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# The Mediterranean Sponge *Dysidea avara* as a 40 Year Inspiration of Marine Natural Product Chemists

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**Abstract**

Marine sponges have been intensively studied by organic chemists, biologists and pharmacologists during last 60 years, due to great biodiversity of their secondary metabolites. A couple of very promising bioactivities of these compounds have been reported. Indeed, some of these metabolites are considered as the leads for the future drugs targeting tumours, viral and bacterial diseases, malaria and inflammations. Herein we briefly report key bioactivities of avarol, the main secondary metabolite of the marine sponge *Dysidea avara* (Schmidt, 1862).

**Keywords:** Marine sponges; *Dysidea avara*; Avarol; Bioactivity; Antimicrobial; Cytotoxic activities; Alzheimer's disease; Antipsoriatic

## The Marine Sponge *Dysidea avara*

The first marine invertebrate group to be studied in any comprehensive way by those searching for new organic compounds was the sponges (sedentary filter-feeding metazoans belonging to the phylum Porifera) [1,2]. Confronted to other organisms, these animals are found to be a rich source of biologically active compounds exhibiting, *inter alia*, cytotoxic, immune-modulatory [3,4] and antifouling effects [5]. Marine sponges are the most ancient metazoan phylum which taxonomy depends on the composition of their skeletal materials [6] and morphological characteristics [7]. Actually, the sponges are divided into three main classes: Calcarea (calcareous spicules), Hexactinellidae (siliceous spicules) and Demospongiae (spicules made of proteinaceous sponging fibres). This phylum encompasses ≈ 6000 taxonomically validated species, with the new ones still being discovered [2,8]. Among the sponges, the class Demospongiae represents the most various and ecologically remarkable group, covering 90% of all species [2]. These sponge species are asymmetric and brightly coloured (being orange, green, yellow, purple or red), due to the presence of pigment granules in their amoebocytes.

The sponges of the genus *Dysidea* belong to the class Demospongiae, order Dictyoceratida and family Dysideidae. Usually lack mineral spicules and have the main skeleton consisting of reticulation of sponging fibers [1]. By the year 2001, this genus was reported to include 17 species [9].

*Dysidea avara* (Schmidt, 1862), a violet-colored, encrusting and heavily conulated dictyoceratid sponge, has been mostly observed at sciaphilic microhabitats of the rocky sublittoral Mediterranean to 80-m depths [10]. This species produces the sesquiterpene hydroquinone avarol (as the main secondary metabolite) and its quinone form avarone [11], as well as some other minor derivatives [12]. Avarol has showed numerous bioactivities such as antitumour, antileukemic and antipsoriasiatic [13-16]. Indeed, high pharmacological potential of avarol has prompted a number of researchers worldwide to address the issue of its obtaining at a large scale, by means of chemical synthesis [17] or the sponge cultivation [18-20].

Different factors have been described to regulate the production of secondary metabolites in marine sessile organisms, primarily in the sponges, both abiotic (temperature, light, salinity and pollutants) and biotic (fouling, space competition, predation, and presence/absence of bacterial symbionts) [21-24]. The spatial variation of these compounds in the sponges has been reported between different localities [25-27] and within the localities in opposing habitats differing in the amount of irradiance [28] or predation pressure [29]. Additionally, the sponge cells that biosynthesise and store these compounds have been identified in a number of cases [30-34]. Interestingly, the concentration of target metabolites is usually higher at the sponge periphery. The favoured storage of bioactives out of the sponge centre zone has been ascribed to a defensive role against the foulers or predators [35-41].

## Avarol and Avarone

Avarol (1) was firstly isolated from the Mediterranean sponge *D. avara* along with a minor amount of its oxidised derivative avarone (2) (Figure 1) [11]. Its chemical structure was firstly established with circular dichroism and spectroscopic studies [42], and later on by chemical synthesis [43]. Avarol and its derivatives are among the most important marine metabolites because of their broad spectrum bioactivity and very low toxicity.

Over 100 articles on avarol and/or avarone recorded in *Chemical Abstracts* show the high level of interest in these compounds. In a mini-review article, it is almost impossible to cover all the bio-effects reported for both compounds so far.

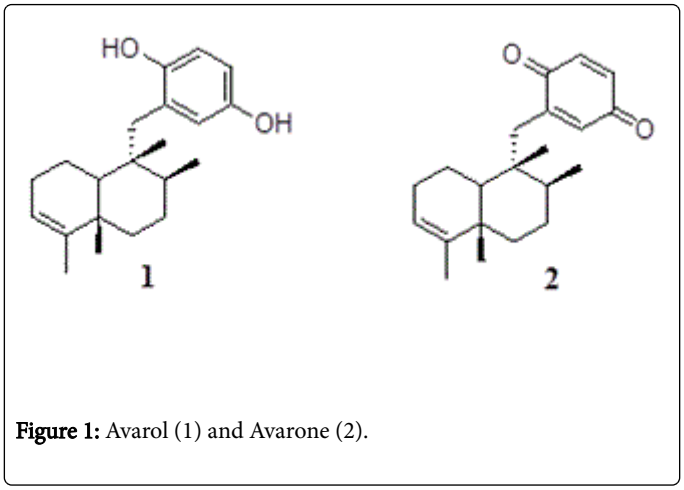


**Figure 1:** Avarol (1) and Avarone (2).

As a consequence, herein the attention will be paid only to more significant ones.

## Antimicrobial activity

Avarone and to a lesser extent avarol are active against a variety of Gram-positive bacterial species. The highest activity was observed for *Streptococcus pneumoniae* and *Erysipelothrix rhusiopathiae* (MIC 0.78 μg/ml) [44]. Recently, it has been reported that avarol exhibits much higher antibacterial activity towards *Bacillus cereus*, *Salmonella typhimurium* and *Enterobacter cloacae* with minimum inhibitory and bactericidal concentrations ranging between 2-8 μg/mL and 4-16 μg/mL, respectively [45]. On the other hand, avarol primarily displayed antifungal activity against *Trichophyton mentagrophytes* (MIC 7.8 μg/ml), *T. rubrum* (MIC 15.6 μg/ml) and *Microsporum canis* (MIC 15.6 μg/ml) [44]. This compound also showed considerable effect on *Aspergillus versicolor* and *Penicillium verrucosum var. cyclopium* exhibiting MIC and MFC values ranging between 4-15 μg/mL and 8-30 μg/mL, respectively.

## Cytostatic activities

In addition, avarol proved to be more active both than ketoconazole and bifonazole [45]. Avarol and avarone also showed potent cytostatic activities on L-5178Y ($ED_{50}$ 0.93 and 0.62 μM, respectively) [13], L-1210 ($ED_{50}$ 13.9 and 15.6 ⊠M, respectively) and C-8166 ($ED_{50}$ 9.2 and 12.9 μM, respectively) cells [46].

