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Review Article

Matrix Metalloproteinase: Inhibitory Effect of Marine Substances on MMP-2 and MMP-9

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Abstract

Marine ecosystems are often characterized by a high biological diversity, and it corresponds to a high chemical diversity. Up to present, more than 20,000 new bioactive substances have been isolated from marine organisms, where considerable numbers of these naturally occurring derivatives are developed as potential candidates for pharmaceutical application. In this process, screening of natural products from marine organisms that could potentially inhibit the expression of metalloproteinases has gained a huge popularity. Cancer is considered as one of the deadliest diseases in the medical field. Matrix metalloproteinase (MMPs) can degrade extracellular matrix (ECM) components and play important roles in a variety of biological and pathological processes. Matrix metalloproteinase inhibitors (MMPIs) have been identified as potential therapeutic candidates for metastasis, arthritis, chronic inflammation and wrinkle formation.

Key words : Matrix Metalloproteinases, Extracellular Matrix, Gelatinases, Matrix Metalloproteinase Inhibitors, Marine Natural Products

1. Introduction

Matrix metalloproteinases (MMPs) comprise a family of at least 28 secreted or transmembrane enzymes collectively capable of processing and degrading both the collagenous and noncollagenous components of the extracellular matrix (ECM). ECM macromolecules are important for creating the cellular environments required during development and morphogenesis. MMPs are a family of zinc-dependent endopeptidases that play important roles in a variety of biological and pathological processes[1]. MMPs are classified mainly into five groups, collagenase, gelatinase, stromelysins, matrisyl, and membrane-type MMPs based on their structure and functions[2]. MMPs regulate the synthesis and secretion of cytokines, growth factors, hormone receptors and cell adhesion molecules. The MMPs influence diverse physiologic and pathologic processes, including aspects of embryonic development, tissue morphogenesis, wound repair, inflammatory diseases, and cancer. They also contribute to the growth and development of angiogenesis, cardiovascular, multiple sclerosis, neurodegenerative diseases[1,3]. Endopeptidases are divided into serine, cysteine, aspartic and metalloproteinases based on their catalytic properties and inhibitor sensitivities. The metzincin superfamily, which belongs to the metalloproteinases, encode a highly conserved zinc-binding motif containing three histidine residues which bind zinc, and a conserved methionine-turn in the active-site helix. The metzincin superfamily includes serralysins, astacins, adamalysins and MMPs[4]. MMPs are destructive, leading to several diseases such as arthritis, cancer and tumor invasion and metastasis[5-7]. Therefore, the development of matrix metalloproteinase inhibitors (MMPIs) to treat and to halt the spreading of some important diseases including cancers, cardiovascular and various kinds of inflammatory diseases have broad prospects[8-10]. Nevertheless, MMPIs entering clinical trial were synthetic inorigin, and their undesirable side-effects led to failure of the trials, mean while discovering the ideal MMPIs from marine natural products is an indispensable job for after studied. Up to present, more than 20,000 new compounds have been isolated from marine organisms, where considerable numbers of these naturally occurring derivatives are developed as potential candidates for pharmaceutical application[11-12].

