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Mini-Review

PROTEASE INHIBITORS FROM MARINE SPONGE AND SPONGE-ASSOCIATED MICROORGANISMS

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Abstract. Protease inhibitors are proteins or peptides inhibiting the activity of protease and constitute a very important mechanism for regulating protease activity. So far, protease inhibitors have been used for the study of enzyme structures and reaction mechanisms, but recently they have also been used in pharmaceutical, agricultural and industrial fields. Compared to terrestrial counterparts, marine environment possesses their unique characters, therefore, they are capable of producing a wide range of novel bioactive compounds including protease inhibitors. In our review, a brief overview of protease inhibitors (e.g., classification, mechanisms, and characteristics of protease inhibitor) and protease inhibitors from marine sponges and sponge-associated microorganisms has been reviewed.

Keywords: action mechanisms, marine sponge, protease, protease inhibitors, sponge-associated microorganisms.

Classification numbers: 1.2.1; 1.5.1; 1.5.3

1. INTRODUCTION OF PROTEASE INHIBITOR

Proteases, also called proteolytic enzymes, are enzymes capable of hydrolyzing peptide bonds of peptide chains and proteins into shorter peptides and amino acids [1]. They are responsible for different physiological functions in the body such as activating zymogene, clotting and degrading fibrin fiber of blood clots, releasing hormones and biologically active peptides from precursors as well as transporting protein across the membrane. Although proteases play a crucial role in host cells, they can be harmful in excess or high concentrations. They can activate cancer and cause many diseases (e.g., neurological disorders, inflammation, and cardiovascular diseases) [1, 2]. Therefore, the function of these proteolytic enzymes should be monitored and controlled strictly. The most important control systems of protease are protease inhibitors (PIs). These inhibition molecules can block the activity of proteases. Furthermore, PIs are also found to have other functions such as activating growth factors, eliminating receptors or promoting cancer [1].

Natural inhibitors were first reported in 1894 by Femi and Pernossi when they discovered antisera activity in human serum [3]. To date, PIs have been found extensively in nature from different sources including animals, plants, and microorganisms (Table 1).

Table 1. Several protease inhibitors from animal, plant, and bacterial sources [4, 5].

Inhibitors	Source	Inhibited protease
A. PIs from animals		
Ov-SPI-l	*Onchocerca volvulus*	Serine
Anisakis simplex inhibitor	*Anisakis simplex*	Serine
AceKI	*Ancylostoma ceylanicum*	Trypsin, Chymotrypsin, Pancreatic elastase
Chagasin	*Trypanosoma cruzi*	Endogenous cysteine
SGTI	*Schistocerca gregaria*	Bovine trypsin
RsTI	*Rhipicephalus sanguineus*	Trypsin protease
CVPI	*Crassostrea virginica*	Thermolysin
CGPI	*Crassotrea gigas*	Thermolysin
PSKP 1 & 2	*Phyllomedusa sauvagii*	Endo peptidase
LTI	*Lymnaea*	Trypsin
B. PIs from plants		
Kunitz trypsin inhibitor	*Glycine max*	Trypsin, Chymotrypsin
Barley subtilisin inhibitor	*Hordeum vulgare*	Subtilisin, α-amylase
Chymotrypsin Inhibitor	*Psophocarpus tetragonolobus*	α-chymotrypsin
Kunitz cysteine peptidase inhibitor 1	*Solanum tuberosum*	Cysteine proteases
Proteinase inhibitor A inhibitor unit	*Sagittaria sagittifolia*	Trypsin, Chymotrypsin, Kallikerin
Kunitz subtilisin inhibitor	*Canavalia lineata*	Subtilisin-type microbial serine proteases
Cathepsin D inhibitor	*Solanum tuberosum*	Cathepsin D, Trypsin
Trypsin inhibitor	*Acacia confusa*	Trypsin, α-chymotrypsin
Ragi seed trypsin/α-amylase inhibitor	*Eleusine coracana*	α-amylase
Barley trypsin/factor XIIa inhibitor	*Hordeum vulgare*	α-amylase, Trypsin
Trypsin/α-amylase inhibitor	*Triticum aestivum*	α-amylase, Trypsin
Trypsin/factor XIIa inhibitor	*Zea mays*	Mammalian trypsin, activated hageman factor
Trypsin inhibitor MCTI-1	*Momordica charantia*	Pancreatic elastase
Trypsin inhibitor MCTI-II	*Momordica charantia*	Trypsin
Macrocyclic squash trypsin inhibitor	*Momordica cochinchinensis*	Trypsin

Trypsin inhibitor CSTI-IV	*Cucumis sativus*	Trypsin
Chymotrypsin inhibitor I	*Solanum tuberosum*	Chymotrypsin, Trypsin
Glutamyl peptidase II	*Momordica charantia*	Glu *S. griseus* protease , Subtilisin
Subtilisin-chymotrypsin inhibitor CI-1A	*Hordeum vulgare*	Subtilisin, Chymotrypsin
Subtilisin/chymotrypsin inhibitor	*Triticum aestivum*	*B. lichenoformis* subtilisin, α-chymotrypsin
Mustard trypsin inhibitor	*Sinapis alba*	Beta-trypsin
Mustard trypsin inhibitor-2	*Brassica hirta*	Bovine beta-trypsin, α-Chymotrypsin
Rape trypsin inhibitor	*Brassica napus*	Trypsin, Chymotrypsin
Metalloprotease inhibitor	*Bothrops jararaca*	Atrolysin C, Jararhagin.
Sarcocystatin	*Sarcophaga peregrina*	Cysteine proteinase
Bowman–Birk plant trypsin inhibitor unit 1	*Glycine max*	Trypsin, Chymotrypsin
Bowman-Birk trypsin/ chymotrypsin inhibitor	*Arachis hypogaea*	Trypsin, Chymotrypsin
Sunflower cyclic trypsin inhibitor	*Helianthus annuus*	Trypsin, Cathepsin G, Elastase, Chymotrypsin and thrombin
Proteinase inhibitor II	*Solanum tuberosum*	Trypsin, Chymotrypsin
Potato peptidase inhibitor II inhibitor unit 1	*Solanum tuberosum*	Trypsin, Chymotrypsin
Tomato peptidase inhibitor II inhibitor unit 1	*Solanum lycopersicum*	Trypsin, Chymotrypsin
Tomato peptidase inhibitor II inhibitor unit 2	*Solanum lycopersicum*	Trypsin, Chymotrypsin
C. PIs from microorganisms		
Marinostatins	*Alteromonas* sp.	Cysteine
Monastatin	*Alteromonas* sp.	Serine
POI	*Pleurotus ostreatus*	Serine proteinase A
Lentinus proteinase	*Lentinus edodes*	Trypsin
SLPI	*Streptomyces lividans*	Subtilisin BPN
SMPI	*Streptomyces nigrescens*	Metalloprotease
Sma PI	*Serratia marcescens*	Metalloprotease

PIs are known as one of the important catalysts in protein purification procedures as they may minimize proteolysis during heterologous expression or protein extraction. Additionally, PIs may support for effective purification of proteases using affinity chromatography. In medicine, PIs can be used for diagnosing and treating different diseases (e.g., viral, bacterial, fungal and parasitic diseases, cancer and immunological, neurodegenerative and cardiovascular diseases [6]. In some circumstances, PIs may be used as drugs for the treatment of diseases using the synthetic inhibitors or the natural inhibitors [7, 8]. Approximately, 32 PIs are currently in clinical use, most of them are synthetic molecules developed by structure-based design [9]. In addition, several protease inhibitors found in natural sources are also in clinical use. For examples, an aspartic protease inhibitor of HIV-1 (ritonavir) has been used since 1996 for the

AIDS treatment, and boceprevir and telaprevir also approved by the FDA in 2011 for the treatment of hepatitis C virus infection [10].