Both compounds exhibited good antileukaemic activity in vivo as well, using L-5178Y cells in NMRI mice. At doses of 10 mg/kg applied to the mice bearing ≈ $10^8$ leukaemia cells during 5 days, avarone was more curative than avarol (70 and 20%, respectively) [13]. Moreover, these compounds are effective antimutagenic agents displaying similar activity as the known cytochrome P-450-dependent mono-oxygenase inhibitor, benzoflavone [47]. The cytotoxicity of avarol was *in vitro* screened by MTT assay against four human tumour cell line [breast (MCF-7), colon (HT-29), prostate (PC-3) and skin (HS-294T)], too. The colon HT-29 tumour cells practically were the only sensitive ones towards this compound ($IC_{50}$ <7 μM), while no toxicity was found against the fetal lung fibroblast MRC-5 cells at the concentrations tested. In comparison with doxorubicin, used as a positive control, avarol actually exhibited at least 588-fold less toxicity towards normal MRC-5 cells. Finally, comet assay indicated that DNA fragmentation was almost 5-fold higher upon the treatment with doxorubicin, compared to avarol [48]. The obtained results have actually confirmed that avarol scaffold may contribute to development of new cytostatics inspired by Nature.

## Anti-HIV virus

Both avarol and avarone showed a dose-related inhibitory effect on HIV (HTLV-III) replication in human H-9 cells measured by determination of RT activity and the expression of HTLV-III gag proteins p24 and p17 by monoclonal antibodies [49]. At the administered dose of 3 mg/kg, no side effects of avarol were observed on healthy test subjects [50].

## Anti-inflammatory activity

Afterwards, it was established that these compounds exhibited a topical anti-inflammatory activity, with $ID_{50}$ values of 97 and 397 μg/ear, respectively, higher than indomethacin, both in the Tetradecanoyl-Phorbol Acetate (TPA) induced ear oedema and the oedema induced by carrageenan ($ID_{50}$ 9.2 and 4.6 mg/kg p.o., respectively) [51]. Avarol also moderately inhibited the human recombinant synovial $PLA_2$ ($IC_{50}$ 158 μM) and COX ($IC_{50}$ 1.4 μM), displaying more effective activity against LO ($IC_{50}$ 0.6 μM) [52]. Furthermore, avarol showed high antioxidant activity ($IC_{50}$ 18 μM), evaluated by the free-radical scavenging assay using DPPH [52]; this compound inhibited *in vitro* induced microsomial lipid peroxidation, too [53]. At the first place, avarone exerted antiplatelet activity both on platelet-rich plasma and washed platelets. It was a stronger inhibitor of platelet aggregation induced by arachidonic acid or A23187 vs. adenosine-5'-diphosphate, platelet-activating factor or U46619 [54].

On the other hand, avarol inhibited tumour factor-α (TNF-α) generation in stimulated human monocytes ($IC_{50}$ 1 μM) and TNF-α-induced activation of nuclear factor-κB (NF-κB)-DNA binding in keratinocytes.

In the psoriasis-like model of TPA-induced mouse epidermal hyperplasia, topical administration of avarol (0.6-1.2 μmol/site) reduced oedema, myeloperoxidase activity, IL-1β, IL-2 and eicosanoid levels in skin. Avarol was also found to suppress NF-κB nuclear translocation *in vivo*, determined in mouse skin [55]. Psoriasis is a known skin disease typified by epidermal hyperplasia, inflammation of the dermis and epidermis and leukocyte infiltration [56]. It is firstly a keratinocyte proliferation disorder caused by the pathological development of a hypertrophic physical defence barrier. The classic topical treatments are founded mainly on anti-proliferative treatment or differentiation modifying activity [57]. NF-κB is a crucial factor for the immuno-inflammatory responses which is also implicated in different skin diseases including psoriasis [58]. A crucial link between high levels of TNF-α and NF-κB activation has been found in psoriatic patients. Indeed, the potential mechanism of action of TNF-targeting agents lies in the down-regulation of NF-κB transcriptional activity [56].

The anti-inflammatory treatments based on the blocking of TNF-α signalling have been recently shown to be valid and highly promising alternative for this skin condition.

The bioactivity of both compounds has been mainly correlated with their redox chemistry and ability to affect radical production. In other words, the terpenoid moiety is believed to play just a marginal role in these processes [59-61].

## Natural and Synthetic Derivatives of Avarol and Avarone

The interesting pharmacological properties of avarol and avarone together with previous findings that avarone, the quinone of avarol, reacts towards protein sulfhydryl groups, have prompted the researchers to prepare further sulfhydryl derivatives of these compounds and extend the bioactivity evaluation to other effects [52,62,63].

### Antipsoriatic

Avarol-3'-thiosalicylate (3) (Figure 2) is a potent inhibitor both of superoxide generation in human neutrophils and $PGE_2$ generation in the human keratinocyte HaCaT cell line ($IC_{50}$ 2.5 μM) [64]. This compound also reduced LTB4 ($IC_{50}$ 1.79 μM), prostaglandin E2 ($IC_{50}$ 17.30 nM) and TNF-α ($IC_{50}$ 4.18 μM) production in activated leukocytes, in a concentration-dependent manner. Its oral administration in the mouse air pouch model gave a dose-dependent reduction of all aforementioned inflammatory mediators. The same compound inhibited human synovial recombinant $PLA_2$ ($IC_{50}$ 5.9 μM) as well as the binding of NF-κB to DNA (at 5 μM) in HaCaT keratinocytes. These findings have indicated that avarol-3'-thiosalicylate represents a promising antipsoriatic agent due to both *in vitro* and *in vivo* inhibition of different biomarkers related to the inflammatory response of psoriatic skin [65].

### Anti-acetylcholinesterase

A number of semisynthetic thio-avarol derivatives have been assayed on the inhibition of the enzyme acetylcholinesterase (AChE) [66].

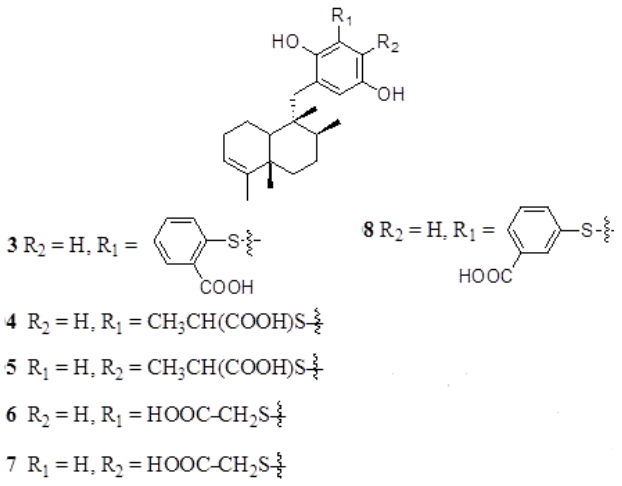


**Figure 2:** Avarol-3'-thiosalicylate (3); Avarol-3'-thiolactate (4); Avarol-4'-thiolactate (5); Avarol-3'-thioglycolate (6); Avarol-4'-thioglycolate (7); Avarol-3'-thiobenzoate (8).

