

Sofia Ortlepp (Autor) Bastadins and Related Compounds from the Marine Sponges lanthella basta and Callyspongia sp:

Structure Elucidation and Biological Activities



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1 Introduction

1.1 Pharmacognosy

Information about man using plants as remedies was found to go back in time to the Sumarians and Akkadians third millennium BC (Samuelsson 1999) and fossil records indicate medicinal usage of plants already in the Mid Paleolithic age approximately 60,000 years ago (Solecki & Shanidar 1975). Natural products were for a long time the only drugs available and among the drugs used today approximately 20% are unmodified natural products, 10% are semi-synthetic derivatives of natural products and over 30% are synthetic drugs based on natural pharmacophores (Jones *et al.* 2006). Renowned examples of natural products are different forms of antibiotics, antitumour agents like paclitaxel and the vinca alkaloids, as well as analgetics ranging from morphine to salicylic acid (Samuelsson 1999).

Secondary metabolites are compounds not directly participating in the metabolic processes crucial for the survival of the organism (primary metabolism), but are believed to have been formed to fulfil ecological tasks like defence, reproduction and interspecies competition. These properties of secondary metabolites make them interesting candidates for new drug leads. And the drug resemblance of secondary metabolites is probably due to their production in living systems which have evolved for millions of years (Firn & Jones 2003, Wink 2003).

The big screening programs of natural products have encountered problems and have not been as successful as one had hoped for. This has been ascribed to the synergistic effects between secondary metabolites in an organism or a biological activity which gets lost when the metabolites are isolated in their pure form. It has also been pointed out that the chemical diversity is much greater than the functional diversity (Tulp & Bohlin 2002).

1.2 The sea as a source of natural products.

Compared to traditional medicine and pharmacognosy, marine natural products chemistry is still a very young area of research. Even though already the Romans used sponges in their treatment of illnesses the development of new drugs from marine sources is relative poor. The use of sponges in medicine though goes back far in time. Plinius (79-23 BC) wrote that sponges should be saturated with iodine and used for stimulation of blood coagulation. Sponges were also used for heartaches by putting them on the chest soaked in wine or, soaked in urine to treat bites of poisonous animals. (Pliny 1938)

In the early 1950s the first natural products were isolated from a marine sponge. The sponge was *Cryptotethia crypta* and the substances were the nucleosides spongothymidine and spongouridine (Bergmann & Feeney 1950,

1951). These nucleosides later became lead structures for the anti-viral drug ara-A and the anti-leukemia drug Ara-C respectively (Muller *et al.* 1977). The same structures also underlie the important HIV antivirus drug azidothymidine (AZT) (Newman *et al.* 2000).

Ten years after the isolation of the sponge nucleotides, prostaglandins were discovered in the gorgonian *Plexaura homomalla* (Weinheimer & Spraggin 1969). Since then the interest in the marine natural products chemistry has remarkably increased.

However today, almost forty years later, only one marine natural product has reached the pharmaceutical market, Prialt[®], an analgesic nerve toxin derived from the marine snail *Conus magus*.

The main reason for the low output from the marine environment probably is the supply problem. Bioactive components in marine organisms give extremely low yields often less than 10^{-6} % based on the wet weight (Fusetani 2000). Aquaculture of marine invertebrates has not been successful to date and marine derived compounds are often structurally complex, which in many cases makes synthesis impossible. The hope now is to focus on associated micro-organisms, which in many cases seem to be the original source of marine derived compounds. They can be cultivated in large scale to provide the pharmaceutical industry with sufficient amounts required for the therapeutic field of application. The wish is also that genetic engineering would help up the supply issue.

Although the medicinal output of the marine research today is very small there are several interesting marine derived compounds present in clinical trials or used as molecular tools. A number of the marine derived products of interest are described below.

1.2.1 Brominated marine natural products

Among the thousands of natural products isolated from sponges so far (Blunt *et al.* 2006) halogenated compounds are frequently encountered. These products found in nature have often been explained to be anthropogenic. In recent years though it has become clear that halogenated compounds are widespread in nature and in several cases the natural organohalogens are more abundant than their synthetic counterparts (Gribble 1999). Most of the organohalogens found in nature originated from marine sources, but have also been found in terrestrial plants, fungi, lichen, bacteria, insects, in some higher animals and, in humans (Gribble 1996, 1999, Gribble 2000, Winterton 2000, Gribble 2003b). The organochlorides are the most predominant natural organohalogens. In the year 2002 they were estimated to count up to 2,200, the organobromines to 1,900, organoiodines to 100, and the organoflourines to only 30. (Gribble 2003a)

