Review

Anti-inflammatory agents from marine sponges: an update

Nadia Ruocco1,2,3, Luisa Albarano1, Francesca Capone4, Susan Costantini1, Maria Costantini1,*

1Department of Biology and Evolution of Marine Organisms, Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Napoli, Italy
2Department of Biology, University of Naples Federico II, Complesso Universitario di Monte Sant’Angelo, Via Cinthia, 80126, Napoli, Italy
3Bio-Organic Chemistry Unit, Institute of Biomolecular Chemistry-CNR, Via Campi Flegrei 34, Pozzuoli, Naples 80078, Italy
4Unità di Farmacologia Sperimentale, Istituto Nazionale Tumori “Fondazione G. Pascale”, IRCCS, Napoli, Italy

Correspondence: maria.costantini@szn.it; Tel.: +39-081-583-3315; Fax: +39-081-764-1355.

Abstract: Inflammation is a dynamic process, in which pro-inflammatory cytokines, such as tumor necrosis factor, interleukins and vascular endothelial growth factor have central roles. A number of drugs or active agents have been developed to treat inflammation, able to reduce the activity of specific cytokines or their receptors to block lymphocyte trafficking into tissues, to prevent the binding of monocyte-lymphocyte co-stimulatory molecules, or to deplete B lymphocytes. Furthermore, inflammation is a critical component of tumor progression, fostering new anti-inflammatory therapeutic approaches against cancer development. In the few last decades marine environment have been recognized to be a rich sources of bioactive metabolites with varied biological and pharmacological activities, including anti-inflammatory effects. A lot of marine phyla were considered for their significance respect to pharmacological active compounds, including bacteria, fungi, algae, soft corals, nudibranchs, bryozoans, tunicates and especially sponges. In particular, marine sponges have been considered as a gold mine because they contain different secondary metabolites and provide novel natural products with a remarkable chemical diversity.

Considering that the last review on anti-inflammatory agents from marine sponges dates back to 2005, and after this many studies report results on the identification of natural compounds from sponges with this activity, here we report an update on anti-inflammatory agents, focusing our attention on marine sponges as promising their sources.

Keywords: anti-inflammatory agents; cancer; sponges
1. Introduction to anti-inflammatory process.

Inflammation is a pattern of response to injury and it is considered a protective response controlled by a huge number of humoral and cellular mediators to combat its effects on the body. Inflammation involves the accumulation of cells and exudates in irritated tissues, which allows protection from further damage [1]. In fact, the function of inflammation is to eliminate the initial cause of cell injury, clearing out damaged cells and tissues from the inflammatory process with following tissue repair. The classical signs of inflammation are heat, pain, redness, swelling, and loss of function.

At the beginning of the inflammation process an harmful stimulus induces epithelial cells such as mast cells to immediately release histamine and nitric oxide (NO), which, in turn, cause vasodilation of arteries and the increase of vessels permeability (Figure 1).

![Figure 1. Schematic representation of anti-inflammatory process.](image)

At the same time, the external injury binds the toll-like receptors (TLRs), activating the MAP-kinase cascade and the biosynthesis of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB), which in turn moves inside the nucleus and promotes the transcription of tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1beta (ILβ). These cytokines stimulate the binding of leukocytes to the internal receptor of the epithelial cells of the vessel, and their adhesion to the wall of vessels, their accumulation, diapedesis (the movement of leukocytes out of the circulatory system and towards the site of tissue damage or infection) and the migration to the damaged site [2]. At this point this reaction provokes the leak of a protein-rich liquid, called edema. Finally, leukocytes such as macrophages and granulocytes can phagocytize the damaging agent though the ligation of many receptors.
of the cell-surface receptors, followed by its elimination and death. In parallel, during the inflammatory response there is a release of endogenous mediators. In fact, several enzymes such as phospholipase A2 (PLA2) are activated and induce the release of arachidonic acid (AA) from membranes. AA can be, in turn, modified by cyclooxygenase (COX), resulting in the synthesis of prostaglandins (PGs) and thromboxane (TBX) or through the activity of lipoxygenase (LOX) with the production of leukotrienes (LTs). These mediators are important for the maintenance of cellular homeostasis and inflammatory tone, promoting the resolution of damage [3].

