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Bioactive Peptides from Marine Molluscs – A Review

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Authors' contributions

This review was carried out in collaboration among all authors. Author QAE managed the literature searches and wrote the first draft of the manuscript. Authors ORO, NM and SW reviewed and corrected the Manuscript. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Marine organisms make up approximately half of the total global biodiversity, with the Mollusca containing the second largest number of species, including snails and bivalves. The marine environment is highly competitive, hostile and aggressive, which has led to the production of specific and potent bioactive compounds by the mollusca and their associated microorganisms, in a bid to protect themselves and ensure their survival. A diverse array of bioactive compounds can be isolated from the extracts of marine molluscs of which linear, cyclic, and conjugated peptides and depsipeptides form some of the most important bioactive compounds that have been well characterized and some of have already reached clinical trials or been approved for use as therapeutic agents and supplements. This review highlights some of the bioactive peptides that have been obtained from marine molluscs as well the challenges facing bioprospecting of valuable peptides from marine mollusc sources.

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1. INTRODUCTION

The pharmaceutical industry is growing rapidly and continuously. In spite of the large number of drugs produced each year, the demand for the discovery of new drugs is imperative. The motivation to discover new drugs include the advent of new diseases and infections such as Middle East respiratory syndrome (MERS) and Severe Acute Respiratory syndrome (SARS); an increase in the occurrence of old diseases such as hemolytic fevers and tuberculosis; the occurrence of new challenges in old diseases such as AIDS; the growing number of drug–resistant infectious disease as well as the highly toxic nature of some currently used drugs.

The Marine environment represents an excellent source for bioactive compounds [1,2,3] due to the magnitude of the oceans and the high biodiversity of the organisms therein. Oceans cover more than 70% of the world surface and are inhabited by 34 out of the 36 known phyla of living organisms, with more than 300,000 known species of fauna and flora [2,4]. Hence, the marine environment may offer a high possibility for the discovery of novel compounds. The marine environment has a wide range of physico-chemical parameters including light, thermal, pressure, oxygen, pH and nutrients. Hence, the marine environment is home to some of the most diverse creatures both in their biology and chemistry. Several marine organisms are generally soft-bodied and completely immersed in their environment. Some organisms are sedentary. Hence, they have developed biological defense systems, including the secretion of mucus containing bioactive compounds in order to protect themselves from the harsh nature of their environment. These defense systems can be exploited and used in rational drug design [5]. Several molecules isolated from a vast number of marine species have clinical application as supplements and drugs while other molecules are currently in clinical trials or are under study [2]. Many of them have novel chemical structures which may lead to the development of entirely new drugs and therapeutic agents. Bioactive compounds isolated from marine sources include secondary metabolites which have been produced by sponges, algae, scale-less fishes, seaweed, marine molluscs and even associated microorganisms. These secondary metabolites include nitrogen heterocyclics, and sulphur-containing nitrogen heterocyclics as well as terpenoids, quinones, steroids and isoprenoids) [3]. Bioactive low molecular weight peptides and depsipeptides, as well as lectins, have also been obtained from marine sources [6]. Exploitable biological activity that marine organisms have been shown to possess include: antimicrobial activity [3,7], anti-cancer and anti-proliferation activity [1,6,8], free radical scavenging and antioxidant activity [9,10] and analgesic and anti-inflammatory effects [11].

2. MARINE MOLLUCS AS SOURCES FOR BIOACTIVE COMPOUNDS

The phylum Mollusca is the second largest animal phylum on earth, representing an enormous diversity of species both in terms of the vast number of species as well as in a wide range of morphologies and ecological niches. Molluscs constitute approximately 7% of living animals (with 100,000 – 200,000 species) and there are approximately 52,000 named species of marine molluscs alone [1,12,13].

In many cultures molluscs, especially shelled gastropods and bivalves, are regarded as food delicacies [14,15,16,17]. Furthermore, they provide a wide range of human resources including: the use of the shells for improvising mixed aggregates for building construction [15, 17] and the production of dyes especially from molluscs such as the Muricid whelk, *Trunculariopsis trunculus* [1]. Molluscs have also been used in a variety of traditional natural remedies [18,19,20] although the active ingredients are typically unknown, this is due to the fact that, very few scientific studies had been undertaken to evaluate and verify the health benefits of the molluscs [18,21].

