Review

The use of marine-derived bioactive compounds as potential hepatoprotective agents

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The marine environment may be explored as a rich source for novel drugs. A number of marine-derived compounds have been isolated and identified, and their therapeutic effects and pharmacological profiles are characterized. In the present review, we highlight the recent studies using marine compounds as potential hepatoprotective agents for the treatment of liver fibrotic diseases and discuss the proposed mechanisms of their activities. In addition, we discuss the significance of similar studies in Oman, where the rich marine life provides a potential for the isolation of novel natural, bioactive products that display therapeutic effects on liver diseases.

Introduction

The prevalence of liver diseases, including chronic hepatitis and cirrhosis, necessitates effective and cost-efficient treatments. The marine environment may be explored as a rich source for novel antifibrotic drugs. A number of marine-derived compounds are shown to prevent ROS formation, possess anti-inflammatory activities, interfere with the TGF-β and PDGF-β pathways, or affect the NF-κB pathway. Furthermore, most of the compounds have negligible immunogenicity and side effects. This review provides an overview of the recent studies using marine compounds as potential hepatoprotective agents for the treatment of fibrotic liver diseases and discusses the proposed mechanisms of their activities.

## Liver inflammation, fibrosis, and cirrhosis

Although liver disease is caused by a variety of different etiologies, such as alcohol abuse, viral infections (*eg*, hepatitis B or C virus), and metabolic syndrome, resulting in non-alcoholic steatohepatitis, autoimmune diseases, cholestasis, genetic metabolic diseases, drug abuse, or toxin intoxication, the pathogenesis of chronic liver disease is relatively uniform. An inflammatory process is initiated in which the parenchymal cells (*ie*, hepatocytes) are affected by necrosis or apoptosis. This cellular damage leads to hepatic release of molecular mediators, infiltration of immune cells, and inflammation-induced activation of normally quiescent hepatic stellate cells (HSC) and their transdifferentiation into myofibroblasts (MFB) (Figure 1). HSC (formerly also known as vitamin A storage cells, Ito cells, or fat storage cells) are pericytes of the liver, which together with sinusoidal endothelial cells (SEC) and Kupffer cells (KC) line the hepatic sinusoid[1, 2]. As well as storing vitamin A, this cell type is important for the intrahepatic presentation of antigens[3], the removal of apoptotic parenchymal cells, the secretion of important apolipoproteins and growth factors, and the control of blood flow, in addition to acting as a progenitor for damaged hepatocytes and endothelial cells[4]. In liver fibrosis, HSC play a central role because they are stimulated to leave their quiescent phenotype and transform into extracellular matrix (ECM) producing cells (*ie*, MFB). Signs of their activation include the loss of their vitamin A store, the expression of typical activation markers (*eg*, α-smooth muscle actin), the acquisition of contractile properties, and an increase in the synthesis of a variety of pro-inflammatory cytokines, chemokines, as well as other growth factors. All of these mediators initiate a complex network of autocrine and paracrine activities resulting in an increased net synthesis and intrahepatic deposition of ECM components, such as collagens. The quantities of cells capable of forming ECM are further increased by fibrocytes (immigrating from the bone marrow), portal fibroblasts and potentially by cells dedifferentiated by an epithelial-mesenchymal transition in which hepatocytes acquire MFB-like properties via a well programmed

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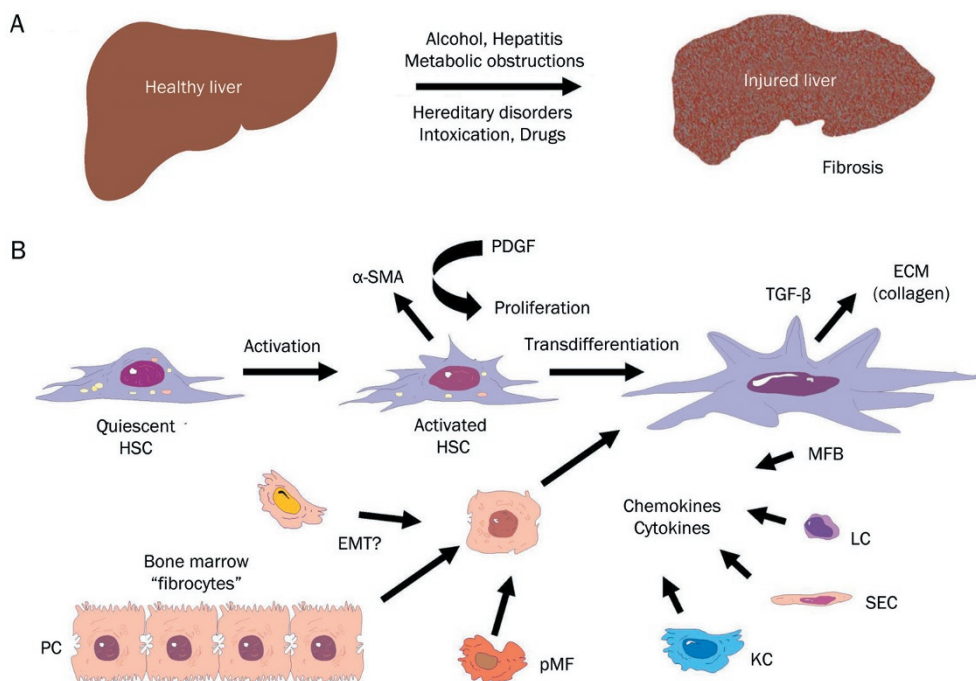
Figure 1. Pathogenesis of hepatic fibrosis. (A) The healthy liver is prompted by various triggers (extensive alcohol abuse, hepatitis infection, metabolic disorders, genetic diseases, intoxication, and drug consumption) to undergo hepatic fibrogenesis resulting in an injured, fibrotic liver; (B) At the cellular level, hepatic fibrogenesis is a complex process. Toxic triggers in the liver promote the activation of quiescent hepatic stellate cells (HSC) that become positive for α-smooth muscle actin (α-SMA), start to proliferate and transdifferentiate into contractile myofibroblasts that have the capacity to synthesize large quantities of extracellular matrix (ECM). Hepatic fibrogenesis is markedly stimulated by platelet-derived growth factor (PDGF) and TGF-β that control collagen expression and cellular proliferation. The events are further triggered by other chemokines and cytokines that are secreted by liver residential cells (KC, Kupffer cells; SEC, sinusoidal endothelial cells) or infiltrating leukocyte (LC) subsets. Moreover, ECM synthesis is further driven by portal (myo)fibroblast (pMF) and bone marrow derived fibrocytes. In addition, whether hepatocytes can transit into cells that are capable of ECM synthesis in a process that is commonly known as epithelial-to-mesenchymal-transition (EMT), is under debate.