Inflammation can be classified as acute and chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation, such as mononuclear cells, and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. It is well known that inflammation is strongly related to development of pre-cancerous lesions, considering that chronic inflammation is characterized by infiltration of mononuclear immune cells (including macrophages, lymphocytes and plasma cells), tissue destruction, increased genomic damage, cellular proliferation, disruption of DNA repair pathways, inhibition of apoptosis and the promotion of angiogenesis. Pro-inflammatory molecules, such as cytokines, inducible nitric oxide synthase (iNOS), reactive oxygen species (ROS), and NF-kB are also up-regulated. All these factors create favorable microenvironment for the initiation and progression of cancer. Anti-inflammatory agents can modulate different phases of the inflammatory cascade, which is involved in a large number of human diseases and, for this reason, the scientific community focused the attention on the discovery of potential new drugs. For many years, salicylate-containing plants were applied therapeutically, leading to the production of one of the major anti-inflammatory drug, the aspirin [4]. Aspirin derives from natural sources and it is used extensively in current clinical practice. Many other aspirin-like drugs are now available, such as the non-steroid anti-inflammatory drugs (NSAIDs). Natural products with anti-inflammatory activity have long been used as a folk remedy for inflammatory conditions such as fevers, pain, migraine and arthritis.

An enormous demand exists for new and potent anti-inflammatory drugs because inflammation underlies a multitude of human diseases, including cancer, Alzheimer’s disease, and arthritis [5]. In this context the marine environment has been shown to be the source of a great diversity of chemical structures with promising biological activities. Among marine organisms, sponges have been considered a rich source of natural compounds synthetized by themselves or their symbiotic organisms. Most of the studies have been done on sponge’s derived compounds to examine its pharmacological properties. These compounds showed antibacterial, antiviral, antifungal, antimalarial, antitumor, immunosuppressive, and cardiovascular activity [6-7]. Marine sponges are known for anti-inflammatory activities of their compounds. In fact, in literature there are a lot of examples on this already since the last twenty years [8]. The compound cavernolide isolated from Fasciospongeia cavernosa, inhibited human synovial secretory phospholipase A2 (sPLA2) in a concentration-dependent manner [9]. The contignasterol isolated from Petrosia contignata had anti-inflammatory effect on allergen-induced plasma exudation in the tracheobronchial airways [10], whereas the compound cyclolinteinone from the sponge Cacospongia linteiiformis, was able to prevent iNOS and inducible cyclo-oxygenase protein expression by blocking NF-kB activation in J774 macrophages [11]. The compound halipeptin A was isolated from the marine sponge Haliclona sp., showing an anti-inflammatory activity on mouse paw edema assay [12]. Petrosaspogloliode M, isolated from the Caledonian marine sponge Petrosaspongia nigra, resulted a potent inhibitor of phospholipase A2 (PLA2) with anti-inflammatory activity in models of acute and chronic inflammation [13].

Since the last review collects all the literature until 2005 [14], here we provide an update of the most recent anti-inflammatory compounds found in sponges, underlying the specific activity in which they
are involved. In addition, we also focus on newly discovered sponge-derived chemicals exhibiting anticancer properties.

2. Anti-inflammatory agents from marine sponges

The most recent sponge-derived compounds with anti-inflammatory activity (Table 1) are chronologically listed in this section.

Bioassay-guided fractionation led to the isolation of carteramine A, a styloguanidine derivative, from the methanol/ethanol extracts of the marine sponge *Stylissa carteri*. The molecular structure was described through Nuclear Magnetic Resonance (NMR) analysis. This compound was found a potent neutrophil chemotaxis inhibitor ($IC_{50} = 5 \mu M$), as well as synthetic compounds, such as cyclic peptides, phenyl benzylamide derivatives and glutarimide derivatives already well-known in literature for their activity on leucocyte chemotaxis. Since carteramine A displayed a different chemical structure in comparison to synthetic compounds, this study can offer new perspectives for the formulation of new anti-inflammatory drugs [15].

