Review

Bioactive compounds from zoanthids (Cnidaria: Anthozoa): A brief review with emphasis on alkaloids

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The marine diversity has been the source of unique chemical compounds with the potential for industrial development as cosmetics, nutritional supplements, molecular probes, fine chemicals and agrochemicals, besides the production of pharmaceuticals. Their secondary metabolites derive from two main biosynthetic pathways (the route of pyruvic and acetic acids and shikimic acid pathway) and belong to large groups such as alkaloids, phenolic compounds and terpenoids. Zoanthidea consists mostly of sessile colonial anthozoans with individuals united by the basal portion. They have no skeleton, but the body wall is usually encrusted with sediment. Zoanthids produce two main types of alkaloids: a unique class of heteroaromatic amines known collectively as zoanthoxanthins responsible for their bright pigmentation, and the zoanthamine type with an original skeleton identified mainly from the genus Zoanthus. Recently, was reported the isolation and structural characterization of a new class of alkaloids named parazoanthines. These alkaloids were isolated as the major constituents of the Mediterranean Sea anemone Parazoanthus axinellae.

Keywords: Alkaloids; secondary metabolites; zoanthids.

INTRODUCTION

Alkaloids are nitrogen-containing compounds that occur naturally not only in plants but also in microorganisms, marine organisms, and animals. Although it is not clear why alkaloids show significant biological activity, they are often useful as drugs or biological probes for physiological studies. As new and more complicated diseases are encountered worldwide, the importance of bioactive alkaloids has increased due to their potential application in chemotherapy. As the application of alkaloids has expanded, the definition of alkaloids has become less restricted (Kuramoto et al., 2004).

Plants produce most of the bioactive alkaloids, including the anticancer action. One of the most important is the Catharanthus roseus (L.) G. Don, also known as Vinca, which is used by the population in Madagascar in treating diabetes. During testing of hypoglycemic activity, extracts of this species produced granulocytopenia as a result of suppression of bone marrow of animals, suggesting evaluation in models of leukemias and lymphomas. Confirmation of activity in these models led to the isolation of the alkaloids vinblastine and vincristine, which currently are of great utility in the treatment of Hodgkin's lymphoma, Kaposi's sarcoma, ovarian and testicular cancers and childhood acute lymphoblastic leukemia (Brandão et al., 2010).

Marine environments are among the richest in biodiversity and most complex ecosystems. Marine invertebrates are rich sources of bioactive compounds and their biotechnological potential attracts scientific and economic interest worldwide. Although nearly 20,000 compounds have been discovered since the field of marine bioactive compound biochemistry began in the mid-1960s, only a very limited number have seen industrial application. It has been clear since marine bioprospecting began that the world’s oceans and their diverse biota represent a significant resource, perhaps the greatest resource on Earth, for the discovery of new bioactive compounds (Rocha et al., 2011).

In the dearth of information about the ethnopharmacology of species of marine invertebrates, it is worth considering comments on its ecology and evolutionary history to make inferences about the presence of chemical defense mechanisms. Soft-bodied, lacking in obvious physical defense structures and often colorful that would be easy prey are strong candidates to holders of chemical defense (Faulkner, 2000).
Cnidarians, sponges, molluscs, sea squirts and algae make up the group of chemically most prolific marine representatives (Wilke, 2009). Among the cnidarians, the class anthozoans hitherto been the most studied, observing a reasonable number of biologically active substances, particularly in soft corals and gorgonians (Teixeira, 2009).

Cnidaria is a diverse group of marine invertebrates includes over 11,000 species, 7500 of them belonging to the class Anthozoa (Rocha et al., 2011). The order Zoanthidea consists mostly of sessile colonial anthozoans with individuals united by the basal portion. They have no skeleton, but the body wall is usually encrusted with sediment and, usually, harbor zooxanthellae symbionts (Longo, 2002).

Antitumor activity has been the major area of interest in the screening of cnidarian compounds, the most promising ones being terpenoids (monoterpenoids, diterpenoids, sesquiterpenoids) (Rocha et al., 2011). Apart from anticancer activity, these compounds have proven to be an abundant source of pharmacologically active agents for the production of therapeutic entities (Glaser and Mayer, 2009) against AIDS, inflammatory conditions and microbial diseases.

Besides the production of pharmaceuticals, this marine diversity has been the source of unique chemical compounds with the potential for industrial development as cosmetics, nutritional supplements, molecular probes, fine chemicals and agrochemicals (Kijjoa and Sawangwong, 2004).