transition process[5]. All of these alterations are favored by the soluble mediators that are released by the resident macrophages of the liver (*ie*, KC), SEC, or infiltrating lymphocytes. The modes of action of these molecular messengers are quite different. Transforming growth factor beta (TGF-β), for example, is a pleiotropic effector molecule that triggers the expression of several ECM proteins, while reducing its degradation by inhibiting the so-called matrix metalloproteinases and the synthesis of specific inhibitors of these enzymes. TGF-β further induces the apoptosis of hepatocytes and inhibits cell proliferation and thus is essential to rebuild damaged liver tissue. Other cytokines, such as the platelet-derived growth factor (PDGF)-β, drive the proliferation of activated HSC and thus increase the quantities of cells able to synthesize ECM components[6]. A large set of other cytokines and chemokines are also released by the invaded cells and influence HSC biology through propagating the inflammatory process and thus the fatal sequence of organ damage[1, 7]. The result is liver fibrosis, which is mainly characterized by the intrahepatic deposition of collagen fibers resulting in liver malfunction, the abolition of liver architecture, or even the impairment of liver function. Fibrosis causes a lack of synthesis of important hepatic biomolecules (transport proteins, coagulation factors, and globulins), a deficiency in the detoxification of endogenous (bilirubin,

ammonia, and steroid hormones) and exogenous (drugs) toxic substances, and deficiencies in the regulation of vitamin and trace element metabolism. At this stage, the liver is unable to restore the damaged parenchymal tissue, while the connective tissue is simultaneously increased, resulting in the destruction of the lobular structure. This final phase is called cirrhosis and is regarded as an optional precancerous lesion from which hepatocellular carcinoma may develop.

Currently available drugs/treatments, their efficiency and drawbacks

The molecular characterization of the processes involved in liver fibrosis, including liver inflammation and tissue remodeling, resulted in the development of diverse therapeutic means to prevent or cure the disease. In this regard, two major available approaches have been discussed for several decades[8]. The simplest treatment is the removal of the causal agent, which is a major therapy for liver inflammatory diseases except autoimmune diseases, where the full removal of immune cells is impossible[9]. The second therapeutic avenue consists of modulating the key activities and phenotypic attributes of HSC, including averting their proliferative capacity or their tendency to undergo apoptosis[2, 8]. Tissue fibrogenesis has been considered as obstinately progressive; however, a

more detailed knowledge of the pathogenetic events underlying fibrogenesis and recent research findings suggest potential reversibility[10]. Direct antifibrotic therapies are still lacking with organ transplantation, the sole, major curative option[10]. There has been extensive progress in the treatment of chronic liver diseases caused by the hepatitis C virus (HCV), with approximately 170 million people infected worldwide[11]. Two direct acting antivirals (DAAs), telaprevir and boceprevir, along with pegylated interferon, have been the focus of treatments for HCV genotype 1 chronic infection[12]. However, a need to develop new DAAs to improve the sustained virological response[12] still exists. Also, because IFN has several side effects, DAA combinations without IFN would be beneficial[11].

A clinical trial by Nelson *et al* showed that long-term interleukin-10 therapy in chronic hepatitis C patients has an anti-inflammatory and proviral effect[13]. However, with reduced disease activity, there was an increase in HCV viral loads[13]. Although several of the clinical trials for liver fibrosis failed to provide any conclusive outcomes, they helped increase knowledge that may facilitate future clinical trials and ultimately lead to a cure for liver fibrosis[14].

Therapies against liver injury

The direct relationship between liver inflammation and the progression of fibrosis is well documented. Several anti-inflammatory drugs have been tested for their antifibrotic effects during liver inflammation with corticosteroids representing a major group. However, anti-inflammatory corticosteroids showed mixed outcomes in *in vitro* experiments and clinical trials, and may have undesirable side effects[8, 15]. The methods targeting cytokines, including TGF-β inhibitors that represent the most anti-fibrogenic agent in hepatic fibrogenesis, are being investigated as potential drug candidates in animal models of hepatic injury[1, 14]. However, further research, including clinical studies, has to be done for therapeutic applications.

Therapies targeting HSC proliferation, apoptosis, and phenotypic alteration

Studies have shown that stellate cells contribute to the majority of the total fibrogenic population during liver fibrosis and play an important role in orchestrating hepatic immune responses[16]. Several therapeutic agents targeting HSC proliferation, intracellular signaling, and cell growth have been studied. Several antioxidant molecules, such as glycyrrhizin, exhibit antifibrotic effects *in vitro* or in animal studies[17]. Growth factors that augment HSC proliferation, including PDGF, are potential targets for drug development, which is discussed in detail in the later part of this review. However, the effects of these compounds in the context of human liver fibrosis are yet to be fully characterized. Vascular endothelial growth factor (VEGF) and its cognate receptors are shown to have roles in hepatic fibrosis development[16]. The increase in VEGF concentration may contribute to the progression of fibrosis in smokers who have hepatitis C[16].

Animal experimentation using vasoconstrictive agents

and hepatocyte growth factor revealed that some of these substances may have side effects as potential carcinogenic molecules[8]. Studies were reported on the control of liver fibrosis through the modulation of peroxisome proliferator-activated receptor-γ receptor signaling, or through the use of compounds, such as the histone deacetylase inhibitor Trichostatin A, which are effective in manipulating HSC proliferation/activation in *in vitro* systems and in animal models[18, 19]. However, future clinical trials have to be carried out on these compounds to prove their efficiencies as antifibrotic drugs.

The preliminary processes in stellate cell activation generally occur simultaneously with progressive changes in the surrounding ECM in the space of Disse[16]. The expression of collagens I and III are usually enhanced during liver fibrosis. Synthetic compounds, including HOE077 (*ie*, pyridine-2,4-dicarboxylic-di(2-methoxyethyl)amide), were reported to prevent rat HSC proliferation and collagen synthesis[20]. In experimental models, adenoviral expression of TGF-β anti-sense mRNA was shown to prevent liver fibrosis[21], but again the potential benefits of manipulating this pathway in human liver disease conditions have yet to be fully determined.

## Novel, marine bioactive compounds as drugs to control liver diseases

With the current understanding of the molecular aspects of liver inflammation and fibrosis, the development of novel therapeutic interventions to control liver diseases is possible. Current therapies have several major hurdles to overcome for the consideration of marine bioactive compounds as ideal drugs. Accordingly, efforts to develop novel, effective anti-fibrotic drugs presently include the identification of novel compounds that are liver specific with negligible immunogenicity and side effects. Furthermore, these drugs should enhance liver function and the overall health of the affected patients and should be made in abundance at a low cost[8, 14].