The AChE inhibition test pointed out a moderate inhibitory activity (1μg) for all thio-avarol derivatives with a carboxylic acid group in the molecule, namely avarol-3'-thiosalicylate (3), avarol-3'-thiolactate (4), avarol-4'-thiolactate (5), avarol-3'-thioglycolate (6), avarol-4'-thioglycolate (7) and avarol-3'-thiobenzoate (8) (Figure 2). In comparison, the alkaloid galanthamine used clinically for the treatment of Alzheimer's disease (AD) inhibited the enzyme at 0.01 μg [52].

In AD, the most common cause of senile dementia in later life, a deficiency in cholinergic neurotransmission has been observed. AChE is the enzyme involved in the metabolic hydrolysis of acetylcholine at cholinergic synapses in the central and peripheral nervous systems. Currently, AChE inhibitors are still the best drugs used for the management of this disease [67]. Most inhibitors of AChE are alkaloids that often possess several side effects; therefore it is important to search for new AChE inhibitors not belonging to this chemical class.

Recently, Sladić and co-workers have reported the synthesis and biological activity of further thio-avarone derivatives. After preliminary cytotoxic bioassays, only 4'-isobutylthio-avarone (9), 3',4'-ethylenedithio-avarone (10) and 4'-phenylthio-avarone (11) (Figure 3) were selected by NIH-NCI for *in vitro* screening in a panel of human tumour cell lines (Table 1) [68].

| | **Mean GI50 (μM) of compounds*** | | |
|---|---|---|---|
| Cell lines | 9 | 10 | 11 |
| IGROV-1 | 10.0 | 32.4 | 16.2 |
| RIPMI-8226 | 3.80 | 14.10 | 20.40 |
| T-47D | 2.63 | >100 | 20.40 |
| RXF-393 | 6.76 | 18.60 | 16.20 |
| SK-MEL-2 | 20.90 | 1.70 | 20.00 |
| CCRF-CEM | 1.35 | 11.00 | 3.89 |
| K-562 | 4.47 | 20.00 | 19.50 |
| MOLT-4 | 2.29 | 21.40 | 2.57 |
| HOP-92 | 4.79 | 31.60 | 17.00 |
| NCI-H23 | 5.50 | 13.20 | 14.80 |
| LOX-IMVI | 4.07 | 11.20 | 13.50 |
| MCF-7 | 7.24 | 20.40 | 22.40 |
| MDA-MB-435 | 15.50 | 8.13 | 17.00 |

**Table 1:** Cytotoxicity of Avarol derivatives against a panel of human tumour cells. *(9) 4'-Isobutylthio-avarone, (10) 3',4'-Ethylenedithio-avarone and (11) 4'-Phenylthio-avarone.

### Cytotoxicity

The finding that 3'-methylamino-avarone (12) and 4'-methylamino-avarone (13) (Figure 3) inhibited the cell cleavage of fertilized eggs from the sea-urchin *Sphaerechinus granularis* [69], has prompted a research group to prepare a series of amino derivatives of avarone and estimate their biological activity [45,70]. 4'-Methylamino-avarone (13) and 4'-ethylamino-avarone (15) (Figure 3) were found to be the most toxic among the compounds tested in brine shrimp lethality assay (as an indicator of cytotoxicity), with the activity (LC50 0.23 and 0.34 ppm, respectively) comparable to the avarol one (LC50 0.18 ppm) [70]. The brine shrimp assay is an in-house assay substituting the KB-9, PS-9, L-5178y and L-1210 cytotoxicity [71].

The amino derivatives of avarol were also tested *in vitro* as cytostatic and antiviral agents (Table 2) [46]. Generally, the introduction of a methylamino or an ethylamino group in the 3' position of the quinone ring of avarone has resulted in the chemical structures endowed with higher potency against L-1210 and B- and T-lymphoblast cells.

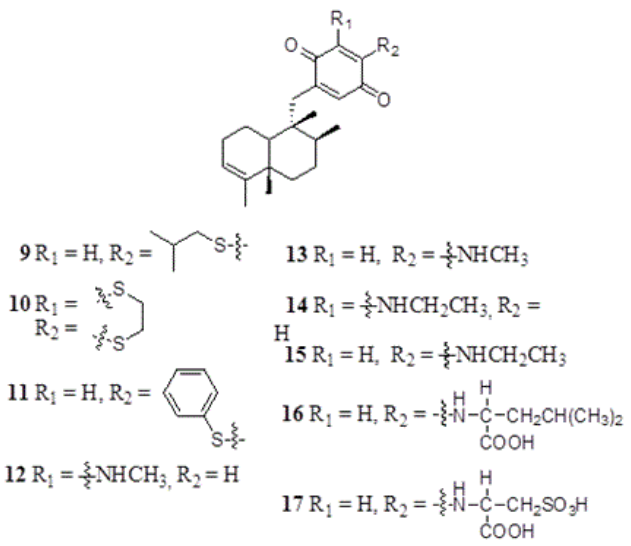


**Figure 3:** 4'-isobutylthio-avarone(9); 3',4'-Ethylenedithio-avarone (10); 4'-Phenylthio-avarone (11); 3'-Methylamino-avarone (12); 4'-Methylamino-avarone (13); 3'-Ethylamino-avarone (14); 4'-Ethylamino-avarone (15); 4'-Leucine-avarone (16); 4'-Serine-avarone (17).

On the other hand, the same introduction in the 4' position has offered the compounds (13 and 15) with cytotoxicity comparable to the avarone one (2). The modifications in the quinone ring of avarone have always resulted in the loss of anti-HIV activity with two exceptions, 4'-leucine-avarone (16) and 4'-serine-avarone (17). Indeed, both avarone deivatives have retained the potency of the parent one [46].

A couple of amino-avarone derivatives were also tested as potential antipsoriatic agents. 3'-methylamino-avarone (12) displayed the best antiproliferative profile, inhibiting the $^3$H-thymidine incorporation in HaCaT cells ($IC_{50}$ 4.5 μM) with potency similar to anthralin, followed by lack of any cell toxicity. Due to its antioxidant properties, the same compound (12) was also able to decrease the undesirable effects derived from respiratory bursts of neutrophils infiltrated in the psoriatic skin [64].

The additional derivatives were isolated from the sponge *D. avara* (collected from different places in the Gulf of Naples, Italy), namely 5'-acetyl avarol (18) [12], diacetyl avarol (19) and 6'-hydroxy-5'-acetyl avarol (20) (Figure 4) [72].