This review attempted to bring together several recent publications that focus on some of the most promising marine bioactive alkaloids isolated from zoanthids. Printed materials were used, as well as studies obtained from scientific databases on the internet, such as: (1) Scielo (www.scielo.br), (2) BIREME/BVS - Biblioteca Virtual em Saúde [Virtual Health Library] (www.bireme.br), and (3) PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi).

**Main groups of secondary metabolites**

The products of secondary metabolism are substances belonging to a single type of organism, or a small group of genetically related organisms (Mann, 1987). According to Teixeira (2009), these products mediate ecological interactions between organisms and environment and between various organisms of the same species or between different species. Such substances, which allowed the success of the current representatives, have been studied by chemists of natural products and have also been a source of food, fragrances, pigments, insecticides and medicines. Natural products or secondary metabolites derive from two main biosynthetic pathways (the route of pyruvic and acetic acids and shikimic acid pathway) and belong to large groups such as alkaloids, phenolic compounds and terpenoids (Figure 1).

The term alkaloid was first proposed by Meissner in 1818 referring to their alkaline properties. In general, the nitrogen atom present in these molecules is from amino acids and the heterocyclic ring formed provides the basis for classification (Bruneton, 1999). The alkaloids are derived from aromatic amino acids (tryptophan, tyrosine), which are derivatives of shikimic acid, as well as aliphatic amino acids (ornithine, lysine).

Phenolic compounds are a large and diverse group of molecules, which includes many different families of aromatic secondary metabolites in plants. These phenolics are the most abundant secondary metabolites in plants and can be classified into non-soluble compounds such as condensed tannins, lignins, cell-wall bound hydroxycinnamic acids, and soluble...
compounds such as phenolic acids, phenylpropanoids, flavonoids and quinones. All these groups are involved in many processes in plants and animals. One family, the flavonoids, is of particular interest because of its multiple roles in plants and its impact on human health (Harborne and Williams, 2000).

Terpenoids (also called “isoprenoids”) constitute one of the largest families of natural products accounting for more than 40,000 individual compounds of both primary and secondary metabolisms. Several terpenoids have been shown to be available for pharmaceutical applications, for example, artemisinin and taxol as malaria and cancer medicines, respectively (Goto et al., 2010).

Isoprene or Isopentyl diphasphate (IPP) and its isomer dimethylallyl diphasphate (DMAPP) are the universal five-carbon precursors of all terpenoids. After the discovery of the mevalonate (MVA) pathway in yeast and animals, it was assumed that IPP was synthesized from acetyl-CoA via MVA and then isomerized to DMAPP in all eukaryotes and some Gram-positive prokaryotes (Withers and Keasling, 2007).

Monoterpenoids (C₁₀H₁₆), made up of two isoprene units, are the smallest and simplest type of terpenoids; sesquiterpenoids (C₁₅H₂₄) are made up three units; diterpenoids (C₂₀H₃₂), triterpenoids (C₃₀H₄₈) and tetraterpenoids (C₄₀H₆₄) are made up four, six and eight units, respectively.

Ahmed et al. (2005) isolated three new oxygenated sesquiterpenoids, (gibberodione, peroxygibberol, and sinugibberodiol), along with sarcophytol L from a Formosan soft coral, Sinularia gibberosa. The structures of the new metabolites were determined on the basis of extensive spectroscopic analyses and by comparison of NMR data with those of related metabolites. Peroxygibberol and sarcophytol L were found to exhibit moderate cytotoxicity toward a human liver carcinoma cell line.

Other secondary metabolites from cnidarians

Palytoxin

Palytoxin (PLTX), found in Palythoa zoanthids and Ostreopsis dinoflagellates, has also been detected in crabs and fish, through which it can enter into the food chain. Indeed, PLTX is considered the causative agent of several cases of human seafood poisoning resulting in systemic symptoms. Available epidemiological data on PLTX human toxicity suggest that the intestinal tract may be one of its in vivo targets and its potential site of access into the bloodstream (Pelin et al., 2012). It is a complex molecule polyether initially isolated from the soft coral Palythoa toxica (Moore and Scheuer 1971).

Research on palytoxin action generally falls into two major areas. One broad class of studies focuses on how palytoxin affects ion flux, which is the immediate effect of this compound on the cell. Another broad class of studies, discussed later, focuses on subsequent cellular effects stimulated by palytoxin that may be related to tumor promotion. Palytoxin stimulates sodium influx and potassium efflux, and thus depolarization of the membrane, in a wide range of systems (Habermann, 1989). In excitable systems, palytoxin-stimulated depolarization can modulate calcium channel activity, resulting in a rise in intracellular calcium, which can then stimulate events that are regulated by calcium-dependent pathways.

