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Teaser This paper reviews the most recent findings in the search for anti-MMP molecules from marine invertebrates regarding their use in the management and treatment of neuroinflammation.

Marine pharmacology: therapeutic targeting of matrix metalloproteinases in neuroinflammation

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Alterations in matrix metalloproteinase (MMP) expression and activity are recognized as key pathogenetic events in several neurological disorders. This evidence makes MMPs possible therapeutic targets. The search for substances that can inhibit MMPs is moving progressively toward the screening of natural products. In particular, marine bioprospecting could be promising for the discovery of marine natural products with anti-MMP activities. Despite recent advances in this field, the possibility of using marine MMP inhibitors (MMPIs) for the treatment of neuroinflammation is still under-investigated. Here, we review the latest findings in this promising research field and the potential that marine MMPIs can have in the management and treatment of various neurological diseases.

Eugenia Gentile received her Master's Degree in Environmental Science at "Ca' Foscari" University of Venice (2011) and her PhD in Environmental Science at the University of Bari (2016). Her field of interest covers marine ecology and management of marine resources. Her research is focused on the isolation and biochemical characterization of biologically active compounds extracted from marine *Demospongiae*, with particular emphasis on the identification of biologically active compounds that can exert anti-MMP activity.

Grazia Maria Liuzzi obtained her PhD in Biochemistry at the University of Bari and is currently Associate Professor of Biochemistry at the University of Bari. Her research focuses on the role of proteolytic enzymes in the pathogenesis of neurological diseases such as multiple sclerosis and HIV-associated neurological diseases, with particular attention to their role as therapeutic targets. Recently, her scientific interest is also addressed in studying the impact of environmental factors on glial cells by investigating how inflammation and oxidative stress can be related to the cellular response under exogenous stimuli.

Matrix metalloproteinases (MMPs) are neutral enzymes that can degrade most components of the extracellular matrix (ECM), playing a key role in physiological tissue remodeling as well as in wound healing and inflammatory states [1]. Experimental evidence underlines how these enzymes are pivotal in central nervous system (CNS) development and physiopathology; moreover, they are involved in recovery after injury as well as in the pathogenesis of some brain diseases [2] therefore MMPs have been proposed as therapeutic targets. To date, the scientific community has described and designed many compounds that can inhibit MMPs, which can be beneficial in the management and treatment of various diseases [3]. However, most of the synthetic MMP inhibitors designed until now showed some downsides such as poor selectivity, low oral bioavailability, improper metabolism and side effects. For these reasons they have failed in clinical trials [4]. Therefore, the search for substances that can inhibit MMPs is moving progressively toward the screening of natural compounds. Until now, some natural MMP inhibitors (MMPIs) extracted from terrestrial sources have been reported [5]. However, in recent years marine bioprospecting (see Glossary) for the identification of compounds with anti-MMP activity appears to be more promising [6]. On the basis of these considerations, we review the marine natural MMPIs discovered so far, with regard to a future application for the treatment of neuroinflammation.

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MMPs: an overview

MMPs are a family of calcium-dependent endopeptidases that include 23 human and 23 murine members [7]. These enzymes have the ability to degrade most components of the ECM, having a central role in tissue remodeling with important implications in fetal development as well as in wound healing and inflammatory states. In recent years, an increasing number of scientific papers have reported that MMPs could act on a variety of substrates such as peptide growth factors, tyrosine kinase receptors, cell adhesion molecules, cytokines and chemokines [8]. MMPs can induce the proteolytic activation or the degradation of these molecules influencing cell functions at different levels – inducing cellular differentiation or migration and regulating growth factor activity, apoptosis, angiogenesis, inflammation and signaling [9].

On the basis of their structure and substrate affinity, MMPs can be divided into six groups: collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT) MMPs and other MMPs. Structurally, most MMPs share a conserved domain structure consisting of a signaling sequence, a propeptide, a catalytic domain, a hinge region and a hemopexin-like domain (Fig. 1) [10]. The signaling sequence (pre-domain), localized at the amino-terminal end of the protein, targets the enzymes after secretion; the propeptide domain (also called the pro-domain) contains a cysteine switch motif that keeps pro-MMPs in the inactive form by a cysteine–zinc binding interaction; the catalytic domain contains a zinc-binding motif in the active site in which three histidines bind the catalytic zinc ion; a proline-rich hinge region links the catalytic domain to the C-terminal hemopexin-like domain – the latter determines the specificity to substrate or ligands, contributing to subcellular localization and inducing activation or inhibition of various MMPs. Beyond this archetypal structure, the family of mammalian MMPs has evolved into different groups by removing some domains or by incorporating others that are absent in the previously described basic core (Fig. 1).

MMP regulation and inhibition

Cells possess multiple strategies to regulate extracellular proteinases: transcriptional regulation, trafficking of membrane-bound forms (secretion and endocytosis); activation of latent proenzymes; extracellular-binding proteins; and endogenous inhibitors. In physiological conditions, healthy tissues show a low proteolytic activity of MMPs. Several factors could induce the production of MMPs whereas the proteolytic activity of these enzymes is, in turn, regulated by various activators and inhibitors. MMP expression is upregulated at the transcriptional level by several inflammatory cytokines and growth factors including tumor necrosis factor (TNF)-α, interleukin (IL)-1, epidermal growth factor (EGF) and transforming growth factor (TGF)-β. Moreover, chemical agents, physical stress and oncogene products, as well as a wide range of hormones and tumor promoters, can induce MMP activity or expression [11].

MMPs are synthesized as inactive zymogens, with the cysteine residue in the pro-domain that binds the zinc ion present at the catalytic site keeping the enzyme in a latent pro-form. Activation requires removal of the propeptide domain through a conformational change, the so called 'cysteine switch', which allows the exposition of the active catalytic site. Several mechanisms that lead to the activation of pro-MMPs have been described, most of them involving proteolytic cleavage of the pro-domain carried out by
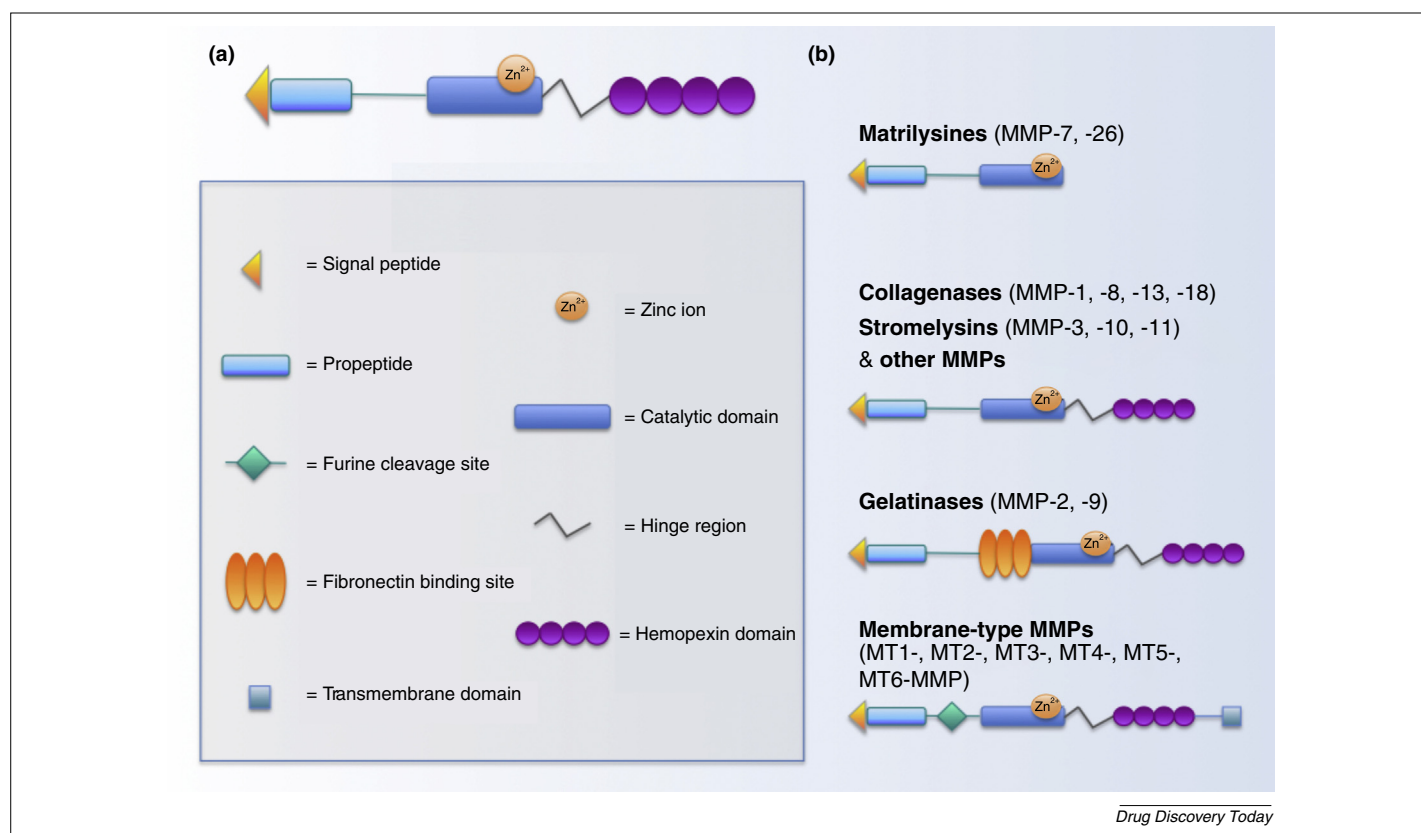
FIGURE 1

