. These alkaloids have attracted much attention due to their uniqueness in some species of *Zoanthus*, but also due to their unprecedented architecture embedded with interesting biological activities. Since the review on zoanthamines published in 2008, other analogues have been isolated that have helped to shed some light on the metabolic pathways possibly involved in their biosynthesis.[62] Synthetic strategies were reviewed in 2012, but, overall, they are not inspired by the biosynthesis.[140]

When zoanthamine (**64**), the first representative of this family, was isolated in 1984 from a *Zoanthus* collected in India, Rao, Faulkner, Clardy and co-workers proposed a triterpenoid origin for this alkaloid due to the presence of 30 carbons, even though "the carbon skeleton is far from a normal polyisoprenoid system".[64] In 1995, the only hypothesis was still a terpene origin and the presence of oxyzoanthamine (**70**) suggested that norzoanthamines would be produced by decarboxylation after oxidation of the methyl C-26.[66] Uemura was the first to propose an alternative to the triterpenoid origin of zoanthamines when he suggested in 1998 a polyketide origin with a starting unit containing a primary amine like glycine, similar to the one present in other marine toxins, like palytoxin congeners (Fig. 2).[68,141]

In 2014, the structures of zoaramine (**80**) and zoarenone (**81**) from a *Zoanthus* collected in the Canary Islands by Daranas' group were in agreement with the polyketide origin, even though *in vivo* experiments are still missing to confirm this assumption (Fig. 10). Isoprenoid-like alkylations at the β positions of polyketide chains are now well established and they could explain the presence of the three methyls at C-25, C-28 and C-30 (the black dots in Fig. 10).[142] Once the polyketide alkyl chain is built with one terminal carboxylic acid (or thioester) and one primary amine on the other end, cyclization can occur, including a Diels–Alder reaction, followed by an electrocyclization providing the three fused six-membered ring. The following steps would then involve a condensation of the primary amine to an electrophilic carbon C-6, leading to a highly reactive imine. The electrophilic imine could then react with the alcohol at C-2, and the subsequent nucleophilicity of the nitrogen could finally explain the attack on the carbonyle at C-10, leading to a highly reactive iminium. A subsequent attack of the terminal carboxylic acid at C-10 would finally lead to the bisaminal core of the zoanthamines according to pathway A. Following pathway A, the nucleophilic enamine could cyclize on the carboxylic acid to yield zoaramine and zoarenone. Interestingly, the chemical diversity of the zoanthamine mainly arises from oxidations at the methyl

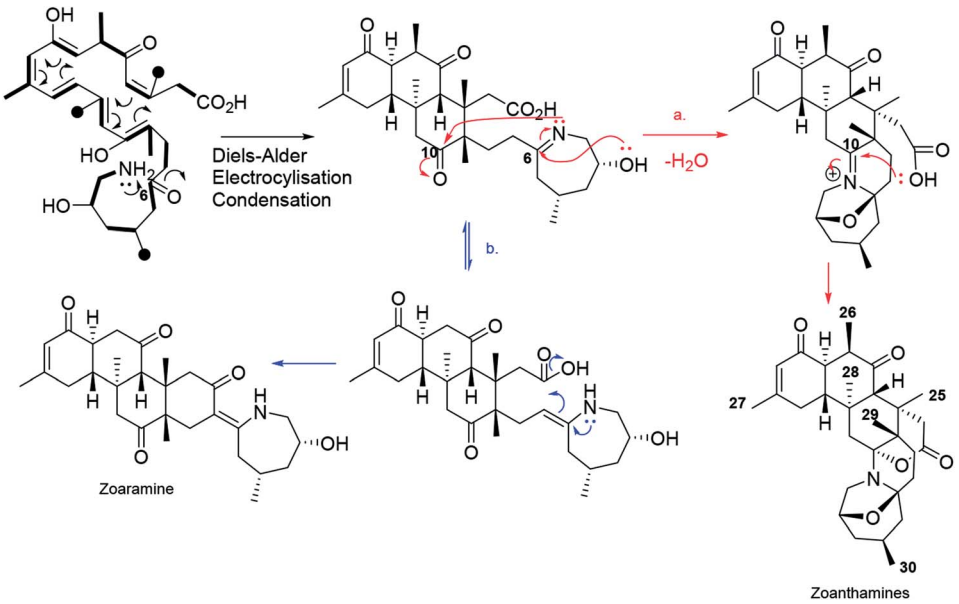


Fig. 10 Biosynthetic hypothesis for zoanthamines.