Furthermore, protease inhibitors can be involved in crop protection against plant pathogens and herbivorous pests in agriculture [11]. Exploration and use of novel PIs with protective function are one of the important tools in crop protection and the development of environmentally friendly pest and pathogen management strategies. The genetically modified plants expressing inhibitors of the digestive enzymes of their insect pests are already under study [12, 13].

2. CLASSIFICATION OF PROTEASE INHIBITORS

PIs may be classified based on different ways: their structure (primary and three-dimensional), the source organism (microbial, fungal, plant, animal), their inhibitory profile (broad range, specific), and action mechanism (competitive, non-competitive, uncompetitive, reversible or irreversible) or based on the class of protease they inhibit (aspartic, cysteine or serine protease inhibitors).

Currently, PIs are commonly grouped into two groups: (1) small molecule inhibitors and (2) proteinaceous inhibitors.

Small molecule inhibitors (SMIs)

SMIs are low molecular mass peptides and synthetic inhibitors from microorganisms. They are inhibitors that are not proteins, include natural compounds (e.g., pepstatin, bestatin and amastatin) as well as synthetic inhibitors generated in a laboratory [14]. To date, most of the natural SMIs have been isolated from bacteria and fungi [15]. Each SMI is named by an initial J followed by a five-digit number. For example, pepstatin is J00095 [16].

Several SMIs have proved useful in inhibition of diseases such as retropepsin of the HIV virus [17], thrombin of thrombosis, dipeptidyl-peptidase IV (18, 19, 20], γ-secretase of Alzheimer's disease [21], renin [22] and angiotensin-converting enzyme of blood pressure [23], and peptidases from the malarial parasite *Plasmodium* [24]. Furthermore, some SMIs are also found as anticancer and antinutritional agents [20, 25, 26, 27, 28].

Proteinaceous inhibitors

Proteinaceous inhibitors are ubiquitous inhibitors and isolated from different sources (e.g., microorganisms, plants, and animals). Natural proteinaceous inhibitors are known as templates for the modification of natural control mechanisms and as a source of basic design principles [29]. Proteinaceous inhibitors are usually classified based on the kind of inhibited protease. Currently, hundreds of protein inhibitors of peptidases are known [30].

According to lastest update of MEROPS database (http://www.ebi.ac.uk/merops/), PIs are grouped into 83 families based on comparisons of protein sequences. However, molecular weight and mechanism of inhibition of PIs are dissimilar. Therefore, these families are further grouped into clans based on comparisons of their tertiary structure. Each clan, family and biochemically characterized peptidase inhibitor is assigned a unique identifier. A family is identified by a letter "I" followed by a number and two-letter clan identifier starts with "I" or "J" [14, 30]. Some families of proteinaceous inhibitors from microbes and fungi are listed in Table 2.

3. MECHANISM OF PROTEASE INHIBITORS

Competitive inhibition

The majority of PIs are known as competitive inhibitors. Generally, these inhibitors often bind to the active sites of target proteases in a substrate-like manner (Figure 1). In some case, the competitive inhibitors bind in and block access to the active site of target proteases, but do not bind in a strictly substrate-like manner. They may interact with protease subsites and catalytic residues in a non-catalytically competent manner [31]. Although the competitive mechanism is considered as an effective strategy of competitive inhibitors, the proteases often have a high degree of homology in the active sites, substrate-like binding may, therefore, lead to inhibitors that can inhibit many different proteases [31]. For example, the activity of 612 known human proteases is regulated by about 115 human protease inhibitors [9]. The inhibitors of serine protease including the Kazal, Kunitz, and Bowman-Birk family are examples of competitive inhibitors [32].

Table 2. Families of proteinaceous inhibitors of microbial and fungal origin [29].

Family	Common name	Families of peptidases inhibited
I1	Kazal	M10, S1A, S1D, S8A, S9A
I2	Kunitz-BPTI	S1A, S7
I4	Serpin	C1A, C14A, S1A, S7, S8A, S8B
I9	YIB	S8A
I10	Marinostatin	S1A, S8A
I11	Ecotin	S1A
I16	SSI	M4, M7, S1A, S8A, S8B
I31	Thyropin	A1A, C1A, M10A
I32	IAP	C14A
I34	IA3	A1A
I36	SMI	M4
I38	Aprin	M10B
139	-	A1A, A2A, C1A, C2A, C11, M4, M10A, M10B, M12A, M12B, S1A, S1B, S8A
I42	Chagasin	C1A
I43	Oprin	M12B
I48	Clitocypin	C1A, C13
I51	IC	S1A, S10
I57	Staphostatin B	C47
I58	Staphostatin A	C47
I63	-	M43B, S1A
I66	Cnispin	S1A
I69	-	C10
I75	CIII	M41
I78	-	S1A, S8A
I79	AVR2	C1A
I85	Macrocypin	C1A, C13, S1A
I87	Hf1KC	M41

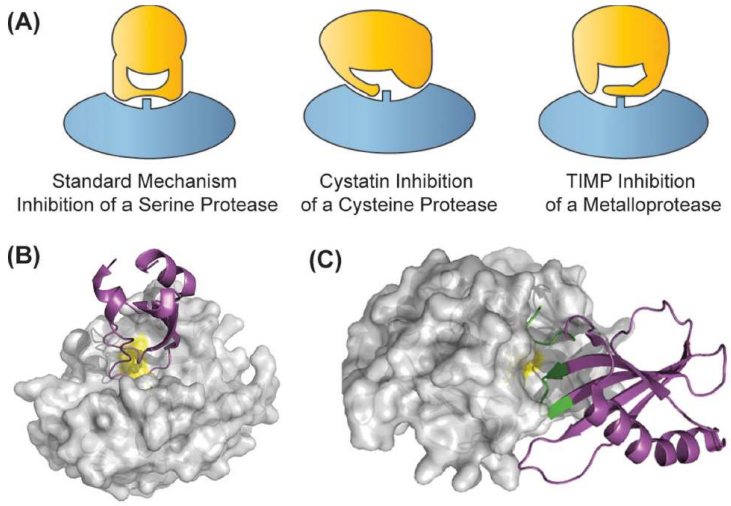

Figure 1. Competitive inhibitors of proteases. (A) Inhibitors bind in the active site, but not in a substrate-like manner. Peptide extensions bind in specificity subsites and can interact with the catalytic residues (rectangle). (B) Crystal structures of a serine protease in complex with the standard mechanism inhibitor aprotinin, and (C) the cystatin stefin A in complex with a cysteine protease. The portion of stefin A that interacts with the protease is coloured in green. Both inhibitors bind in the active site groove of their targets [31]. The reuse of this figure was permitted by John Wiley and Sons publisher under licensed number: 4390911096682.

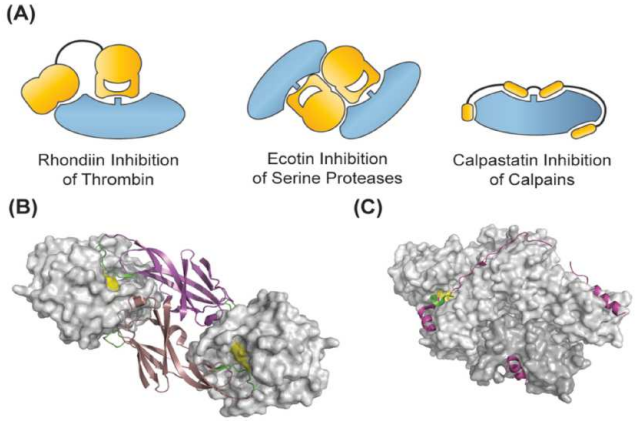

Figure 2. The competitive inhibitors with exosite binding (A). Most exosite inhibitors are competitive inhibitors that prevent substrate binding at the active site. In the case of (B) ecotin (bound to trypsin), the exosites provide binding energy and allow for broad specificity, while (C) calpastatin gains binding energy and specificity by forming critical interactions across the calpain protease surface [31]. The reuse of this figure was permitted by John Wiley and Sons publisher under licensed number: 4390911096682.