Palytoxin is a novel skin tumor promoter, which has been used to help probe the role of different types of signaling mechanisms in carcinogenesis. The multistage mouse skin model indicates that tumor promotion is an early, prolonged, and reversible phase of carcinogenesis. Understanding the molecular mechanisms underlying tumor promotion is therefore important for developing strategies to prevent and treat cancer (Wattenberg, 2007).

Prostaglandins

A class of lipids with much pharmacological potential is composed by prostaglandins. These compounds are derivatives of prostanoid acid. The first structures of prostaglandins were elucidated in 1962 and so named because it was believed they were synthesized in prostate. All natural prostaglandins have a hydroxyl group at C-15, the trans double bond between C-13 and C-14 and an oxygenated function at C-9 (Vieira et al., 2002). Han et al. (2006) isolated two prostaglandins, PGA₂ and PGB₂, from the Okinawan zoanthid Palythoa kochii, during a search for paclitaxel-like neurite-degenerating compounds from natural sources using a cell-based assay method. In the presence of PGA₂ at 30 µM, the neuronal processes induced in rat pheochromocytoma cell line (PC12) by the nerve growth factor (NGF) degenerated over 24 h, whereas PGB₂ had no effect on the neuronal processes of PC12 cells. This activity of PGA₂ was similar to that of the microtubule-stabilizing agents, paclitaxel (Taxol) and epothilone A, unlike the microtubule-depolymerizing agent, colchicine, which brought about quick neurite degeneration within 3 h. PGA₂ stimulated tubulin polymerization although less potently than paclitaxel.

Lipidic α-amino Acids

Lipidic α-amino acids (LAAs) have been described as non-natural amino acids with long saturated or unsaturated aliphatic chains. In the continuing prospect to discover anticancer agents from marine sources, Wilke et al. (2010) have obtained a mixture of two cytotoxic LAAs
from the zoanthid Protopolythoa variabilis. The anti-
proliferative potential of 14 synthetic LAAs and of the two
natural LLAs was evaluated on four tumor cell lines
(HCT-8, SF-295, MDA-MB-435, and HL-60). Five of the
synthetic LAAs showed high percentage of tumor cell
inhibition, while the two natural LLAs completely inhibited
tumor cell growth. Additionally, apoptotic effects of these
LLAs were studied on HL-60 cell line. Treated cells
showed apoptosis morphology, loss of mitochondrial
potential, and DNA fragmentation.

Bioactive alkaloids from zoanthids

Alkaloids are only rarely found in marine coelenterates
and they also appear to be restricted to animals within
the order Zoanthidea. These organisms produce two
types of alkaloids: a unique class of heteroaromatic
amines known collectively as zoanthoxanthins
responsible for their bright pigmentation, and the
zoanthamine type with an original skeleton identified
mainly from the genus Zoanthus (Daranas et al., 2000).

Zoanthoxanthins

Zoanthoxanthin alkaloids are yellow fluorescent
substances emanate solely from colonial anthozoans in
both major families (Epizoanthidae and Zoanthidae) of
the order Zoanthidea. Their molecules can vary among
three skeletal types: 3H-zoanthoxanthin, 3H-
pseudozoanthoxanthin and 4H-pseudozoanthoxanthin
(Jiménez and Creurs, 1993).

Turk et al. (1995) obtained an ethanolic extract from a
zoanthid crust coral Parazoanthus axinellae, which was
lethal to mice and crabs and exhibited anticholinesterase
activity. The isolation of several acetylcholinesterase
(AChE) inhibitors was made with the aid of RP-HPLC.
The most abundant of the inhibitors present in the P.
axinellae extract was identified as pseudozoanthoxanthin
or an almost identical compound which belongs to the
chemically well-characterized series of
tetrazacyclopentazulene natural pigments from the
genera Parazoanthus, Epizoanthus, Zoanthus and
Polythoa. The inhibitor has a molecular weight of 242
and acts as a competitive inhibitor with a Ki of 4 µM. The
inhibitor exhibited a strong blue fluorescence. In vivo
action of crude extract and the isolated inhibitor showed a
typical picture of systemic AChE inhibition. Atropinization
of experimental animals prior to injection of the inhibitor
almost entirely neutralized its activity.

In the Turk et al. (1995) study, the most abundant of
the inhibitors characterized as a methylated
pseudozoanthoxanthin variant, and some other pigments
not well characterized, expressed a potent
anticholinesterase activity in vitro but was inactive with
trypsin or alkaline phosphate. Sepčić et al. (1998) have
taken advantage of the chemical synthesis of the
representative of zoanthoxanthins, parazoanthoxanthin
A, to provide evidence for anticholinesterase activity of
the linear form of these natural pigments.