Molluscs are quite vulnerable to predators and pathogens [22,23] as they are essentially soft-bodied and often live in microbe-rich habitats. The presence of a hard external shell in a vast number of the molluscan species, does not offer much of a physical barrier against microbial infection as the organisms must often come out of their shells to enable them move or feed. Thus, in order to survive, molluscs must have developed defense strategies to protect themselves against microbial assault, including perhaps the production and circulation of antimicrobial and antiparasitic secondary metabolites in the haemolymph, mucus

secretions on body surfaces and body openings [1,3,5].

3. BIOACTIVE PEPTIDES ISOLATED FROM MARINE MOLLUSCS

A vast number of bioactive compounds have been isolated from the mollusca, including metabolites like complex alkaloids, macrolides and terpenes. However among the most active of them are the cyclic peptides and depsipeptides or linear peptides. Currently, peptides isolated from molluscs as well as their synthetic structural analogues are in clinical trials as anticancer compounds [1,6,8,24] and have been approved for use in pain management [25,26,27].

3.1 Peptides with Anti-cancer Activity

Marine organisms have been shown to possess bioactive compounds with anticancer activity and bioactive peptides from a host of marine molluscs as well as their synthetic analogs have been discovered and are under investigation for their anticancer properties. An example is Kulokekahilide-2, a cyclic depsipeptide derived from the marine *mollusc Philinopsis speciosa* which has been found to be cytotoxic, *in vitro,* against the leukemia cell line P388, the human ovarian cancer cell line SKOV-3, the melanoma cell line MDA-MB-435 and A-10 embryonic rat thoracic aorta medial layer myoblast cells with IC50 values ranging from 4.2 to 59.1 nM [28]. Another peptide is Keenamide A (Fig. 1) isolated from the marine mollusc *Pleurobranchus forskalii* [29]. Keenamide A, a cyclic hexapeptide, exhibited significant *in vitro* activity against the lymphocytic leukemia cells (P-388) and human adenocarcinoma cells (A-549) with and IC_{50} of 2.5 µg/ml was well as the human colorectal adenocarcinoma tumor cells (HT-29) with an IC_{50} of 5.0 µg/ml [29]. A 15 kDa linear peptide designated Mere15 was isolated from *Meretrix meretrix* [30,31,32]. Mere15 inhibited the growth of K562 leukemia cells *in vitro* (IC_{50} 38.2 µg/ml) and inhibited the growth of A549 cells xenograft in nude mice *in vivo* (IC_{50} 31.8 µg/ml). The cytotoxicity of Mere 15 is due to the peptide's ability to induce apoptosis, cause microtubule disassembly and arrest the cell cycle [30,31,32]. SCH-P9 (Leu-Pro-Gly-Pro) and SCH-P10 (Asp-Tyr-Val-Pro) peptides were obtained from *Sinonovacula constricta* hydrolysates and demonstrated to inhibit the growth of the prostate cancer cell lines, DU-145 and PC-3, in a dose- and time-dependent manner [33].

The most widely investigated and publicized set of marine mollusc peptides which have been shown to possess anti-cancer activity however, are the dolastatins and kahalalides.

3.1.1 Dolastatins

The Dolastatins are a group of cytotoxic cyclic and linear peptides with cell growth suppressing activity [34]. They were initially isolated from the marine mollusc *Dolabella auricularia*, although they have also been isolated from two microbial species (*Symploca sp* and *Lyngbya majuscula)* that form part of the diet of *Dolabella auricularia* [35,36,37]. Preclinical research studies indicate that the dolastatins show potency against breast cancers, liver cancers and solid tumours. At the time of their discovery, dolastatin 10 (Fig. 2) and dolastatin 15 (Fig. 3) were reported as the most active anticancer natural substance of the time with an ED_{50} of 4.6×10^{-5} g/ml against the P-388 lymphocytic leukemia cell line [38]. The dolastatins are essentially inhibitors of mitosis. They interfere with tubulin formation by inhibiting tubulin polymerization and tubulin dependent GTP hydrolysis thereby inhibiting microtubule assembly, thus disrupting mitotic cell division and inducing apoptosis and Bcl-2 (an oncoprotein that is overexpressed in some cancers) phosphorylation in cancerous cells. Dolastatins also act as noncompetitive inhibitors of vincristine and vinblastine [36,38,39]. Dolastatin 10 is a unique pentapetide (dolavaline-valine-dolaisoleuine-dolaproine-dolaphenine) that exists in two different conformations, which are essentially a cis-trans isomerization of a central amide bond [36,40]. Dolastatin 15 is a seven subunit depsipeptide (dolavaline-valine-dolavaline-proline-proline-o valine-unk). Dolastatin 10 is chemically similar to symplostatin 1 and 3, also potent microtubule inhibitors isolated from *Symploca hynoides* found in Hawaii and Guam.