Three sesquiterpenoid quinones, deriving from marine sponges, ethylsmenoquinone, smenospongiarine and smenospongeidine, were reported to possess anti-inflammatory activities, causing PLA₂ inhibition with 73.2% at 269 μM, 61.5% at 242 μM and 41.0% at 224 μM, respectively [16]. These compounds induced stronger effects than ilimaquinone (inhibition %= 36.4 at 279 μM), a sesquiterpene quinone already described by Potts and Faulkner [17] for its anti-inflammatory properties. The biological activity was evaluated through colorimetric assay, using red phenol as pH indicator and lecithin as enzyme substrate.

Anti-inflammatory effects of aqueous extracts from the Brazilian sponge *Aphysina caissara* were investigated against formalin induced mice paw edema. Thirty minutes after the administration of the extract (60 and 90 mg/kg body weight), acute inflammatory edema was induced by sub-plantar injection of formalin into the right hind paw of mice. The intra-peritoneal pre-treatment with *A. caissara* extracts visibly reduced edema respect to the control group injected with saline solution (0.1 mL/10 g body weight) [8].

Another compound, plakortide P, a polyketide, was isolated from the marine sponge *Plakortis angulospiculatus*. Dried sample were extracted with methanol and partitioned petroleum ether and EtOAc. ¹H NMR of both fractions revealed the presence of polyketides. Further purifications through normal-phase High Performance Liquid Chromatography (HPLC) led to the isolation of plakortide P. This compound showed potential pharmacological properties with anti-leishmanial, anti-trypanosomal and anti-inflammatory activities. It has been observed in vitro anti-inflammatory effects in LPS-stimulated peritoneal macrophage cells with the inhibition of NO production after 24 hours of treatments. In addition, plakortide P displayed anti-neuroinflammatory activities, inhibiting the release of thromboxane B₂ (TXB₂) in murine microglia cells with IC₅₀ of 0.93 μM [18].

Purification and NMR analyses of the polar extract from the marine sponge *Coscinoderma mathewsi* led to the isolation of two novel nitrogen-containing cheilanthane sesterterpenoids, coscinolactams A and B, and the well-known suvanine [19]. These compounds together with suvanine alcoholic and aldehyde derivatives were tested against four different secretory PLA₂ and LPS-stimulated murine macrophage RAW 264.7 cells. Among them, only suvanine aldehyde derivative showed a moderate activity against human synovial sPLA₂ and bee venom sPLA₂. On the contrary, anti-inflammatory effects were detected for all compounds in LPS-stimulated RAW 264.7 cells with the inhibition of PGE₂ and NO production. In particular, suvanine aldehyde derivative displayed the strongest activity on iNOS expression.
Table 1. Sponge-derived species, compound name or active fraction, chemical isolation methods, mechanism of action and references for the anti-inflammatory compounds from marine sponges.