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Gelatinase (MMP-2 and MMP-9) activity and plays an important role in cancer invasion and metastasis, which have been most consistently detected in malignant tissues. This subgroup of metalloproteinases was originally described as type IV collagenases, because of their ability to cleave type IV collagen. One of them, MMP-2 (gelatinase-A, 72 kDa type IV collagenase) was originally purified from highly a metastatic murine tumour[13-17]. MMP-2 binds to type I collagen through the fibronectin domain, which stabilises it from autolysis, there by controlling its activity[18]. MMP-2 expression is dependent on extracellular matrix metalloproteinase inducer, growth factors, cytokines, and hormones. Pro-MMP-2 activation needs MT1-MMP and TIMP-2 contribution. MMP-2 is changed in distribution and increased in amount in the ventral cochlear nucleus after unilateral cochlear ablation. A low level of MMP-2 is linked to favorable prognosis in patients with a hormone receptor-negative tumor, usually associated with high risk. As a zymogen requiring proteolytic activation for catalytic activity, MMP-2 has been implicated broadly in the invasion and metastasis of many cancer model systems, including human breast cancer[19-21]. MMP-9 (92 kDa type IV collagenase, gelatinase B) is produced in human macrophages and polymorphonuclear leukocytes. It has also been localized into the endothelial cells and synovial fibroblasts in rheumatoid arthritis synovium[16,22]. MMP-9 is expressed by osteoclasts in the human normal bone tissues, implicating a role in the bone remodeling. Mature human intact odontoblasts also express MMP-9. In addition, it has been identified in human dental caries lesion and saliva. However, MMP-9 is not expressed by human gingival fibroblasts. Like MMP-2, MMP-9 may existin the ECM bound totype I collagen, gelatin or laminin[23-27]. The ability of MMP-2 and MMP-9 to degrade denatured collagen I was developed into a relatively easy yet powerful technique to detect their presence in biological samples. This technique, known as gelatin zymography, identifies gelatinolytic activity in biological samples using sodium dodecyl sulfate (SDS)-polyacrylamide gels impregnated with gelatin[28-30].

2. Matrix Metalloproteinase Inhibitors from Marine Substances

2.1. Compounds of Chitosan and Chitin

Chitooligosaccharides (COS) are partially hydrolyzed products of chitosan (Fig. 1a). Kim et al. studied the inhibitory effect of chitooligosaccharides (COS) on activation and expression of MMP-2 in human dermal fibroblasts (HDFs) cells. In addition, Authors were investigated that COS with 3-5 kDa exhibited the highest inhibitory effect on MMP-2 activity in HDFs cells, and protein expression of MMP-2 was also inhibited by COS with same molecular weight[31]. This inhibition was caused by the decrease in gene expression and transcriptional activity of MMP-2. In a subsequent publication, Ta et al. investigated the effect of COS on activity and expression of MMP-9 in human fibrosarcoma (HT1080) cells by gelatin zymography, reverse transcription polymerase chain reaction (RT-PCR), gene reporter assay, and western blot analysis. They found that MMP-9 inhibition in the presence of COS was clearly observed in gelatin zymography. Specifically, COS-I (1- to 3 kDa) exhibited the highest inhibitory effect on MMP-9 activity in HT1080 cells among tested molecular mass fractions, and COS-I was capable of inhibiting both gene and protein expression of MMP-9[2].

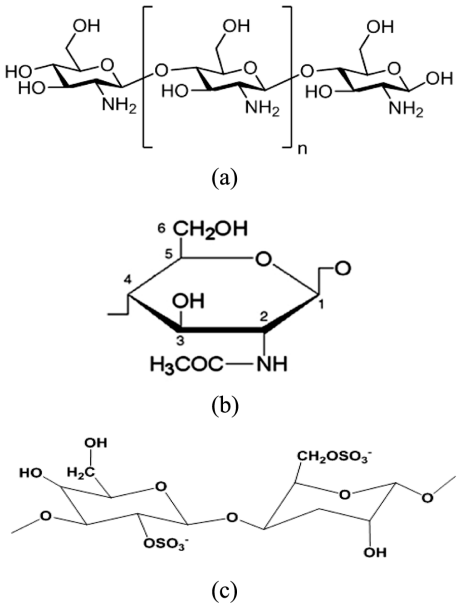


Fig. 1. Chemical structure of oligosaccharides. (a) Chitooligosaccharides (COS), (b) carboxylated COS (CCOS), (c) -carrageenan.

The novel low molecular-weight carboxylated Chitooligosaccharides (CCOS) has been evaluated for

MMP-9 inhibitory effect on human fibrosarcoma cell line (Fig. 1b). A clear dose-dependent inhibition on MMP-9 mediated gelatinolytic activities were observed in HT1080 cells following the treatment with CCOS in zymography experiments. They conclude that CCOS inhibit MMP-9 expression in HT1080 cells through transcriptional down-regulation of c-Fos subunit of activator protein-1 (AP-1) that inhibits degradation and cellular invasion of extracellular matrix (ECM) and basement membrane. Thus, control of MMP-9 expression by CCOS has considerable significance for the regulation of tumor progression[32]. However, no sifnificant inhibitory effect on nuclear factorκB (NF-κB) and TIMP-1 expression with presence of CCOS.