Molecular structure of matrix metalloproteinases: **(a)** basic molecular structure of MMPs; **(b)** structural classification of major MMPs based on their domain arrangement.

some proteinases, for example furin, plasmin, tissue kallikrein, trypsin and MMPs themselves [12]. Other than being regulated at the transcriptional level and by post-translational modifications, MMP activity is influenced by endogenous inhibitors such as tissue inhibitors of metalloproteinases (TIMPs), α2-macroglobulin, α1-antiprotease, heparin and reversion-inducing cysteine-rich protein with Kazal motifs (RECK) [13]. TIMPs can be considered the key inhibitors in the tissues. The TIMP family consists of four members (TIMP-1, -2, -3, -4) of small (20–21 kDa) multifunctional proteins, variably glycosylated, that are expressed by cells in various tissues and body fluids. TIMPs inhibit MMPs by binding to their catalytic site to form a tight 1:1 noncovalent complex that keeps the enzyme in a latent form [13]. The four known TIMPs competitively and reversibly inhibit the activity of all MMPs; moreover they share many properties but also have distinct activities, suggesting that they might have other specific physiological roles. Many authors attributed the biological functions of TIMPs to sequences within the N-terminal domain, although the C-sub-domain mediates interactions with the catalytic domains of some MMPs and with the hemopexin domains of MMP-2 and MMP-9 [13]. Some authors highlighted that MMP/TIMP balance is a crucial factor in controlling the overall proteolytic activity *in vivo* and therefore in the maintenance of normal physiological conditions [2].

Physiological role of MMPs in the CNS

Experimental evidence underlines the important role of MMPs in CNS development and in maintaining normal physiological functions such as synaptic plasticity, learning and memory. During ontology and early development, MMPs seem to be involved in different processes such as neurogenesis, angiogenesis, axonal guidance and in the development of oligodendrocytes and their formation of myelin (Fig. 2) [14,15].

In many processes of nervous system development, including migration of neuronal precursors, axonal growth, myelinogenesis and angiogenesis, it is necessary for substantial rearrangements of the ECM, with the digestion of some components that are replaced by new matrix. ECM, which is composed of molecules synthesized by neurons and glial cells, affects many aspects of nervous system development and function [16]. During early development, ECM gives structural and functional support to neural cells and has crucial roles in their proliferation, migration and differentiation. These phenomena take place with an important contribution of MMPs [17]. By contrast, in the mature brain, ECM supports multiple physiological processes and undergoes a slow turnover, restraining structural plasticity. In fact, the mature ECM environment seems to play an inhibitory part in plasticity and remodeling of the neural network [18]. Therefore, the remodeling of ECM, regulated through precise proteolytic processes, is crucial for the health and function of neurons and for the structural plasticity of neuronal circuits [19]. In this context, the ability of MMPs to regulate synaptic plasticity in the healthy mature CNS is relevant, affecting learning and memory [20]. These rearrangements are regulated by proteolytic disassembly of the ECM through an intricate process involving cleavage of specific sites by extracellular proteinases [14].

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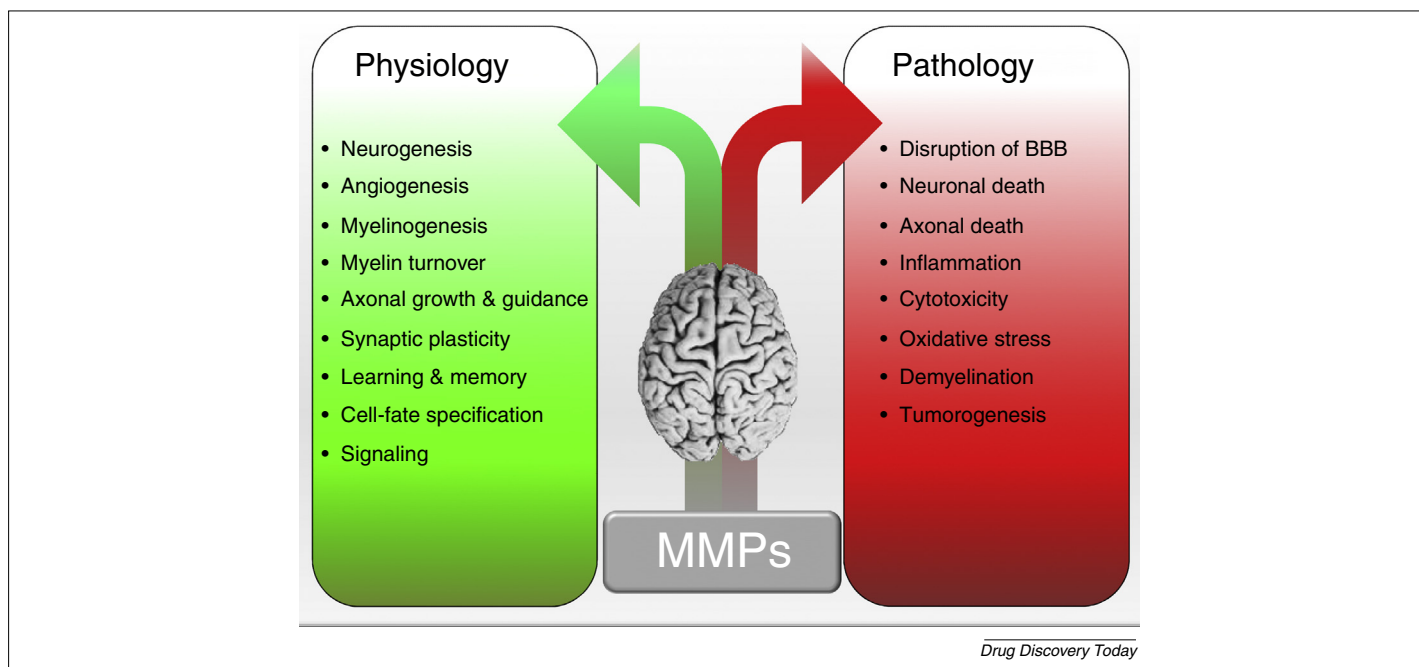
FIGURE 2

Beneficial and detrimental roles of matrix metalloproteinases within the central nervous system in physiological and pathological conditions.

MMP substrates possess well-known roles in synaptogenesis, synaptic plasticity and long-term potentiation [21].