positions of the polycyclic system. Oxidation at methyl C-26 would afford a primary alcohol that could lead to some kuroshines after lactonization but also to norzoanthamines after decarboxylation at this position. Oxidation at C-25 would afford the spiro γ-lactone at C-22 of kuroshines and zoanthenamines, while oxidation at C-28 would lead to additional cyclizations at the highly reactive C-11. Bisaminals are highly unstable entities and can produce iminium ions in equilibrium with their corresponding enamine, especially at C-11. This phenomenon is also demonstrated by deuterium exchange at this position. Additional oxidations have been observed at C-1, C-3, C-7, C-18 and also on the methyls C-27 and C-30, but not at C-29 so far. Even if the only *in vivo* feeding experiment using radiolabelled precursors did not lead to any conclusive results, the polyketide origin is almost certain, but experimental proof is still awaited.[143]

3.4 Palytoxins

Palytoxins belong to a class of complex linear polyether toxins. Palytoxin analogues were also isolated in 1995 from a benthic dinoflagellate of the genus *Ostreopsis* that might be the actual producer of the compound, or other associated bacteria or cyanobacteria.[144] No experimental data are available for the biosynthesis of these compounds so far, but they are urgently needed to gain some insights into the understanding of the metabolic pathways involved. Due to structural similarities with other linear polyether toxins, like okadaic acids or dinophysistoxins, we can assume a polyketide origin and the involvement of acetate/propionate units as elongation units together with nitrogenated starting units, like glycine.[145,146] The complexity of the structure together with the presence of possible Favorskii rearrangements prevent any definitive conclusion regarding the biosynthesis of this family of compounds and further research is necessary to provide some reliable evidence. Interestingly, a transcriptomic study was performed on *Ostreopsis ovata*, resulting in the identification of a new type of monofunctional non-ribosomal peptide synthase that could be involved in the biosynthesis of ovatoxins, which are very close analogues of palytoxin.[147] This outstanding result should pave the way for the elucidation of their metabolic pathway.

4 Systematics and ecological roles

4.1 Chemotaxonomic markers

Drug discovery but also chemotaxonomy probably represent the oldest applications of natural products. To help in the taxonomy of living organisms, several research efforts were conducted on the metabolites isolated from the organisms, with the main aim to identify some potential synapomorphic chemical markers at different taxonomic levels.[148,149] This approach has been applied with limited success to some marine invertebrates[133,150,151] and plants.[152,153] The main limitations were associated with the lack of a comprehensive description of metabolites in the species as natural products will only be published if they have new structures or some interesting bioactivities. The recent development of metabolomics allows a broader assessment of the metabolic content of marine invertebrates. Such holistic methods are now being used, not only to include metabolites as an additional information for the systematics, but also to give an overview of the metabolomic content of a single species in the search for possible new compounds. In this part, we discuss the main families of specialized metabolites that could possibly represent chemical markers at different taxonomic levels: the whole order Zoantharia, and the suborders Brachycnemina or Macrocnemina, with a final emphasis on some specific metabolites that could serve at the genus level. Metabolites will be considered as