Carboxymethyl-chitosan (CM-chitosan) and carboxymethyl-chitin (CM-chitin) were synthesised by means of carboxymethylation reaction (Fig. 2). Their antioxidative and MMP-2 and MMP-9 inhibitory effects were investigated in HT1080 cells. Treatment with CM-chitosan and CM-chitin suppressed the formation of intracellular reactive oxygen species (ROS), protein oxidation and lipid peroxidation in a concentration-dependent manner. In addition, a protective effect against oxidative damage of purified genomic DNA was observed in the presence of CM-chitosan and CM-chitin. Moreover, CM-chitosan and CM-chitin reduced the expression levels of MMP-2 and MMP-9 in gelatin

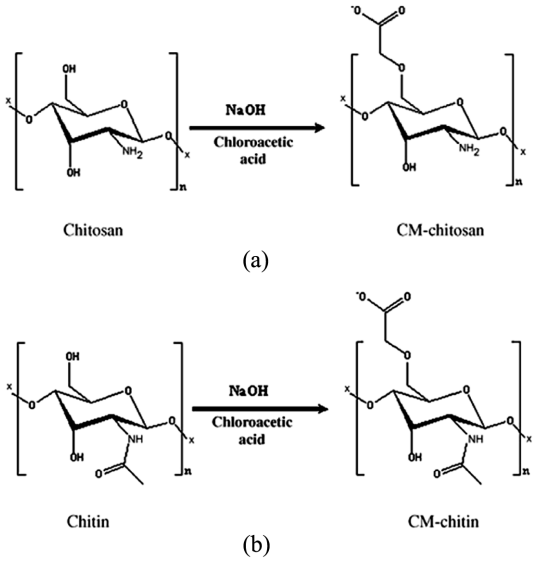


Fig. 2. Synthesis pathway. (a) CM-chitosan, (b) CM-chitin. Adapted from Kong et al. (2010).

zymography, RT-PCR and western blot analysis without any cyctotoxic influence. CM-chitin exhibited higher inhibition via down-regulations of AP-1 and NF-κB than CM-chitosan[33].

Chen et al. obtained highly sulfated λ-carrageenan oligosaccharides (λ-CO) by carrageenan depolymerization (Fig. 1c). They have demonstrated that λ-CO could effectively inhibit angiogenesis in the CAM (chick chorioallantoic membrane) model and human umbilical vein endothelial cells[34]. Significant inhibition of vessel growth was observed at 200 μg/pellet. A histochemistry assay also revealed a decrease of capillary plexus and connective tissue in λ-CO treated samples. λ-CO inhibited the viability of cells at the high concentration of 1 mg/mL, whereas it affected the cell survival slightly at alow concentration (<250 μg/mL). In addition, the inhibitory actionof λ-CO was also observed in the endothelial cell invasion and migration at relatively low concentrations (150-300 μg/mL), through down-regulation of intracellular MMP-2 expressionon endothelial cells.

2.2. Compounds of Glucosamine

Brito et al. investigated on the shrimp heparin-like glycosaminoglycan isolated from *Litopenaeus vannamei,* which was reduced almost 90% the activity of MMP-9 secreted by human activated leukocytes. In addition, heparinand shrimp heparin-like compound were able to reduce this enzyme activity, either in alower or higher concentration (10 and 100 μg/mL), but the shrimp compound had a pronounced effect on this enzyme activity, reducing almost 90% of its activity[35].

Glucosamine was chemically modified to obtain Carboxylated glucosamine (CGlc) (Fig. 3a). The inhibitory results obtained in the presence of CGlc were in well agreement with zymography results, where dose-dependent MMP-9 inhibition was observed with increment of concentration of CGlc. At 500 μg/mL concentration of CGlc, relative fluorescence intensity was decreased by 66%[36].