Dolastatin 10 and 15 are undergoing phase I and II clinical trials for use in the treatment of solid tumours and have been evaluated in various phase I clinical trials reporting good tolerability and identifying myelosuppression as the dose limiting toxicity. Preclinical toxicity evaluations in which Dolastatin-10 was administered as a single intravenous bolus dose to CD2F1 mice, Fischer-344 rats and beagle dogs to determine the the maximum tolerated dose (MTD; i.e. the highest dose that produces significant toxicity but does not cause lethality), the dose-limiting toxicity and target organ of toxicity as well as its reversibility

demonstrated that the primary target organ of toxicity in all three species was the bone marrow, as indicated by decreases in the numbers of erythroid cells, myeloid cells, and megakaryocytes in the femoral bone marrow and by decreased white blood cell (WBC) and reticulocyte counts in peripheral blood [41]. The MTD was approximately 1350 μg/m^2 body surface area (450 μg/kg) in mice, 450 μg/m^2 (75 μg/kg) in rats and ≤400 μg/m^2 (≤20 μg/kg) in dogs. Adverse signs were observed at doses ≥1350 μg/m^2 in mice, ≥150 μg/m^2 in rats and ≥400 μg/m^2 in dogs. A decrease in weight gain or actual weight loss was observed at doses ≥1350 μg/m^2 in mice, ≥600 μg/m^2 in rats and ≥450 μg/m^2 in dogs [41]. A Phase II clinical trial in 16 patients (with ages ranging from 59–79 years) with hormone-refractory prostate cancer in which Dolastatin-10 was administered at a dose of 400 μg/m^2 *i.v.* every 3 weeks for a total of 56 cycles showed that Dolastatin-10 is very well tolerated. Only 2 patients needed a dose adjustment because of toxicity, and in 5 patients, dose increase was possible to 450 μg/m^2 [42]. Other side effects observed were peripheral sensory neuropathies, pain, swelling, and erythema at the injection site [6].

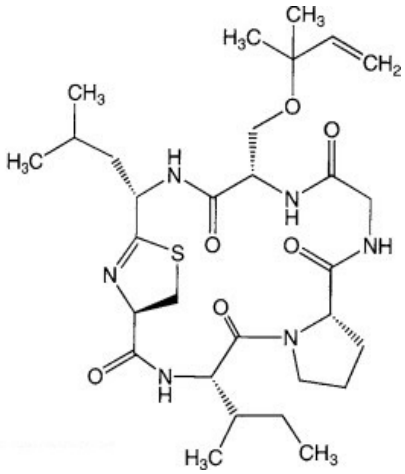

Fig. 1. Keenamide [43]

Numerous synthetic derivatives of the dolastatins have also been produced. Tasidotin and soblidotin, two synthetic analogues of the dolastatins, have advanced into clinical trial for cancer therapy. Auristatins (monomethyl auristatin E; MMAE) are also potent synthetic analogues of dolastatin 10. Due to their toxicity, they are linked to a monoclonal antibody that can be targeted against cancer cell-specific antigens [39].

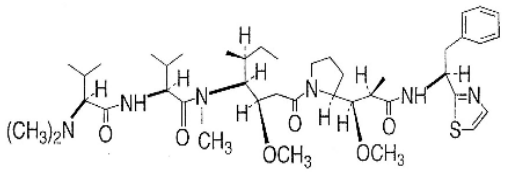

Fig. 2. Dolastatin 10 [44]

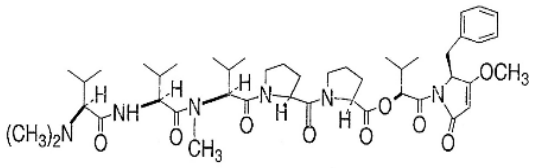

Fig. 3. Dolastatin 15 [44]