Competitive inhibition with exosite binding

In some PIs, they are not only competitive and bind to the protease active site, but also bind to secondary sites outside the active site [31]. This inhibition mechanism has two benefits. Firstly, it may increase the surface area of the protein-protein interaction, which results in a greater affinity. Secondly, it can provide a significant effect on the specificity of the inhibitor

[31]. Some inhibitors such as Rhondiin, Ecotin, and Calpastatin are examples of this inhibition mechanism (Figure 2).

Irreversible inhibition

Generally, irreversible inhibitors activate the proteolysis by the enzymes they inhibit, leading to a covalent modification of the enzyme. In this mode, inhibitors act as substrates in order to trap and inhibit the enzyme using the enzyme's catalytic machinery [31]. The serpins, a family of inhibitors act as the irreversible inhibitors (Figure 3).

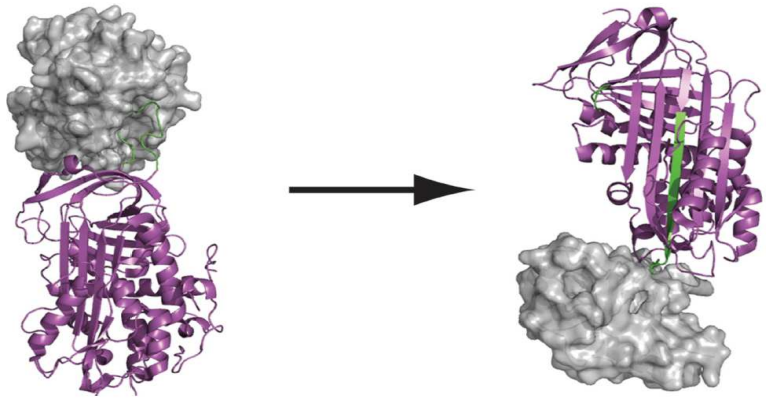

Figure 3. Serpins inhibit serine proteases by binding a reactive centre loop in the active site, forming a covalent complex with the enzyme, undergoing a large conformational change, and irreversibly distorting the active site of the protease [31]. The reuse of this figure was permitted by John Wiley and Sons publisher under licensed number: 4390911096682.

4. CHARACTERIZATION OF PROTEASE NHIBITORS

PIs are one of the most important class of proteins that can be applied to various fields. Therefore, characterizing PIs is crucial for determining their scope and application. The determination of the physicochemical properties and the structural stability of PIs is key to select effective and stable inhibitors for their application.

pH and temperature stability

Thermal stability of PIs is one of the important properties for their biotechnological applications. The extreme conditions of pH and temperature might cause the distortion of the structure of the PIs. Therefore, the combination of inhibitors with the enzymes or their substrates may be broken [33]. Ryan et al. [34] reported that many PIs exhibiting anti-feedant activity are active against the neutral serine proteases such chymotrypsin and trypsin. The pH and temperature stability of the PIs can also be involved in the presence of disulfide linkages [35]. The functional stability of Kunitz type PIs are related to the intra-molecular disulphide bridges in the presence of physical and chemical denaturants (e.g., temperature, pH and reducing agents) [36].

Effect of metal ions

The presence of metal ions is essential for the activity of PIs. The metal ions play an important role in maintaining the structure of PIs. The structural stability of proteins is enhanced by divalent metal ions as the metal ions can attain the critical conformation that is needed for

biological activity of the protein [29]. For example, cysteine protease inhibitor from pearl millet needs the Zn^{2+} for the protease inhibitory and antifungal activity of the protein [37].

Effect of oxidizing agents

The proteins may be oxidized when they expose to oxidising agents (e.g., H_2O_2, periodate, dimethyl sulfoxide, N-chlorosuccinamide, chloramine-T), and to oxidants released by neutrophils (e.g. superoxide, hydroxyl radical) [38, 39]. The oxidation of methionine residues may result in a decline in the biological activity of the protein. A report of the influence of methionine oxidation on α1-protease inhibitor (α1-PI) showed that the oxidation of one of the methionine residues (Met358) lead to a complete loss of inhibitory activity of the α1-protease inhibitor [40].

Effect of reducing agents

In some proteins, the covalent linkage of cysteine residues by disulfide bonds is one of the crucial elements in maintaining their conformational stability and biological activity. This covalent linkage plays a crucial role in the proper folding, stability and function of many proteins [41, 42, 43]. Conformational destabilization of the protein may result from the removal of the covalent link of cysteine residues caused by reduction or substitution of another amino acid residue [44, 45].

Effect of detergents

Some detergents (e.g., cationic, anionic, zwitterionic and non-ionic) are used for solubilizing proteins from lipid membranes. Therefore, proteinase inhibitors are often combined with detergents in cell lysis buffers to inhibit undesired proteolysis and facilitate membrane protein solubilization in protein purification procedures [29]. Normally, nonionic detergents are considered as mild detergents and not interact extensively with the protein surface, while ionic detergents (e.g., SDS) generally bind unwanted to the protein surface, which results in protein unfolding [46]. Additionally, Triton X-100, Tween 20 and Tween 80 are nonionic detergents and the majority of their interaction with proteins are hydrophobic [47].

Effect of chemical modifiers

Chemical modification is a useful method for changing undesired characteristics of a protein related to stability and catalytic activity. In a chemical modification, the chemical reagents bind covalently to specific amino acid chains of proteins and produce changes in the biological property of the protein [48, 49]. For example, Urwin et al. (1995) reported that chemical modification might enhance the activity of PIs against proteinases of the pests [50]. In other studies, chemical modification of soybean cystatin scN and tomato multicystatin reveals the considerable influences of the substitution of individual amino acid residues in the N-terminal portion of one of multicystatin domains on its ability to inhibit diverse proteinases [51, 52].

5. PROTEASE INHIBITORS FROM SPONGE AND SPONG-ASSOCIATED MICROORGANISMS

Protease inhibitor from marine sponges

Although marine sponges are known as the most simple organisms, they are able to produce a great number of biologically active compounds, including PIs. A summary table of compounds from marine sponges with protease inhibitory activity is shown in Table 3. The compounds with protease inhibitory activity from marine sponge are diverse and exhibit inhibitory activity

against many different proteases. For example, cyclotheonellazoles isolated from sponge *Theonella* inhibited various proteases such as chymotrysin, elastase, malaria parasite from *Plasmodium falciparum*, thrombin, plasmin [53]. Cyclotheonamides from sponge *Theonella swinhoei* and *Theonella* sp. also inhibited two proteases thrombin (IC_{50} = 5.2 - 13 nM) and trypsin (IC_{50} = 7.4 - 370 nM) [54, 55]. Interestingly, other compounds from the same sponge species *Theonella swinhoei* such as nazumazoles, pseudotheonamides, dihydrocyclotheonamide A showed inhibitory activity against proteases (e.g., thrombin, RCE-protease, chymotrypsin) [65, 70]. To date, many new compounds extracted from various sponge have been known as protease inhibitors (see Table 3), indicating that marine sponge is one of the potential sources for mining protease inhibitors.

Table 3: Protease inhibitors from marine sponges.