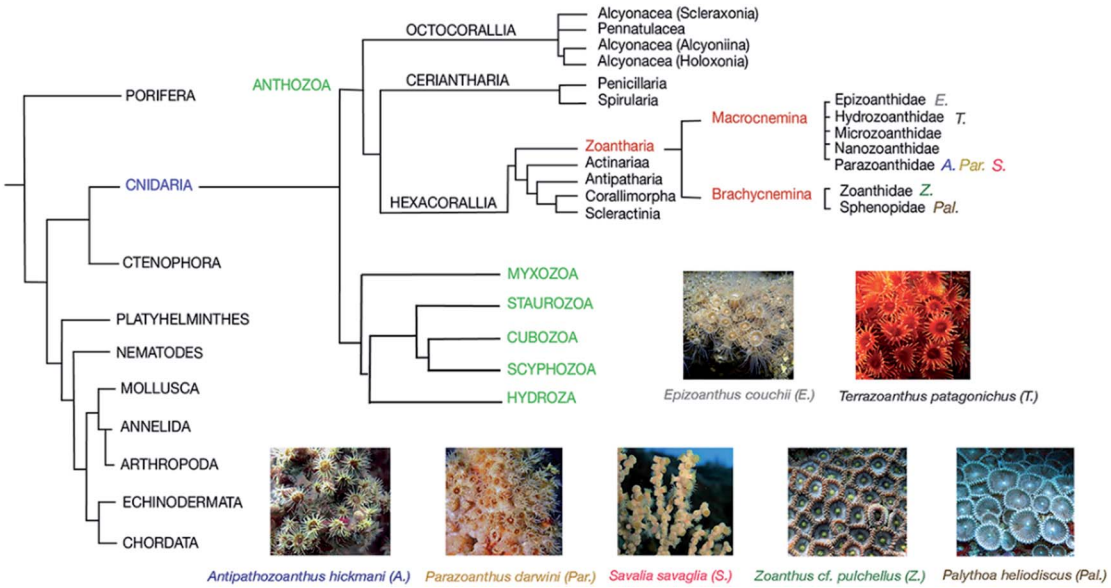


Fig. 11 Classification of the zoantharian genera studied for their chemical diversity: *Epizoanthus* (*E.*), *Terrazoanthus* (T.), *Antipathozoanthus* (*A.*), *Parazoanthus* (*Par.*), *Savalia* (*S.*), *Zoanthus* (*Z.*) and *Palythoa* (*Pal.*).

chemotaxonomic markers if and only if (i) they are present in all species of the group and (ii) they are absent in species outside the defined group. In the literature, compounds have been reported from the seven following genera: *Palythoa*, *Zoanthus*, *Savalia*, *Parazoanthus*, *Epizoanthus*, *Terrazoanthus* and *Antipathozoanthus* (Fig. 11). Some key families of natural products are quite redundant, and others restricted to some groups, allowing for some chemotaxonomic considerations.

4.1.1 Order Zoantharia

4.1.1.1 Ecdysteroids. Ecdysteroids are well-known moulting hormones from arthropods. However, reports of their presence in marine invertebrates are more scarce.

Remarkably, members of this family have been described in 6 out of the 7 chemically studied zoantharians: *Palythoa*, *Zoanthus*, *Parazoanthus*, *Savalia*, *Antipathozoanthus* and *Terrazoanthus* (Fig. 3).[59,108,133] We suspect that ecdysteroids could also be present in species of *Epizoanthus*, even if, so far, only three chemical studies have been performed on species of this genus and no ecdysteroid has been reported. Unexpectedly, unusual ecdysteroids, acylated on the oxygen at the C-22 position, were identified from the Caribbean sponge *Iotrochota birotulata*.[154] However, this sponge is known to have small specimens of the zoantharian *Parazoanthus swiftii*, and the ecdysteroids might actually come from the zoantharian epibiont. More common ecdysteroids were found in the Caribbean sponge *Agelas dispar*,[155] but, here also, the sponge has been reported to be covered with the zoantharian *Parazoanthus puertoricense*.[156] Finally, another ecdysteroid was isolated from the Eastern Atlantic sponge *Ptilocaulis spiculifer* that was also described to live in close association with a *Palythoa* sp.[157] Remarkably, some unusual ecdysteroids with an opposite configuration at C-14 were isolated from an Antarctic tunicate and this is, so far, the only report from a tunicate.[158] These metabolites may be specific to this particular species. All other steroids produced by sessile invertebrates do not contain the characteristic 6-keto, $\Delta^{7,8}$, 14-hydroxy adjacent system and therefore cannot be considered as ecdysteroids. In conclusion, we feel it reasonable to propose ecdysteroids as chemical markers of the entire order Zoantharia. Finding this family of compounds during the chemical study of other marine invertebrates should encourage researchers to closely look at the studied specimen in order to identify possible epibiont zoantharians.