Mendis et al. identified that a novel Glc derivative having quaternized amino functionality (QAGlc) (Fig. 3b) suppresses MMP-9 and MMP-2, gelatinases in HT1080 cells at 40 μg/mL, following stimulation with phorbol 12-myristate 13-acetate (PMA)[37]. Reporter gene assay results revealed that, expression and activity of above MMPs studied suggested QAGlc as a potent

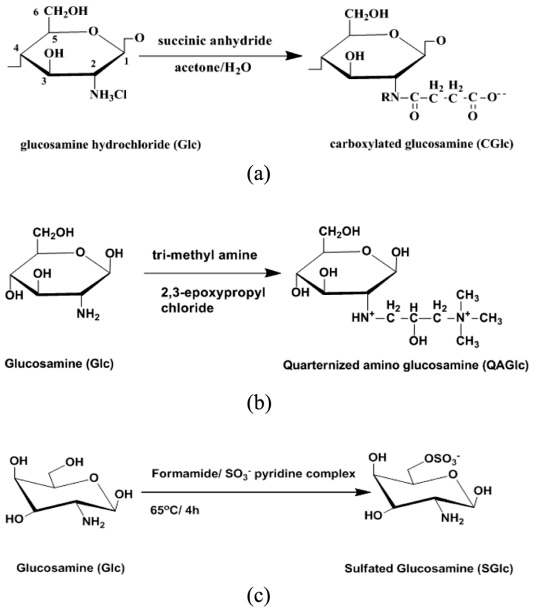


Fig. 3. Synthesis of substances from glucosamine. (a) Carboxylated glucosamine, (b) Quartemized amino glucosamine, (c) Sulfated glucosamine.

MMP inhibitor, and inhibition of MMP-2 and MMP-9 was due to down-regulation of NF-κB and AP-1. However based on western blot results, QAGlc did not attenuate the nuclear translocation of both NF-κB and AP-1. Moreover, the ability of QAGlc to inhibit gelatinases was confirmed by its ability to act against invasiveness of HT1080 cells through invasion assay.

Rajapakse et al. studied for sulfated glucosamine (SG) inhibitory effects on MMP-2 and MMP-9 in HT1080 cells (Fig. 3c). The mechanism of suppression involves decreased transcriptional activation of MMP-9 and MMP-2 via transcription factors NF-κB[38]. However, expression of activator protein-1 was not affected by SG treatment. Moreover, down-regulation of NF-κB resulted in production of low levels of both NF-κB p50 and p65 proteins and directly affected activation process of MMP-2 and MMP-9 expressions.

2.3. Compound of Sulfated Polysaccharide

Wang et al. investigated that SIP-SII is the sulfated S. maindroni ink polysaccharide (SIP) isolated from cuttlefish *Sepiellamaindroni*[39]. SIP-SII weakly inhibited tumor cell growth without cytotoxicity invitroassay. Herein, they examined the effects of SIP-SII on the

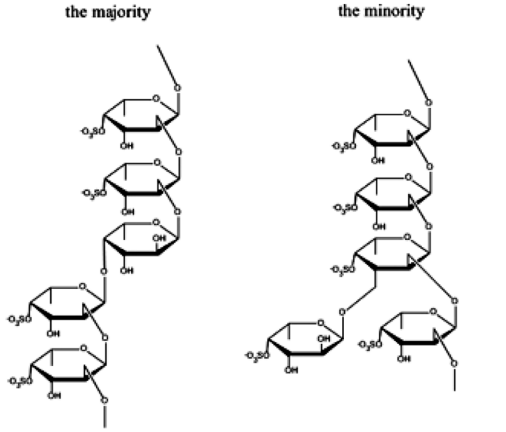


Fig. 4. Structure unit of fucoidan with two kinds the majority and the minority.

expression of matrix metalloproteinase MMP-2 and MMP-9 as well as tumor cell invasion and migration. SIP-SII (0.8-500 μg/mL) signficantly decreased the expression of MMP-2 activity in human ovarian carcinoma cells SKOV3 as evidenced by the gelatin zymography analysis. No significant decrease of MMP-9 was detected in the cell line after SIP-SII treatment. The expression of MMP-2 was also evaluated using Western blot analysis. The results showed that SIP-SII inhibited the expression of MMP-2 in SKOV3 and human umbilical vein vascular endothelial cells ECV304 after 24 h incubation.