Compounds	Sponge	Inhibited protease and activity	Ref.
Cyclotheonellazole A	*Theonella aff. swinhoei*	Chymotrypsin (IC50 = 0.62 nM) Elastase (IC50 = 0.034 nM) Malaria parasite (IC50 > 20 μg/mL)	[53]
Cyclotheonellazole B	*Theonella aff. swinhoei*	Chymotrypsin (IC50 = 2.8 nM) Elastase (IC50 = 0.10 nM) Malaria parasite (IC50 > 20 μg/mL)	[53]
Cyclotheonellazole C	*Theonella aff. swinhoei*	Chymotrypsin (IC50 = 2.3 nM) Elastase (IC50 = 0.099nM) Malaria parasite (IC50 > 20 μg/mL)	[53]
Cyclotheonamide A	*Theonella* sp.	Thrombin (IC50 = 0.076 μg/mL) Trypsin (IC50 = 0.2 μg/mL) Plasmin (IC50 = 0.3 μg/mL)	[54]
Cyclotheonamide C	*Theonella swinhoei*	Thrombin (IC50 = 8.4 nM) Trypsin (IC50 = 7.4 nM)	[55]
Cyclotheonamide D	*Theonella swinhoei*	Thrombin (IC50 = 5.2 nM) Trypsin (IC50 = 63 nM)	[55]
Cyclotheonamide E	*Theonella swinhoei*	Thrombin (IC50 = 28 nM) Trypsin (IC50 = 370 nM)	[55]
Cyclotheonamide E2	*Theonella* sp.	Thrombin (IC50 = 13 nM) Trypsin (IC50 = 55 nM)	[56]
Cyclotheonamide E3	*Theonella* sp.	Thrombin (IC50 = 9.5 nM) Trypsin (IC50 = 52 nM)	[56]
Plakortide E	*Plakortis halichondroides*	Cathepsin B, cathepsin L, falcipain, rhodesain, SARS M^{pro}, SARS PL^{pro}, DENV-2^{pro}, Chymotrypsin (inhibition 10 – 90%)	[57]
Miraziridine A	*Theonella swinhoei*	Cathepsin L (inhibition 60%)	[58]
Miraziridine A	*Theonella aff. mirabilis*	Trypsin, cathespin L, cathespin B, pepsin	[59]
Tokaramide A	*Theonella aff. mirabilis*	Cathepsin B (IC50 = 29.0 μg/mL)	[60]
1-methylherbipoline salts of halisulfate-1	*Coscinoderma mathewsi*	Thrombin(IC50 > 100 μg/mL) Trypsin (IC50 = 25 μg/mL)	[61]
1-methylherbipoline salts of sulvanine	*Coscinoderma mathewsi*	Thrombin (IC50 = 27 μg/mL) Trypsin (IC50 = 12 μg/mL)	[61]
Sodium salt of halisulfate-1	*Coscinoderma mathewsi*	Thrombin (IC50 = 35 μg/mL) Trypsin (IC50 = 2 μg/mL)	[61]
Sodium salts of suvanine	*Coscinoderma mathewsi*	Thrombin (IC50 = 9 μg/mL) Trypsin (IC50 = 27 μg/mL)	[61]

N,N-dimethylguanidium salts of suvanine	*Coscinoderma mathewsi*	Thrombin (IC50 = 25 μg/mL) Trypsin (IC50 = 23 μg/mL)	[61]
Dysinosin A	*Lamellodysidea chlorea*	Thrombin (Ki = 0.108 μM) FVIIa (Ki = 00.452 μM)	[62]
Dysinosin B	*Lamellodysidea chlorea*	Thrombin (Ki = 0.090 μM) FVIIa (Ki = 0.170 μM)	[62]
Dysinosin C	*Lamellodysidea chlorea*	Thrombin (Ki = 0.124 μM) FVIIa (Ki = 0.550 μM)	[62]
Dysinosin D	*Lamellodysidea chlorea*	Thrombin (Ki = 1.320 μM) FVIIa (Ki > 5.1 μM)	[62]
Crude extracts	*Jaspis stellifera*	*E. coli protease* (MIC = 0.08%) *S. aureus* protease (MIC = 0.08%)	[63]
Crude extracts	*Plakortis nigra*	*S. aureus* protease (MIC = 0.12%)	[63]
Esculetin-4-carboxylic acid ethyl ester	*Axinella cf. corrugata*	SARS-coronovirus 3CL (ID50 = 46mM/L)	[64]
Pseudotheonamide A1	*Theonella swinhoei*	Thrombin (IC50 = 1.0 μM) Trypsin (IC50 = 4.5 μM)	[65]
Pseudotheonamide A2	*Theonella swinhoei*	Thrombin (IC50 = 3.0 μM) Trypsin (IC50 > 10 μM)	[65]
Pseudotheonamide B2	*Theonella swinhoei*	Thrombin (IC50 = 1.3 μM) Trypsin (IC50 = 6.2 μM)	[65]
Pseudotheonamide C	*Theonella swinhoei*	Thrombin (IC50 = 0.19 μM) Trypsin (IC50 = 3.8 μM)	[65]
Pseudotheonamide D	*Theonella swinhoei*	Thrombin (IC50 = 1.4 μM) Trypsin (IC50 > 10 μM)	[65]
Dihydrocyclotheonamide A	*Theonella swinhoei*	Thrombin (IC50 = 0.33 μM) Trypsin (IC50 = 6.7 μM)	[65]
Barangcadoic acid A	*Hippospongia* sp.	RCE-protease (IC50 = 10 μg/mL)	[66]
Rhopaloic acid A	*Hippospongia* sp.	RCE-protease (IC50 = 10 μg/mL)	[66]
Rhopaloic acid B	*Hippospongia* sp.	RCE-protease (IC50 = 10 μg/mL)	[66]
Rhopaloic acid C	*Hippospongia* sp.	RCE-protease (IC50 = 10 μg/mL)	[66]
Rhopaloic acid D	*Hippospongia* sp.	RCE-protease (IC50 = 10 μg/mL)	[66]
Rhopaloic acid E	*Hippospongia* sp.	RCE-protease (IC50 = 10 μg/mL)	[66]
Crude extract C-29EA	*Amphimedon* sp.	NS3 protease (IC50 = 10.9 μg/mL)	[67]
Toxadocial A	*Toxadocia cylindrical*	Thrombin (IC50 = 6.5 μg/mL)	[68]
5,9,23-Triacontatrienoic methyl ester	*Chondrilla nucula*	Elastase (ID50 = 10 μg/mL)	[69]
Nazumazole D	*Theonella swinhoei*	Chymotrypsin (IC50 = 2 μM)	[70]
Nazumazole E	*Theonella swinhoei*	Chymotrypsin (IC50 = 3 μM)	[70]
Nazumazole F	*Theonella swinhoei*	Chymotrypsin (IC50 = 10 μM)	[70]
Asteropterin	*Asteropus simplex*	Cathepsin B (IC50 = 1.4 μg/mL)	[71]
Shishicrellastatin A	*Crella (Yvesia) spinulata*	Cathepsin B (IC50 = 8 μg/Ml)	[72]
Shishicrellastatin B	*Crella (Yvesia) spinulata*	Cathepsin B (IC50 = 8 μg/mL)	[72]
Xestosaprol F	*Xestospongia* sp.	BACE1 (IC50 = 135 μM)	[73]

Xestosaprol G	*Xestospongia* sp.	BACE1 (IC50 = 155 µM)	[73]
Xestosaprol H	*Xestospongia* sp.	BACE1 (IC50 = 82 µM)	[73]
Xestosaprol I	*Xestospongia* sp.	BACE1 (IC50 = 163 µM)	[73]
Xestosaprol J	*Xestospongia* sp.	BACE1 (IC50 = 90 µM)	[73]
Xestosaprol K	*Xestospongia* sp.	BACE1 (IC50 = 93 µM)	[73]
Xestosaprol L	*Xestospongia* sp.	BACE1 (IC50 = 98 µM)	[73]
Xestosaprol M	*Xestospongia* sp.	BACE1 (IC50 = 104 µM)	[73]
Ancorinoside B	*Penares sollasi*	MT1-MMP (IC50 = 500 µg/mL) MMP2 (IC50 = 33 µg/mL)	[74]
Ancorinoside C	*Penares sollasi*	MT1-MMP (IC50 = 370 µg/mL)	[74]
Ancorinoside D	*Penares sollasi*	MT1-MMP (IC50 = 180 µg/mL)	[74]
Ancorinoside A	*Penares sollasi*	MT1-MMP (IC50 = 440 µg/mL)	[74]
Ageladine A	*Agelas nakamurai*	MMP-1 (IC50 = 1.2 µg/mL) MMP-2 (IC50 = 2.0 µg/mL) MMP-8 (IC50 = 0.39 µg/mL) MMP-9 (IC50 = 0.79 µg/mL) MMP-12 (IC50 = 0.33 µg/mL) MMP-13 (IC50 = 0.47 µg/mL)	[75]
Psammaplin A	*Poecillastra* sp. *Jaspis* sp.	mammalian aminopeptidase N	[76]
Aeroplysinin-1	Marine sponge	MMP-2	[77]