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<th>Sponge specie</th>
<th>Compound name</th>
<th>Isolation method</th>
<th>Mechanism of action</th>
<th>Reference</th>
</tr>
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<td><em>Stylissa carteri</em></td>
<td>Carteramine A</td>
<td>MeOH/EtOH extractions, CHCl₃/H₂O partition, further extraction with n-BuOH, additional fractionation with n-Hexane/Methanol-H₂O (9:1), ODS HPLC purification, NMR data</td>
<td>Neutrophil chemotaxis inhibitors</td>
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<td><em>Smenospongia sp.</em></td>
<td>Ethylsmenoquinone</td>
<td>Theoretical prediction using chemometric approaches</td>
<td>Bee venom PLA₂ inhibition</td>
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<td></td>
<td>smenospongiarine</td>
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<tr>
<td></td>
<td>smenospongidine</td>
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<tr>
<td><em>Plakortis angulospiculatus</em></td>
<td>Plakortide P</td>
<td>MeOH extraction, HPLC purification, NMR analysis</td>
<td>Inhibition of TXB₂ in murine microglia cells</td>
<td>[18]</td>
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<tr>
<td><em>Plakortis angulospiculatus</em></td>
<td>Plakortide P</td>
<td>MeOH extraction, HPLC purification, NMR analysis</td>
<td>NO inhibition in LPS-stimulated macrophage cells</td>
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<td><em>Aplysina caissara</em></td>
<td>Aqueous extract</td>
<td>EtOH/MeOH extractions, hexane (1:1) partition</td>
<td>Reduction of edema in mice models</td>
<td>[8]</td>
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<td><em>Coscinoderma mathewsi</em></td>
<td>Coscinolactams A-B, suvanine</td>
<td>MeOH extraction, purification through chromatography and reversed-phase HPLC, NMR analysis</td>
<td>PGE₂ and NO inhibition in LPS-stimulated RAW 264.7 cells</td>
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<td><em>Tedania ignis</em></td>
<td>Tedanol</td>
<td>MeOH extraction, purification through reversed-phase HPLC, MS and NMR analyses</td>
<td>Reduction of edema/NO inhibition in mice models</td>
<td>[20]</td>
</tr>
<tr>
<td>Organism</td>
<td>Compound</td>
<td>Extraction/Purification Method</td>
<td>Activity/Molecular Target</td>
<td>Reference</td>
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<tr>
<td><em>Tedania ignis</em></td>
<td>Tedanol</td>
<td>MeOH extraction, purification through reversed-phase HPLC, MS and NMR analyses</td>
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<td>Sponge-derived fungus <em>Acremonium</em> sp.</td>
<td>Ascofuranone, ascochlorin, ilicicolin C and LL-Z1272 ε</td>
<td>Extraction with EtOAc, CH₂Cl₂/water and MeOH/n-hexane partition, NMR, CD, and optical activity data</td>
<td>NO, IL-6 and TNF-α inhibition in murine macrophage cells</td>
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<td><em>Theonella swinhoei</em></td>
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<td><em>Plakortis angulospiculatus</em></td>
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<td>CH₂Cl₂/MeOH extraction, C18 flash chromatography, reversed-phase HPLC, MS analysis, ¹H and ¹³C NMR spectral data</td>
<td>Inhibition of iNOS and NF-κB activity in LPS-induced RAW 264.7 and SW1353 cells, respectively</td>
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<td><em>Callyspongia siphonella</em></td>
<td>Callysterol</td>
<td>MeOH/CH₂Cl₂ (1:1), ⁵-n-Hexane and CH₂Cl₂ extractions, purification on silica gel column, NMR data</td>
<td>Reduction of TBX2 in LPS-activated rat neonatal brain microglia</td>
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<td><em>Hyrtios</em> sp.</td>
<td>Dibromoindoles</td>
<td>MeOH extraction, solvent partitions using CH₂Cl₂ and MeOH/H₂O (1:1), chromatography on C18 column, reversed-phase HPLC, NMR data</td>
<td>Bee venom PLA₂ inhibition</td>
<td>[26]</td>
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<td><em>T. swinhoei</em></td>
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<td>HR ESIMS mass spectrometry, NMR analysis</td>
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<td><strong>Aplysina fistularis</strong></td>
<td>11-oxoaerothionin</td>
<td>EtOH/MeOH extractions, EtOAc partition, chromatography and HPLC purification, MS and NMR analyses</td>
<td>Inhibition of iNOS protein, pro-inflammatory cytokines and PGEs in LPS-stimulated RAW 264.7 cells</td>
<td>[1]</td>
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<td><strong>Theonella swinhoei</strong></td>
<td>Perthamides H-K</td>
<td>Extraction with n-BuOH, chromatography and reversed-phase HPLC purification, MS and NMR analyses</td>
<td>Reduction of edema in mice models</td>
<td>[28]</td>
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<tr>
<td><strong>Spongia officinalis</strong></td>
<td>Methanol extract</td>
<td>Methanol extraction, purification through C18 column using gradient elution with MeOH-H2O mixture</td>
<td>Reduction of edema in mice models</td>
<td>[30]</td>
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<td><strong>Stylissa massa</strong></td>
<td>Stylissatin A</td>
<td>Methanol extraction, bioassay-guided fractionations, reversed-phase HPLC purification, NMR data</td>
<td>NO inhibition in LPS-stimulated macrophage cells</td>
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<td><strong>Pseudoaxinyssa cantharella</strong></td>
<td>Girolline</td>
<td>Extraction, solvent partition, HPLC, NMR data</td>
<td>Inhibition of IL-8 and NF-kB/AP-1 in THP1-derived macrophage cells; decrease of IL-8 and IL-6 in primary human mononuclear cells</td>
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<td><strong>Phorbas sp.</strong></td>
<td>Phorbaketal A</td>
<td>MeOH extractions, CH2Cl2/H2O and MeOH/n-Hexane partitions, HPLC and spectral data</td>
<td>NO and NF-kB inhibition in LPS-stimulated macrophage cells</td>
<td>[35]</td>
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Tedanol, a brominated and sulfated pimarane diterpene isolated from the Caribbean sponge *Tedania ignis*, also exhibited dose-dependent anti-inflammatory activity, after intraperitoneal administration (0.1-1 mg/kg) in carrageenan-induced mouse paw edema. Its potent effect was evaluated in vivo as reduction of edema and through the decrease of mieloperoxidase (MPO) levels after 4 and 48 hours of carrageenan injection [20]. Tedanol pre-treatments induced a strong reduction of iNOS expression at 2 h and 48 hours of inflammatory process. Furthermore, a significant COX-2 depletion both in the early phase (2 and 4 hours) and in the second edema phase (48 and 72 hours) was detected. On the contrary, tedanol did not affect COX-1 levels in the early phase but only in the second phase.
Costantino et al. [21] isolated two glycosphingolipids, terpioside A-B from the sponge *Terpios* sp. These compounds were purified through a series of HPLC purifications and the chemical structure was elucidated by Mass Spectrometry (MS) and NMR analyses. Anti-inflammatory activities were evaluated on LPS-stimulated macrophage cells. Incubation at different concentrations (1-30 μM) of terpioside B significantly reduced NO production in a dose dependent manner, while terpioside A resulted less active with a weak NO inhibition.