There are wide variations of MMP expression in different neuronal developmental phases [22]. Moreover, in the nervous system the expression profiles of constitutive and inducible MMPs in adult healthy brain vary enormously between regions, cell types and species. In particular, in the adult brain MMP-2 and MMP-9 have been found in astrocytes, microglia and neurons of humans and rodents. MMP-9 can additionally be found in myelinated fibers. MMP-1 has been immunolocalized in neurons, whereas microglia is also immunoreactive for MMP-7 and several MT-MMPs [8,14]. Although there are many studies on the role of MMP expression in the nervous system, so far many functions of MMPs in the healthy CNS still remain undefined.

Role of MMPs in neuroinflammation

CNS injuries such as brain trauma, ischemic injuries, immunological reactions and infections trigger a cascade of events, broadly defined as neuroinflammation, that involve cytokine and chemokine response associated with production of free radicals and proteases [23]. Experimental evidence indicates that the neuroinflammatory process plays a major part in the pathogenesis of various diseases of the CNS leading to neural damage and death. MMPs are actively involved in all these phenomena, thus playing a key part in various neuroinflammatory and neurodegenerative diseases of the CNS as well as in response to injury [2]. Many authors [24–26] have extensively reviewed the implication of MMPs in the pathogenesis and development of acute and chronic neurological diseases. Here, we want to highlight the relevance of MMPs in acute neuroinflammation as well as in the most common neurodegenerative diseases that affect the brain.

In the initial phases of the acute inflammatory process of hypoxia–ischemia, MMPs and free radicals attack proteins of the tight junctions (TJs) and components of the basal lamina that surround cerebral blood vessels, causing edema, hemorrhage and cell death (Fig. 3a) [27]. There are indications that, during transient focal ischemia, MMP-2, -3 and -9 increase the permeability of the blood–brain barrier (BBB) by degrading the components of the basal lamina and the TJ proteins and that inhibitors of MMPs can reduce BBB damage [28,29]. A recent study demonstrated that MMP-12 is upregulated in rats subjected to ischemia and that its suppression inhibits the degradation of TJ proteins and protects BBB integrity [30]. Similarly, caveolin-1, an integral membrane protein located at caveolae, can prevent the degradation of TJ proteins and protects BBB integrity by inhibiting MMP activity [31]. Activated MMP-9 actively contributes to cerebral vascular damage as demonstrated by the reduction of the cerebral infarct size in MMP-9 knockout mice and after treatment with MMP inhibitors [32]. Other authors demonstrated that, after a stroke injury, MMP inhibition reduces the migration of neuroblasts from the subventricular zone to the injured area [33].

Multiple sclerosis (MS) is a chronic inflammatory disorder of the CNS characterized by demyelination in the brain and spinal cord and axonal loss within the CNS. MS is manifested through the breakdown of the BBB associated with infiltration of various types of peripheral blood immune cells such as T cells, dendritic cells and monocytes/macrophages into the brain parenchyma. Although several studies demonstrated that alteration of various MMPs contributes to the development of MS, a convergence of data indicate MMP-9 as the key factor involved in different steps of MS pathogenesis (Fig. 3b).

During the acute MS phase, MMP-9 levels are elevated in the cerebrospinal fluid (CSF) and are related to magnetic resonance imaging (MRI) activity [34,35]. Liuzzi et al. [36] demonstrated the intrathecal synthesis of MMP-9 and found a significant inverse correlation between MMP-9 and its endogenous inhibitor TIMP-1,
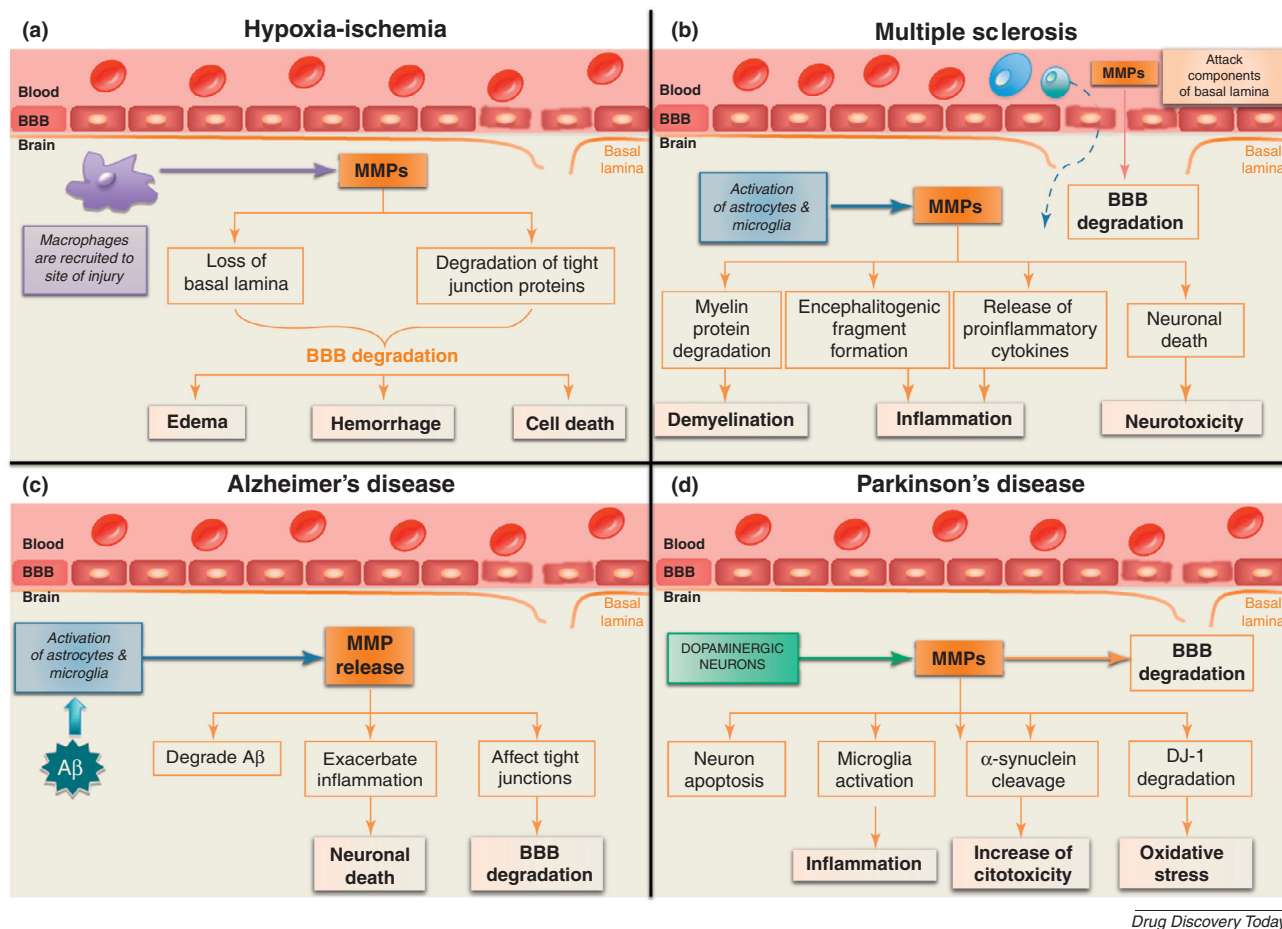
FIGURE 3

Role of matrix metalloproteinases (MMPs) in the pathogenesis of neurological diseases. **(a)** In the acute inflammatory process of hypoxia–ischemia, macrophages and microglia, recruited to the injury site, activate MMPs, contributing to blood–brain barrier (BBB) disruption by degrading the tight junction proteins and the components of the basal lamina. BBB disruption leads to edema, hemorrhage and cell death. **(b)** MMPs contribute to several steps of multiple sclerosis pathogenesis: (i) the breakdown of BBB associated with infiltration of peripheral blood immune cells into the brain parenchyma; (ii) the release and activation of proinflammatory cytokines; (iii) the degradation of myelin proteins resulting in the formation of encephalytogenic fragments; (iv) neuronal death. **(c)** In Alzheimer's disease the deposition of amyloid β (Aβ) plaques in the nervous tissue results in the activation of microglia and astrocytes which, in turn, induce the production of MMPs which contribute to the degradation of the BBB. **(d)** MMPs participate in the pathophysiology of Parkinson's disease contributing to dopaminergic apoptosis, microglia activation, cleavage of α-synuclein and DJ-1 degradation events that lead to inflammation, cytotoxicity, oxidative stress and BBB degradation.