Protease inhibitor from sponge-associated microorganisms

Marine sponges are one of the most potential producers of bioactive agents among marine organisms. They have been proven to be a rich source of novel secondary metabolites with diverse bioactive activities (e.g., anticancer, antibiotic, protease inhibitory activity) [78, 79, 80, 81, 82]. However, there is still an ongoing debate about whether known bioactive compounds from sponges are originated from sponges or from their associated symbionts. Recent studies have evidenced that many previous compounds isolated from sponges are from their associated microorganisms [83, 84].

Although PIs can be found from different sources (e.g., microorganisms, plants, animals), there are a few number studies of PIs from the marine environment, especially from sponge-associated microorganisms. Recent studies have shown the potential protease inhibitors isolated from sponge-associated microorganisms (Table 4). The crude extracts from bacteria associated with Caribbean sponges exhibited inhibitory activity against different proteases such as cathepsin B, rhodesain, falcipain-2. In addition, these crude extracts showed immunomodulatory activity via induction of cytokine release by human peripheral blood mononuclear cells [85]. In another study, teromycins extracted from *Streptomyces axinellae* associated with sponge *Axinellae polypoides* also inhibited various proteases such as rhodesain, falcipain-2, cathepsin-L, cathepsin-B, SARS-CoV-PL^{pro} [86]. Furthermore, the crude extracts from bacteria associated with other sponge species (e.g., *Jasis* sp., *Plakortis nigra*, *Jasis stellifera*, *Xestospongia testudinaria*, *Aplysina aerophoba*) showed protease inhibitory activity against subtilisin, thermolysin as well as proteases from *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* [87, 88, 89].

Table 4: Protease inhibitors from sponge-associated microorganisms.

Compounds	Sponge	Bacteria	Inhibited protease and activity	Ref.
Crude extract	Caribbean Sponges	*Nocardioides* sp.	Rhodesain (inhibition 40 ± 1 %)	[85]
Crude extract	Caribbean Sponges	*Agrococcus jenensis*	Cathepsin B (inhibition 41 ± 1 %) Falcipain-2 (inhibition 44 ± 2 %)	[85]
Crude extract	Caribbean Sponges	*Micromonospora coxensis*	Falcipain-2 (inhibition 42 ± 2 %)	[85]
Crude extract	Caribbean Sponges	*Saccharopolyspora shandongensis*	Rhodesain (inhibition 52 ± 1 %)	[85]
Crude extract	Caribbean Sponges	*Rhodococcus* sp.	Cathepsin L (inhibition 44 ± 4 %)	[85]
Crude extract	Caribbean Sponges	*Micromonospora coxensis*	Cathepsin B (inhibition 45 ± 3 %) Cathepsin L (inhibition 43 ± 2 %) Falcipain-2 (inhibition 41 ± 2 %) Rhodesain (inhibition 57 ± 5 %)	[85]
Crude extract	Caribbean Sponges	*Sphingobium* sp.	Rhodesain (inhibition 53 ± 3 %)	[85]
Crude extract	Caribbean Sponges	*Sphingomonas mucosissima*	Cathepsin B (inhibition 49 ± 5 %) Falcipain-2 (inhibition 45 ± 1 %)	[85]
Tetromycin B	*Axinella polypoides*	*Streptomyces axinellae*	Rhodesain (Ki = 0.62 ± 0.03 µM) Falcipain-2 (Ki = 1.42 ± 0.01 µM) Cathepsin L (Ki = 32.5 ± 0.05 µM) Cathepsin B (Ki = 1.59 ± 0.09 µM) SARS-CoV-PL^{pro} (Ki = 69.6 ± 7.2 µM)	[86]
Tetromycin 3	*Axinella polypoides*	*Streptomyces axinellae*	Rhodesain (Ki = 2.1 ± 0.9 µM) Falcipain-2 (Ki = 1.65 ± 0.25 µM) Cathepsin L (Ki = 15.0 ± 1.95 µM) Cathepsin B (Ki = 0.57 ± 0.04 µM)	[86]
Tetromycin 4	*Axinella polypoides*	*Streptomyces axinellae*	Rhodesain (Ki = 4.0 ± 0.3 µM) Falcipain-2 (Ki = 3.1 ± 0.2 µM) Cathepsin L (Ki = 22.4 ± 0.8 µM) Cathepsin B (Ki = 1.6 ± 0.1 µM) SARS-CoV-Pl^{pro} (Ki = 40 ± 6.5 µM)	[86]
Diazepinomicin	*Aplysina aerophoba*	*Micromonospora*	Rhodesain (Ki = 98 µM) Cathepsin L (IC50 = 72.4 ± 5.3 Mm)	[87]
Crude extract	*Jaspis* sp.	*Providencia* sp.	Subtilisin (inhibition 91.57 %) Thermolysin (inhibition 59.4 7%) *E. coli* protease (inhibition 98.84 %)	[88]
Crude extract	*Jaspis* sp.	*Bacillus* sp.	Subtilisin (inhibition 57.23 %) Thermolysin (inhibition 70.37 %) *S. aureus* protease (inhibition 51.29 %)	[88]
Crude extract	*Jaspis* sp.	*Paracoccus* sp.	Subtilisin (inhibition 30.78 %) Thermolysin (inhibition 50.52 %) *P. aeruginosa* protease (inhibition 23.52 %)	[88]
Crude extract	*Jaspis* sp.	Unidentified bacteria	*P. aeruginosa* protease (inhibition 72.7 %)	[89]
Crude extract	*Plakortis nigra*	Unidentified bacteria	*E. coli* protease (inhibition 93.5 %)	[89]

Crude extract	*Jaspis stellifera*	Unidentified bacteria	*S. aureus* protease (inhibition 40.0 %)	[89]
Crude extract	*Xestospongia testudinaria*	*Chromohalobacter* sp.	*P. aeruginosa* protease (inhibition 95.5 %)	[89]

In spite of continuous attempts of discovering novel PIs from sponge-associated microorganisms, it is still a big challenge as an only minor fraction of sponge-associated microorganisms can be culture *in vitro*. Fortunately, the new advance approaches (e.g., metagenomics) provide powerful tools for discovering the biosynthetic gene clusters related to polyketide synthases and PIs from uncultured microorganisms [90]. This opens the new avenues for detecting novel bioactive metabolites including PIs in future. For example, a novel serine protease inhibitor (serpin) gene was detected and cloned from a metagenomic library of uncultured marine microorganisms. The phylogenetic analysis and the deduced amino acid sequence comparison of this gene indicated that it was closely related to Spi1C and some partial proteinase inhibitor I4 serpins. Furthermore, functional analyses demonstrated that the recombinant Spi1C protein could inhibit a series of serine proteases [91].

6. CONCLUSION

In this review, we summarised protease inhibitors with focusing on their classification, action mechanism, and characters as well as protease inhibitors from marine sponge and sponge-associated microorganisms. The marine environment poses unique characters and provides a prolific resource for novel bioactive compounds. Therefore, continuous efforts in the discovery of structure, functions, biophysical characterization, and mode of action of PIs from marine environment such marine sponge and sponge-associated microorganisms can open up opportunities for their potential role in medicine, biotechnology and agriculture.