Bacteria and microalgae associated with sponges are generally known to biosynthesize several of the metabolites found in sponges. This is also the case with several organohalogenes. Halide salts of the four major halogens are abundant on earth, in oceans, in sedimentary rocks and volcanoes (Gribble 1996). Three major types of halogenating enzymes have been discovered to date, the halogenases, bromoperoxidases and perhydrolases. The first step in the biosynthesis of organohalogens is the enzymatic oxidation by peroxidises of chloride, bromine, and iodide ions. Neither the haloperoxidases nor the perhydrolases are regioselectively when halogenating and van Pée and Unversucht state that it is very unlikely that these enzymes are involved in the biosynthesis of halogenated metabolites (van Pee & Unversucht 2003). The halogenases are flavin-dependent and show both a substrate specificity and a regioselectivity. FADH₂ is required for the halogenating activity and due to their selectively the halogenases cannot substitute each other. All halogenases discovered have been cloned from different bacteria and interestingly they do all contain a tryptophan residue. (van Pee et al. 2006). Bromoperoxidases have been found in several species of marine algae (Murphy & Heocha 1973b, 1973c, 1973a, Manley & Chapman 1978, 1979) and in one sponge, Iotrochota birotlata (Baden & Corbett 1979). The first bromoperoxidase was isolated from the red alga Cystoclonium purpureum and was found to be a heme protein (Pedersen 1976). Further haloperoxidases have been isolated and it has turned out that they are either heme- or vanadium-dependent and generate HOX (X=Cl, Br, I, of F) from H₂O₂ (Hasan *et al.* 2006). Another group of halogenating enzymes are the perhydrolases. They require hydrogen peroxide for the halogenation but are no peroxidases. They belong to the α/δ hydrolase family and the serine residue at its active site forms an intermediate by reaction with a short-chained carboxylic acid. A peracid is formed, which can oxidize halide ions that in turn act as halogenating agents (van Pee 1996).

All these findings make it clear that halogenated compounds are actually generated by nature. The function of these indicates defensive roles (Walker *et al.* 1985) and have also shown to be involved in larval settlement in the life cycle of marine invertebrates (Pawlik 1992).

1.3 Marine natural products as drugs

As already mentioned above, after 40 years of research, only one marine derived product has arrived at the pharmaceutical market. This compound is a peptide named **ziconotide**. The compound, which consists of twenty five amino acids with three interlocking cystinyl bridges, was first isolated from the marine snail *Conus magus* (McIntosh *et al.* 1982). *C. magus* contains a series of different conotoxins which include the α -, ϖ -, μ -, and δ -forms. The ϖ -form of these toxins has become the first drug originating from a marine source. The substance is marketed under the name Prialt[®] with the indication "chronical pain in patients who fail to obtain adequate analgesia and/or suffer intolerable adverse effects with systemic opioids". Zinconotide blocks N-type calcium channels, which regulate the release of neurotransmitters in specific neuronal populations responsible for the spinal processing of pain. Through this binding, ziconotide inhibits the voltage sensitive calcium current into the primary nociceptive afferents terminating in the dorsal horn of the spinal cord. This

inhibits neutrotransmittors (including substance P) and through that the signalling of pain through the spine. An almost complete analgetic effect is achieved within a couple of hours and zinconotide has until now not shown any development of pharmacological tolerance. This makes zinconotide an excellent alternative to opioid analgetics (European-Medicines-Agency 2006). Another marine toxine, the **tetrodotoxin**, is also a promising drug candidate for the analgesic market. TetrodinTM is in Phase II for treatment of morphine withdrawal pain. Its synthetical derivative, TectinTM is in Phase IIb/III for chronic pain in patients with advanced cancer (Wex-Pharmaceuticals-Inc.).