3.1.2 Kahalalides

The kahalalides are a group of cyclic peptides isolated from the Indo-Pacific mollusc *Elysia rufescens*. Kahalalides are probably secondary metabolites synthesized by the mollusc from peptides produced from its diet of green algae notably *Bryopsis pennata* [45,46]. There are seven kahalalide peptides (six cyclic peptides designated A-F and one acyclic analogue designated G). However, kahalalide F (KF; Fig. 4), a depsipeptide, is the largest and most active [27]. KF exhibits potent *in vitro* antitumour activity against a host of solid tumours including human prostate (with IC_{50} ranging from 0.07 μM against PC3 cells to 0.28 μM against DU145 and LNCaP), breast cancer cell lines (with IC_{50} of 0.28 μM against SKBR-3, BT474, and MCF7 cell lines) and the liver hepatocellular carcinoma HepG2 (IC_{50} of 0.3 μM). KB also exhibited significant non-activity on non-tumour human cells (MC10A, HUVEC, HMEC-1, and IMR90) [45,47]. In *in vivo* animal models, KF showed activity against human prostate cancer xenografts and has exhibited cytotoxicity against human ovarian, colon, and breast carcinomas [45]. It was demonstrated that treatment of malignant cells with Kahalalide F caused mitochondrial damage; vesiculation and enlargement of the endoplasmic reticulum; severe cytoplasmic swelling and vacuolization and led to eventual plasma membrane rupture [32]. Hence, Kahalalide F induces oncosis in cancer cells by lysosomal induction and an increase in cell membrane permeability [27]. It has also been reported that Kahalalide F induces cell death similar to necrosis via inhibition of Akt Signaling and depletion of ErbB3, thus inhibiting tumour metastasis and proliferation [47]. KF was developed by Pharmamar, Madrid, Spain, a

biopharmaceutical company and underwent phase II clinical trials [27,45,48] where it exhibited clinical benefits in treated patients and low toxicity. Side effects reported were fatigue, headache, vomiting, and pruritus limited to the hands [6]. Hematological toxicities have not been observed on treatment with Kahalalide F thus showing its suitability for trials in combination with other anticancer agents [6,49]. Unfortunately, due to a lack of efficacy, Kahalalide F was removed from phase II clinical trials in spite of the fact that a few patients achieved a positive response [32]. Pharmacokinetic studies indicated that Kahalalide F has a short half-life of about 30 minutes and thus a narrow volume of distribution and this may explain its low efficacy [50]. However, due to the cytotoxic effect exhibited by KF, several efforts have been attempted to modify KF in a bid to produce analogues with either higher potency or increased half-life. Furthermore, there is evidence that suggests that Kahalalide F may be active against several other tumor types and justifies further studies and clinical testing on it, either as a single agent or in combination.

3.2 Antimicrobial Peptides

The majority of marine organisms fend off a wide range of microorganisms by producing antimicrobial peptides (AMPs). Bioactive peptides isolated from marine molluscs specifically those isolated from bivalves and abalone, have displayed potent antimicrobial efficacy [37,51]. A saccharothrixmicine peptide with antimicrobial activity against *Candida albicans* and *Xanthomonas sp* was isolated from the marine mollusc *Anadara broughtoni* in association with *Saccharothrix espanaensis* AN 113 [37,52]. A cysteine-rich peptide, myticin, has been isolated from the Mediterranean mussel (*Mytilus galloprovencialis*) Myticin has potent antibacterial activity against both gram- negative and positive bacteria [37,52]. Peptides with antifungal properties have also been isolated from the blue mussel (*Mytilus edulis*) [52,53] and from *Mytilus coruscus* [54].

3.3 Peptides with Antioxidant Activity

Antioxidants are natural or sometimes synthetic substances that may prevent or delay cell damage caused by free radicals notably Reactive Oxygen Species (ROS). These free radicals arise mainly from metabolic activities of the human body during aging and as a result of lipid peroxidation which is the principal reason for the decline of quality in lipid-containing foods during storage.