indicating that in MS patients the increase in MMP-9 and the decrease in TIMP-1 serum levels could contribute to BBB disruption and T lymphocyte entry into the CNS. The involvement of MMP-9 in mechanisms of BBB disruption is also supported by the demonstration that the treatment of MS patients with steroids reduces levels of MMP-9 and restores BBB integrity [37]. Similarly, interferon (IFN)-β treatment reduces MMP-9 serum levels, suggesting that the clinical efficacy of IFN-β in MS patients could also result from the ability of this drug to interfere with the production of MMP-9 [38]. The increase of MMP-2 serum levels during the chronic progression of MS was also reported [39]. In the brain lesions of MS patients, microglia and astrocytes show an increased expression of MMPs [40,41]. By using an *in vitro* model it was also demonstrated that IFN-β significantly inhibited the expression of MMP-2 and MMP-9 in lipopolysaccharide (LPS)-activated astrocytes and microglia [42]. The role of MMPs in the pathogenesis of MS also includes the direct destruction of myelin proteins and the

activation of cytokines. There are several experimental reports that show myelin basic protein (MBP) is a direct proteolytic substrate for MMP-9, suggesting a pathogenetic role for this enzyme in the mechanism of demyelination [43,44]. Finally, another mechanism by which MMPs might promote inflammation is by the conversion of pro-TNF-α into its mature soluble form [45].

Vascular cognitive impairment (VCI) refers to a broad spectrum of diseases (from early cognitive decline to dementia) related to vascular causes, resulting in the progressive damage of the deep white matter (WM) often accompanied by BBB disruption and demyelination. Although the etiology of VCI is still not clearly defined, different studies suggest the involvement of MMPs in WM lesion formation [46]. By using a rat model of chronic cerebral hypoperfusion, an increase in MMP-2 levels was shown in endothelial cells and microglia in the WM [47]. Nakaji *et al.* [48] investigated the involvement of MMP-2 in BBB disruption and the subsequent WM lesions after chronic cerebral hypoperfusion

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of rats and demonstrated that the treatment with AG3340, a selective MMP-2 inhibitor, reduced the severity of WM lesions and the number of activated astroglia and microglia. Similar results were obtained in MMP-2 knockout mice, suggesting that MMP-2 plays a crucial part in BBB disruption, glial cell activation and WM lesion formation, indicating its role in myelin damage. Pathological studies on brain tissue from patients with VCI showed that patients present gliotic regions with reactive astrocytes that overexpress MMP-2 and MMP-3 immunopositive macrophages around damaged blood vessels [48], suggesting that MMPs might damage blood vessels through disruption of the BBB, activation of microglia and recruitment of macrophages [49]. Taken together, these results suggest that MMPs could be biomarkers and therapeutic targets of VCI.

Alzheimer's disease (AD) is a neurodegenerative disease of the elderly characterized by gross atrophy of affected cerebral cortex caused by neuronal cell death and synaptic degeneration. The hallmark of AD is the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles, which are linked to cerebral atrophy. The amyloid plaques result from the aggregation of small peptides of about 40 amino acids called amyloid-β peptides (Aβ) formed by the combined action of β- and γ-secretases. The deposition of Aβ in tissues around the plaques results in the activation of microglia and astrocytes which, in turn, induces the production of MMPs. An in vitro study showed that astrocytes exposed to Aβ were induced to the secretion of MMP-2, MMP-3 and MMP-9 [50]. Other authors demonstrated that MMP-9 was expressed in neurons and plasma of AD patients [51,52], whereas MMP-3 expression was detected in hippocampal neurons around amyloid plaques [53]. It was suggested that the increase of MMP expression in blood and brain tissue of AD patients exacerbates the inflammatory response and contributes to neuronal death [54] (Fig. 3c).

Parkinson's disease (PD) is a common neurodegenerative disorder resulting from selective degeneration of dopaminergic neurons in the substantia nigra (SN), associated with microglia activation. PD is characterized by motor symptoms such as weakness, tremor, rigidity, bradykinesia and postural instability. Several studies demonstrated that MMPs and TIMPs are disregulated in the SN of PD patients suggesting a correlation with the death of dopaminergic neurons [55]. The contribution of MMPs to the pathophysiology of PD includes microglia activation, inflammation, dopaminergic apoptosis, BBB disruption and modulation of α-synuclein (Fig. 3d). Studies with MMP-3 knockout mice suggested that MMP-3 is a key player in dopaminergic neuronal degeneration [56]. Expression and activity of MMP-3 have been shown in the SN of postmortem PD brain and in Lewy bodies (LBs), which represent a pathologic hallmark of PD [57]. In an in vitro study, apoptotic dopaminergic neurons released MMP-3 which was able to activate microglia, suggesting an important role as a signaling molecule mediating the interaction between apoptotic neurons and microglia [58,59]. MMP-3-mediated activation of microglia promotes the release of proinflammatory cytokines which induce neuronal death [2]. Activated MMP-3 could cleave α-synuclein into fragments that aggregate increasing neurotoxicity [57] and could also degrade the antioxidant DJ-1 protein resulting in increased oxidative stress [60]. MMP-9 has also been implicated in PD development because its higher promoter activity as a result of C(1562)T polymorphism was observed in a recent study [61].

Here, we have shown that in many pathologies of the CNS the upregulation of certain MMPs, shortly after an acute insult or in the active state of a chronic injury, is detrimental and contributes to the exacerbation of the disease. However, emerging direct and indirect experimental evidence suggests that when some MMPs are expressed at discrete levels by specific cell types during the repair or the recovery phase of the disease they might have beneficial effects. For example, in brain ischemia, late-phase repairing by MMPs is thought to promote angiogenesis and neurogenesis. This is highlighted by the observation that treatment with MMPIs at 7 days after stroke suppresses neurovascular remodeling, increases ischemic brain injury and impairs functional recovery [62]. Hsu et al. [63] observed a delayed expression of MMP-2 after 7–14 days from traumatic spinal cord injury suggesting that this enzyme is necessary for ECM remodeling and functional recovery. In AD, MMPs participate not only in the formation of plaques but also in clearance of Aβ [64]. Indeed, experimental evidence demonstrated that the degradation of Aβ by MMPs results in the reduction of Aβ deposit [51,65].

Other studies indicated that oligodendrocytes utilize MMP-9 to extend their processes, which is a prerequisite for remyelination [66]. In this respect, other authors, using a MMP-9 null mice model, demonstrated that MMP-9 is necessary to promote maturation of oligodendrocytes and remyelination 7 days after lysolecithin-induced demyelination of the spinal cord [67]. However, other studies on knockout mice and the amelioration of disease pathology in response to the inhibition of MMPs suggest that the overall effect of MMPs in CNS pathologies is detrimental. Therefore, the use of drugs or natural compounds able to counteract the increased expression and activity of MMPs in tissues and body fluids represents a valid therapeutic approach for the treatment of CNS diseases.

Natural MMPIs from marine invertebrates

Many studies have demonstrated that the excessive production of MMPs is involved in the pathology of many inflammatory and malignant diseases. For these reasons the scientific community, including the pharmaceutical industry, has focused attention on the design of synthetic substances that can be used as inhibitors of MMPs for therapeutic purposes [68]. The first-generation MMPIs consisted mostly of peptides and their derivatives that, by simulating MMP substrate, chelated Zn^{2+} in the active site of the catalytic domain. In the design of this kind of inhibitor chelating groups such as hydroxylamine, carboxyl, thiol were chosen. Among those, initially British Biotech's batimastat (BB-94) and marimastat (BB-2516) were very successful. Both present a strong Zn^{2+}-chelating group, hydroxamate, providing them with strong MMP inhibitory activity. Despite initial interest, these compounds soon showed some downsides such as poor selectivity, low oral bioavailability, improper metabolism and side effects like musculoskeletal pain and inflammation, leading to the failure of clinical trials [4].