In fact most of the marine research has been concentrated on the area of cancer (Blunt *et al.* 2006). One promising anti tumor drug candidate is the depsipeptide **kahalalide F** (Figure 1) from the Sacoglossan mollusc *Elysia ornata* and *E. rufenscens* both grazing on the alge *Bryopsis* sp, also containing kahalalide F (Hamann & Scheuer 1993, Hamann *et al.* 1996, Horgen *et al.* 2000). Kahalalide F has shown potent anti-tumour activity in several solid tumour models including colon, breast, non-small cell lung, and prostate carcinoma as well as activity against opportunistic infections in HIV/AIDS patients (Hamann & Scheuer 1993). The mechanism of action is still unclear, nevertheless the depsipeptide is currently in clinical trials phase II against androgen-independent prostate cancer (Brown *et al.* 2002, Ciruelos *et al.* 2002, Rademaker-Lakhai *et al.* 2005)

Another antitumor agent, ecteinascidin 743 (ET-743) named Yondelis TM (Figure 1), initially isolated from the ascidian *Ecteinascidina turbinata* in 1969 was not reported together with its anti-tumor activity until the early 1990s (Rinehart et al. 1990). The mode of action of ET-743 is still under investigation whereas the compound is in clinical trials phase II for advanced soft tissue sarcomas (Le Cesne et al. 2005) and resistant ovarian carcinomas (Sessa et al. 2005). The mode of action has been proposed in numerous reports where several point in the direction of interactions of ET-743 with the DNA adduct (Bonfanti et al. 1999, Takebayashi et al. 2001, Zewail-Foote et al. 2001, van Kesteren et al. 2003, Dziegielewska et al. 2004). ET-743 can be semisynthesized based on the fermentation product cyanosafracin B (Cuevas et al. 2000). This is decisive for continued preclinical and clinical trials as the yield from the ascidian is extremely low (Newman & Cragg 2004). One of the reasons why ET-743 has not come further in its development process is because of the reported hepatoxicity in toxicology studies. However, it seems that the human hepatoxicity is easier to control compared to the toxicity shown in rats (Beumer et al. 2005).

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Figure 1. Exaple of marine natural products in clinical trials.

A third compound currently in clinical trials is the macrocyclic lactone **bryostatin 1** (Figure 1) isolated from the bryozoan *Bugula neritina* (Pettit *et al.* 1982). Bryostatin 1 exhibits anti-tumor effect through binding and modulating protein kinase C isoenzymes (Isakov *et al.* 1993, Galron *et al.* 1994, Zhang *et al.* 1996, Yoo *et al.* 2001) without, or to a very little extent, promoting tumor formation (Jalava *et al.* 1993, Stanwell *et al.* 1994). The effect of bryostatin 1 as anticancer chemotherapeutics has been tested in clinical trials phase I and II, alone or in combination with other anticancer agents, showing promising effects. The main adverse event observed is myalgia. (Dowlati *et al.* 2003, Haas *et al.* 2003, Madhusudan *et al.* 2003, Haas *et al.* 2006).

From the Okinawan sponge Agelas mauritianus potent antitumor and immunostimulatory glycosphingolipids, namned agelaspins, have been isolated (Natori *et al.* 1993, Natori *et al.* 1994). It has been proven that the α galactosylceramide structure is decisive for the antitumour activity and a synthetic analogue to the agelaspins, **KRN7000** (Figure 1), has been developed for treatment of various types of metastasis in combination with radiotherapy (Natori *et al.* 2000). Clinical trials phase I have given promising results in humans both as antitumour agent and immunostimulator in patients with solid tumours (Crul *et al.* 2002, Ishikawa *et al.* 2005, Motohashi *et al.* 2006).

The above mentioned compounds are only a few examples of substances that could have a future as medicinal agents. Others are summarized in several reviews (Newman & Cragg 2004, Simmons *et al.* 2005, Sipkema *et al.* 2005, Blunt *et al.* 2006, Newman & Cragg 2006).

1.4 Chemistry of marine sponges of the order Verongida

Sponges (phylum Porifera) are primitive metazoans and were probably the starting point for the metazoan explosion in the Precambrian. They share many of their features with unicellular protozoa (e.g. nutrition, gas exchange, reproduction, response to external stimuli), but they also have some similarities with higher eukaryotes (e.g. genes and proteins) (Costantino *et al.* 2004). Sponges are soft bodied and sessile animals, which lack spine and shell (Armstrong & Quigley 1999). For their defence against fouling organisms, predators, and neighbours competing for space, sponges rely on bioactive secondary metabolites instead (Proksch *et al.* 2002).