4.1.1.2 2-Aminoimidazole alkaloids. Bis(2-aminoimidazole) alkaloids named zoanthoxanthins and pseudozoanthoxanthins were first described from the following genera of the suborder Macrocnemina, *Epizoanthus*, *Savalia* and *Parazoanthus* (Fig. 5). Then, some analogues were found in *Palythoa* and *Zoanthus* species, demonstrating the wide distribution of these pigments in zoantharians. Recent studies on *Epizoanthus* and *Terrazoanthus* species evidenced the presence of non-fully aromatic analogues, likely produced through a similar biosynthetic pathway, confirming that this pathway is present in most zoantharians species (Fig. 9).[125,126] However, the only chemical study performed on a species of *Antipathozoanthus* did not evidence the presence of compounds from this metabolic pathway, but the annotation of the metabolites was maybe not exhaustive.[133] On the other hand, zoanthoxanthin derivatives have also been isolated from non-zoantharian invertebrates, like the octocoral *Echinogorgia pseudosassapo*[159] or the black coral *Antipathes dichotoma*.[160]

Species of Macrocnemina are commonly found overgrowing octocorals, like *S. savaglia* on the Mediterranean octocoral *Paramuricea clavata*, or black corals of the genus *Antipathes*, like *A. hickmani* in the Eastern Pacific, so the origin of these compounds could be questioned in these two cases. In conclusion, with very few exceptions, we could consider bis(2-aminoimidazole) derivatives as chemotaxonomic markers of the whole order Zoantharia.

4.1.2 Suborder Brachycnemina. Species of this suborder are generally found in shallow waters (mainly tropical waters) and, as such, they are characterized by the presence of associated zooxanthellae that can contribute to the production of their chemical diversity. Two families of zoantharians have been the focus of chemical investigations: the family Sphenopidae (Hertwing, 1882), with the most studied genus *Palythoa* (Lamourox, 1816), and the family Zoanthidae (Rafinesque, 1815), with the genus *Zoanthus* (Lamarck, 1801). Different families of metabolites have been proposed as biomarkers for this suborder: zoanthamines alkaloids for the genus *Zoanthus*, palytoxins for the genus *Palythoa* and some unique sterols for the entire suborder.

4.1.2.1 Sterols. Historically, chemotaxonomic analyses were first performed on the sterol composition of animals or plants. Some variability was early detected in the sterol composition of species from the genus *Palythoa*, whereby some of them were found to contain a mixture of C_{28} sterols called palysterol and others the main sterol chalinasterol (**7**). This difference suggested a division of the genus *Palythoa* in two subgenera (Fig. 1).[6,8] However, the study of Miralles *et al.* in 1988 expressed some reservations on this division, as they found a *Palythoa* species with a distinct sterol profile, and cholesterol (**1**) as the main component.[10] They proposed that the sterol composition would in fact reflect the association with zooxanthellae or other microorganisms and therefore should not be used as a chemotaxonomic marker for this genus. Due to strong similarities between the sterols found in *Zoanthus sociatus* in Jamaica with those known to be produced by zooxanthellae, Kelecom proposed that the host animal would only biotransform the sterols provided by the associated microalgae.[13]

4.1.2.2 Zoanthamines. These metabolites are mostly found in zoantharian of the genus *Zoanthus*.[62] The genus *Zoanthus* is well known as one of the most challenging groups to identify specimens at the species level when using only traditional taxonomic characters, so the inclusion of chemical markers would be highly acknowledged to help in their identification.[131,133] Even though most of the species producing the current 39 reported zoanthamines have been described only at the genus level,[66,74,79,161] some species have been well described.[81] During a metabolomic study of Ecuadorian zoantharians, the presence of zoanthamines was evidenced in *Z. cf. pulchellus* but not in the closely related *Z. cf. sociatus*, therefore precluding the use of zoanthamines as a chemical markers of the whole genus *Zoanthus*. Nevertheless, some zoanthamines might be specific to some *Zoanthus* species, such as *Z. kuroshio*, which is the only known producer of kuroshines.[80,81] The presence of zoanthamine derivatives detected by MS analyses in Brazilian species of *Palythoa* could either be due to the presence of *Zoanthus* in the same environment or some common zooxanthellae.[96] Lobozoanthamine is the only example of a zoanthamine derivative isolated from a non-zoantharian invertebrate, and it was described in good quantity from the Indonesian soft coral *Lobophytum* sp.[162] The authors ruled out the possibility of zoantharians present as epibionts of this soft coral due to the high amount of metabolites isolated. At the same time, they proposed that the actual producer of the zoanthamine derivative might be an associated zooxanthella. This hypothesis could also explain why zoanthamines would be restricted to some species of *Zoanthus* containing the producer zooxanthella. Another proof of the microalgal origin of zoanthamines was demonstrated when a zoanthamine derivative was produced in a culture of *Symbiodinium* microalgal species in 1998.[163] Even though zoanthamines are produced by the associated zooxanthella, this family of compounds could be of some taxonomic relevance as they reveal the presence of a unique type of zooxanthellae in the zoantharian species. Zoanthamine producers could therefore form a distinct group of *Zoanthus*.