Bioactive antioxidant peptides have been isolated from marine molluscs. A bioactive antioxidant peptide (leu-lys-gln-glu-leu-glu-asp-leu-leu-glu-lys-gln-glu) has been isolated from the Oyster, *Crassostrea gigas* and which exhibits significant inhibitory effect against polyunsaturated fatty acid peroxidation as compared to α-tocopherol and also scavenged hydroxyl radical(IC_{50} 28 μM) and superoxide radical (IC_{50} 78.97μM) [37,55]. Furthermore, peptides obtained by hydrolysis with proteolytic enzymes of the viscera and body of the clam (*Meretrix casta*), resulted in significant DPPH radical scavenging activities and reducing power (measured as the ability of the hydrolysate to reduce iron III) [32]. A peptide Hydrolysate derived from blue mussel (Mytilus edulis) and designated BMCH has also been reported to exhibit antioxidant activities as indicated by DPPH radical scavenging (IC_{50} 0.35 mg/ml) and ABTS+ radical scavenging activity (117 μM TE/Mg sample) [52]. BMCH also showed a protective effect against H_2O_2-mediated oxidative injury in human umbilical vein endothelial cells (HUVECs) by increasing cellular antioxidant capacities, including increasing levels of catalase, glutathione (GSH), glutathione peroxidase and superoxide dismutase, thus indicating that BMCH may be useful in in protecting against endothelial dysfunction and related diseases [56].

Two peptide hydrolysate fractions from the Marine Bivalve Mollusc, *Tergillarca granosa,* have also been demonstrated to exhibit antioxidant activity [57]. The peptides with amino acid composition (and thus designated) MDLFTE and WPPD exhibited strong DPPH free radical scavenging (EC50 = 0.53 and 0.36 mg/mL, respectively), hydroxyl radical scavenging (EC50 = 0.47 and 0.38 mg/mL respectively), superoxide anion radical (EC50 = 0.75 and 0.46 mg/mL, respectively) and ABTS cation radical scavenging (EC50 = 0.96 and 0.54 mg/mL, respectively) activities, although these antioxidant activities were not sustained at basic pH conditions (pH > 9 for 2.5 h), high temperatures (>80ºC for 0.5 h) nor during simulated GI digestion [57].

Mud creepers such as the Matah Merah Snail, *Cerithidea obtuse* and Ipong-Ipong, *Fasciolaria salmo* have also been reported to exhibit potent antioxidant activity [9,10] although this activity has not been attributed to peptides.

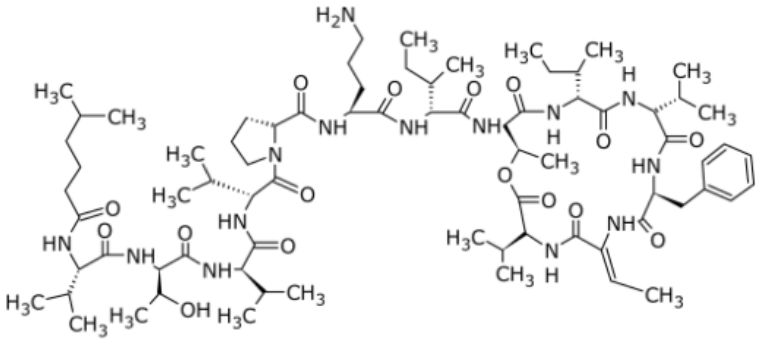

Fig. 4. Kahalalide F [69]

Lower molecular weight peptides possess stronger antioxidant activity compared to peptides with higher molecular weight [32] and this may be due to improved permeability and/or better contact ability with membrane lipids by the lower molecular weight peptides [32,56]. In addition, the presence of histidine and aromatic amino acids in the peptide structure may be an advantage as these amino acids can easily donate protons to electron-deficient radicals while maintaining their stability via resonance structures [32,58].

3.4 Conopeptides: Peptides with Analgesic and Anti-inflammatory Effect

Conopeptides (Conotoxins) are short (10 – 30 amino acids) neurotoxic peptides that are typically crosslinked with multiple disulphide bonds. They are isolated from the venom of the marine cone snail (genus *Conus*). Conotoxins are extremely fast acting toxins [25] containing several different compounds and are thus hypervariable in their composition (even within the same species) [25,26,58].

Conotoxins are essentially potent ion channel modulators. Some conotoxins are also pain-reducing, thus, they show promise for providing a non-addictive pain reliever for use in treating neuropathic pain [25,37]. The U.S. Food and Drug Administration in December 2004 approved ω-conotoxin MVIIA (Ziconotide), as a painkiller for limited clinical use under the name Prialt® (Elan Pharmaceuticals Inc, Dublin, Ireland) [25,26,27]. Another peptide, AVC1 (from *Conus victoria*) has been reported to also provide effective pain relief from neuropathic pain and post-surgical [25,27].