Marine bioactive compounds

Drugs from the marine environment have the potencies to serve as an excellent alternative for established antifibrotic drugs. The oceans comprise more than 70% of the earth's surface and the diversity of the marine environment contributes to the presence of potent bioactive molecules[17, 22]. The marine-derived bioactive compounds are structurally and biologically intriguing, and more than 30 000 compounds with unique structures and diverse pharmacological activities have been isolated from the marine environment, including plants and invertebrates[23]. Some of these compounds that have been extracted and studied show effects (Figure 2) against deadly diseases, including HIV and cancer[24, 25]. However, only a few marine-based drugs are currently in the market, including Prialt®, a potent analgesic; Yondelis®, Cytosar-U®, Adcetris®, and Halaven®, antitumour agents; Vira-A® and Carragelose®, antiviral agents; and Lovaza® for treating hypertriglyceridemia[26]. Other potential candidates include Scytonemin, a pigment isolated from cyanobacteria (Figure 3), which inhibits cell cycle-regulatory kinases with the potential to cure hyperprolifera-
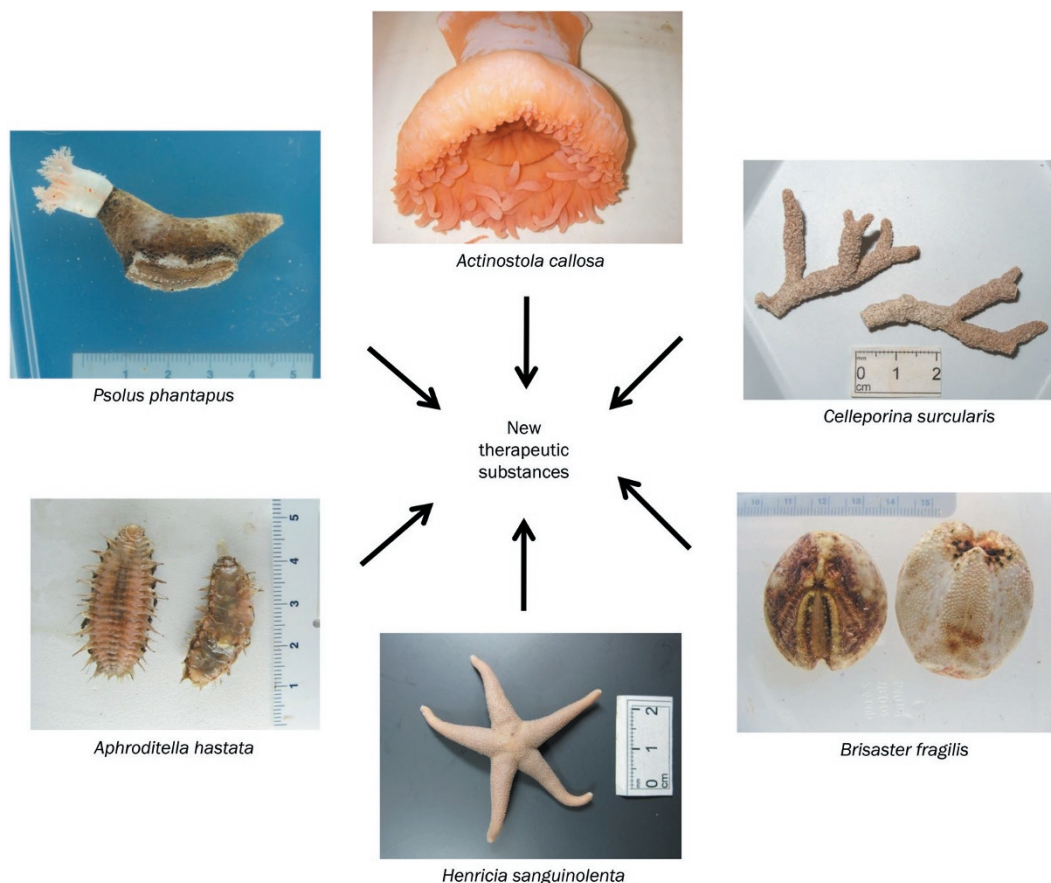
Figure 2. Potential sources of novel marine bioactive antifibrotic compounds. There are several marine organisms, including *Actinostola callosa*, *Celleporina surcularis,* and *Psolus phantapus,* that might be useful for the isolation of novel therapeutic drugs for the treatment of hepatic fibrosis. The individual figure panels were published with the kind permission of Mr Claude NOZÈRES, Government of Canada, Department of Fisheries and Oceans (www.marinespecies.org/carms/photogallery.php) without any change or modification.
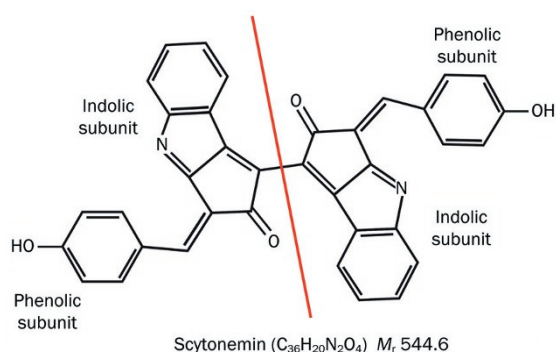
Figure 3. Structure of Scytonemin. The biological pigment Scytonemin is an effective, photo-stable ultraviolet shield in prokaryotes that is composed of two identical subunits (indicated by a red separation line) that each consists of indolic and phenolic subunits[78].

tive disorders, with no chemical toxicity[27]. The difficulties in the collection of marine samples and the difficulties in culturing marine microorganisms may underlie the main reasons for the slow progress in this field. Recent advances in technolo-gies to i) isolate and maintain less-culturable marine microbes, ii) to bypass culture-dependent issues through development in the field of metagenomics, and iii) to isolate and character-ize secondary metabolites using new robust technologies may promote faster progress[28].

Previous reports on marine-derived compounds with hepatoprotective roles

Although multiple studies have been carried out to character-ize marine bioactive compounds with an aim to cure human disease conditions, the focus on liver diseases is very limited. The hepatoprotective role of brown algae (*Padina boergesenii*) extract against carbon tetrachloride (CCl_4)-induced oxidative damage and liver fibrosis in Wister rats was reported[29]. The authors suggest that the carotenoids present in the extract may contribute to the hepatoprotective effects, as there is a strong correlation between carotenoid content and antioxidant activ-ity[29]. The mechanism of action seems to be through cellular membrane stabilization or through antiperoxidase activity, however, the bioactive compounds responsible are unknown and are yet to be isolated and characterized[29]. Similarly, ethanolic extract of red algae (*Hypnea muciformis*) also showed

hepatoprotective activity against CCl₄-induced liver damage in male albino rats; again, the active compounds involved are not isolated or characterized[30]. Zhao *et al* demonstrated the antioxidative and hepatoprotective activities of low molecular fucoidan oligosaccharides (LMFO) isolated from *Laminaria japonica* in mice with liver injury[31]. CCl₄ and *D*-galactosamine (*D*-Gal)/lipopolysaccharides (LPS) were used as two models of liver injury. Pretreatment with LMFO showed protective effects on the hepatocytes and the potential mechanism of action is through their *in vivo* antioxidant activities[31]. Studies by Hong *et al* have shown the protective effect of fucoidan on acetaminophen-induced liver damage in rats[32]. Administration of fucoidan 2 h prior to acetaminophen reduced liver damage and cell death induced by acetaminophen via antioxidant, anti-inflammatory, and anti-apoptotic effects[32].