Furthermore, the activity of invasion and migration of SKOV3 and ECV304 cells were measured. SIP-SII displayed an inhibitory effect on the penetration of SKOV3 cells through Matrigel-coated membrane in transwell chamber. A significant inhibition of ECV304 cell migration was observed in the presence of SIP-SII. These results suggest that SIP-SII might suppress invasion and migration of carcinoma cells via inhibition of MMP-2 proteolytic activity.

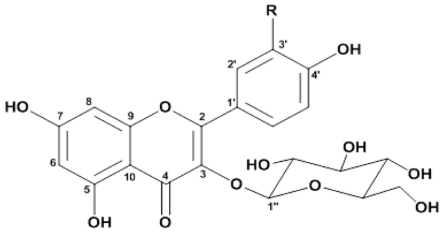
Fucoidan is a uniquely-structured sulfated polysaccharide found in the cell walls of several types of brown seaweed that has recently, especially as enzyme-digested fucoidan extract, attracted a lot attention due to its anti-tumor potential in Fig. 4[40]. Enzyme-digested fucoidan extracts prepared from seaweed, *Mozuku* of *Cladosiphon novae-caledoniae kylin* showedi *nvitroin* vasion and angiogenesis abilities of human tumor cells. The mechanism of significant inhibition of HT1080

cells invasion by fucoidan extracts, possibly via suppressing MMP-2 and MMP-9 activities. Further, they investigated the effects of the fucoidan extracts on angiogenesis of human uterine carcinoma HeLa cells, and found that fucoidan extracts suppressed expression and secretion of vascular endothelial growth factor, resulting in suppressed vascular tubules formation of tumor cells.

2.4. Compounds of Flavonoid

Isorhamnetin 3-O-β-D-glucoside and quercetin 3-O-β-D-glucoside inhibitory effects on MMP-9 and MMP-2 were evaluated in HT1080 cells, and they were isolated from *Salicornia herbacea* (Fig. 5a). These flavonoid glycosides led to the reduction of the expression levels and activities of MMP-9 and MMP-2 without any significant difference between these flavonoid glycosides by zymography experiments. Protein expression levels of both MMP-9 and MMP-2 were inhibited and TIMP-1 (tissue inhibitor of metalloproteinase-1) protein level was enhanced by these flavonoid glycosides. Therefore, these results suggested that these flavonoid glycosides have a potential as valuable natural chemopreventive agents for cancer[41].

Luteolin is a flavonoid which is part of our daily nutrition in relatively low amounts (Fig. 5b). Nevertheless, some epidemiological studies suggest an inverse correlation between luteolin intake and the risk of some cancer types[42]. The epidermal growth factor increased the levels of MMP-2 and MMP-9, while luteolin appeared to suppress the secretion of these two MMPs in A431 cells.



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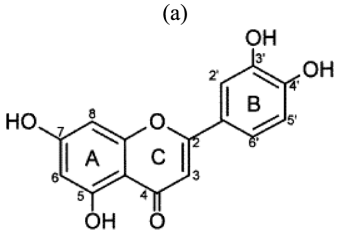


(b)

Fig. 5. SChemical structure of flavonoid. (a) Flavonoid glycosides. SF-A: isorhamnetin 3-O-β-D-glucoside. SF-B: quercetin 3-O-β-D-glucoside, (b) Luteolin.

Huang et al. investigated that luteolin were the most potent of eight flavonoids to inhibit cell proliferation and secretion of matrix metalloproteinases MMP-2 and MMP-9, two gelatinases involved in metastasis[43]. Luteolin reduced activity of MMP-2 by 73% and of MMP-9 by 94%.