Monoacetyl avarol (18) showed a cytotoxicity ($LD_{50}$ 0.09 ppm) twice of the avarol one (1) [12], while diacetyl avarol (19) ($LD_{50}$ 0.15 ppm) and the compound 20 ($LD_{50}$ 1.30 ppm) exhibited the activity comparable to avarol, in the brine shrimp assay [72]. Afterwards, Shen and co-workers reported the synthesis and cytotoxicity of several acylated derivatives of avarol. Diacetyl avarol (19) displayed a marked antitumour activity against KB cells ($IC_{50}$ 1.35 μg/ml), while di-p-bromobenzoyl avarol (21) (Figure 4) was active against Hepa cells ($IC_{50}$ 1.30 μg/ml) [73].

| | Compounds* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cell lines | 1 | 2 | 12 | 13 | 14 | 15 | 16 | 17 |
| Cytostatic | Mean ID50 (μM) | | | | | | | |
| C-8166 | 9.2 | 12.9 | 1.7 | 16.2 | 2.5 | 18.6 | 8.8 | 22.3 |
| L-1210 | 13.9 | 15.6 | 2.3 | 15.2 | 3.4 | 28.2 | 11.6 | 26.3 |
| Raji | 11.7 | 18.1 | 2.0 | 18.1 | 3.9 | 20.3 | 9.1 | 24.0 |
| H-9 | 13.5 | 14.2 | 2.3 | 22.0 | 3.7 | 28.0 | 9.1 | 30.1 |
| Vero | 19.5 | 17.0 | 14.0 | 10.2 | 12.5 | 12.0 | 23.5 | >100.0 |
| Antiviral | Mean ED50 (μM) | | | | | | | |
| Polio | 0.8 | 0.8 | 0.6 | 2.0 | 0.8 | 3.3 | 2.7 | 3.9 |
| ASFV | 19.0 | 17.4 | 14.0 | 10.5 | 12.5 | 12.0 | 23.5 | >100.0 |
| HSV-1 | 10.5 | 9.5 | 4.4 | 10.2 | 12.5 | 12.0 | 23.5 | >100.0 |

**Table 2:** Cytostatic and antiviral activities of Avarol derivatives. *Avarol (1), Avarone (2), 3'-Methylamino-avarone (12), 4'-Methylamino-avarone (13); 3'-Ethylamino-avarone (14); 4'-Ethylamino-avarone (15); 4'-Leucine-avarone (16); 4'-Serine-avarone (17).

## Microorganisms Associated with the Marine Sponge *Dysidea avara*

Till date the sponges have been in the focus for the reason of two main and often interrelated factors: they are a rich source of biologically active secondary metabolites and form close association with a wide variety of microorganisms. Indeed, the surfaces and internal spaces of marine sponges make a unique microhabitat for these organisms. As more rich in nutrient than seawater and most sediment, the sponge environments do represent a very promising niche for the isolation of diverse bacteria and fungi.

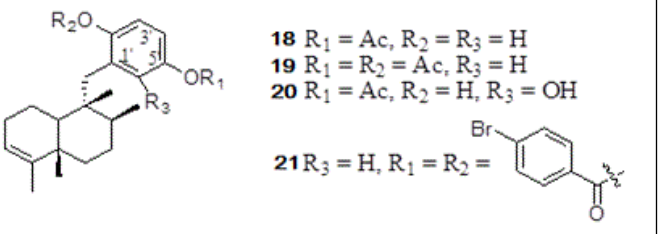


**Figure 4:** 5'-Acetyl avarol (18); 2',5'-Diacetyl avarol (19); 6'-Hydroxy-5'-acetyl avarol (20); Di-p-bromobenzoyl avarol (21).

In fact, high microbial diversity within some marine sponges and intriguing 'host-bacterial symbiont' distributions have been recently reported [74].

Certain demosponges are known to be associated with enormous amounts of microorganisms contributing up to 40-60% of the animal biomass, with density in excess of 109 microbial cells per mL of the sponge tissue, several orders of magnitude higher than those typical for

sea water [75]. The vast majority of these microorganisms are located extracellularly within the mesohyl matrix [76].

It appears that a given sponge species contains a mixture of generalist and specialist microorganisms with the associated microbial communities being fairly stable both in space and time [77]. To date, the bacterial roles in the sponge biology and ecology have not been elucidated in details. More precisely, a few reports describe the contribution of symbiotic microbes to the sponge well-being or survival [78]. The symbiont community may serve as a source of nutrition by transferring products of metabolite processes to the host. For example, symbiotic cyanobacteria have been shown to transfer organic carbon obtained through photosynthesis to the host [79]. The other suspected benefits of the symbionts include contribution to the structural rigidity of the sponges and production of halogenated marine natural products [80].

Generally, little is known about the mechanisms that regulate association and communication between the sponges and associated microorganisms. Recently, it has been demonstrated that multispecies bacterial communities can communicate with each other via small secreted molecules, such as N-acyl homoserine lactones (AHLs) and diketopiperazines (DKPs), through the process known as quorum sensing (QS) [81,82].

The specimen of *D. avara* sponge (collected in the Gulf of Naples, Italy) was used for the isolation of bacteria. The identified strains by PCR analysis were found to belong to the genera *Vibrio* and *Pseudoalteromonas*. These strains were tested for AHL production by means of a TLC-overlay (with *Agrobacterium tumefaciens* NTL4) and Lux screen assays. The dichloromethane extract of cell-free medium of Vibrio sp. (isolated from the sponge) activated QS both in TLC overlay and Lux screen assays. Furhermore, *Pseudoalteromonas* sp. inhibited QS in a 'T' streak assay with *Chromobacterium violaceum*. More precisely, a negative gradient of violacein production (that is under control of AHL molecules) was observed. This indicated either AHL inhibition or degradation by *Pseudolateromonas* sp. To identify the AHL bioreporter activating signal, the aforementioned extract of *Vibrio* sp. was purified by means of chromatographic techniques affording four compounds characterised by NMR ($^{1}H$, $^{13}C$) [83]. The isolated compounds displayed typical spectra of cyclic dipeptides and were identified as cyclo-(L-prolyl-L-phenylalanine) (22), cyclo-(cis-4-hydroxy-D-prolyl-L-leucine) (23), cyclo-(trans-4-hydroxy-L-prolyl-L-phenylalanine) (24) and cyclo-(L-prolyl-L-leucine) (25) (Figure 5) [84]. The finding of two different bacteria associated with *D. avara* which display opposed responses on AHL synthesis adds an interesting dimension to the study of signalling interactions in the sponge microcosms. This peculiar behaviour may be part of the mutual control of the growth of different microorganisms in the same host. In addition, other cyclic dipeptides, namely cyclo-(L-prolyl-L-leucine) and cyclo-(L-prolyl-L-phenylalanine), isolated from *Vibrio* sp. associated with the marine sponge *D. avara*, were also able to activate QS bioreporters. These experimental data should be useful for studying cross-kingdom chemical communication [83].

## Biotechnologies for the Production of Marine Natural Products

Although the marine environment is a plentiful source of novel marine natural products with pharmaceutical potential, only a few of them have reached the stage of commercial production so far. Arabinofuranosyladenine (Ara-A, isolated from the gorgonian Eunicella cavolini) [85], classifying among the most potent antiviral drugs, is one of the compounds currently in clinical use [86]. Avarol [11,87] has been also commercialised, as a cream against skin disorders including psoriasis.