In the past 20 years, many MMPIs have been formulated, but only a few are still being investigated. Therefore, design and development of selective MMPIs remain at early stages. This is mainly due to the poor selectivity toward specific MMP members to the nonspecific blocking of unrelated zinc proteases along with the evidence that the high homology within the MMP family

impedes the advancement in specific inhibitor development [3,69] (Box 1).

In recent years, increasing attention has been given to the search for natural inhibitors, with the identification of several

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BOX 1

Shaping the perfect MMPI: challenges and perspectives

Matrix metalloproteinases (MMPs) play a pivotal part in the pathogenesis of cancer, arthritis, neurodegenerative diseases as well as inflammatory states. This has led the scientific community to identify MMPs as an important therapeutic target. Over the past 30 years, there has been an intense search for inhibitors able to block the detrimental activity of these enzymes and several synthetic compounds have been proposed as MMP inhibitors (MMPIs); some of them entered clinical trials for cancer and other pathologies but, despite high expectations, the results were disappointing. Indeed, most of the clinical trials failed owing to low selectivity, side effects and lack of effectiveness. One of the reasons for this failure is attributable to the discovery that, not only in physiological processes but also in the recovery from injury, proteolysis by MMPs can have beneficial effects. Therefore, only more-precise information on the specific part played by the single MMPs during disease progression could allow therapeutic intervention without blocking beneficial actions of MMPs. MMP-deficient mice have been extensively used to obtain such information, although none of the available animal models resembles perfectly the complex human situation and this makes it difficult to extrapolate the outcome of MMP inhibition from animal models to humans.

Greater efforts should be made to design and develop more-selective MMPIs. A possible approach might be the use of new proteomic techniques that allow the determination of a finer structural characterization of MMPs. This, together with a precise knowledge of their physiological role as modulators in biological processes, would result in a better discrimination between detrimental and essential MMPs. The perfect MMPI should present high selectivity, good oral bioavailability and convenient pharmacokinetics without showing toxicity. This is particularly true in the treatment of chronic diseases, which require continuous drug supply.

To increase selectivity, the new generation of inhibitors should be designed on the basis of substrate-binding specificity. In this respect, strategies for MMP inhibition have progressed beyond the development of antibody-based MMPIs that are highly selective and possess great potential for therapy [111]. Another proposed approach is based on the concept of 'tailoring' tissue inhibitors of metalloproteinases (TIMPs) to selectively inhibit specific MMPs [112]. However, although these approaches showed promising results in preclinical studies, the bioavailability of these compounds still represents an unresolved problem.

To avoid toxicity and increase safety, future directions should focus on the improvement of delivery systems targeted to specific tissues to reduce drug dosage. In this respect, nanotechnology-based tools could be useful for the treatment of neurological diseases, allowing selective delivery of the drug across the blood–brain barrier (BBB) to specific areas of the central nervous system (CNS), increasing drug efficacy. In addition, another promising approach to modulate MMP expression is based on the use of nanoparticles to act as non-viral gene delivery vectors for MMP gene silencing. In the light of these considerations, MMP inhibition certainly represents a feasible therapeutic approach for the treatment of CNS diseases, but the success of this pharmacological strategy is strictly related to a better understanding of the physiological and pathological roles of these enzymes and, consequently, to the availability of more-selective MMPIs.

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compounds extracted from terrestrial sources that can inhibit MMPs. In particular, 90 kinds of extracts from clinical application herbal medicines have been screened [70], demonstrating that the extracts from Baicalin, Cinnamon, Euonymus and Magnolia have strong inhibitory effects toward MMPs. However, in recent years marine bioprospecting appears to be more promising for the identification of compounds with anti-MMP activity. In 2010 Thomas and Kim [71] reviewed the past research work carried out on MMPIs derived from the different classes of marine organisms, outlining the specific areas of metalloproteinase research in a perspective manner. The properties of some marine MMPIs have already been described and on the basis of their structures they can be divided into three main classes: marine saccharoid MMPIs; marine flavonoids and polyphenol MMPIs; and marine fatty acid MMPIs [6]. A recent review discusses a remarkable number of MMPIs extracted from edible seaweed together with their applications in the pharmaceutical sector [72].

Here, instead, we have taken into account the compounds extracted from marine invertebrates that, for their particular adaptation to environmental conditions (Box 2), produce various substances that can show biological activities such as inhibitory ability against MMPs as well as anti-inflammatory properties in general. In particular, we reviewed the latest findings in this promising research field, considering the beneficial role that marine MMPIs can have in the management and treatment of various diseases. The compounds from marine invertebrates found in the literature are summarized in Table 1. In addition, the chemical structures of the known compounds reported in this review are shown in Table 2.

Anti-MMP compounds from Porifera

So far the anti-MMP compounds isolated from marine sponges are mostly represented by lipophilic organic molecules, which can exert their anti-MMP inhibitory activity with high selectivity. Among them, the MMP inhibitor ageladine A, with antiangiogenic activity, was isolated for the first time from the marine sponge *Agelas nakamurai* and tested *in vitro* on endothelial cells [73]. This compound not only inhibits MMP-2 but also MMP-1, -8, -9, -12 and -13, whereas its *N*-methylated derivatives did not inhibit MMP-2. Many potent MMPIs exert their action by binding the Zn^{2+} in the catalytic domain; instead, ageladine A seemed not capable of chelating Zn^{2+}, suggesting a different mechanism of inhibition.

Bioassay-guided fractionation resulted in isolation of three new tetramic acid glycosides related to ancorinoside A (*i.e.*, ancorinosides B–D) that could inhibit MT1-MMP [74]. These new metabolites have been extracted from the marine sponge *Penares sollasi* Thiele, collected in southern Japan, and contain two carboxylic acids and a tetramic acid group. The authors suggested that the latter might have an effective role in the inhibition of MMPs.

In another study, during a blinded screening of a number of extracts and bioactive compounds isolated from marine organisms, (+)aeroplysinin-1, extracted from the sponge *Aplisina aerophoba*, was selected by means of its ability to inhibit endothelial cell differentiation and proliferation *in vitro* [75]. This compound, which was able to decrease levels of MMP-2 and urokinase in conditioned medium from endothelial cells, was shown to possess antiangiogenic activity and to inhibit migration and invasion of

BOX 2

Marine invertebrates: a treasure from the depths

Marine invertebrates contribute greatly to the deep-sea life and are characterized by a wide range of morphologies, adaptations and ecological behaviors. Most of them are sessile, with soft bodies and a sedentary lifestyle. Porifera, which means pore-bearing, are organisms commonly known as sponges. These organisms are multicellular primitive animals. Marine sponges are sessile and suspension-feeding organisms, which are able to pump water through their porous bodies. They typically use specialized flagellated cells to drive water into their body. By maintaining a constant water flow through their bodies, sponges obtain food such as microorganisms and remove waste. Porifera do not present true tissues and organs. Most of their body cells are totipotent with a high mobility, being able to change position, form and function. Also, for this reason, sponges possess a high level of phenotypic plasticity.

Corals belong to the phylum Cnidaria and they are colonial organisms composed of hundreds of thousands individuals, called polyps, which originate in reef structures. Cnidaria possess a peculiar cell type, nematocyte, with an extrusive organelle used for predation and defense. Many cnidarians, including corals, contain algae called zooxanthellae. These symbiotic organisms are well protected within cnidarian tissues and use metabolic waste products for photosynthesis. In return, corals benefit of organic products derived from photosynthesis to grow and build barriers.

Ascidians (mostly known as sea squirts) belong to Tunicates, a subphylum of the phylum Chordata. These animals are filter feeders, indeed water flows through their basic bodies allowing animals to filter marine suspensions. Sea squirts include solitary and colonial species. Adult organisms are sessile and can be attached to several kinds of substrates.