4.1.2.3 Palytoxins. A total of 15 species belonging to the genus *Palythoa* have been studied chemically, with 12 of them well described at the species level,[16,17,31,35,49,59] 2 remaining as unconfirmed species (*cf.* or aff.)[36,38] and only 1 at the genus level.[57] Between the several families of natural products discovered in zoantharian species of the genus *Palythoa*, one of the most important families is certainly the palytoxins, due to their extreme structural complexity and high level of toxicity (Fig. 2). First, this toxin has only been found in species of Brachycnemina collected in Hawaii or more generally in the Pacific.[6] While the presence of palytoxin was assumed to be exclusive to the genus *Palythoa*, it was later reported in other zoantharians, such as *Zoanthus* spp., space competitors of *Palythoa* in the coral reef, and also in other marine invertebrates from the same habitat, like crustaceans, sea anemones or species that feed or find shelter on *Palythoa*.[6,40,41] Therefore a contamination cannot be excluded. In 1982, Moore *et al.* proposed a bacterial origin for the toxic compound, as an associated bacterial strain was able to produce palytoxin, but the origin is still a matter of debate.[164] Here also, zooxanthellae could be the first producers of the toxins. Indeed, palytoxin and analogues have been identified in diverse dinoflagellates, most of them from the genus *Ostreopsis*.[165] Remarkably, and in a similar way as for zoanthamines, the biosynthesis of palytoxins is most probably of polyketide origin with a glycine as the starter unit. For both palytoxins and zoanthamines, the zooxanthella origin is highly supported, and therefore the use of these compounds as chemotaxonomic markers seems at the very least questionable. However, in both cases and to some extent, these compounds might be considered as proxys of unique zooxanthellae that might be of some taxonomic relevance. As discussed before, recent metabolomics approaches allow a broader cover of the metabolic content of specimens. This approach has been applied to study the chemical variability of two *Palythoa* species along the Atlantic coast of Brazil.[96] This study resulted in a high geographical variability when compared to species variability, ruling out some possible chemical markers for species of *Palythoa*. The main drawback of metabolomic studies using mass spectrometry (MS) is the ionization potential of the metabolites, which can largely vary between metabolites, rendering MS non-universal. In the case of *Palythoa* species, the main families of metabolites are zoanthoxanthins and palytoxins. Zoanthoxanthins are easily detected with little expected variation, while palytoxins are usually

found in traces and might not be detected in a single small specimen. Other lipids and sterols are quite difficult to ionize and therefore can be overlooked in the metabolomic analysis. Overall, *Palythoa* might therefore not represent ideal candidates for metabolomic studies as easily ionizable minor compounds produced by associated microorganisms could lead to contradictory conclusions.

4.1.3 Suborder Macrocnemina. Species of this suborder are usually found in subtidal waters and in some cases overgrowing black coral or octocorals. They are devoid of any associated zooxanthellae, but some other symbionts can be involved in the biosynthesis of the metabolites. Up to now, three families of Macrocnemina have been chemically investigated with a total of 12 well-described species, but many others are still to be investigated in this suborder. In the family Epizoanthidae (Delage & Hérouard, 1901), only the genus *Epizoanthus* (Gray, 1867) with 2 described species, namely *Epizoanthus arenaceus*,[123] and *Epizoanthus illoritcatus*,[125] has been studied, while in the family Hydrozoantidae (Sinniger, Reimer & Pawlowski, 2010), only the species *Terrazoanthus patagonichus* has been investigated recently.[126] Finally, Parazoanthidae is the family with the highest number of studied genera (Delage & Herouard, 1901): *Parazoanthus* (Haddon & Shackleton, 1891) (3 spp.), *Antipathozoanthus* (Sinniger, Reimer & Pawlowski, 2010) (1 sp.) and *Savalia* (Nardo, 1844) (1 sp.).