Four sesquiterpenoids, ascofuranone, ascochlorin, ilicicolin C and LL-Z1272 ε, were isolated from a sponge-derived fungus *Acrenonium* sp. [22]. The absolute configurations, determined by modified Mosher’s method and Circular Dichroism (CD) spectroscopy, showed that these compounds are a class of cyclic terpenes containing functional groups, for instance, chlorine or hydroxyl residues. Anti-inflammatory activity of sesquiterpenoids was detected by measuring the levels of pro-inflammatory mediators (NO, IL-6, and TNF-α) after incubation in LPS-stimulated RAW 264.7 cells. Ascofuranone and ascochlorin resulted able to inhibit the production of NO and TNF-α at the concentration of 100 μM, whereas ilicicolin C and LL-Z1272 ε was specifically active on NO expression.

Chemical investigations on CH₂Cl₂-MeOH (1:1) extracts from two sponges, *Plakortis angulospiculatus* and *P. halichondrioides* collected from the Bahamas and Cayman Islands, revealed the isolation of six polyketides and three new aromatic compounds [23]. These compounds have been purified through reversed-phase HPLC and the chemical structures were deducted by MS and NMR analyses. Anti-inflammatory activity was detected using in vitro assays in chondrosarcoma cell line SW1353. The polyketides 24-nor-spiculoic acid B and 27-nor-zyggomphic acid B showed a strong inhibition of NF-kB with IC₅₀ values of 0.47 and 2.28 μM, respectively, whereas 22-nor-zyggomphic acid B resulted totally inactive. On the contrary, 27-nor-zyggomphic acid B (IC₅₀= 18.6 μM) together with 22-nor-zyggomphic acid B (IC₅₀= 19.9 μM) inhibited iNOS expression in LPS-induced RAW 264.7 cells.

Youssef and colleagues [24] defined the chemical structure of callysterol, isolated from the marine sponge *Callyspongia siphonella*. Structure determination, based on extensive NMR and MS studies, showed a correspondence to the steroidal structure. Callysterol was previously found a potent anti-inflammatory compound for its strong capability to reduce hind paw edema in comparison to the drug cortisone [25]. The authors also demonstrated a dose-dependently reduction of TXB₂ in LPS-activated rat neonatal brain microglia after treatments for 70 minutes with callysterol at 0.1 μM, 1 μM and 10 μM.