Very recently, Esmat and colleagues showed the hepatoprotective effect of bioactive phenolic compounds, including chlorogenic acid, pyrogallol, rutin, and coumaric acid, from the sea cucumber (*Holothuria*), in thioacetamide (TAA)-induced liver fibrosis in rats[33]. High-performance liquid chromatographic analysis (HPLC) of the phenolic compounds revealed the presence of nine components, with chlorogenic (92.86%) and ascorbic (0.067%) acids as the major and minor component, respectively[33]. The subchronic administration of the sea cucumber extract showed no side effects, whereas TAA-administration showed toxic effects, including a reduced percentage of body weight gain, an increased relative weight of body organs, and a low survival rates in animal models[33]. The toxic effects of TAA-administration were counterbalanced by the administration of the sea cucumber extract[33]. Further, the coadministration of the sea cucumber extract and TAA substantially reduced the hepatotoxic effect of TAA as indicated by the normalization of serum bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, and the AST/ALT ratio, as well as the decrease in serum alkaline phosphatase (ALP) activity, and hepatic triacylglycerol content. Under TAA-induced toxic conditions, the sea cucumber extract also aided in the maintenance of serum total protein, hepatic total protein, DNA and RNA contents, and the protein/DNA ratio, and nullified oxidative stress and decreased liver fibrosis patterns[33]. Bioactive compounds/mixtures extracted from holothurians were also shown to have anti-inflammatory, antitumor, and fungicidal activities[34].

Additionally, another recent study by Makhmoor *et al* showed the hepatoprotective roles of chemical constituents from the marine brown algae *Spatoglossum variabile* in animal models with CCl₄-induced liver disease[35]. The antiradical and hepatoprotective effects of the compounds were tested after analyzing the radical scavenging potential and β-glucuronidase inhibition using *in vitro* tests. The tested compounds exhibited antiradical activity against 1,1-diphenyl-2-picrylhydrazyl radicals along with superoxide anion scavenging effects[35]. In animal models, the orally pre-administered compounds 2–4 significantly blocked the CCl₄-induced elevation of serum biochemical markers[35]. These studies provide basic scientists and clinicians with evidence that novel, bioactive, and antioxidant molecules or compounds isolated from these organisms may assist in the development of novel drugs/means to treat liver diseases.

Marine-derived compounds worthy of investigation for potential therapeutic benefit in liver diseases

A repertoire of marine bioactive molecules has been shown to have profound effects in manipulating human pathophysiological conditions. Because liver disease also generally involves inflammation and fibrosis, compounds affecting common inflammatory pathways may also have significance in hepatic fibrogenesis. Some of the marine compounds affecting these pathways that will be later discussed have the potential to be tested in liver disease or can act as a lead for therapeutic agents.

Compounds with anti-inflammatory properties
COX pathway inhibitors

COX-1 and COX-2 enzymes are the targets of several non-steroidal anti-inflammatory drugs which are commonly used but generally have some severe side effects, such as gastric ulcerations. Natural compounds, in contrast, generally have the advantage of less adverse side effects, such as the terpenoid Pacifenol (Figure 4) that was isolated from seaweeds of the marine alga *Laurencia claviformis* and later found naturally in *Laurencia tasmanica*[34]. Likewise, Epitaondiol (Figure 4), another terpenoid from seaweeds of *Stypopodium flabelliforme*, has an antioxidant effect, inhibiting eicosanoid production
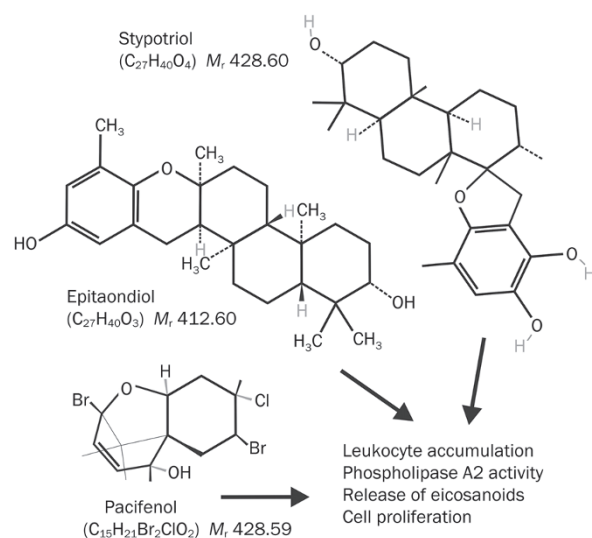
Figure 4. Structure and anti-inflammatory activity of Pacifenol, Epitaondiol, and Stypotriol. Epitaondiol is a polycyclic meroditerpenoid containing two fused six-membered rings forced into the twist-boat conformation[79]. Likewise, Stypotriol (also known as Stypotriol triacetate) is a pentacyclic ichthyotoxin with a meroditerpenoid structure. Pacifenol is a polyhalogenated sesquiterpene consisting of three isoprene units with a total of 15 C atoms[80]. All three of these drugs interfere to a different degree with leukocyte accumulation, phospholipase A2 activity, release of eicosanoids and cell proliferation. The precise mode of action for each drug is described elsewhere[81, 82].

through the inhibition of phospholipase A2 and COX-2[36]. Stypotriol triacetate (Figure 4) is a meroditerpenoid from the same seaweed that inhibits phospholipase A2 and the expression of elastase, and also has antibacterial and anti-proliferative activities[36].

Marine steroids

Several uncommon steroids with anti-inflammatory properties are abundantly found in marine organisms (Figure 5), including i) the polyoxygenated steroid contignasterol isolated from the marine sponge *Petrosia contignata* that inhibits platelet aggregation, ii) the pentacyclic polyhydroxylated steroid xestobergsterol isolated from the marine sponge *Xestospongia bergquisicta,* and iii) clathriols A and B that are isolated from the marine sponge *Clathria lissosclera*[36].