4.1.3.1 2-Aminoimidazole alkaloids. An interesting observation can first be made from the structures of zoanthoxanthins isolated from Macrocnemina species. While fully aromatized metabolites were mainly isolated from these species, zoamides and terrazoanthines were isolated as non-fully aromatic metabolites only from a *Parazoanthus* and a *Terrazoanthus* species (Fig. 5).[122,126,127] A subsequent acylation seems favoured for these compounds. If we consider that the identification of the *Parazoanthus* producing zoamides A–D is doubtful, and it was in fact a *Terrazoanthus*, we could propose these acylated non-aromatic bis(2-aminoimidazole) derivatives as chemical taxonomic markers of the genus *Terrazoanthus*.

4.1.3.2 Halogenated tyrosine derivatives. Very few tyrosine derivatives have been isolated from zoantharians and, in all cases, they are halogenated at the ortho position of the phenol. These metabolites have only been reported in species from the family Parazoanthidae. From the genus *Parazoanthus*, a new type of alkaloids named parazoanthines were isolated from the Mediterranean *Parazoanthus axinellae*, but they were absent in another morphotype of this species (Fig. 7).[102,128] Then, two simple brominated and iodinated tyramine derivatives were isolated from *Parazoanthus darwini* collected off the mainland coast of Ecuador.[127] Finally, a chemical study on another species of the same family and collected in the same area, *Antipathozoanthus hickmani*, led to the isolation of four new tyrosine dipeptides named valdiviamides A–D (Fig. 7).[127] Due to the absence of such metabolites in some species of this group, like *Savalia*, it is difficult to propose halogenated tyrosine metabolites as chemical markers at the family or genus level and additional studies are needed to confirm this assumption.[102,129,130]

4.2 Ecological roles

The number of specialized metabolites reported from the order Zoantharia is lower than the number of metabolites found in sponges, ascidians or octocorals and only a few studies have assessed their ecological roles.[6,15,166] The production of specialized metabolites by marine invertebrates has since early on been regarded as a chemical defence mechanism where slow-growing species persist when they face organisms competing for space in a dynamic and marine ecosystem, such as the coral reefs.[167–173] Up to now, only a few studies in chemical ecology have focused on species of the order Zoantharia. Overall, the ecological significance of zoantharians metabolites remains therefore largely overlooked and underestimated.

4.2.1 Ecdysteroids. The first ecdysteroid isolated from a zoantharian was described in 1982 from *S. savaglia*.[103] Pietra and co-workers ruled out the possibility that this ecdysteroid could behave as a hormone due to the high concentration of the metabolite in the organism. Like for plants, the authors postulated a defensive role for ecdysteroids in zoantharians. This family of natural products is largely present in most of the zoantharians studied and we could indeed consider a defensive role for these molecules even if no experimental data have evidenced this role so far.[133]

4.2.2 2-Aminoimidazole alkaloids. Zoanthoxanthin derivatives have since early on been recognized as pigments, largely distributed among species of zoantharians. They could contribute to the bright colours usually characteristic of species of zoantharians, but they could also represent defensive compounds, as explained in a very comprehensive review published on the topic.[174] However, in the case of zoantharians, no experimental proof has been reported to confirm this hypothesis.

4.2.3 Suborder Brachycnemina

4.2.3.1 Palytoxin. The variability in the production of zoantharian metabolites was first studied in species of *Palythoa*, and the easy assessment of the toxicity of their extracts was considered as a proxy for the presence of palytoxin. The toxicity of *P. tuberculosa* has been found to vary with the season and, importantly, it was linked to the reproductive cycle of the organism.[166] Indeed, the toxicity appeared to be associated with the maturation of female polyps and located in the eggs, whereas the male and sterile polyps were all non-toxic. Later, research was conducted in the Caribbean Sea, where the relationship between the content in palytoxin and the fertility/egg production of *Palythoa* polyps was examined.[41] In this case, no clear correlation between the reproductive cycle of *Palythoa* and the content in palytoxin was evidenced. The eggs of certain polyps did not contain palytoxin, and the sterile polyps presented a considerable amount of palytoxin. The variability in the content of palytoxin might also explain the low toxicity of the specimen of *P. toxica* collected in September at the Halona Blowhole, Oahu, since all previous specimens had shown toxicities comparable to those of *P. toxica* from Muolea, Maui.[6] The variability in the content of palytoxin was also investigated for two other species of *Palythoa*, namely *P. caribaeorum* and *P. mammillosa*, in Jamaica.[16] Because these species live in