MS and 1D and 2D NMR data of the CH₂Cl₂ extract and successive HPLC fractions from the sponge *Aplysina fistularis* [1]. RAW264.7 cells and primary macrophages from rats were pre-incubated for 2 h with 11-oxoaerothionin (15 to 60 μM) and stimulated with LPS for 24 h. Then, cells were lysed by sonication in ice-cold lysis buffer and a protease inhibitor cocktail. Western blots using antibodies against iNOS revealed a dose-dependent decrease of iNOS protein, confirmed by low levels of NO₂ estimated by the absorbance at 540 nm. Pro-inflammatory cytokines (IL-1α, TNF-α) and PGE₂ expression were also inhibited by 11-oxoaerothionin pre-treatments. In fact, ELISA tests on culture supernatants showed a reduction of these proteins in a dose dependent manner.
New peptides, the perthamides, have been isolated from the marine sponge *Theonella swinhoei*. Their complete chemical structure was obtained through the interpretation of extensive spectroscopic and spectrometric data [27]. These compounds exhibited potent in vivo anti-inflammatory activity when tested in carrageenan-induced mouse paw edema. Perthamides were injected intraperitoneally before the inflammatory reaction activated by the administration of carrageenan. Perthamide C significantly reduced paw edema both in the early phase (0-6 h) and in the late phase (24-96 h) with a dose-dependent (0.1, 0.3 and 1 mg/kg) activity, causing about 60% of reduction at the dose of 300 mg/kg. Moreover, perthamide D showed an anti-inflammatory activity in both phases of edema but, due to the scarcity of natural compound availability, it was tested only at the dose of 0.3 mg/kg, generating a 46% of edema inhibition. New perthamide derivatives, perthamides G-K, were also tested for anti-inflammatory activity in the same mice models. Intraperitoneal administration of perthamides H, I and K at the dose of 0.3 mg/kg, showed the same behaviour of perthamide C reducing mouse edema at early phase (0-6 hours) and at the late phase (24-96 hours). Perthamide G, did not show anti-inflammatory activity whereas, perthamide J, which contains a ADAA residue replacing a g-methylproline, resulted the most active of the group [28].

Similar studies have been developed with solomonamide A, a peptide isolated from the polar extracts of *T. swinhoei*. The molecular formula, deducted by MS analyses and 2D NMR experiments, revealed the presence of the three amino acid residues, alanine, glycine, and serine. Additional information, obtained by $^1$H NMR and $^{13}$C NMR data, allowed to reconstruct the absolute configuration of the complete molecule. This compound displayed a dose-dependent anti-inflammatory activity causing about 60% reduction of edema in carrageenan-induced mouse paw edema at the dose 100 μg/kg [29].

Dellai and co-authors [30] have performed a methanol extraction from the Mediterranean sponge *Spongia officinalis*. The extract was then purified on a C18 cartridge using a gradient of water/methanol mixture with 0%, 50% and 80% of methanol. The methanol extract and its semi-purified fractions (F2/F3) were tested on carrageenan-induced paw edema. Increasing concentrations (25, 50 and 100 mg/kg) of the methanol extract and a single concentration (50 mg/kg) of F2/F3 were injected in the left hind paw before carrageenan administration. Treatments with the methanol extract showed a visible dose-dependent reduction of edema (42.5, 52.7 and 61.71%) at 3 hours after carrageenan injection. Regarding the semi-purified fractions, F2 reduced the 62.3% of edema, whereas F3 resulted the most efficacious with edema inhibition of 72.85%. These results were compared to a standard drug, ASL, which decreased the 62.3% of edema, suggesting a potent anti-inflammatory activity of these sponge-derived extracts.

Stylissatin A, a cyclic heptapeptide, was isolated from the Papua New Guinean marine sponge *Stylissa massa* [31]. The 80% aqueous methanol extract of *S. massa* was partitioned with EtOAc and H$_2$O and the organic phase was further separated with hexane/CH$_3$Cl/60% aqueous methanol. Then, a bioassay-guided fractionation led to the identification of the active compound and MS/NMR analyses elucidated the chemical structure. It has been observed that stylissatin A inhibited in vitro NO production in LPS-stimulated murine macrophage cells with $IC_{50}$ value of 87 μM.