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REFERENCES

1. Barrett A. J., Rawlings N. D., Woessner J. J. F. - Hand book of proteolytic enzymes. Academic Press. London (1998).
2. Chambers R. C., Laurent G. J. - Coagulation cascade protease and tissue fibrosis, Biochem. Soc. Trans. **30** (2) (2001) 194-200.
3. Fermi C., Pernossi L. - Untersuchungen uber die enzyme, Vergleichende Studie, Z. Hyg. Infektionskr. **18** (1) (1894) 83-89.
4. Bijina B. - Isolation, purification and characterization of protease inhibitor from Moringa oleifera LAM. Ph.D. Dissertation, Cochin University of Science and Technology, (2006).
5. Habib H., Fazili K. M. - Plant protease inhibitors: a defense strategy in plants, Biotechnology and Molecular Biology review **2** (3) (2007) 68-85.
6. Sabotic J., Kos J. - Microbial and fungal protease inhibitors-current and potential applications, Appl. Microbiol. Biotechnol. **93** (4) (2012) 1351-1375.
7. Grant P., Mackie A. - Drugs from the sea-facts and fantasy, Nature **267** (1977) 786-788.
8. Hamilton S. C., Farchaus J. W., Davis M. C. - DNA polymerases as engines for

biotechnology, Biotechniques **31** (2) (2001), 370-393.

9. Turk B. - Targeting proteases: successes, failures and future prospects, Nat. Rev. Drug. Discov. **5** (9) (2006) 785–799.
10. Fitzpatrick F. - Cyclooxygenase enzymes: Regulation and function, Curr. Pharma. Des. **10** (6) (2004) 577-588.
11. Sabotič J., Kos J. - Microbial and fungal protease inhibitors—current and potential applications, Appl. Microbiol. Biotechnol. **93** (4) (2012) 1351–1375.
12. Samac D., Smigocki A. - Expression of oryzacystatin I and II in alfalfa increases resistance to the root-lesion nematode, Phytopathology **93** (7) (2003) 799–804.
13. Telang M., Patankar A., Harsulkar A., Joshi V., Damle A., Deshpande V., Sainani M., Ranjekar P., Gupta G. - Bitter gourd proteinase inhibitors: potential growth inhibitors of *Helicoverpa armigera* and *Spodoptera litura*, Phytochemistry **63** (6) (2003) 643–652.
14. Umezawa H. - Low molecular weight enzyme inhibitors of microbial origin, Ann. Rev0 Microbiol. **36** (1982) 75-99.
15. Rawlings N. D. - Peptidase inhibitors in the MEROPS database, Biochimie **92** (11) (2010) 1463-1483.
16. Rawlings N. D., Barrett A. J. - MEROPS: the peptidase database, Wellcome Trust (2017).
17. Kempf D. J., Sham H. L., Marsh K. C., Flentge C. A., Betebenner D., Green B. E., Mcdonald E., Vasavanonda S., Saldivar A., Wideburg N. E., Kati W. M., Ruiz L., Zhao C., Fino L., Patterson J., Molla A., Plattner J. J., Norbeck D. W. - Discovery of ritonavir, a potent inhibitor of HIV protease with high oral bioavailability and clinical efficacy, J. Med. Chem. **41** (4) (1998) 602-617.
18. Feng J., Zhang Z., Wallace M. B., Stafford J. A., Kaldor S. W., Kassel D. B., Navre M., Shi L., Skene R. J., Asakawa T., Takeuchi K., Xu R., Webb D. R., Gwaltney S. L. - Discovery of alogliptin: a potent, selective, bioavailable, and efficacious inhibitor of dipeptidyl peptidase IV, J. Med. Chem. **50** (10) (2007)2297-2300.
19. Hughes T. E., Mone M. D., Russell M. E., Weldon S. C., Villhauer E. B. - NVPDPP728 (1-[[[2-[(5-cyanopyridin-2-yl) amino]ethyl] amino] acetyl]-2-cyano-(S)-pyrrolidine), a slow-binding inhibitor of dipeptidyl peptidase IV, Biochemistry **38** (36) (1999) 11597-11603.
20. Kim J. H., Mullin C. A. - Antifeedant effects of proteinase inhibitors on feeding behaviors of adult western corn rootworm (*Diabrotica virgifera* virgifera), J. Chem. Ecol. **29** (4) (2003) 795–810.
21. Imbimbo B. P. - Alzheimer's disease: gamma-secretase inhibitors, Drug Discov. Today Ther. Strat. **5** (3) (2008) 169-175.
22. Wood J. M., Maibaum J., Rahuel J., Grutter M. G., Cohen N. C., Rasetti V., Ruger H., Goschke R., Stutz S., Fuhrer W., Schilling W., Rigollier P., Yamaguchi Y., Cumin F., Baum H. P., Schnell C. R., Herold P., Mah R., Jensen C., O'Brien E., Stanton A., Bedigian M. P. - Structure-based design of aliskiren, a novel orally effective renin inhibitor, Biochem. Biophys. Res. Commun. **308** (4) (2003) 698-705.
23. Sybertz E. J., Watkins R. W., Ahn H. S., Baum T., Rocca P. L., Patrick J., Leitz F. - Pharmacologic, metabolic, and toxicologic profile of spirapril (SCH 33844),a new angiotensin converting inhibitor, J. Cardiovasc. Pharmacol. **10** (Suppl 7) (1987) S105-

S108.

24. Andrews K. T., Fairlie D. P., Madala P. K., Ray J., Wyatt D. M., Hilton P. M., Melville L. A., Beattie L., Gardiner D. L., Reid R. C., Stoermer M. J., Adams T. S., Berry C., McCarthy J. S. - Potencies of human immunodeficiency virus protease inhibitors in vitro against *Plasmodium falciparum* and in vivo against murine malaria, Antimicrob. Agents. Chemother. **50** (2) (2006) 639-648.
25. Hanada K., Tamai M., Yamagishi M., Ohmura S., Sawada J., Tanaka I. - Isolation and characterization of E-64, a new thiol protease inhibitor, Agr. Biol. Chem. Tokyo **42** (3) (1978) 523–528.
26. Frlan R., Gobec S. - Inhibitors of cathepsin B, Curr. Med. Chem. **13** (19) (2006) 2309–2327.
27. Oppert B., Morgan T. D., Kramer K. J. - Efficacy of *Bacillus thuringiensis* Cry3Aa protoxin and protease inhibitors against coleopteran storage pests, Pest. Manag. Sci. **67** (5) (2011) 568–573.
28. Amirhusin B., Shade R. E., Koiw H., Hasegawa P. M., Bressan R. A., Murdock L. L., Zhu-Salzma, K. - Protease inhibitors from several classes work synergistically against *Callosobruchus maculatus*, Journal of Insect Physiology **53** (7) (2007) 734–740.
29. Sapna K. - Isolation, purification, characterization and application of proteinaceous protease inhibitor from marine bacterium *Pseudomonas mendocina* BTMW 301. Ph.D. Dissertation, Cochin University of Science and Technology, (2006).
30. Rawlings N. D., Barrett A. J., Thomas P. D., Huang X., Bateman A., Finn R. D - The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database, Nucleic Acids Research **46** (D1) (2018) D624-D632.
31. Farady C. J., Craik C. S. - Mechanisms of macromolecular protease inhibitors, Chembiochem. **11** (17) (2010) 2341-2346.
32. Laskowski M. J., Kato I. - Protein inhibitors of proteinases, Ann. Rev. Biochem. **49** (1980) 593-626.
33. Veselovsky A. V., Ivanov Y. D., Ivanov A. S., Archakov A. I., Lewi P., Janssen, P. - Protein–protein interactions: mechanisms and modification by drugs, J. Mol. Recognit **15** (6) (2002) 405–422.
34. Ryan C. A. - Protease inhibitors in plants: genes for improving defenses against insects and pathogens, Ann Rev Phytopatho **28** (1990) 425–449.
35. Vernekar J. V., Ghatge M. S., Deshpande V. V. - Alkaline protease inhibitor: a novel class of antifungal proteins against phytopathogenic fungi, Biochem. Biophys. Res. Commun. **262** (3) (1999) 702−727.
36. Kridric M., Fabian H., Brzin J., Popovic T., Pain R. - Folding, stability and secondary structure of a new cysteine dimeric proteinase inhibitor. Biochem. Biophys. Res. Commun. **297** (4) (2002) 962–967.
37. Joshi B.N., Sainani M.N., Bastawade K.B., Deshpande V.V., Gupta V.S., Ranjekar P.K. - Pearl millet cysteine protease inhibitor. Evidence for the presence of two distinct sites responsible for anti-fungal and anti-feedant activities, Eur. J. Biochem., **265** (1999) (2) 556-563.