2.5. Compounds of Phlorotannin

In the brown algae, the only group of tannins present is the phlorotannins. They are polymers of phloroglucinols (1,3,5-trihydroxybenzene) and may constitute up to 15% of the dry weight of brown algae[44-46]. The relationship of phenolic substances to phloroglucinol in brown algae was mentioned and confirmed numerous times by Ragan et al. (1976)[47]. Quite similar results are presented in the work of Boettcher and argett. (1993) and McClintock and Baker. (2001)[48-49]. However, they studied the molecular weights of phlorotannins vary from 126 Da to 650 kDa, but are most commonly found in the 10 to 100 kDa range.

Kim et al. studied on the inhibitory effects of phlorotannins in brown algae *Ecklonia cava*(EC) on MMP activities in cultured human cell lines[50]. A novel gelatin digestion assay could visualize complete inhibition of bacterial collagenase-1 activity at 20 μg/mL of EC extract during preliminary screening studies. Sensitive fluorometric assay revealed that EC extract can specifically inhibit both MMP-2 and MMP-9 activities significantly at 10 μg/mL. In addition, artificially induced activities of MMP-2 and MMP-9 in HDFs and HT1080 cells were inhibited by EC extract in a more or less similar manner to the positive control doxycycline. Even though the expression levels of MMPs differ from one cell type to the other, gelatin zymography clearly revealed that both MMP expression and activity in cells could inhibited by EC extract. More interestingly, EC extract did not exert any cytotoxic effect even at 100 μg/mL anticipating its potential use as a safe MMP inhibitor.

Corallina pilulifera methanol extract shown to exert a potent antioxidant activity and protective effect on

ultraviolet A-induced oxidative stress of HDFs cells have been discussed by Ryu et al. (2009)[51]. Antioxidant evaluated by various antioxidant assays. These include reducing power, total antioxidant, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, hydroxyl radical scavenging and protective effect on DNA damage caused by hydroxyl radicals generated. Further, the ROS level was detected using a fluorescence probe, 2',7'-dichlorofluorescein diacetate, which could be converted to highly fluorescent dichlorofluorescein with the presence of intracellular ROS on HT-1080 cells. Those various antioxidant activities were compared to standard antioxidants such as a-tocopherol. In addition, the invitro activities of MMP-2 and MMP-9 in HDFs cells were inhibited by Corallina pilulifera methanol extract dose dependently by using gelatin zymography method. The results obtained in the present study suggested that the *Corallina pilulifera* methanol extract may be a potential source of natural anti-photoaging.

Antioxidant and matrix metalloproteinase inhibitory effects of methanolic extract from *Amphiroa dilatata* were investigated in HT1080 cells. Radical simulated oxidation of membrane proteins and lipids were also inhibited by treatment with *Amphiroa dilatata* extract in a concentration-dependent manner. In addition, these results revealed that *Amphiroa dilatata* has an excellent scavenging ability on ROS-induced oxidative damage. Moreover, this extract reduced the expression levels of MMP-2 and MMP-9 in gelatin zymography, and RT-PCR analysis without any cytotoxic influence[52].

Zhang et al. studied phlorotannin derivative 6,6'-bieckol isolated and characterized from an edible marine brown alga *Ecklonia cava* (EC), according to the comprehensive spectral analysis of MS and NMR data in Fig. 6[53]. Here the influence of 6,6'-bieckol on expressions of MMPs was examined by zymography and western blot analysis via HT1080 cells. It is shown that 6,6'-bieckol significantly down regulated the expressions of MMP-2 and MMP-9 in dose-dependent manner.

The influence of 6,6'-bieckol on the cell viability and cell behavior of HT1080 cells were also investigated, our dates shown that it suppressed the migration and 3D culture in HT1080 cells. Meanwhile, they explored several signal pathways that may contribute to this process, and found the suppressing of MMPs expressions in HT1080 cells might be due to the suppression of NF-κB signal pathway.

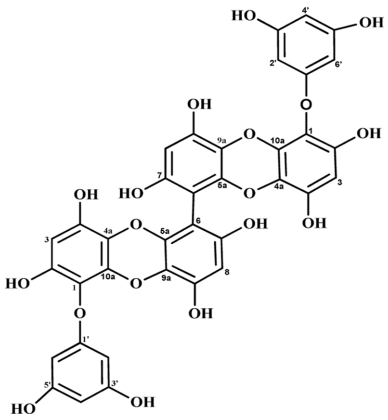


Fig. 6. Chemical structures of 6,6'-bieckol.