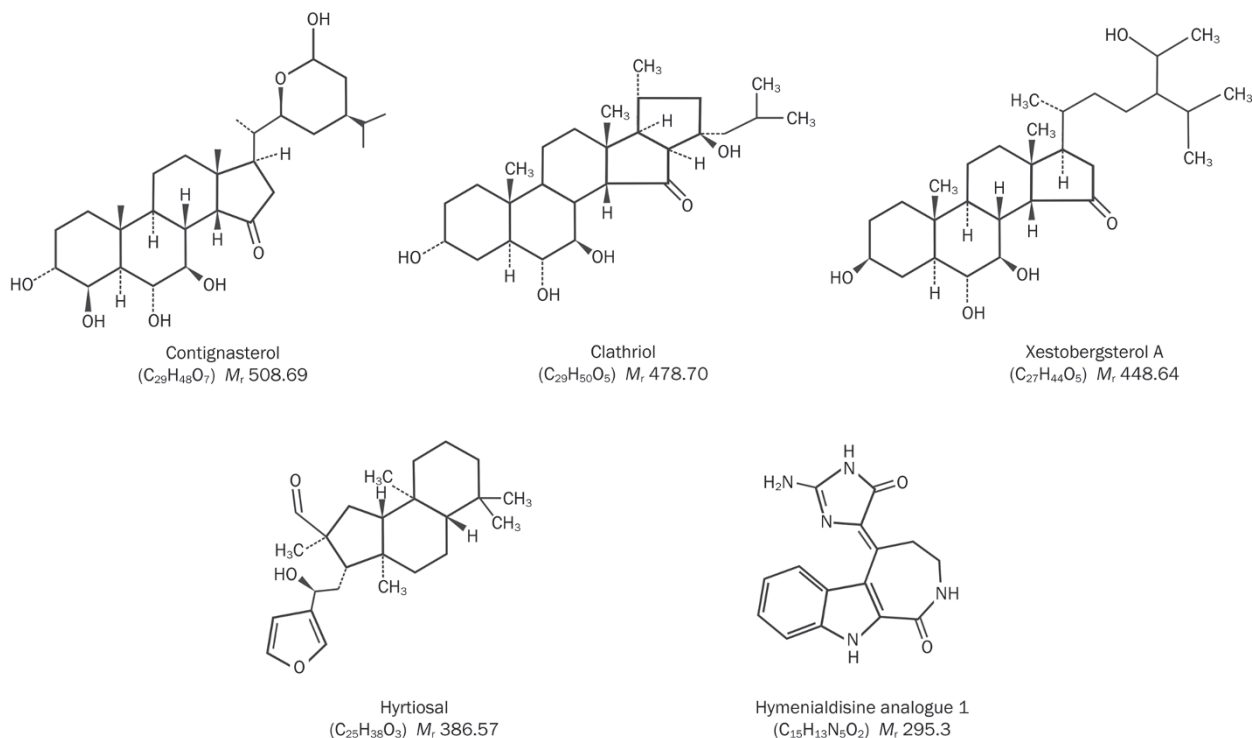### Nuclear factor kappa beta (NF-κB) and TGF-β pathway inhibitors

The NF-κB pathway is strongly activated following the transformation of quiescent to activated HSCs during liver injury[37]. NF-κB responsive genes that are induced in activated HSC include diverse cytokines, chemokines, and adhesion molecules[37]. The effect of Hymenialdisine (Figure 5), a marine-derived natural product, on inhibiting the NF-κB pathway and preventing IL-8 expression after stimulation with tumor necrosis factor-α, lipopolysaccharide, or phorbol myristate acetate was comprehensively reported in the human histio-cytic leukemia cell line U937[37]. Speculation that this molecule or its derivatives will evolve the same therapeutic activity during HSC activation and transdifferentiation is worth consideration.

TGF-β is a key regulator in chronic liver disease, influencing the plasticity of HSC and contributing to all of the stages of disease progression[1]. The biological effects of TGF-β are exerted through a distinct network of different receptors that are structurally similar serine/threonine kinases and several intracellular signaling mediators commonly known as Smad proteins[1]. The TGF-β pathway is further controlled by antagonistic acting regulators and negative feedback inhibitor loops, which offers a large variety of attractive drug targets[38]. Hyrtiosal, an inhibitor of protein tyrosine phosphatase 1B, isolated from the marine sponge *Hyrtios erectus*, affects TGF-β/Smad2 activation and signaling[39]. The identification of novel marine-derived molecules that exhibit similar effects on the TGF-β pathway or its target gene expression would be very useful.

Compounds with antioxidant properties

The important role of oxidative stress in the development of liver injury and activation of HSC is well documented[40]. Antioxidative active substances, such as the traditional herbal Chinese medicine Sho-saiko-to, are effective natural drugs in preventing hepatic fibrosis[41]. The antifibrotic effect of Sho-saiko-to and its ingredients, confirmed in many independent

Contignasterol
($C_{29}H_{48}O_7$) M_r 508.69

Clathriol
($C_{29}H_{50}O_5$) M_r 478.70

Xestobergsterol A
($C_{27}H_{44}O_5$) M_r 448.64

Hyrtiosal
($C_{25}H_{38}O_3$) M_r 386.57

Hymenialdisine analogue 1
($C_{15}H_{13}N_5O_2$) M_r 295.3

Figure 5. Structure of contignasterol, xestobergsterol A, and clathriols A/B. Contignasterol is a highly oxygenated steroid with a rare 14 beta configuration[83] that dose-dependently inhibits histamine release[84]. Likewise, the pentacyclic polyhydroxylated sterol Xestobergsterol A interferes with histamine release and further inhibits inositol triphosphate (IP3) generation, membrane-bound phosphatidylinositol-specific phospholipase C (PI-PLC) activity, and Ca^{2+}-mobilization from intracellular Ca^{2+} stores[85]. The polyoxygenated steroid Clathriol has the capacity to inhibit the production of superoxide from human peripheral blood neutrophils, which is a key factor implicated in the pathogenesis of inflammatory disorders[86].