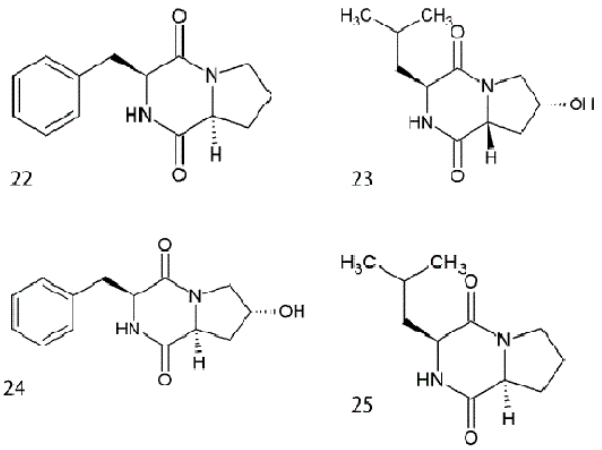


**Figure 5:** Cyclo-(L-prolyl-L-phenylalanine) (22); Cyclo-(cis-4-hydroxy-D-prolyl-L-leucine) (23); Cyclo-(trans-4-hydroxy-L-prolyl-L-phenylalanine) (24); Cyclo-(L-prolyl-L-leucine) (25).

Nowadays the patent applications on these natural products represent less than 10% of the total number of the relevant papers. Additionally, the number is very little compared with that one covering the patents on terrestrial organisms. The limited availability of larger quantities of a particular organism to be extracted is one of the major causes for low commercial utilisation. On the other hand, the recovery from marine organisms is unacceptable, due to the devastating impact on the natural environment. Actually, four different strategies may be attempted to obtain marine bioactives in enhanced amounts: chemical synthesis, aquaculture, cultivation of marine organisms in bioreactor and cell culture.

Generally, pharmaceutical companies need a strong patent position before starting the long and rather expensive path of a drug development. This approach has successfully been undertaken especially for the compounds with industrial application that can be easily synthesised. High structural and stereochemical complexity of the marine metabolites make their chemical synthesis not realistic, primarily for the numerous steps followed by low yield.

The first effort for *in situ* aquaculture of marine sponges (bath sponges) was achieved in Adriatic Sea in 1870, but no detailed statement of the applied methods was reported. Indeed, such reports were firstly published during the late 19th and early 20th centuries [88,89]. The technique is based on the capacity of sponges to regenerate themselves and create new colonies starting with small fragments. Later on, the large-scale sponge aquaculture has been developed in more countries [90-92].

Some researchers have considered the possibility of producing sponge biomass under controlled conditions, because the in situ conditions in the aquaculture cannot be affected. The supply of an adequate food source and accumulation of waste products represent the main difficulties. Recently, Osinga and co-workers [93] have reported about the growth of the sponge *Pseudosuberites andrewsi* in a

closed system, using the microalgae *Chlorella sorokiniana* and *Rhodomonas* sp. as food source. These two microalgae were selected due to ingestion and digestion by the sponge cells (microscopic observation). High growth rates of this sponge species indicate a promising future for the sponge cultivation in the closed systems.

The sponge cells are characterised by significant proliferation capacity which may facilitate the establishing of their cell cultures *in vitro*. In analogy with the production of bioactive metabolites from bacteria and fungi, the sponge secondary metabolites might be produced in bioreactor using their cell cultures. In the last few years, the production of axenic sponge cell cultures has been developed. However, till date the maintenance of sponge cells has been reported only for *in vitro* conditions [94-96]. Primary cell cultures have been obtained from several sponges, with a low cell density in the cultures. This low proliferation could depend on the culture condition and/or relevant experimental approach. The optimisation of some physical parameters (pH, temperature and light) together with the improved commercial medium (using different supplements, such as cholesterol, fatty acids and glucose) has led to the promotion of the sponge cell proliferation [97,98].

The single cells in suspension do not proliferate readily [95], since they loose telomerase activity and consequently the potency for cell division [99]. The formation of multicellular aggregates (primmorphs) from dissociated single sponge cells both regains telomerase activity and their growth potential [100,101].

Another promising method for the cultivation of sponges is based on the fragmentation of the whole organisms. Actually, in vitro cultivation of sponge fragments (without additional dissociation and reaggregation) has been reported. However, there is a limitation – only the species with high capability of wound healing may be used [102].

The morphological changes observed in all aforementioned methods might indicate non optimal culture conditions. Therefore, additional ecological parameters have to be considered in the optimisation of the sponge cell culture conditions and bioreactor design. Other studies have demonstrated the ability of these cultures to produce secondary metabolites [18,103,104]. If an appropriate growth medium and bioreactor system for primmorphs can be developed, this system may represent the most promising biotechnological approach.

## Future perspectives

The Mediterranean sponge *D. avara* has been attracting the attention of a marine research community since 1974, with Dr. Salvatore De Rosa (CNR-ICB, Pozzuoli-Naples, Italy) playing one of very special roles in the field. In terms of sourcing novel therapeutics, marine sponges are currently recognised as one of the most promising source of bioactive substances for drug discovery research. As previously stated, avarol and avarone, two sesquiterpenes (hydroquinone and quinone, respectively) possessing a rearranged drimane skeleton, were firstly isolated from this marine species. Their penetration of the blood-brain barrier and lack of neurotoxicity, together with a broad range bioactivity *in vitro* and *in vivo*, make this scaffold an excellent platform for AD drug development. The rationale for selecting a drug development platform based on derivatives and analogues of avarol and avarone, and in particular thio- and amino-derivatives, for the prevention and treatment of AD rests on several basic elements including the forementioned anti-AChE and antioxidant activities.