The phylum Mollusca includes a broad spectrum of organisms with characteristics that make them very different from each other, such as bivalves, gastropods and marine snails. These animals present a soft body and can present an internal or external shell. Mollusca possess well-developed tissues and organs with nervous, circulatory and respiratory systems. Most molluscs have a well-developed muscular foot that presents different morphological adaptations that can be used for clinging to surfaces, digging, anchoring to substrates, swimming and grasping and for locomotion.

Shrimp belong to Decapoda (Crustacea). Animals of this order possess well-developed, hard and calcified exoskeleton (carapace) that covers the head and thorax and protects gills. They are very active and usually are omnivore predators.

Because of their peculiar ecological and morphological features, marine invertebrates have developed defense strategies based on the production of biologically active compounds that serve as chemical weapons against predators, competitors and pathogens [113,114]. These bioactive compounds are usually secondary metabolites that, once released into the water, are quickly diluted and therefore must be very powerful to be really effective [115]. Consequently, the metabolites produced from marine invertebrates can have a significantly higher potential than those from terrestrial habitats regarding pharmacological use.

cells. Taken together these data indicate that aeroplysinin-1 inhibits several essential steps of the angiogenic process, making it a promising drug for further evaluation in the treatment of angiogenesis-related pathologies.

Callysponginol sulfate A is a fatty acid extracted from the marine sponge *Callyspongia truncate* with the ability to inhibit recombinant MT1-MMP with an IC_{50} value of 15.0 µg/ml [76].

Because the desulfated callysponginol A did not show any inhibitory activity against MT1-MMP, the authors assumed that the enzyme inhibition activity was probably a consequence of the presence of sulfate. Halichondramide (HCA), a trisoxazole-containing macrolide isolated from the marine sponge *Chondrosia corticata*, belonging to Demospongiae, has been shown to exhibit cytotoxicity and antifungal activities. It has been demonstrated that HCA exhibits antiproliferative activity *in vitro* against a variety of cancer cells [77,78]. Moreover, the same authors identified the antimetastatic activity of HCA in highly metastatic PC3 human prostate cancer cells [79]. Further analysis revealed that the antimetastatic effect of HCA was correlated with the downregulation of MMPs and the modulation of cadherin switches.

Recently, Di Bari *et al.* [80] evaluated whether water-soluble compounds present in aqueous extracts from seven sponges exert biological activity toward MMPs. The species screened were seven common Mediterranean demosponges: *Tethya aurantium*, *Tethya citrina*, *Hymeniacidon perlevis*, *Ircinia variabilis*, *Chondrilla nucula*, *Aplysina aerophoba* and *Sarcotragus spinosulus*. The results demonstrated that the studied extracts contain water-soluble compounds able to inhibit MMP-2 and MMP-9 activity as well as expression in LPS-activated astrocytes. The sponge compounds with inhibitory activity against MMPs have not yet been determined. However, the authors, as a result of an extensive analysis, assumed that the MMP inhibitory effect was attributed to protein compounds present in crude extracts. Moreover, comparing the anti-MMP activities present in the aqueous extracts from wild and reared specimens of *T. aurantium* and *T. citrina*, the authors reported that the reared sponges maintain the production of bioactive compounds with anti-MMP inhibitory effect for the duration of the rearing period. Taken together, these results indicate that the aqueous extracts from the studied demosponges possess some bioactive anti-MMP compounds, which might have possible pharmacological applications for the treatment of neuroinflammation.

Anti-MMP compounds from Cnidaria

11-Epi-sinulariolide acetate (11-epi-SA) has been isolated from the soft coral *Sinularia querciformis* [81]. This compound was able to significantly inhibit *in vivo* expression of proinflammatory proteins in a rat model of adjuvant induced arthritis. The same compound, isolated from the cultured soft coral *Sinularia flexibilis*, was tested *in vitro* on human hepatoma HA22T cells. Authors showed that 11-epi-SA was able to inhibit cell migration and invasion in hepatocellular carcinoma and alter HA22T cell metastasis by reducing MMP-2, MMP-9 and urokinase-type plasminogen activator (uPA) expression through the suppression of mitogen-activated protein kinases (MAPKs), phosphoinositide 3 kinase (PI3K)/Akt and the focal adhesion kinase (FAK)/Grb2 signaling pathways. Meanwhile, the expression of TIMP-1 and TIMP-2 were increased in a concentration-dependent manner [82]. These findings suggest that sinulariolide could be a good candidate for potential pharmaceutical applications and needs further evaluation as a chemotherapeutic agent for human hepatocellular carcinoma.

Lee *et al.* [83] investigated the effects of lemnalol, a sesquiterpenoid with anti-inflammatory proprieties extracted from the soft coral *Lemnalia* sp., on mast cell (MC) function and osteoclast activity in rats with monosodium urate (MSU) crystal-induced

TABLE 1

Natural matrix metalloproteinase inhibitors from marine invertebrates

Taxon	Compound	Species	Target	Activity	Model	Refs
Porifera	Aeroplysinin-1	*Aplisina aerophoba*	MMP-2 and urokinase	Inhibition	*In vitro* endothelial cells	[75]
	Ageladine A	*Agelas nakamurai*	MMP-2, -1, -8, -9, -12, -13	Inhibition	*In vitro* endothelial cells	[73]
	Ancorinosides B–D	*Penares sollasi*	MT1-MMP	Inhibition	*In vitro* enzyme inhibition assay	[74]
	Callysponginol sulfate A	*Callyspongia truncate*	MT1-MMP	Inhibition	*In vitro* enzyme inhibition assay	[76]
	Halichondramide	*Chondrosia corticata*	Various MMPs	Downregulation	*In vitro* PC3 human prostate cancer cells	[79]
Cnidaria	Lemnalol	*Lemnalia* sp.	TGF-β1, MMP-9, cathepsin K	Downregulation	*In vivo* rat MSU-induced gouty arthritis	[83]
	Sinulariolide (11-epi-SA)	*Sinularia querciformis*	MMP-2, MMP-9	Suppression	*In vivo* rat adjuvant induced arthritis	[81]
		Sinularia flexibilis	TIMP-1, TIMP-2	Increase	*In vitro* human hepatoma HA22T cells	[82]
Tunicates	Nano-heparin	*Styela plicata*	MT1-MMP	Inhibition	*In vitro* breast cancer cells	[85]
Mollusca	Mere15	*Meretrix meretrix*	MMP-2 and MMP-9	Downregulation	*In vitro* human lung adenocarcinoma A549 cells	[86]
	Abalone oligopeptide	*Haliotis discus hannai*	MMP-2 and MMP-9	Inhibition	*In vitro* human fibrosarcoma HT1080 cells	[87]
Crustacea	Heparin-like compound	*Litopenaeus vannamei*	MMP-9	Inhibition	*In vitro* human leukocytes	[88]

gouty arthritis. Immunohistochemical analysis showed that administration of lemnalol reduces MSU-induced TGF-β1, MMP-9, cathepsin K and tartrate-resistant acid phosphatase protein expression suggesting that lemnalol treatment could be beneficial for the attenuation of MC infiltration and degranulation and for the suppression of osteoclast activation in gouty arthritis.

Anti-MMP compounds from Tunicates
Few works to date have reported the presence of MMP inhibitory activity in sea squirt. Ascidian tunicate extracts, orally administered in a mouse model of collagen-induced arthritis, have shown the ability to alleviate paw edema and to improve the histological hind leg cartilage status through the reduction of MMP-9 and prostaglandin E synthase levels [84]. These findings suggest that the ascidian extracts contain not yet identified anti-MMP-9 compounds with potential therapeutic effects for the treatment of rheumatoid arthritis.

Piperigkou et al. [85] demonstrated that a nano-heparin formulation isolated from the sea squirt *Styela plicata* has inhibitory effects on cell proliferation, invasion and proteasome activity in a breast cancer cell model. Moreover, nano-Styela regulates cell apoptosis, expression of inflammatory molecules, such as IL-6 and IL-8, and reduces the expression levels of MT1-MMP. These findings indicate that ascidian heparin is an effective agent for heparin-induced effects in important cancer cell functions, providing an important possibility in pharmacological targeting.