studies and animal models, enabled its availability in North America without a prescription as a natural, alternative approach for patients who are non-responsive to standard medical treatment or are not candidates for interferon based treatments. In addition, the anti-inflammatory, anti-fibrogenic, and immune modulating activities of this natural component have enforced studies aiming to identify novel antioxidant natural substances. Although there were reports of hepatic injury caused by this compound, the case reports were few and it was difficult to determine whether the hepatic injury was entirely due to the Sho-saiko-to or due to the coadministration of interferon[42]. Silymarin, isolated from a medicinal plant *Silybum marianum*, is another well characterized natural compound with hepatoprotective effects mainly due to its antioxidant effect[43]. Edible seaweeds are generally a rich source of antioxidants. The compounds derived from these marine plants show anti-inflammatory, anticoagulant, and antitumoral activities and are known to reduce oxidative stress via their free radical scavenging properties. Kelman *et al* analyzed the antioxidant activities of 30 species of Hawaiian algae and found that the major active compound is the carotenoid, fucoxanthin[44]. This unique carotenoid, primarily isolated from brown algae and diatoms, shows a higher antioxidant activity than other antioxidants, including tocopherols, potentially due to the presence of the allenic moiety[44]. Fucoxanthin also shows an *in vivo* antioxidant effect on obese/diabetic mouse models and is worth being tested in an experimental model for liver disease[34]. Another major antioxidant found in algae is Astaxanthin from *Haematococcus pluvialis*[34]. Balaji and coworkers demonstrated the antihepatotoxic nature of *Ulva reticulata* extract on acetaminophen-induced liver damage in rats[45]. A number of studies have demonstrated that peptides derived from different marine protein hydrolysates act as potential antioxidants. They have been isolated from marine organisms, such as the jumbo squid, sea cucumber, and tuna, and show greater antioxidant properties than α-tocopherol (vitamin E) in certain cases[46]. Similarly, polysaccharide from *Apostichopus japonicus* shows *in vitro* free radical (hydroxyl and superoxide) scavenging activity[47], again demonstrating that novel therapeutic, antioxidant agents can be identified from the marine world with a potential to be used as pharmaceuticals, nutraceuticals, or as substitution for synthetic antioxidants[47].

Compounds affecting PDGF receptor-β signaling

The PDGF signaling network that triggers a complexity of different cellular pathways consists of a family of four homodimers (PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD); one heterodimer (PDGF-AB); and three types of dimeric glycoprotein receptors (PDGFR-α, PDGFR-αβ, and PDGFR-β) (Figure 6). The elevated expression of the PDGF isoforms and its receptors has been found in fibrotic liver tissue[48]. In particular, similar to intestinal smooth muscle cells during inflammation, the fundamental role of PDGF-BB and one of its receptors, ie, PDGFR-β, in the formation of hepatic fibrosis was reported[48, 49].
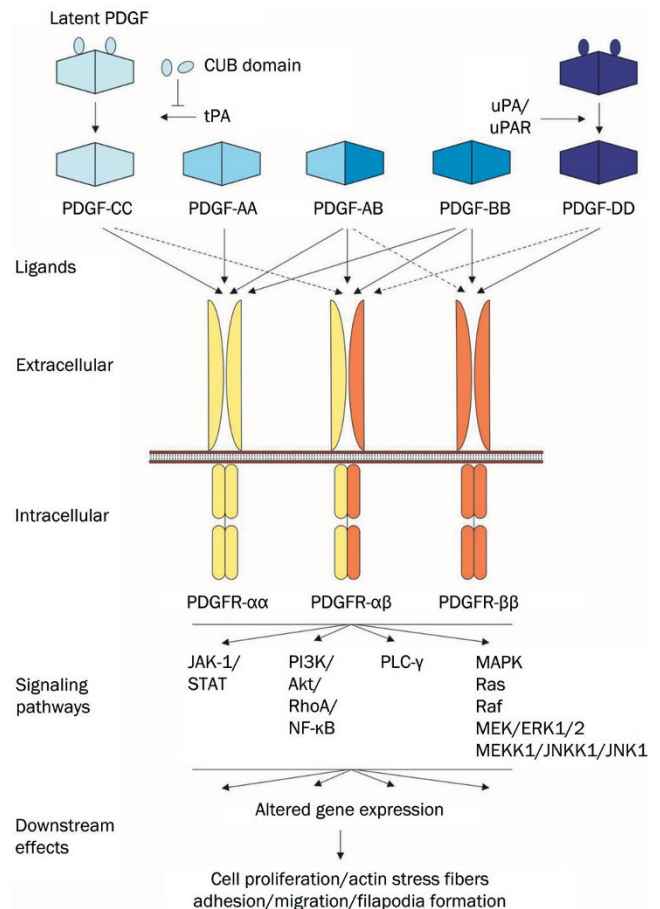
Figure 6. PDGF signaling in hepatic fibrosis. While PDGF-A and -B are secreted as bioactive homo- or heterodimers, PDGF-C and -D are secreted as latent homodimers that become activated by proteolytic separation of the inhibitory CUB domains, a process that is mediated by the activity of the tissue plasminogen activator (tPA) or urokinase plasminogen activator and its receptor system (uPA/uPAR). The PDGF ligands bind to specific tyrosine kinase receptors that, upon ligand binding, lead to the formation of three different dimeric receptor combinations, namely PDGFR-αα, PDGFR-αβ, and PDGFR-ββ. During activation, the receptors become phosphorylated at specific tyrosine residues and subsequently trigger the activation of a wide variety of intracellular signaling cascades that leads to altered gene expression. As a consequence, the increase of PDGF during liver insult results in increased cell proliferation and actin stress fiber formation. In addition, the different PDGF isoforms modulate cellular adhesion, migration, and filapodia formation.

Substances isolated from sponges

Recently, several reports were published describing the effects of marine bioactive compounds on PDGF-BB signaling. A recent study by Choi *et al* demonstrated that Sponge-derived acetylenic alcohols and petrosiols inhibit PDGF-induced proliferation and the migration of vascular smooth muscle cells[50]. These drugs likely have the capacity to inhibit the phosphorylation of PDGFR-β and its downstream effectors phospholipase-γ1, AKT/PKB, and extracellular signal-regulated kinases ERK1/2. In similar studies, Pinchuk and col-

leagues showed the inhibitory effects of the pyrazin-2(1H)-one scaffold from hamacanthins, which were also isolated from a marine sponge and are highly potent in binding and inhibiting PDGFR-β[51]. Based on the outstanding role of the PDGF system in liver fibrosis, it will be extremely worthwhile to study the effects of these molecules on the setting of experimental liver disease models.

Substances isolated from the sea cucumber

Philinopside A is a sulfated saponin isolated from the sea cucumber *Pentacta quadrangularis*. It shows a potent inhibitory activity against receptor-tyrosine kinases (RTKs), including PDGFR-β, and dose-dependently inhibited the proliferation, migration, and tube formation of human microvascular endothelial cells[52]. Further studies will help to better understand the mechanism of its action and the potential modifications on the saponin backbone may enhance the specificity for individual RTKs.

Additional applications of marine-derived compounds to control liver fibrosis or to enhance recovery

Marine polysaccharides: for hepatic cell therapy and tissue engineering

Marine polysaccharides already have several important medical, pharmaceutical, and biotechnological applications, which include the fabrication of wound dressings, high polymer biomaterials, and tissue scaffolds[53]. However, these compounds, potentially safer than mammalian polysaccharides, are still under-exploited for their potential applications in hepatology or drug discovery and should be given further consideration[53].