Parazoanthoxanthin A (ParaA) is a strongly fluorescent
pigment occurring in zoanthids, which has been found to
inhibit electric eel (Electrophorus electricus)
acetylcholinesterase in the micromolar range.

Nicotinic acetylcholine receptors are implicated in
different nervous system-related disorders, and their
modulation could improve existing therapy of these
diseases. Since Parazoanthoxanthin A (ParaA) is a
potent acetylcholinesterase inhibitor, it may also bind to
nicotinic acetylcholine receptors (nAChRs). For this
reason its effect on Torpedo nAChR (α12βγδ)
transplanted to Xenopus laevis oocytes was evaluated by
Rozman et al. (2010), using the voltage-clamp technique.
Para A dose-dependently reduced the acetylcholine-
induced currents. This effect was fully reversible only at
lower concentrations. ParaA also reduced the Hill
coefficient and the time to peak current, indicating a
channel blocking mode of action. On the other hand, the
combined effect of ParaA and d-tubocurarine (d-TC)
on acetylcholine-induced currents exhibited only partial
additivity, assuming a competitive mode of action of
ParaA on nAChR. These results indicate a dual mode of
action of ParaA on the Torpedo AChR.

Zoanthamines

Zoanthamine alkaloids, the first of which were isolated
over 20 years ago, are of particular interest to the
synthetic community because they feature a novel
structural framework and exhibit a broad range of
biological activities. Zoanthamine (1) was isolated by
Faulkner et al. in 1984 from an unidentified colonial
zoanthid Zoanthus sp. collected at the Visakhapatnam
coast of India and this compound was identified as the
first member of a new class of alkaloids (Behenna et al.,
2008).

In 1995, Uemura and co-workers identified five new
zoanthamine natural products isolated from a Zoanthus
species collected off the Ayamaru coast of the Amami
Islands south of Japan: norzoanthamine, norzoanthaminone, oxyzooanthamine, cyclozoanthamine
and epinorzoanthamine. The authors reported that these
zoanthamine natural products display significant
cytotoxicity against P388 murine leukemia cells (Table 1).
The most potent cytotoxicity was displayed by
norzoanthaminone and oxyzooanthamine (Fukuzawa et
al., 1995).

Norzoanthamine has also been reported as a
promising candidate for an osteoporotic drug as an IL-6
inhibitor. IL-6 is known to stimulate osteoclast formation,
and the suppression of IL-6 secretion can be effective in
the prevention of osteoporosis. Norzoanthamine and
norzoanthamine hydrochloride inhibit IL-6 induction at values of 13 and 4.7 μg/mL, respectively (Kuramoto et al., 1997). Furthermore, norzoanthamine and norzoanthamine hydrochloride, both of which counteract decreases in bone weight and strength in ovariectomized mice, could be good candidates for osteoporotic drugs (Kuramoto et al., 2004).

Villar et al. (2003) evaluated ten zoanthamine-type alkaloids from two marine zoanthids belonging to the Zoanthus genus (Zoanthus nymphaeus and Zoanthus sp.) along with one semisynthetic derivative for their antiplatelet activities on human platelet aggregation induced by several stimulating agents. 11-Hydroxyzoanthamine and a synthetic derivative of norzoanthamine showed strong inhibition against thrombin-, collagen- and arachidonic acid-induced aggregation, zoanthenol displayed a selective inhibitory activity induced by collagen, while zoanthaminone behaved as a potent aggregant agent. These evaluations allowed to deduce several structure–activity relationships and suggested some mechanisms of action for this type of compounds.

Recently, Cachet et al. (2009) reported the isolation and structural characterization of a new class of alkaloids named parazoanthines A-E. These alkaloids were isolated as the major constituents of the Mediterranean sea anemone Parazoanthus axinellae. Their structural elucidation was achieved through NMR spectroscopic and mass spectrometric analyses. This family of alkaloids represents the first example of natural 3,5-disubstituted hydantoins which do not exhibit a methyl at N-3. All compounds were tested for their antitumor (MDA-MB-231, HT-29, and A-549) and antimalarial (FcB1) activities and none of them exhibited significant bioactivity.

During a screening of marine organisms for their natural toxicity, P. axinellae was found to exhibit the most bioactive extract among the cnidarians (Marti et al., 2005). Cachet et al. (2009) then decided to evaluate all the isolated compounds in this Microtox® assay. Parazoanthine C showed the highest natural toxicity (EC₅₀ = 1.64 μM) and may consequently be responsible for the results obtained with the extract.

REFERENCES