2.6. Other Marine Compounds

2.6.1. *Floridoside*, and *D-Isofloridoside*

In the exploration of abundant marine biological resources, edible red alga *Laurencia undulata* led to two bioactive isolates, floridoside and D-isofloridoside (Fig. 7). The antioxidant properties of both derivatives (floridoside and D-isofloridoside) were characterized via free radical scavenging using the ESR technique, reactive oxygen species (ROS) inhibition, membrane protein

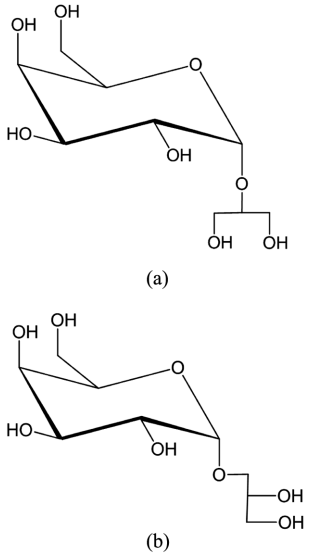


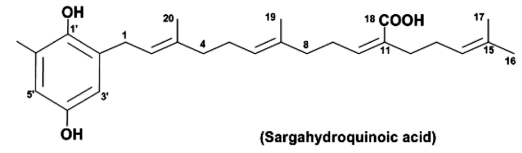
Fig. 7. Chemical structures of floridoside [α-D-galactopyranosyl-(1-2)-L-glycerol] (a) and D-isofloridoside [α-D-galactopyranosyl-(1-1)-D-glycerol] (b).

oxidation, myeloperoxidase inhibition, gene expression levels of glutathione (GSH) and superoxide dismutase, and protein expression of MMP-2 and MMP-9[54].

The results demonstrate that floridoside and D-isofloridoside possess significant antioxidant capacity and are potential inhibitors of MMP-2 and MMP-9. These results clarified that these components may be responsible for the relative activities of crude extract from this genus, which is used as folk medicine. Furthermore, the structure-activity relationships were also suggested. Both isomers could be effective candidates for applications in food and pharmaceutical fields as natural marine antioxidants.

2.6.2. *Sargahydroquinoic acid, Sargachromanol E, D*

Systematic separation by diverse chromatographic methods led to the isolation of sargahydroquinoic acid and sargachromanols (1-3) (Fig. 8). Antioxidative and matrix metalloproteinase (MMP) inhibitory effects of methanolic extract from *Sargassum thunbergii* were investigated in HT1080 cells. This extract suppressed the electron spin resonance (ESR) signal intensity on generation of DPPH radicals and intracellular reactive oxygen species formation by 2',7'-dichlorofluorescin diacetate method. In addition, treatment with this extract



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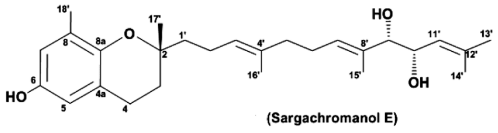

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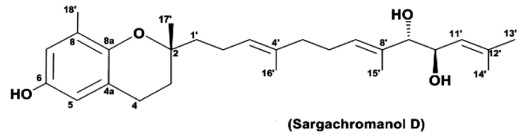

(c)

Fig. 8. Chemical structures of compounds 1,3 from S.thunbergii. (a) Sargahydroquinoicacid. (b) SargachromanolE. (c) SargachromanolD.

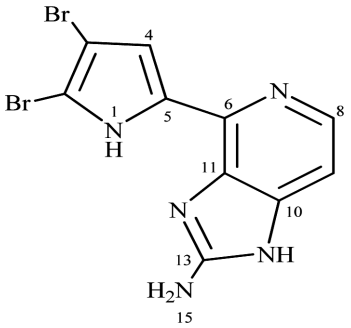

Fig. 9. Chemical structures of ageladine A.

inhibited radical simulated oxidation of membrane lipids and proteins in a dose-dependent manner[55]. These results revealed that *S. thunbergii* extract has excellent scavenging abilities in ROS-induced damage. Moreover, this extract led to the reduction of the expression levels of MMP-2 and MMP-9 in gelatin zymography, RT-PCR and western bolt analysis.