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## References

1. Bergquist PR (1978) Sponges. London: The Anchor Press Ltd.
2. Lévi C (1998) What is a sponge? In: Lévy C (ed) Sponges of the New Caledonian lagoon. Paris: Orstrom editions 27-34.
3. Belarbi EH, Gómez AC, Christi Y, Camacho FG, Grima EM (2003) Producing drugs from marine sponges. Biotechnol Adv 21: 585-598.
4. Debitus C (1998) The fascinating world of sponge chemistry & chemical ecology. In: Lévy C (ed) Sponges of the New Caledonian Lagoon. Paris: Orstrom editions 61-64.
5. Armstrong E, McKenzie JD, Goldworthy GT (1999) Aquaculture of sponges on scallops for natural product research and antifouling. J Biotechnol 70: 163-174.
6. Mather P, Bennett I (1992) Phylum Porifera. In: Mather P, Bennett I (eds) A Coral Reef Handbook NSW, Australia: Surrey Beatly & Sons PTY Limited. 35-46.
7. Hooper J (1998) How can you identify a sponge? In: Lévy C (ed) Sponges of the New Caledonian Lagoon. Paris: Orstrom editions 39.
8. Brusca RC, Brusca GJ (2003) Phylum Porifera: The Sponges. In: Invertebrates (2ndedn), USA: Sinauer Associates, Inc.179-208.
9. Munro MH, Blunt JW (2001) Marine Literature Database (MarinLit). University of Canterbury, New Zealand.
10. Uriz MJ, Rosell D, Martin D (1992) The sponge population of the Cabrera archipelago (Balearic Islands): characteristics, distribution and abundance of the most representative species. PSZN I: Mar Ecol 113: 101-117.
11. Minale L, Riccio R, Sodano G (1974) Avarol, a novel sesquiterpenoid hydroquinone with a rearranged drimane skeleton from the sponge Dysidea avara. Tetrahedron Lett 38: 3401-3404.
12. Crispino A, de Giulio A, De Rosa S, Strazzulio G (1989) A new bioactive derivate of avarol from the marine sponge Dysidea avara. J Nat Prod 52: 646-648.
13. Müller WEG, Maidhof A, Zahn RK, Schröder HC, Gasic MJ, et al. (1985) Potent antileukemic activity of the novel cytostatic agent avarone and its analogues in vitro and in vivo. Cancer Res 45: 4822-4827.
14. Amigo M, Paya M, Braza-Boils A, De Rosa S, Terencio MC (2008) Avarol inhibits TNF-alpha generation and NF-kappa B activation in human cells and in animal models. Life Sci 82: 256-264.
15. Pietschmann R, Shatton M, Schatton W (2004) Process for preparation of compositions with a high content in avarol and their use. Eur. Pat EP1391197.
16. Gordaliza M (2010) Cytotoxic terpene quinones from marine sponges. Mar Drugs 8: 2849-2870.
17. Sakurai J, Oguchi T, Watanabe K, Abe H, Kanno S, et al. (2008) Highly efficient total synthesis of the marine natural products (+)-avarone, (+)-avarol, (−)-neovarone, (−)-neoavarol and (+)-aureol. Chem Eur J 14: 829-837.
18. Müller WEG, Böhm M, Batel R, De Rosa S, Tommonaro G, et al. (2000) Application of cell culture for the production of bioactive compounds from sponges: synthesis of avarol by primmorphs from Dysidea avara. J Nat Prod 63: 1077-1081.