Anti-MMP compounds from Mollusca
Two peptides with anti-MMP activity have been isolated from Mollusca. In particular, Mere15 has been purified from the marine bivalve *Meretrix meretrix* Linnaeus, a mollusk that has been used in traditional Chinese medicine for the treatment of cancer. In particular, Wang et al. [86] evaluated the effects of this novel antitumor polypeptide on cell adhesion, migration, invasion, as well as secretion and expression of MMPs in human lung adenocarcinoma A549 cells. Results revealed that Mere15 can downregulate the secretion and mRNA expression of MMP-2 and MMP-9. This study demonstrated that Mere15 is able to inhibit tumor

growth *via* proapoptotic and antimetastatic pathways, proving to be a potential multi-target therapeutic agent for the treatment of human lung cancer.

Nguyen et al. [87] purified Abalone oligopeptide (AOP) with anti-MMP activity from the intestine digests of marine gastropod Abalone (*Haliotis discus hannai*). The results of this study indicated that AOP could inhibit the expression of MMP-2 and -9 in HT1080 cells *in vitro via* the nuclear factor (NF)-κB-mediated pathway, suggesting that AOP might possess therapeutic and preventive potential for the treatment of MMP-related disorders. These findings are particularly interesting because Abalone represents a relevant fishery resource, widely reared for food consumption, which could be exploited for pharmaceutical purposes.
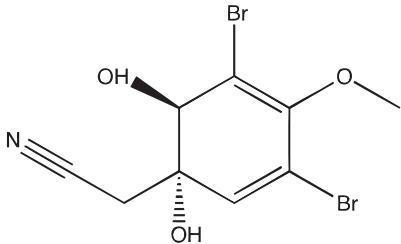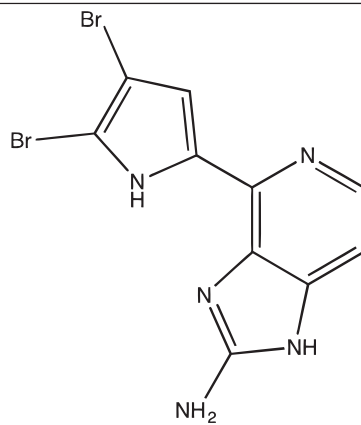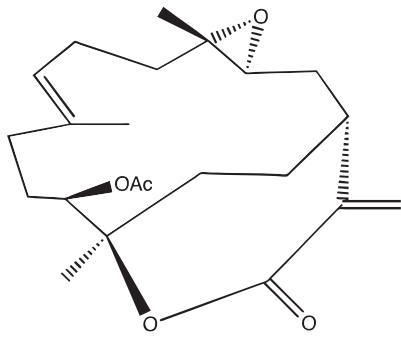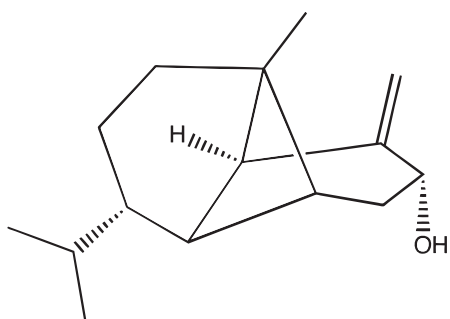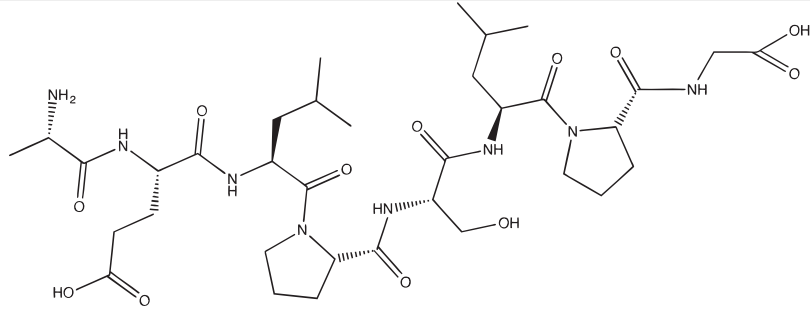
Anti-MMP compounds from Crustacea
Brito et al. [88] studied the anti-inflammatory properties of a heparin-like compound from the shrimp *Litopenaeus vannamei*. This compound has been extracted and purified from shrimp heads. Besides reducing significantly the influx of inflammatory cells to an injured site in an *in vivo* rat model of acute peritoneal inflammation, shrimp heparin-like compound was able to reduce MMP activity in the peritoneal lavage of inflamed animals. Moreover, in another set of experiments, carried out on human activated leukocytes, this compound affected cell migration and inhibited MMP-9 activity, demonstrating that it could interfere with different inflammatory response events.

Nervous system marine pharmacology
To date, the use of marine natural products to treat neuroinflammation is largely underexploited in comparison with other sectors of application. Indeed, only a few papers reported that molecules of marine origin have been tested on neuronal models [89,90]. Pharmacological studies with marine natural products (MNPs) affecting the nervous system mostly involve four areas of neuropharmacology [91,92]: (i) the stimulation of neurogenesis; (ii) the targeting of receptors; (iii) ion channel pharmacology; and (iv) other miscellaneous activities on the nervous system.