38. Brot N., Weissbach H. - Biochemistry and physiological role of methionine sulfoxide residues in proteins, Arch. Biochem. Biophys **223** (1) (1983) 271–281.
39. Vogt W. - Oxidation of methionyl residues in proteins: tools, targets, and reversal, Free Radical Biol. Med **18** (1) (1995) 93-105.
40. Johnson D., Travis J. - The oxidative inactivation of human alpha-1-proteinase inhibitor. Further evidence for methionine at the reactive center, J. Biol. Chem. **254** (10) (1979) 4022-4026.
41. Creighton T. E., Zapun A., Darby N. J. - Mechanisms and catalysts of disulfide bond formation in proteins, Trends Biotechnol **13** (1) (1995) 18–23.
42. Frand A. R., Cuozzo J. W., Kaiser C. A. - Pathways for protein disulphide bond formation, Trends Cell Biol **10** (5) (2000) 203–210.
43. Hogg P. J. - Disulfide bonds as switches for protein function, Trends Biochem. Sci **28** (4) (2003) 210–214.
44. Kawamura S., Ohkuma M., Chijiiwa Y., Kohno D., Nakagawa H., Hirakawa H., Kuhara S., Torikata T. - Role of disulfide bonds in goose-type lysozym, FEBS J**. 275** (11) (2008) 2818–2830.
45. Zavodszky M., Chen C. W., Huang J. K., Zolkiewski M., Wen L., Krishnamoorthi R. - Disulfide bond effects on protein stability: designed variants of *Cucurbita maxima* trypsin inhibitor-V, Protein Sci **10** (1) (2001) 149–160.
46. Mogensen J. E., Sehgal P., Otzen D. E. - Activation, Inhibition, and destabilization of *Thermomyces lanuginosus* lipase by detergents, Biochemistry **44** (5) (2005) 1719-1730.
47. Salameh M. D. A., Wiegel J. - Effects of detergents on activity, thermostability and aggregation of two alkalithermophilic lipases from *Thermosyntropha lipolytica*, The Open Biochemistry Journal **4** (2010) 22-28.
48. Jonossen I. B., Svendson I. B. - Identification of the reactive sites in two homologous serine prteinase inhibitors isolated from barley, Corlsberg Res Commun **47** (1982) 199-203.
49. Yang S. Q., Wang C. I., Gillmor S. A., Fletterick R. J., Craik C. S. - Ecotin: a serine protease inhibitor with two distinct and interacting binding sites, J. Mol. Biol. **279** (4) (1998) 945-957.
50. Urwin P. E., Atkinson H. J., Waller D. A., Mcpherson M. J. - Engineered oryzacystatinI expressed in hairy roots confers resistance to *Globodera pallida*, Plant Journal **8** (1) (1995) 121-131.
51. Kiggundu A., Goulet M. C., Goulet C., Dubuc J. F., Rivard D., Benchabane M., Pepin G., Vyver C. V. D., Kunert K., Michaud D. - Modulating the proteinase inhibitory profile of a plant cystatin by single mutations at positively selected amino acid sites, Plant J **48** (3) (2006) 403–413.
52. Koiwa H., D'Urzo M. P., Assfalg-Machleidt I., Salzman K. Z., Shade R. E., Murdock L. L., Machleidt W., Bressan R. A., Hasegawa P. M. - Phage display selection of hairpin loop soyacystatin variants that mediate high affinity inhibition of a cysteine proteinase, Plant J. **17** (5) (2001) 383–391.
53. Issac M., Aknin M., Gauvin-Bialecki A., Voogd N. D., Ledoux A., Frederich M., Kashman Y., Carmeli S. - Cyclotheonellazoles A–C, Potent Protease Inhibitors from the

Marine Sponge *Theonella aff. Swinhoei*, J. Nat. Prod. **80** (4) (2017) 1110–1116.

54. Fusetani N., Matsunaga S., Matsumoto H., Takebayashi Y - Bioactive marine metabolites. 33. Cyclotheonamides, potent thrombin inhibitors, from a marine sponge *Theonella* sp., J. Am. Chem. Soc. **112** (19) (1990) 7053–7054.
55. Nakao Y., Matsunaga S., Fusetani N. - Three more cyclotheonamides, C, D, and E, potent thrombin inhibitors from the marine sponge *Theonella swinhoei*, Bioorganic and Medicinal Chemistry **3** (8) (1995) 1115-1122.
56. Nakao Y., Oku N., Matsunaga S., Fusetani N. - Cyclotheonamides E2 and E3, new potent serine protease inhibitor from the marine sponge of the genus *Theonella*, J. Nat. Prod. **61** (5) (1998) 667-670.
57. Oli S., Abdelmohsen U. R., Hentschel U., Schirmeister T. - Identification of Plakortide E from the Caribbean Sponge *Plakortis halichondroides* as a Trypanocidal Protease Inhibitor using Bioactivity-Guided Fractionation, Mar. Drugs **12** (5) (2014) 2614-2622.
58. Tabares P., Degel B., Schaschke N., Hentschel U., Schirmeister T. - Identification of the protease inhibitor miraziridine A in the Red sea sponge *Theonella swinhoei*, Phcog Res **4** (1) (2012) 63-66.
59. Schaschke N. - Miraziridine A: natures blueprint towards protease class-spanning inhibitors, Bioorg Med Chem Lett. **14** (4) (2004) 855-857.
60. Nakao Y., Fujita M., Warabi K., Matsunaga S., Fusetani N. - Miraziridine A, a novel cysteine proteae inhibitor from the marine sponge *Theonella aff.mirabilis*, J Am Chem Soc **122** (42) (2000) 10462-10463.
61. Kimura J., Ishizuka E., Nakao Y., Yoshida W. Y., Scheuer P. J., Kelly –Borges M. - Isolation of 1-methylherbipoline salts of halisulfate-1 and of suvanine as serine protease inhibitors from a marine sponge, Coscinoderma mathewsi, J Nat Prod **61** (2) (1998) 248-250.
62. Carroll A. R., Buchanan M. S., Edser A., Hyde E., Simpson M., Quinn R. J. - Dysinosins B−D, Inhibitors of Factor VIIa and Thrombin from the Australian Sponge *Lamellodysidea chlorea*, J. Nat. Prod. **67** (8) (2004) 1291–1294.
63. Nurhayati T., Suptijah P., Suhartono M. T., Febrian dan I. - Penapisan Inhibitor Protease Yang Dihasilkan Oleh Sponge Asal Kepulauan Seribu, Buletin Teknologi Hasil Perikanan **8** (2) (2004) 45-59.
64. de Lira S. P., Seleghim M. H. R., Williams D. E., Marion F., Hamill P., Jean F., Andersen R. J., Hajdu E., Berlinck R. G. S. - A SARS-coronovirus 3CL protease inhibitor isolated from the marine sponge *Axinella cf. corrugata*: structure elucidation and synthesis, Journal of the Brazilian Chemical Society **18** (2) (2007) 440-443.
65. Nakao Y., Masuda A., Matsunaga S., Fusetani N. - Pseudotheonamides, Serine Protease Inhibitors from the Marine Sponge *Theonella swinhoei*, J. Am. Chem. Soc. **121** (11) (1999) 2425–2431.
66. Craiga K. S., Williams D. E., Hollander I., Frommer E., Mallon R., Collins K., Wojciechowicz D., Tahir A., Van Soest R., Andersena R. J. - Novel sesterterpenoid and norsesterterpenoid RCE-protease inhibitors isolated from the marine sponge *Hippospongia* sp, Tetraedron Letters **43** (27) (2002) 4801-4804.
67. Fujimoto Y., Salam K. A., Furuta A., Matsuda Y., Fujita O., Tani H., Ikeda M., Kato N., Sakamoto N., Maekawa S., Enomoto N., de Voogd N. J., Nakakoshi M., Massayoshi T.,