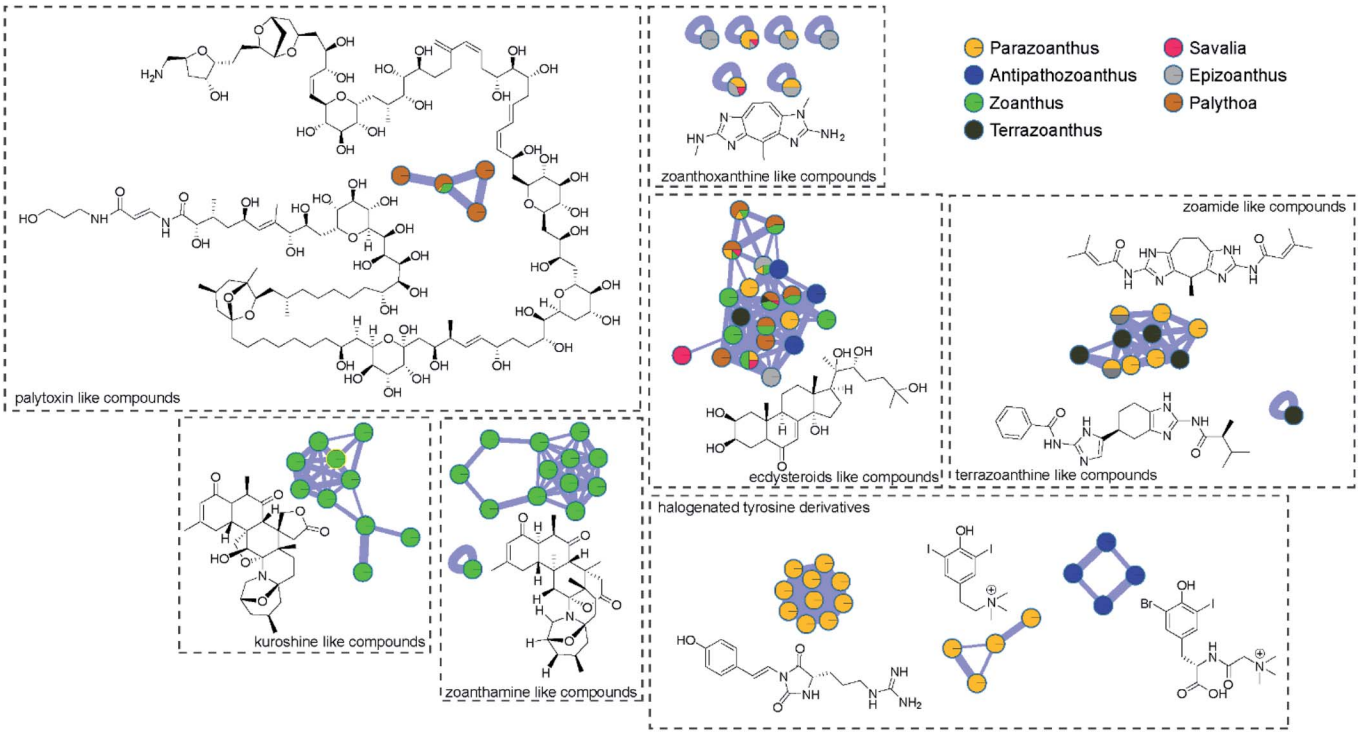


Fig. 12 Molecular network (MN) showing the compounds distribution according to the zoantharian genera. The MN was realized using CF-MID *in silico* fragmentation spectra of the metabolites described in this review and the GNPS platform. All molecules have been uploaded in https://pubchem.ncbi.nlm.nih.gov/.

symbiosis with zooxanthellae, the authors assumed that the toxicity levels vary with the intensity of solar radiation. The ecological role of palytoxin has never been clearly demonstrated and several hypotheses are still debated. As the real producer of this toxin seems to be a dinoflagellate, most studies on the ecotoxicology of these compounds were performed on the metabolites produced by species of the *Ostreopsis* genus and not *Palythoa*. However, as highlighted in a comprehensive review by Kubanek and co-workers, the true role of phycoplanktonic toxin remains a subject of controversy and a defensive role has not been demonstrated unambiguously.[175]