19. Sipkema D, Heilig HGHJ, Akkermans ADL, Osinga R, et al. (2003) Sponge-cell culture? A molecular identification method for sponge cells. Mar Biotechnol 5: 443-449.
20. De Caralt S, Sánchez-Fontenla J, Uriz MJ, Wijffels RH (2010) In situ aquaculture methods for Dysidea avara (Demospongiae, Porifera) in the Northwestern Mediterranean. Mar Drugs 8: 1731-1742.
21. Wulff JL (2006) Ecological interactions of marine sponges. Can J Zool 84: 146-166.
22. Hay ME (1996) Marine chemical ecology: what's known and what's next? J Exp Mar Biol Ecol 200: 103-134.
23. Becerro MA, Thacker RW, Turon X, Uriz MJ, Paul VJ (2003) Biogeography of sponge chemical ecology: comparisons of tropical and temperate defenses. Oecologia 135: 91-101.
24. Martí R, Uriz MJ, Turon X (2004) Seasonal and spatial variation of species toxicity in Mediterranean seaweed communities: correlation to biotic and abiotic factors. Mar Ecol Prog Ser 282: 73-85.
25. Abdo DA, Motti CA, Battershill CN, Harvey ES (2007) Temperature and spatiotemporal variability of salicylihalamide A in the sponge Haliclona sp. J Chem Ecol 33: 1635-1645.
26. Noyer C, Thomas OP, Becerro MA (2011) Patterns of chemical diversity in the Mediterranean sponge Spongia lamella. PLoS One 6: 1-11.
27. Sacristan-Soriano O, Banaigs B, Becerro MA (2011) Relevant spatial scales of chemicals variation in Aplysina aerophoba. Mar Drugs 9: 2499-2513.
28. Becerro MA, Turon X, Uriz MJ (1995) Natural variation of toxicity in encrusting sponge Crambe crambe (Schmidt) in relation to size and environment. J Chem Ecol 21: 1931-1946.
29. Ruzicka R, Gleason DF (2008) Latitudinal variation in spongivorous fishes and the effectiveness of sponge chemical defenses. Oecologia 154: 785-794.
30. Thompson JE, Barrow KD, Faulkner DJ (1983) Localization of two brominated metabolites, aerothionin and homoaerothionin, in spherulous cells of the marine sponge Aplysina fistularis (Verongia thiona). Acta Zool 64: 199-210.
31. Uriz MJ, Turon X, Galera J, Tur JM (1996) New light on the cell location of avarol within the sponge Dysidea avara (Dendroceratida). Cell Tissue Res 285: 519-527.
32. Uriz MJ, Becerro MA, Tur JM, Turon X (1996) Location of toxicity within the Mediterranean sponge Crambe crambe (Demospongiae, Poecilosclerida). Mar Biol 124: 583-590.
33. Turon X, Becerro MA, Uriz MJ (2000) Distribution of brominated compounds within the sponge Aplysina aerophoba: coupling of X-ray microanalysis with cryofixation techniques. Cell Tissue Res 301: 311-322.
34. Salomon CE, Deerinck T, Ellisman MH, Faulkner DJ (2001) The cellular localization of dercitamide in the Palauan sponge Oceanapia sagittaria. Mar Biol 139: 313-319.
35. Sacristan-Soriano O, Banaigs B, Becerro MA (2012) Temporal trend in the secondary metabolite production of the sponge Aplysina aerophoba. Mar Drugs 10: 677-693.
36. Turon X, Becerro MA, Uriz MJ (1996) Seasonal patterns of toxicity in benthic invertebrates: the encrusting sponge Crambe crambe (Poecilosclerida). Oikos 75: 33-40.
37. Becerro MA, Paul VJ, Starmer J (1998) Intracolonial variation in chemicals defenses of the sponge Cacospongia sp. and its consequences on generalist fish predators and the specialist nudibranch predator Glossodoris pallida. Mar Ecol Prog Ser 168: 187-196.
38. Schupp P, Eder C, Paul V, Proksch P (1999) Distribution of secondary metabolites in the sponge Oceanapia sp. and its ecological implications. Mar Biol 135: 573-580.
39. Furrow FB, Amsler CD, McClintock JB, Baker BJ (2003) Surface sequestration of chemical feeding deterrents in the Antartic sponge Latrinculia apicalis as an optimal defense against sea star spongivory. Mar Biol 143: 443-449.
40. Rhode S, Schupp PJ (2011) Allocation of chemical and structural defences in the sponge Melophlus sarasinorum. J Exp Mar Biol Ecol 399: 76-83.
41. Sirirak T, Intaraksa N, Kaewsuwan S, Yuenyongsawad S, Suwanborirux K, et al. (2011) Intracolonial allocation of trisoxazole macrolides in the sponge Pachastrissa nux. Chem Biodivers 8: 2238-2246.
42. De Rosa S, Minale L, Riccio R, Sodano G (1976) The absolute configuration of avarol, a rearranged sesquiterpenoid hydroquinone from a marine sponge. J Chem Soc Perkin Trans 1: 1408-1414.
43. Sarma A, Chattopadhyay P (1982) Synthetic studies of trans-clerodane diterpenoids and congeners: stereocontrolled total synthesis of (±)-avarol. J Org Chem 47: 1727-1731.
44. Seibert G, Raether W, Dogović N, Gašić MJ, Zahn RK, et al. (1985) Antibacterial and antifungal activity of avarone and avarol. ZBL Bakt Hyg A 260: 379-386.
45. Pejin B, Iodice C, Tommonaro G, Stanimirovic B, Ciric A, et al. (2014) Further in vitro evaluation of antimicrobial activity of the marine sesquiterpene hydroquinone avarol. Curr Pharm Biotechnol 15: 583-588.
46. De Giulio A, De Rosa S, Strazzulo G, Diliberto L, Obino P, et al. (1991) Synthesis and evaluation of cytostatic and antiviral activities of 3′ and 4′-avarone derivatives. Antiviral Chem Chemother 2: 223-227.
47. Kurelec B, Zahn RK, Gasić MJ, Britvić S, Lucić D, e al. (1985) Antimutagenic activity of the novel antileukemic agents, avarone and avarol. Mutat Res 144: 63-66.
48. Pejin B, Iodice C, Kojic V, Jakimov D, Lazovic M, Tommonaro G In vitro evaluation of cytotoxic and mutagenic activity of avarol. Nat Prod Res DOI: 10.1080/14786419.2015.1052067 (In Press)
49. Sarin P, Sun D, Thornton A, Miller W (1987) Inhibition of replication of the etiologic agent of acquired immune deficiency syndrome (human T-lymphotropic retrovirus/lymphadenopathy-associated virus) by avarol and avarone. J Natl Cancer Inst 78: 663-666.
50. Müller WE, Sarin PS, Kuchino Y, Dorn A, Hess G, et al. (1987) In W. Vettermann, M. Schauzu (Edn), AIDS. Bonn: Bundesministerium für Forschung und Technologie 354-378.
51. Ferrándiz ML, Sanz MJ, Bustos G, Payá M, Alcaraz MJ, et al. (1994) Avarol and avarone, two new anti-inflammatory agents of marine origin. Eur J Pharmacol 253: 75-82.
52. Pejin B, Iodice C, Tommonaro G, De Rosa S (2008) Synthesis and biological activities of thio-avarol derivatives. J Nat Prod 71: 1850-1853.
53. Belisario MA, Maturo M, Pecce R, De Rosa S, Villani GR (1992) Effect of avarol and avarone on in vitro-induced microsomal lipid peroxidation. Toxicology 72: 221-233.
54. Belisario MA, Maturo M, Avagnale G, De Rosa S, Scopacasa F, et al. (1996) In vitro effect of avarone and avarol, a quinone/hydroquinone couple of marine origin, on plattelet aggregation. Pharmacol Toxicol 79: 300-304.
55. Amigo M, Paya M, Braza-Boils A, De Rosa S, Terencio MC (2008) Avarol inhibits TNF-alpha generation and NF-kappaB activation in human cells and in animal models. Life Sci 82: 256-264.
56. Lizzul PF, Aphale A, Malaviya R, Sun Y, Masud S, et al. (2005) Differential expression of phosphorylated NF-kappaB/RelA in normal and psoriatic epidermis and downregulation of NF-kappaB in response to treatment with etanercept. J Invest Dermatol 124: 1275-1283.
57. Bayliffe AI, Brigandi RA, Wilkins HJ, Levick MP (2004) Emerging therapeutic targets in psoriasis. Curr Opin Pharmacol 4: 306-310.
58. Banno T, Gazel A, Blumenberg M (2005) Pathway-specific profiling identifies the NF-kappa B-dependent tumor necrosis factor alpha-regulated genes in epidermal keratinocytes. J Biol Chem 280: 18973-18980.
59. De Rosa S, De Giulio A, Strazzullo G (1991) Biologically active metabolites from marine organisms and some semi-synthetic derivatives. Trends Org Chem 2: 127-141.
60. Gašić MJ (1988) Biologically active compounds from marine sponges: an approach to chemical and biochemical characterization of the avarol/avarone redox couple. J Serb Chem Soc 53: 229-249.