Sekiguchi Y., Tsuneda S., Akimitsu N., Noda N., Yamashita A., Tanaka J., Moriishi K. - Inhibition of Both Protease and Helicase Activities of Hepatitis C Virus NS3 by an Ethyl Acetate Extract of Marine Sponge *Amphimedon* sp, PLoS ONE **7** (11) (2012) e48685.

68. Nakao Y., Matsunaga S., Fusetani N. - Toxadocial A: A novel thrombin inhibitor from the marine sponge *Toxadocia cylindrica*, Tetraedron Letters **34** (9) (1993) 1511-1514.
69. Meyer M., Guyot M. - 5,9,23-Triacontatrienoic methyl ester, an elastase inhibitor from the marine sponge *Chondrilla nucula*, Lipids **37** (11) (2003) 1109-1111.
70. Fukuhara K., Takada K., Okada S., Matsunaga S. - Nazumazoles D-F, Cyclic Pentapeptides That Inhibit Chymotrypsin, from the Marine Sponge *Theonella swinhoei*, J Nat Prod **79** (6) (2016) 1694-1697.
71. Murayama S., Imae Y., Takada K., Kikuchi J., Nakao Y., van Soest R. W., Okada S., Matsunaga S - Shishicrellastatins, inhibitors of cathepsin B, from the marine sponge *Crella (Yvesia) spinulata*, Bioorg Med Chem. **19** (22) (2011) 6594-6598.
72. Murayama S., Nakao Y., Matsunaga S. - Asteropterin, an inhibitor of cathepsin B, from the marine sponge *Asteropus simplex*, Tetraedron Letters **49** (26) (2008) 4186-4188.
73. Dai J., Sorribas A., Yoshida W. Y., Kelly M., Williams P. G. - Xestosaprols from the Indonesian marine sponge *Xestospongia* sp., J Nat Prod **73** (6) (2010) 1188-1891.
74. Fujita M., Nakao Y., Matsunaga S., Seiki M., Itoh Y., van Soest R. W. M., Fusetani N. - Ancorinosides B-D, inhibitors of membrane type 1 matrix metalloproteinase (MT1-MMP), from the marine sponge *Penares sollasi Thiele*, Tetrahedron **57** (7) (2001) 1229-1234.
75. Fujita M., Nakao Y., Matsunaga S., Seiki M., Itoh Y., Yamashita J., van Soest R. W., Fusetani N. - Ageladine A: an antiangiogenic matrixmetalloproteinase inhibitor from the marine sponge *Agelas nakamurai*, J Am Chem Soc. **125** (51) (2003) 15700-15701.
76. Shim J. S ., Lee H. S., Shin J., Kwon H. J. - Psammaplin A, a marine natural product, inhibits aminopeptidase N and suppresses angiogenesis in vitro, Cancer Letters **203** (2) (2004) 163-169.
77. Rodríguez-Nieto S., González-Iriarte M., Carmona R., Muñoz-Chápuli R., Medina M. A., Quesada A. R. - Antiangiogenic activity of aeroplysinin-1, a brominated compound isolated from a marine sponge, FASEB Journal **16** (2) (2002) 261-263.
78. Mayer A.M.S., Lehmann V.K.B. - Marine pharmacology in 1998: marine compounds with antibacterial, anticoagulant, antifungal, anti-inflammatory, anthelmintic, antiplatelet, antiprotozoal, and antiviral activities; with actions on the cardiovascular, endocrine, immune, and nervous systems; and other miscellaneous mechanisms of action, Pharmacologist **42** (2000) 62–69.
79. Lee Y. K., Lee J. H., Lee H. K. - Microbial symbiosis in marine sponges, J. Microbiol. **39** (4) (2001) 254–264.
80. Calcabrini C., Catanzaro E., Bishayee A., Turrini E., Fimognari C. - Marine sponge natural products with anticancer potential: an updated review, Mar. Drugs **15** (10) (2017) E310.
81. Mayer A. M. S., Rodríguez A. D., Taglialatela-Scafati O., Fusetani N. - Marine pharmacology in 2012-2013: marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozal, antituberculosis, and antiviral activities;

affecting the immune and nervous systems, and other miscellaneous mechanisms of action, Mar. Drugs **15** (9) (2017) E273.

82. Ruocco N., Costantini S., Palumbo F., Costantini M. - Marine sponges and bacteria as challenging sources of enzyme inhibitors for pharmacological applications, Mar. Drugs **15** (6) (2017) 173.
83. Unson M. D., Faulkner D.J. - Cyanobacterial symbiont biosynthesis of chlorinated metabolites from *Dysidea herbacea* (Porifera), Experientia **49** (4) (1993) 349-353.
84. Fisch K. M., Gurgui C., Heycke N., van der Sar S. A., Anderson S. A., Webb V. L., Taudien S., Platzer M., Rubio B. K., Robinson S. J., Crews P., Piel J. - Polyketide assembly lines of uncultivated sponge symbionts from structure-based gene targeting, Nat Chem Biol **5** (7) (2009) 494-501.
85. Tabares P., Pimentel-Elardo S. M., Schirmeister T., Hünig T., Hentschel U. - Anti-protease and immunomodulatory activities of bacteria associated with Caribbean sponges, Mar. Biotechnol. **13** (5) (2011) 883–892.
86. Pimentel-Elardo S. M., Buback V., Gulder T. A. M., Bugni T. S., Reppart J., Bringmann G., Ireland C. M., Schirmeister T., Hentschel U. - New Tetromycin Derivatives with Anti-Trypanosomal and Protease Inhibitory Activities, Mar. Drugs **9** (10) (2011) 1682-1697.
87. Ramadan A. U., Szesny M., Othman E. M., Schirmeister T., Grond S., Stopper H., Hentschel U. - Antioxidant and Anti-Protease Activities of Diazepinomicin from the Sponge-Associated *Micromonospora* Strain RV115, Mar. Drugs **10** (10) (2012) 2208-2221.
88. Wahyudi A. T., Qatrunnada, Mubarik N. S. - Screening and characterization of protease inhibitors from marine bacteria associated with sponge *Jaspis* sp., HAYATI journal of Biosciences **17** (4) (2010) 173-178.
89. Nurhayati T., Suhartono M. T., Nuraida L., Poerwanto S. B. - Karakterisasi Awal Inhibitor Protease dari Bakteri yang Berasosiasi dengan Spons Asal Pulau Panggang, Kepulauan Seribu, HAYATI Journal of Biosciences **13** (2) (2006) 58-64.
90. Pimentel-Elardo S. M., Grozdanov L., Proksch S., Hentschel U. - Diversity of nonribosomal peptide synthetase genes in the microbial metagenomes of marine sponges, *Mar Drugs* **10** (6) (2012) 1192-1202.
91. Cheng-Jian J., Hao Z., Zeng R., Shen P., Li J., Wu B. - Characterization of a Novel Serine Protease Inhibitor Gene from a Marine Metagenome, Mar. Drugs **9** (9) (2011) 1487-1501.