4.2.3.2 Zoanthamine. An interesting work was reported on the distribution and possible function of norzoanthamine in a *Zoanthus* sp.[72] The authors first noticed that the structure of zoanthamines does not resemble any known toxin and therefore suggested a non-defensive role of this family of compounds. Then, through MALDI-MS analyses, they observed that norzoanthamine was distributed in the epidermal tissue of the polyps. The high concentration of this metabolite measured in the surface of the polyps confirmed the non-toxic effect. Indeed, toxins are normally only found at very low concentration in organisms. Finally, they provided some experimental data proving that norzoanthamine could act as a protective molecule for collagen, enhancing its resistance towards UV light. This ecological role could be related to the anti-osteoporosis activity evidenced for this family of natural products.

4.2.4 Suborder Macrocnemina

4.2.4.1 Parazoanthus. From the suborder Macrocnemina, we could identify only few experimental data on the chemical ecology, essentially on the Mediterranean *Parazoanthus axinellae*.[102] In this study, only one morphotype of this species was shown to produce a unique class of halogenated tyrosine derivatives called parazoanthines. The variability in the production of different types of metabolites, including ecdysteroids, zoanthoxanthins and parazoanthines, was studied over a one-year period and the concentrations of all the families of metabolites were found to be higher in the winter season from December to February. Also, using the Microtox® assay as a proxy for the ecotoxicological activity, parazoanthines were found to express the highest level of antimicrobial activity, suggesting an antibacterial defensive role for these metabolites.

5 Conclusion

In this first review on the chemical diversity of marine zoantharians, we highlighted the untapped reservoir of molecules represented by species of this group. Some unique classes of metabolites have already been uncovered, mostly in species of the suborder Brachycnemina. The structural complexity of the zoanthamines and palytoxins represent true opportunities for the development of new drugs in the pharmaceutical sector. Species of *Palythoa* and *Zoanthus*, also described as coral mats, are usually present in shallow/intertidal waters of tropical areas. This location would justify the presence of symbiotic zooxanthellae, which require solar exposition and nutrients for their

development. Associated zooxanthellae are likely to be involved in the biosynthesis of both families of metabolites. These bioactive molecules may contribute to the ecological success of species of both genera in large areas of the tropical Indian, Atlantic and Pacific Oceans. Because their substrate cover seems to expand in some areas, they could benefit from global warming and might represent health concerns soon due to the presence of toxic metabolites. They seem extremely resistant to environmental changes and are easily maintained in aquaria. Their bright colours and ease of culture make them perfect species for ornamental tropical aquaria. Unfortunately, some studies have already evidenced the presence of palytoxins in the aquaria.[176]

The diversity in terms of species is clearly higher in the suborder Macrocnemina, and the genus *Parazoanthus* has been the focus of most chemical studies to date. Some families of this suborder have been, however, entirely overlooked and efforts should be made to chemically study these groups. The main metabolites ecdysteroids, zoanthoxanthins and halogenated tyrosine alkaloids found in species of this group have not demonstrated a real interest for medical applications yet, but studies are still in their infancy. Unlike species of Brachycnemina, some species of the suborder Macrocnemina have adapted their morphology to inhabit all types of marine ecosystems, from shallow to deep sea waters, driving the species to evolve a unique diversity of specialized metabolites as part of their adaptive processes.[177] Some of the species of this suborder are commonly found as epibionts of octocorals or black corals, and as such, they play important ecological roles shaping marine ecosystems.

The systematics of zoantharian has proven to be extremely challenging and, if they are used with parsimony, some families of metabolites could help in the classification. Some metabolites have been proven to be produced by associated microorganisms and the host might just modify their architecture. Ecdysteroids and 2-aminoimidazole alkaloids seem to be present in a vast majority of zoantharian species. Conversely, other families of metabolites seem restricted to particular groups of zoantharian, as illustrated in the molecular network built through the GNPS platform (Fig. 12).[178]

The biosynthesis and ecological roles of zoantharians metabolites are still largely understudied. Because zoantharians are becoming dominant in certain ecosystems, we highly recommend some collaborative work between marine natural product chemists and biologists to strengthen the field of marine chemical ecology to better understand the functions of these species in ecosystems.

6 Conflicts of interest

There are no conflicts to declare.

7 Acknowledgements

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