TABLE 2

Chemical structures of the reported compounds

Aeroplysin-1	Ageladine A

Sinulariolide	Lemnalol

Abalone oligopeptide

Ancorinoside B
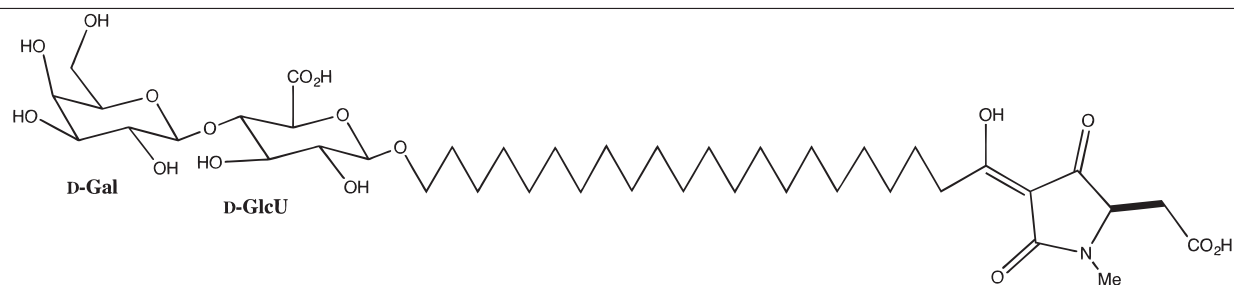
Ancorinoside C

Ancorinoside D

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Halichondramide
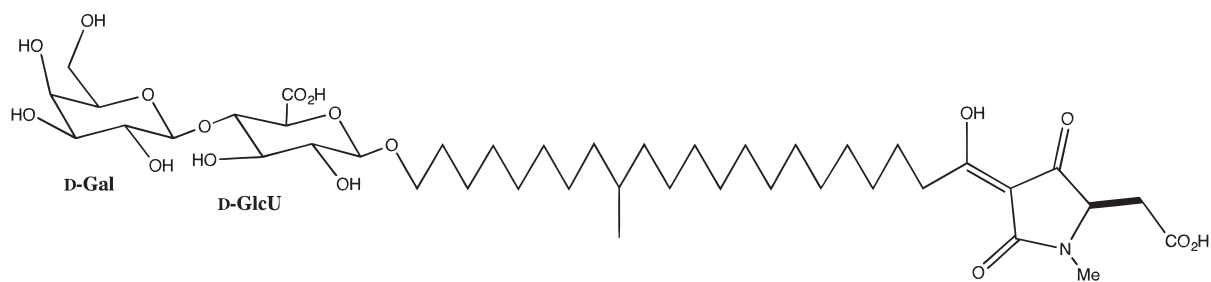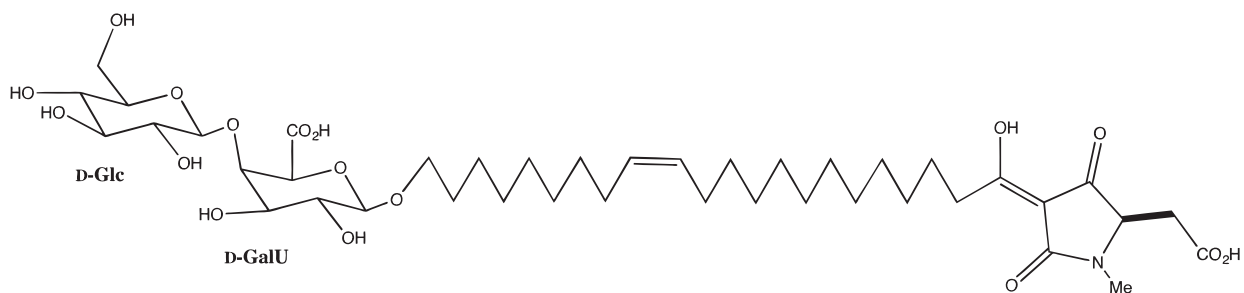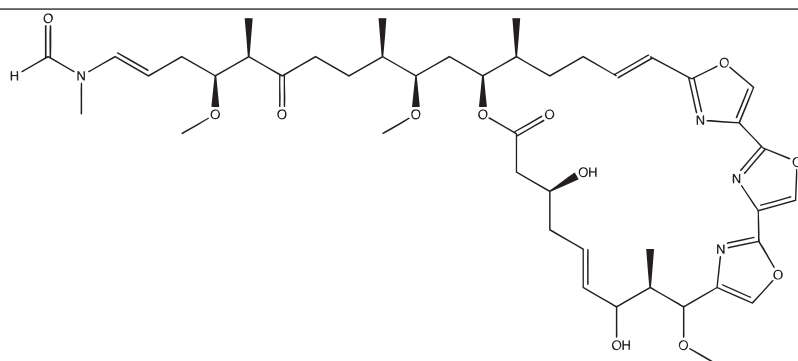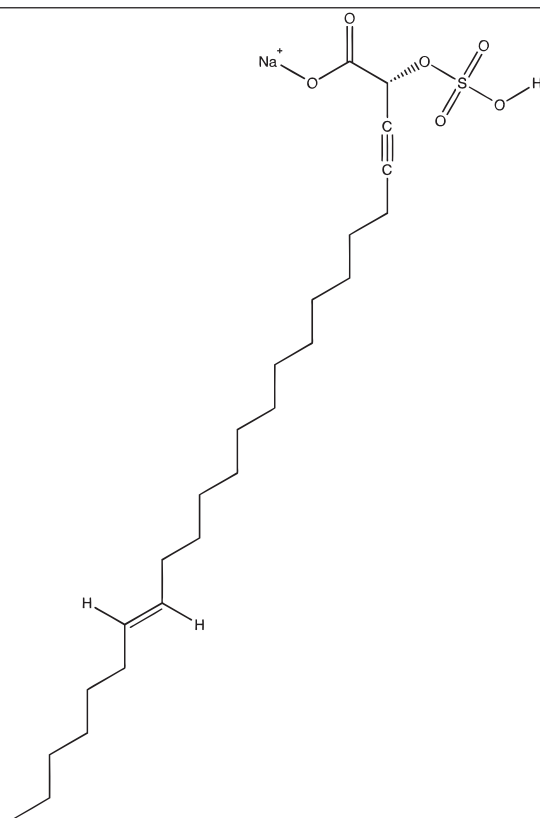

Callysponginol sulfate A

Biologically, active molecules that stimulate neurogenesis and rescue damaged neuronal cells represent potentially promising therapeutic strategies to treat neurodegenerative diseases [93]. The enhancement of the neuritogenic properties of nerve growth factor (NGF), a chemical that has a crucial role in differentiation, survival and neuronal regeneration, was reported for several marine natural compounds. For example, some gangliosides and glycosides from several Echinodermata induced neurite outgrowth and neuritogenic activity in different cell types [94–97].

There are also studies in which MNPs were shown to target receptors present in the nervous system. In this regard, the action of some marine conotoxins as selective antagonists of nicotinic acetylcholine receptors is relevant [98,99]. Another inhibitor of nicotinic acetylcholine receptors is the marine quinolizidine alkaloid pictamine, isolated from the ascidian *Clavelina picta* [100]. Another alkaloid, the 4-acetoxy-plakinamine B, isolated from the sponge *Corticium sp.*, significantly inhibited acetylcholinesterase [101], suggesting its potential use for the treatment of AD.

The outcome of research of MNPs for ion channel pharmacology is interesting [102]. In this respect, a new conopeptide isolated from the marine snail *Conus striatus* selectively targeted *N*-type voltage-sensitive calcium currents in cultured hippocampal neurons

suggesting that it could have therapeutic potential as a novel analgesic agent [103]. Ziconotide (Prialt®) is a widely investigated conotoxin originally isolated from the venom of the marine snail *Conus magus* with potent analgesic properties. It acts by reversibly blocking *N*-type calcium channels located on primary nociceptive afferent nerves in the superficial layers of the dorsal horn of the spinal cord. Ziconotide is used for the treatment of severe chronic pain in patients with cancer or AIDS and could have more potentiality for the management of neuroinflammation [104].

Additional marine compounds were reported to exhibit pharmacological effects on the nervous system with various types of activities such as neurite retraction and neurotransmission inhibition. In this regard, several alkaloids have been isolated from different sponges with promising potential against human neurodegenerative diseases [105–107]. Calyculin, trigonelline and 11-dehydrosinulariolide, molecules extracted from different soft corals, act on different cellular targets with neuroprotective properties. For this reason, they have been proposed for the treatment of nervous system pathologies, such as PD [108,109]. Two hydrocarbons, derived from the soft coral *Capnella imbricate*, showed antineuroinflammatory properties *in vitro* on microglial cells as well as *in vivo* in neuropathic rats; therefore they have been proposed as new therapeutic agents for the treatment of neuroinflammatory diseases [110].

Bryostatin-1 (Bry-1) is a macrolide lactone that deserves attention. Bry-1 was initially isolated from the extract of the brown bryozoans *Bugula neritina* which is exploited in noncorrelated different diseases such as cancer, HIV and neurodegenerative diseases. Preclinical studies showed that Bry-1 is able to enhance spatial learning and long-term memory in rats, mice and rabbits, and to exert neuroprotective effects in a model of AD transgenic mice [90]. Among the new rising pharmacological proprieties of Bry-1 there is the ability to prevent neuronal apoptosis and to enhance synaptogenesis leading to cognitive deficit recovery.

Concluding remarks and future perspectives

The ocean is a treasure trove of biodiversity, hosting most of the global biosphere. Moreover, it holds a number of environments with peculiar conditions that allow the development of special evolutionary adaptations and the production of molecules with unique properties. These MNPs present specific structures and functions that can be exploited in pharmacology. To date, several marine compounds able to inhibit MMPs have been extracted from marine invertebrates, such as Porifera, Cnidaria, Mollusca and others.

Most of these compounds have been studied in cancer models and only a few of them have already been tested for the treatment of neuroinflammation. To overcome this gap, the scientific community should test the marine natural compounds with already proven activity against MMPs on neuronal models. Indeed, as already described in this review, the majority of the MNPs isolated from marine invertebrates exhibit antiangiogenic, antioxidant and antiproliferative properties that represent hallmarks in the pathogenesis of neuronal diseases and therefore might be great allies in the treatment of neuroinflammation.

Other efforts should be made to investigate the anti-MMP potential of MNPs that have already been shown to represent future candidates for the treatment of neurological diseases, such as AD and PD. In this respect, a future therapeutic strategy might focus on the combination of MNPs with anti-MMP activity with well-known anti-inflammatory drugs to exploit their synergistic action for a more specific targeting of MMPs. In conclusion, although still at their infancy, studies examining the possibility of using MNPs to specifically block MMPs in neuronflammation should be strongly encouraged.

Acknowledgements

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