A huge screening of methanol extracts from marine sponges on TLR5 reporter cell line (CHO-K1) led to the isolation of girolline, a 2-aminoimidazole derivative, previously described from the sponge *Pseudoaxinysa cantharella* for its antitumor activity [32]. After a complete NMR analysis of the chemical structure, girolline was synthetized de novo and tested on TLR5 reporter cell line. Girolline displayed inhibitory effects on TLR5 signaling but, interestingly, girolline diastereomer resulted more potent in blocking TLR5 pathway. Anti-inflammatory activity was also tested on macrophage response. The human acute monocytic leukemia cell line (THP1) was transfected with NF-kB/AP-1 reporter and differentiated into a macrophage-like phenotype. Treatments on THP1-derived macrophages with girolline (2 μg/ml) significantly reduced flagellin-induced IL-8 secretion, and almost completely abolished NF-kB/AP-1 activity. Additional experiments on primary human mononuclear cells revealed the decrease of pro-inflammatory cytokines IL-8 and IL-6 to baseline [33].
Phorbaketal A, a tricyclic sesterpenoid, isolated from the methanol extract of the marine sponge Phorbas sp. and characterized through spectroscopic methods [34], was tested for anti-inflammatory activity. Treatments with phorbaketal A (2.5, 5, and 10 μM) in LPS-stimulated macrophage cells significantly reduced NO production with dose-dependent effects. These data were confirmed by Western Blot and Real-Time qPCR, revealing a strong down-regulation of iNOS protein and mRNA levels, respectively. On the contrary, no significant effects were detected on COX-2 expression and PGE2 production. In addition, it has been showed that phorbaketal A inhibited the expression levels of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 in a dose-dependent manner. Furthermore, luciferase assay revealed the reduction of NF-κB activity in LPS-stimulated macrophage cells, suggesting possible negative effects on the expression of pro-inflammatory genes [35].

3. Sponge-derived anti-inflammatory agents with effects in cancer

Even in the area of cancer, the natural products from marine ecosystem provided several molecules which are potent sources for drug discovery and pharmaceutical industries. Marine compounds are used in the treatment of different cancer and in several other activities such as anti-inflammatory, immunomodulatory and anti-tumor [36].

Already in 2010 it was underlined the importance of the compounds from marine sponges to provide novel natural products with a chemical diversity (see Table 2).

Table 2. Sponge-derived species, compound name or active fraction, chemical isolation methods, mechanism of action and references for the anti-inflammatory compounds from marine sponges with effect in cancer.

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<tr>
<th>Sponge species</th>
<th>Compound name</th>
<th>Isolation method</th>
<th>Mechanism of action</th>
<th>Reference</th>
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<td>MeOH extraction</td>
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<td>Haliclonia sp., Pachyrellina sp., Pellina sp. and Xestospongia sp</td>
<td>Manzamine A</td>
<td>MeOH extraction</td>
<td>decrease of phosphorylated p65 NFκB</td>
<td>[41]</td>
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<tr>
<td>Geodia cydonium</td>
<td>Methanol extract</td>
<td>MeOH and CHCL3 extraction, LC-HRMS</td>
<td>decrease of IL-8, CXCL10 and VEGF levels and increase of IL-4 and IL-10 levels</td>
<td>[42-43]</td>
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</table>
Indeed, Dellai et al. [37] identified components from the Mediterranean sponge, Spongia officinalis, which have anti-inflammatory and antiproliferative activities, by methanol extraction and related purification using different gradient elution with methanol–water mixture. They performed some experiments both on animal models and on three human cancer cell lines such as A549 lung cell carcinoma, HCT15 colon cell carcinoma and MCF7 breast adenocarcinoma, using the MTT assay as an indicator of metabolically active cells and clonogenic inhibition assay [37]. Subsequently the same authors have evaluated the potency of other methanol extract form S. officinalis and its semi-purified fractions to inhibit inflammation and growth in animal model and in three human cancer cell lines (A549, HCT15 and MCF7), in order to identify new molecules useful in the pharmacological field [30].

Moreover, the biological effects of heteronemin, a marine sesterterpene isolated from the sponge Hyrtios sp., was purified by a normal phase silica HPLC-column with hexane/ethyl acetate (70/30) solvent system and identified by 1H and 13C NMR spectra. Its biological effects were tested on the resistant chronic myeloid leukemia cancer cell line K562 by reporter gene analysis, electrophoretic mobility shift analysis and I-kB degradation. This molecule was involved in different cellular processes, as well as cell cycle, apoptosis, mitogen-activated protein kinases (MAPKs) pathway, NF-κB activation cascade and anti-inflammatory action. In details, the heteronemin was able to inhibit TNFα-induced NF-κB activation in a dose-dependent manner in K562 cells, to prevent the TNFα-induced degradation of IκBα and the subsequent translocation of p50 and p65 to the nucleus, and, thus, to strongly attenuate the IkBα phosphorylation [38].