61. De Rosa S, Pejin B, Tommonaro G (2008) Anti-inflammatory marine natural products with antipsoriatic effects. 4th International Conference on Oxidative Stress in Skin Medicine and Biology, Andros, Greece, September 11-14, 2008, Book of Abstracts, p. 53.
62. Tommonaro G, Pejin B, Iodice C, Tafuto A, De Rosa S (2015) Further in vitro biological activity evaluation of amino-, thio- and ester- derivatives of avarol. J Enzym Inhib Med Chem 30: 333-335.
63. Pejin B, Iodice C, Tommonaro G, Bogdanovic G, Kojic V, et al. (2014) Further in vitro evaluation of cytotoxicity of the marine natural product derivative 4'-leucine-avarone. Nat Prod Res 28: 347-350.
64. Amigó M, Terencio MC, Mitova M, Iodice C, Payá M, et al. (2004) Potential antipsoriatic avarol derivatives as antioxidants and inhibitors of PGE2 generation and proliferation in the HaCaT cell line. J Nat Prod 67: 1459-1463.
65. Amigó M, Payá M, De Rosa S, Terencio MC (2007) Antipsoriatic effects of avarol-3'-thiosalicylate are mediated by inhibition of TNF-alpha generation and NF-kappaB activation in mouse skin. Br J Pharmacol 152: 353-365.
66. Pejin B, Vitale RM, Tommonaro G, Iodice C, De Rosa S, et al. (2011) Development of semisynthetic inhibitors of acetylcholinesterase (AChE) as potential anti-Alzheimer drugs by an integrated computational-experimental approach. Science and Supercomputing in Europe – Research Highlights in 2011 (DG Research in the Seventh Framework Programme - FP7) p. 93.
67. Scott LJ, Goa KL (2000) Galantamine: a review of its use in Alzheimer's disease. Drugs 60: 1095-1122.
68. Bozić T, Novaković I, Gasić MJ, Juranić Z, Stanojković T, et al. (2010) Synthesis and biological activity of derivatives of the marine quinone avarone. Eur J Med Chem 45: 923-929.
69. Cariello L, De Nicola Giudici M, Zanetti L (1980) Developmental aberrations in sea-urchin eggs induced by avarol and two cogeners, the main sesquiterpenoid hydroquinones from the marine sponge, Dysidea avara. Comp Biochem Physiol C 65: 37-41.
70. Cozzolino R, De Giulio A, De Rosa S, Strazzullo G, Gašić MJ, et al. (1990) Biological activities of avarol derivatives, 1. amino derivatives. J Nat Prod 53: 699-702.
71. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen JB, Nicholsand DE, et al. (1982) Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med 45: 31-34.
72. De Giulio A, De Rosa S, Di Vincenzo G, Strazzullo G (1990) Further bioactive derivative of avarol from Dysidea avara. Tetrahedron 46: 7971-7976.
73. Shen Y-C, Lu C-H, Chakraborty R, Kuo Y-H (2003) Isolation of sesquiterpenoids from sponge Dysidea avara and chemical modification of avarol as potencial antitumor agents. Nat Prod Rep 17: 83-89.
74. Thomas TRA, Kavlekar DP, LokaBharathi PA (2010) Marine drugs from sponge-microbe association - a review. Mar Drugs 8: 1417-1468.
75. Hoffmann F, Rapp HT, Reitner J (2005) Anaerobic world in sponges. Geomicrobiol J 22: 1-10.
76. Becerro MA, PaulVJ (2004) Effects of depth and light on the secondary metabolites and cyanobacterial symbionts of the sponge Dysidea granulosa. Mar Ecol-Prog Ser 280: 115-128.
77. Pita L, Turon X, López-Legentil S, Erwin PM (2013) Host rules: spatial stability of bacterial communities associated with marine sponges (Ircinia spp.) in the Western Mediterranean Sea. FEMS Microbiol Ecol 86: 268-276.
78. Webster NS, Blackall LL (2009) What do we really know about sponge-microbial symbioses? The ISME Journal 3: 1-3.
79. Ridley CP, Faulkner DJ, Haygood MG (2005) Investigation of Oscillatoria spongeliae -dominated bacterial communities in four dictyoceratid sponges. Appl Environ Microb 71: 7366-7375.
80. Hildebrandt M, Waggoner LE, Lim GE, Sharp KH, Ridley CP, et al. (2004) Approaches to identify, clone, and express symbiont bioactive metabolite genes. Nat Prod Rep 21: 122-142.
81. Shiner EK, Rumbaugh KP, Williams SC (2005) Inter-kingdom signalling: deciphering the language of acyl homoserine lactones. FEMS Microbiol Rev 29: 935-947.
82. Tommonaro G, Abbamondi GR, Iodice C, Tait K, De Rosa S (2012) Diketopiperazines produced by the halophilic archaeon, Haloterrigena hispanica, activate AHL bioreporters. Microbial Ecol 63: 490-495.
83. Abbamondi GR, De Rosa S, Iodice C, Tommonaro G (2014) Cyclic dipeptides produced by marine sponge-associated bacteria as quorum sensing signals. Nat Prod Comm 9: 229-232.
84. De Rosa S, Mitova M,Tommonaro G (2003) Marine bacteria associated with sponge as source of cyclic peptides. Biomol Eng 20: 311-314.
85. Cimino G, De Rosa S, de Stefano S (1984) Antiviral agents from a gorgonian, Eunicella cavolini. Experientia 40: 339-340.
86. Cohen SS (1963) Sponges, cancer chemotherapy and cellular aging. Perspect Biol Med 6: 215-227.
87. De Rosa S, Minale L, Riccio R, Sodano G (1976) The absolute configuration of avarol, a rearranged sesquiterpenoid hydroquinone from a marine sponge. J Chem Soc Perkin Trans 1. 1976: 1408–1414.
88. Smith HM (1898) The Florida commercial sponges. Fish Bull 17: 225-240.
89. Moore HF (1910) The commercial sponges and the sponge fisheries. Fish Bull 28: 399-511.
90. Verdenal B, Vacelet J (1990) In: Rützler K (ed) New perspectives in sponge biology Washington D.C.: Smithsonian Institution Press. pp. 416-424.
91. Müller WE, Sarin PS, Kuchino Y, Dorn A, Hess G, et al. (1987) In: Vettermann W, Schauzu M (eds) AIDS Bonn: Bundesministerium für Forschung und Technologie pp. 354-378.
92. Adams C, Stevely JM, Sweat D (1995) Economic feasibility of smallscale sponge farming in Pohnpei, Federated States of Micronesia. J World Aquac Soc 26: 132-142.
93. Duckworth AR, Battershill CN, Bergquist PR (1997) Influence of explant procedures and environmental factors on culture success of three sponges. Aquaculture 165: 251-267.
94. Osinga R, De Beukelaer PB, Meijer EM, Tramper J, Wijffels RH (1999) Growth of the sponge Pseudosuberites (aff.) andrewsi in a closed system. J Biotechnol 70: 155-161.
95. Pomponi SA, Willoughby R (1994) In: Van Soest RWM, Van Kempen TMG, Braekman JC (eds) Sponges in Time and Space Rotterdam: Balkema pp. 395-400.
96. Ilan M, Contini H, Carmeli S, Rinkevich B (1996) Progress towards cell cultures from a marine sponge that produces bioactive compounds. J Mar Biotechnol 4: 145-149.
97. Müller WE, Schäcke H (1996) Characterization of the receptor protein-tyrosine kinase gene from the marine sponge Geodia cydonium. Prog Mol Subcell Biol 17: 183-208.
98. De Rosa S, De Caro S, Tommonaro G, Slantchev K, Stefanov K, et al. (2001) Development in a primary cell culture of the marine sponge Ircinia muscarum and analysis of the polar compounds. Mar Biotechnol 3: 281-286.
99. De Rosa S, De Caro S, Iodice C, Tommonaro G, Stefanov K, Popov S (2003) Development in primary cell culture of demosponges. J Biotechnol 100: 119-125.
100. Koziol C, Borojevic R, Steffen R, Müller WEG (1998) Sponges (Porifera) model systems to study the shift from immortal to senescent somatic cells: the telomerase activity in somatic cells. Mech Ageing Dev 100: 107-120.
101. Custodio MR, Prokic I, Steffen R, Koziol C, Borojevic R, et al. (1998) Primmorphs generated from dissociated cells of the sponge Suberites domuncula: a model system for studies of cell proliferation and cell death. Mech Ageing Dev 105: 45-59.
102. Müller WEG, Wiens M, Batel R, Steffen R, Schröeder HC, et al. (1999) Establishment of a primary cell culture from a sponge: primmorphs from Suberites domuncula. Mar Ecol Prog Ser 178: 205-219.

103. Nickel M, Leininger S, Proll G, Brümmer F (2001) Comparative studies on two potential methods for the biotechnological production of sponge biomass. J Biotechnol 92: 169-178.

104. Andrade P, Willoughby R, Pomponi SA, Kerry RG (1999) Biosynthetic studies of the alkaloid, stevensine, in a cell culture of the marine sponge Teichaxinella morchella. Tetrahedron Lett 40: 4775-4778.

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