2.6.3. *Ageladine A*

Fujita et al. found that the hydrophilic extract of the marine sponge *Agelas nakamurai* inhibited MMP-2 significantly[56]. They reported that the isolation, structure elucidation, and antiangiogenic activity of the new alkaloid. Their results indicate that the ageladine A has considerably inhibited the MMP-2 (Fig. 9). In addition, ageladine A showed 33.3% inhibition of cell migration using bovine aortic endothelial cells at 5 μg/mL and 65.9% inhibition at 25 μg/mL.

3. Conclusions

The third level of restricting the proteolytic activities of MMPs includes endogenous tissue inhibitors of MMPs (TIMPs). TIMPs specifically inhibit active forms of MMPs, and in some cases, latent MMPs as well, and disturbance in this balance may lead to pathological situations in tissues[57]. Recent findings indicate that serine proteinase inibitor, tissue factor pathway inhibitor-2, inhibits, MMP-2, MMP-9[58]. Such as Ageladine A, which inhibit MMP-2 was not competitive judging from the Lineweaver-Burk plot. Thus, the inhibition mechanism of Ageladine A was presumed to be unique. These MMPIs will be the focus of future work. Table 1 lists the major classes about inhibitory effect from marine substances on MMP-2 and MMP-9.

Table 1. Matrix Metalloproteinase Inhibitors (MMPIs) from Marine Substances

Compound name	Origin	Activity
Chitooligosacc harides	Crustaceans, Microorganisms	MMP-2, MMP-9
Carboxylated Chitooligosacc harides	Crustaceans, Microorganisms	MMP-9
Carboxymethylations of chitosan and chitin	Crustaceans, Microorganisms	MMP-2, MMP-9
Glucosamine	Fungus	MMP-2, MMP-9
Carboxylated glucosamine	Fungus	MMP-9
Sulfated glucosamine	Fungus	MMP-2, MMP-9
λ-carrageenan	*Gigartina stellata Chondrus crispus*	MMP-2
Sulfated polysaccharide	*Sepiella maindroni ink*	MMP-2
Fucoidan	Mozuku	MMP-2, MMP-9
Flavonoid Glycosides	*Salicornia herbacea*	MMP-2, MMP-9
Phlorotannins	*Ecklonia cava*	MMP-2, MMP-9
Luteolin	*Zostera marina*	MMP-2, MMP-9
Sargahydroquinoic acid Sargachromanol E, D	*Sargassum thunbergii*	MMP-2, MMP-9
Floridoside, D-Isofloridoside	*Laurencia undulata*	MMP-2, MMP-9
6,6'-bieckol	*Ecklonia cava*	MMP-2, MMP-9
Glycosaminoglycan	*Litopenaeus vannamei*	MMP-9
Phenolic	*Amphiroa dilatata Corallina pilulifera*	MMP-2, MMP-9
Ageladine A	*Agelas nakamurai*	MMP-2

Although many of the synthetic inhibitors of MMPs showed good inhibitory activity. However, the compounds do not have an ideal MMPs selectivity, combined with others limitations such as the low oral bioavailability, unstable metabolism, biological toxicity, and also these inhibitors in clinical trials show excessive side effects[12].

Previously, it has been reported that fibrosarcoma HT1080 cells secrete type IV collagenase, MMP-2 and MMP-9, and that these enzymes play a major role in cancer metastasis. Ecklonia cava extract did not exert any cytotoxic effect even at 100 μg/mL anticipating its potential use as a safe MMP inhibitor[31]. COS-I has an inhibitory effect on MMP-9 invitro. Therefore, it can be suggested that COS-I can prevent metastasis in vivo through inhibition of MMP-9 expression[2].

Acknowledgements

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