During liver failure, the complication of liver transplant necessitates the use of tissue engineering in which autonomous cells have to be seeded for regeneration on a scaffold with an abundance of oxygen, nutrients, and growth factors, such as PDGF. A decade ago, the Cohen group demonstrated the use of porous alginate scaffolds that were prepared from brown sea weed for the efficient seeding and survival of hepatocytes[54].

Apart from its application as a biomedical material, marine-derived chitosan demonstrates protective effects against liver damage. Yan *et al* demonstrated the protective effect of chitosan oligosaccharide and its derivatives against CCl4-induced liver damage by pretreatment[55]. β-Chitosan isolated from the squid *Sepioteuthis lessoniana* showed hepatoprotective activity against CCl4-induced oxidative stress in Wistar rats[56]. Along with the stabilization of various liver marker enzyme activities, histopathological observations also suggested that the hepatoprotective activity of β-chitosan is possibly due to its antioxidant and antilipidemic properties[56]. Recently, another study by Ramasamy *et al* showed the antioxidative and hepatoprotective roles of chitosan from *Sepia kobiensis* against CCl4-induced liver damage in rats[57]. The changes in the circulatory and hepatic antioxidant defense systems were normalized by the administration of chitosan[57].

Chitosan-based microgels for 'smart' drug-delivery

Microgels based on chitosan from shrimp and crab shells can be used *in vivo* for the 'smart' delivery of drugs or growth factors. Recently, Gu *et al* reported a closed-loop insulin delivery platform using injectable self-regulated microgels that facilitate insulin release when subjected to hyperglycemic conditions[58]. Most interestingly, these microgels sufficiently promoted a reduction of blood glucose levels in a mouse model of type 1 diabetes[58]. It is tempting to speculate that similar technology and scaffolds can be developed that allow the administration of anti-inflammation drugs, therapeutic proteins, growth factors, or marine-derived compounds.

Fish oil as a supplement or as a drug

The beneficiary effects of fish oil or its constituents on hepatic protection have gained recent attention. Depner and colleagues showed that menhaden oil decreased several, but not all, high-fat diet-induced markers of hepatic damage, steatosis, inflammation, and fibrosis in mice[59]. N-3 polyunsaturated fatty acid (PUFA), a constituent of fish oil, was shown to regulate hepatic gene transcription[60]. The administration of dietary fish oil resulted in reduced damage in the liver tissue of *Mus musculus*, suggesting that long chain PUFA, such as eicosapentaenoic acid and docosahexaenoic acid, may serve as a preventive agent for hepatic cirrhosis[61]. Similarly, the supplementation of fish oil rich in PUFA showed an *in vivo* protective effect against oxidative stress-induced DNA damage caused by the administration of ferric nitrilotriacetate in rat liver[62]. Studies also showed the hepatoprotective effects of fish oil against liver damage induced by several agents, including lipopolysaccharides[63], cisplatin[64], and acetaminophen[65], in various *in vivo* models. The novel constituents of fish oil as supplements or drugs might therefore be beneficial in controlling critical pathways in liver pathophysiology.

Marine biodiversity in Oman and the potential for identifying novel hepatoprotective therapeutic agents

We have initiated efforts to search for novel bioactive compounds from the unexplored marine environment in Oman. Compared to the neighboring countries in the Arabian Peninsula, Oman has the longest coastal habitats along the Arabian Sea, the Gulf of Oman, and the Arabian Gulf. Oman contributes a rich environment to marine biodiversity, one example is Ras Al-Hadd, one of the largest nesting beaches for marine turtles around the world[66]. A complete taxa of Oman's marine flora and fauna is needed for an extensive investigation, but so far, little has been done. No studies related to the majority of marine organisms have been completed. Fisheries are one of the most vital resources to the Oman economy, after crude oil. Noteworthy, approximately 134 000 metric tons of fish are produced annually in Oman with an average income of 160 million USD[67]. Due to the extensive fishing, it was reported that 20 fish species are already on the verge of extinction[67], while the fate of many other species has not yet been documented. Large scale coral reef damage is irreversible to

some habitats, which has already taken place in Oman. Thus, tremendous efforts must be taken immediately to preserve the marine environment and to explore, identify, and characterize novel bioactive molecules from this rich environment.

Some fungi secrete mycotoxins, such as aflatoxins and fumonisins, that when ingested through contaminated food may cause liver toxicity, which can ultimately lead to cancer. Studies in Oman reported aflatoxin contaminations in spices and black tea[68, 69]. Recent studies in Oman used *Hibiscus sabdariffa* extract and *Nigella sativa* oil to prevent the production of aflatoxin B1 and used baobab (*Adansonia digitata*) extracts as a biocontrol to prevent the growth and aflatoxin production by *Aspergillus flavus* and *A parasiticus*[70, 71]. Similar studies can be carried out using local marine flora and fauna to cure liver diseases. Populations that live in villages near industrial areas have higher rates of liver diseases due to the continuous exposure to heavy metals. Patients with liver diseases were found to have higher levels of Cr[72]. Studies in Oman have shown that treated sewage effluent contains different types of heavy metals that contaminate terrestrial and marine environments and underground water, leading to the accumulation of metals in tomato crops, marine fish and turtles[73–76], which may have long-term impacts on human health. Half of the chronic liver diseases in Oman are reported to be of hereditary origin[77]. Hence, in addition to the ongoing efforts to prevent the incidence of liver diseases in Oman and also worldwide, it is imperative and important to focus on the local, rich marine resources for the identification of novel hepatoprotective agents (Figure 7), most of which have been poorly characterized with respect to liver diseases.