The dideoxypetrosynol A polyacetylene has been isolated from the sponge Petrosia sp. by methanol extraction and purified by HPLC chromatography. This compound resulted to have a significant selective cytotoxicity against several human tumor cell lines by hemocytometer counts, fluorescent microscopy, agarose gel electrophoresis and flow cytometry analysis. In detail, the dideoxypetrosynol A exhibited anti-inflammatory properties through the inhibition of the arachidonic acid (AC) pathway on human leukemia U937 cell line and also proapoptotic activity on human skin melanoma cells through the mitochondrial pathway [39]. Its anti-inflammatory effect was verified by cyclooxygenase-2 downregulation at mRNA and protein level and by decrease of inflammatory mediator prostaglandins in leukemia cells.

Moreover, theopederins K and L from the marine sponge Discodermia sp. and mycalamide A from Mycalia sp. were obtained by methanol extraction. They had an inhibitory activity on IL-8 secretion in various pancreatic cancer cell lines by cell-based enzyme-linked immunosorbent assay [40]. Several and different sponges such as Haliclona sp., Pachypelma sp., Pellina sp. and Xestospongia sp. contain an alkaloid, manzamine A, obtained by methanol extraction, with anti-inflammatory and anti-cancer activities by cell viability and apoptosis assays and morphological changes. In pancreatic cancer cell lines this compound induces a decrease of phosphorylated p65 NF-κB by Western Blot analysis [41].

Also our laboratory has demonstrated the anti-inflammatory effects of a methanol extract from the marine demospongiae Geodia cydonium obtained by methanol and chloroform extraction, on human estrogen-responsive breast cancer cell line, MCF-7. We showed that, in a dose-dependent manner with increasing levels of sponge extract, a decrease in proinflammatory cytokine levels compared to untreated cells was observed [42]. In particular, we demonstrated a reduction of the vascular endothelial growth factor (VEGF) levels and other proinflammatory cytokines as CCL2, CXCL8, CXCL10, IFN-γ, and TNF-α on cellular MCF7 supernatants after treatment in a dose-dependent manner by multiplex biometric ELISA-based immunoassay and a decrease in the expression of two NFkB1 and c-Rel subunits by RT-qPCR experiments. These data highlighted that this extract of G. cydonium possesses an anti-inflammatory activity mediated by NF-κB inactivation. In 2017, our group evaluated the putative anti-inflammatory effect of an active fraction obtained from the same methanol extract of G. cydonium subjected also to LC-HRMS characterization, on three breast cancer cell lines, the estrogen-responsive MCF7 cells, and two triple-negative MDA-MB231 and MDA-
MB468 cells. In this case, the results demonstrated that: i) in MCF-7, MDA-MB231 and MDA-MB468 cells, the levels of pro-inflammatory IL-8 decreased; ii) in MCF-7 and MDA-MB231 cells, the levels of VEGF decreased; iii) only in MCF-7 the level of pro-inflammatory CXCL10 decreased; iv) only in MDA-MB231 cells the level of anti-inflammatory IL-4 increased; and v) only in MDA-MB468 cells the level of anti-inflammatory IL-10 increased. In the context, as in the previous paper, the data evidenced a slight anti-inflammatory effect of this fraction on estrogen-responsive and triple-negative breast cancer cell lines [43].

General Conclusions

Considering that the inflammatory processes lead to the development of several diseases including cancer, the need to discover new drug entities is urgent and represents a strong push to explore the marine environment for new pharmaceuticals. In fact, the marine environment harbour a great number of micro- and macroorganisms, very peculiar in their metabolic mechanisms for the biosynthesis of secondary metabolites. As reported in this review, marine organisms and mainly the sponges represents a very rich source of biologically active compounds with pharmacological applications, claiming considerable attention from the health science communities. Hence the use of bioactive compounds of marine origin could represent attractions in the future years in the management of several diseases for their potential anti-inflammatory and anticancer.

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Conflicts of Interest: The authors declare no conflict of interest.

References