Ianthella basta Oken, 1815, which has been investigated in this project, belongs to the order Verongida Bergquist, 1978. The order is divided into three families: Aplysinidae (Aplvsina and Verongula). Aplysinelliidae (Pseudoceratina, Psammaplysilla and Aplysinella), and Ianthellidae (Ianthella, Anomoianthella and Bajalus). A common pigmentation of the Verongida sponges is sulphur yellow/green. When the sponge dies or gets hurt the colour turns dark blue/purple through oxidation. Species of the order Verongida are biochemically characterised by the production of brominated tyrosine derivatives and aplystane sterols are characteristic products of this order. Terpenes, except for steroids, have never been found present in the Verongida sponges. (Bergquist & Cook 2002)

1.4.1 Brominated tyrosine derivatives

Brominated tyrosine derivatives of a great range were found in Verongida sponges including the relatively simple nitrile, aerophysinin-1 (Fattorus *et al.* 1972), via the isoxazolines aerothionin (Fattorus *et al.* 1972) and isofistularin (Cimino *et al.* 1983), to the more complicated macrocyclic bastadins (Kazlauskas *et al.* 1980) (Figure 2).



Figure 2. Examples of bromotyrosines isolated from Verongida sponges.

Brominated tyrosine derived compounds have proven to possess a great variety of biological activities.

The bastadins, described below (section 1.5), do not only inhibit barnacle larval settlement, thrombocyte aggregation, bacterial growth, protein kinases and provide cytotoxicity, as described in this thesis, but do also show for example antagonistic effects on the ryanodin receptor and calcium channels in the endoplasmatic reticulum. (Mack *et al.* 1994, Franklin *et al.* 1996)

Other bromotyrosine derivatives have previously shown antifouling effects as well (discussed below in section 1.6). The brominated isoxazoline derivative isofistularin-3 is known as a fish deterrent (Thoms *et al.* 2004), purpurealidin B is antibacterial, and aerothionin has shown antimycobacterial and antibiotic activities (Rotem *et al.* 1983, Encarnacion-Dimayuga *et al.* 2003).

Aeroplysinin-1 as well as dienon have been found to be antibacterial and cytotoxic to tumour cells (Fattorus *et al.* 1972, Teeyapant *et al.* 1993, Koulman *et al.* 1996). Aeroplysinin-1 also inhibits the tyrosine kinase activity of epidermal growth factor (EGF), blocks EGF-stimulated proliferation, and induces apoptosis of cancer cell lines (Kreuter *et al.* 1990, Hinterding *et al.* 1998, Rodriguez-Nieto *et al.* 2001). Additional to these activities aeroplysinin-1 has also shown to inhibit Na/K ATPase (Gorshkov *et al.* 1984) and has recently proved to exhibit activity against chloroquine resistant *Plasmodium falciparum* and the intracellular form of *Trypanosoma cruzi* (Gutierrez *et al.* 2005).

Psammaplin A possesses cytotoxic effects in several ways (Arabshahi & Schmitz 1987, Kim *et al.* 1999, Jiang *et al.* 2004, Shim *et al.* 2004, Mora *et al.* 2006). Among other effects it was found to be active as a histone deacetylase inhibitor (HDAC). A myelom cancer drug, NVP-LAQ824, has been developed

based on psammaplin A together with trapoxin and trichostatin as building blocks. NVP-LAQ824 was in clinical tarials phase I by Dana Farber Cancer Institute against hematologic malignancies in 2004 with positive results (Remiszewski 2003). NVP-LAQ824 is now suggested for clinical evaluations for treatment of biliary tract cancer (Wiedmann *et al.* 2006).



Figure 3. Examples of bioactive brominated tyrosine derived secondary metabolites.

1.4.2 Biosynthesis of brominated tyrosine metabolites

Due to the slow growth rate of sponges and their microbe symbiosis biosynthetical studies are difficult to accomplish (Dexter et al. 1993). A few studies on Aplysina fistularis have been conducted for the purpose of investigating the biosynthesis of brominated tyrosine derivatives in the sponges of the order Verongida (Tymiak & Rinehart 1981, Carney & Rinehart 1995). The studies have shown that the sponge either uses tyrosine or phenylalanine [(I), Scheme 1], as a precursor. The tyrosine undergoes bromination by bromoperoxidases (II) and thereafter two possible pathways follow. Either the amine is oxidized to an oxime (III) or the tyrosine goes through O-methylation followed by oxidation of the amine to an oxime (IV). Pathway III results in a free oxime which further can be used as a building block of for example bastadins. Pathway IV forms oxepins like psammaplysins or isoxazolines like aerothionin. (Scheme 1) (Carney & Rinehart 1995). Since the OH free oxime intermediate and intermediates for oxepins, psammaplysin, isoxazolines, and aeropysinin have never been isolated, these pathways remain to be hypothetical.