Conclusions

Liver fibrosis and cirrhosis, induced by a plenitude of different mechanisms, are a major healthcare burden worldwide. The sole removal of the causal agent is not an efficient approach to cure advanced stages of fibrosis. Although there is progress in liver transplantation and viral hepatitis treatment, these techniques have some limitations. Issues regarding organ availability for transplantation, organ compatibility, and the health of the donor and patient complicate the process. Hence, there is a great need for novel therapies for liver fibrosis, which represents a reversible pathological condition. Antifibrotic drugs targeting hepatic stellate cell activation or phenotypic alteration have a high potential to develop as novel therapeutic agents. The marine-derived environment represents an unexplored area for identifying novel hepatoprotective drugs. Although several compounds have been isolated and tested in other human disease conditions (Table 1), there are presently very few studies focused on their efficacies in hepatic lesions. Due to the prevalence of liver diseases and the presence of environmental/other factors contributing to their development, more detailed studies related to liver diseases are urgently needed in Oman and other countries. The effect of water and food contamination and their impact on liver functionality requires further extensive investigation. Because Oman is rich with marine flora and fauna, these organisms could provide an important source of novel biomedical products for the treatment of liver disease, as well as many other diseases. Therefore, successful collaborations of basic scientists across different academic disciplines, clinicians, and industrial partners are compulsory and urgently needed to
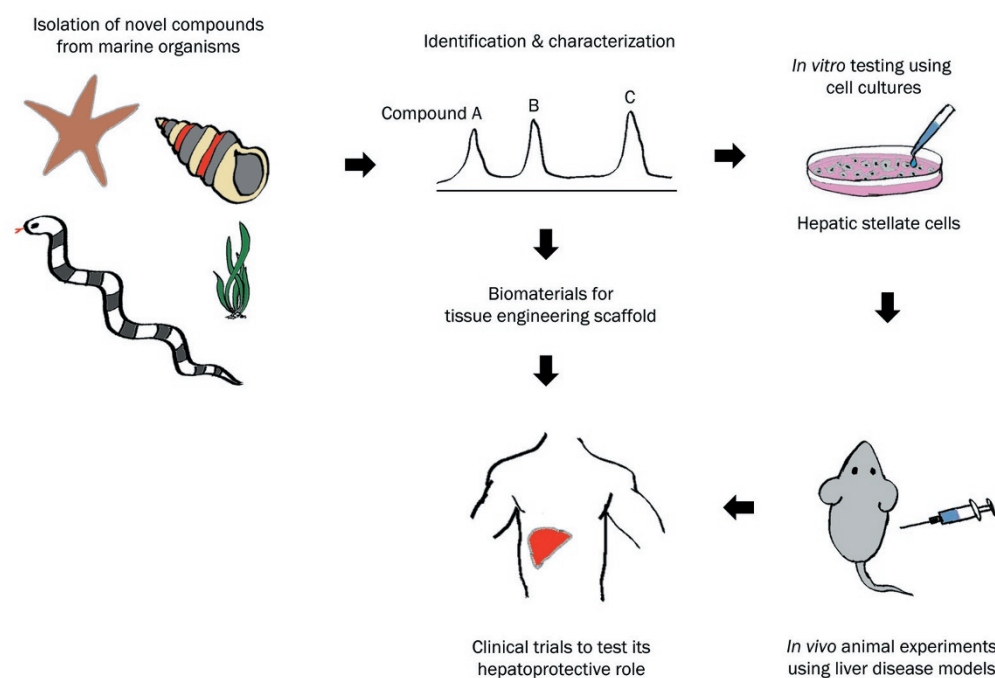
Figure 7. Graphical abstract showing a summary of the review article. Novel bioactive compounds are isolated from the marine environment and are characterized before *in vitro* testing. The compounds showing *in vitro* activities will be screened for *in vivo* activities using animal models and ultimately for their hepatoprotective roles in humans.

Table 1. A list of marine bioactive compounds or extracts with reported or potential hepatoprotective roles.

Activities	Organism		Compound/extract	Reference
Antioxidant	Algae	*Ulva reticulata*	Extract	Balaji *et al*, 2004[45]
		Haematococcus pluvialis	Astaxanthin	D'Orazio *et al*, 2012[34]
		Cymopolia barbata	Cymopol	Takamatsu *et al*, 2003[87]
		Avrainvillea longicaulis	Avrainvilleol	Takamatsu *et al*, 2003[87]
		Halimeda opuntia	Polyphenols	Silva *et al*, 2012[88]
		Undaria pinnatifida	Fucoxanthin	D'Orazio *et al*, 2012[34]
	Hard clam	*Gelonia erosa*	Ethylacetate	Yeh *et al*, 2012[89]
		Paphia undulata	Hydrolysates	He *et al*, 2013[90]
	Sea cucumber	*Apostichopus japonicus*	Polysaccharide	Liu *et al*, 2012[47]
		Holothuria atra	Extract	Esmat *et al*, 2013[30]
		Holothuria edulis and *Stichopus horrens*	Extract	Althunibat *et al*, 2013[91]
		Stichopus japonicus	Peptides	Zhou *et al*, 2012[92]
Antiinflammatory	Algae	*Laureafncia claviformis*	Pacifenol	Balaji *et al*, 2004[45]
		Stypopodium flabelliforme	Epitaondiol, Stypotriol triacetate	Pereira *et al*, 2011[82]
		Undaria pinnatifida	Fucoidan	Hong *et al*, 2012[93]
	Gorgonian	*Junceella fragilis*	Frajunolides	Liaw *et al*, 2010[94]
	Molluscs	*Mytilus galloprovincialis* (L) and *Rapana venosa*	Amino acids	Baidu *et al*, 2010[95]
	Sea cucumber	*Holothuria atra*	Extract	Esmat *et al*, 2013[33]
		Holothuria nobilis	Extract	Whitehouse & Fairlie[96]
	Sponge	*Plakortis angulospiculatus*	Plakortide P	Kossuga *et al*, 2008[97]
		Stylissa carteri	Carteramine A	Kobayashi *et al*, 2007[98]
Interfering PDGF-Rβ signaling	Sponge	*Petrosia strongylata*	Acetylenic alcohols, petrosiols	Choi *et al*, 2013[50]
		Rhaphisia lacazei	Pyrazin-2(1H)-one	Pinchuk *et al*, 2013[51]
	Sea cucumber	*Pentacta quadrangulari*	Philinopside A	Tong *et al*, 2005[52]
Interfering NF-κB pathway	Algae	*Nostoc commune* and *Spirulina platents*	Extract	Ku *et al*, 2013[99]
		Cladophora fascicularis	Porphyrinolactone	Huang *et al*, 2007[100]
	Sponge	*Fasciospongia cavernosa*	Cacospongionolide B	Posadas *et al*, 2013[101]
		Petrosaspongia nigra	Petrosaspongiolide M	Posadas *et al*, 2013[101]
	Cyanobacteria	*Nostoc commune*	Scytonemin	Nagle *et al*, 2004[102]
		Lyngbya majuscula	Curacin A	Catassi *et al*, 2006[103]
Interfering TGF-β pathway	Sponge	*Hyrtios erectus*	Hyrtiosal	Sun *et al*, 2007[39]
	Microalgae	*Navicula incerta*	Peptides (NIPP-1 & NIPP-2)	Kang *et al*, 2013[104]
Interfering cyclooxygenase pathway	Soft coral	*Lobophytum crassum*	Cembranoids & Crassumolides A & C	Chao *et al*, 2008[105]
		Lobophytum duru	Durumolides A–C	Cheng *et al*, 2008[106]

isolate and evaluate these novel drugs.

Abbreviations

HSC, hepatic stellate cells; TGF-β, transforming growth factor β; PDGF-β platelet-derived growth factor β; PPAR-γ receptor, peroxisome proliferator-activated receptor-γ; COX-1/COX-2, cyclooxygenase 1 or 2; NF-κB, Nuclear Factor Kappa Beta; HBeAg, Hepatitis B e-Antigen; CCl_4, Carbon tetrachloride.

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