There are five conotoxins whose activities have been determined so far: α (alpha), δ (delta), κ (kappa), μ(mu), and ω (omega) types [25]. ω-conotoxins are basic peptides with 24 to 30 amino acids residues in length with three disulfide bonds and an amidated *C*-terminus [25,59], found in piscivorous cone snails. ω-conotoxins have emerged as a new class of drugs for the treatment of neuropathic pain. Their analgesic effects are due to their ability to inhibit or block N-type or P/Q type calcium channels which are related to analgesia (pain sensitivity) in vertebrates.

3.4.1 ω-conotoxins SVIB

SVIB ω-conotoxins from *Conus striatus* is a CaV channel blocker that targets the P/Q-type CaV channel [25,59]. The action of SVIB is rather generalized and non-targetable and because the P/Q-type current plays an important role in the regulation of transmitter release at neuromuscular junctions, SVIB may not be a useful drug lead for pain management because it would non-specifically target all neuromuscular junctions and thus lead to an impairment of normal function. Hence, SVIB can be lethal even at low doses [25,59].

3.4.2 ω-conotoxin MVIIA

MVIIA is a 25 amino acid peptide coupled with 3 disulphide bonds, isolated from the venom of the predatory marine mollusc, *Conus Magnus*. Ziconotide (Fig. 5), a synthetic version of MVIIA, has been reported to exhibit analgesic activity that is hundred times higher than standard morphine [25,37] and unlike pain management involving morphine, treatment with MVIIA does not lead to addiction or development of tolerance [59]. During a large-scale study of i.t. ziconotide's long-term safety, clinical experience with MVIIA (Ziconotide) revealed significant treatment-related side effects including abnormal gait, nausea and vomiting, dizziness, nystagmus,

headaches, pain, confusion, somnolence, fever, postural hypotension, memory impairment and urinary retention [60,61,62]. These side effects can be minimized by starting with a low beginning dose of intrathecal MVIIA followed by a slow titration [60]. However, acute cardiovascular toxicity of low doses of *i.t*. Ziconotide has been reported [61].

3.4.3 ω-conotoxins CVID

ω-conotoxins CVID isolated from the fish-hunting *Conus catus*, is the most selective inhibitor of N-type over P/Q-type CaV channels. CVID has undergone clinical trials (Phase I/IIa) for pain management involving morphine-resistance [59].

3.4.4 ω-conotoxin GVIA

ω-conotoxin GVIA, produced from *Conus geographus,* is a selective and virtually irreversible inhibitor of the N-type CaV channel [25,59]. Although studies involving the intrathecal administration of GVIA in animal models showed it to be more potent than morphine, MVIIA and CVID at reducing neuropathic pain [59,63], GVIA has limited clinical usage due to the irreversibility of its binding as well as its slow onset and recovery kinetics [64].

4. STRATEGIES TO ENHANCE PEPTIDE PERMEABILITY, BIOAVAILABILITY AND HALF-LIFE EXTENSION

Natural peptides generally do not have a long circulating plasma half-life. Due to the amide bonds in their structure, peptide drugs are susceptible to proteolysis by proteases or peptidases enzymes. Hence to enhance the half-life of peptide drugs many strategies have been developed to enhance peptide permeability and limit enzymatic degradation, including cyclization to increase rigidity, N-methylation to reduce hydrogen bonding and introducing intramolecular hydrogen bonds to reduce intermolecular hydrogen bonds and flexibility [65,66,67].

Many strategies have been proposed to enhance the stability of peptides from proteolysis through structural modification. In general, the N-terminus residue of a peptide correlates to its half-life in plasma [66]. Peptides with N-terminus containing Val, Ser, Met, Thr, Ala, or Gly typically have longer half-lives. Peptides with N-terminus containing Arg, Phe, Lys, Asp, or Leu usually have shorter half-lives while peptides rich in Pro, Glu, Ser, and Thr are more prone to enzymatic degradation [65]. Since a number of proteolytic enzymes in the blood or plasma, liver and kidney are exopeptidases, peptide stability can be achieved by modification of the N- or/and C-termini. Several reports have shown that N-acetylation, and C-amidation increase resistance to proteolysis [65]. Furthermore, substituting natural L-amino acids with their non-natural D-amino acids decreases the substrate recognition and binding affinity of proteolytic enzymes thus increasing peptide stability [65,66]. Also, the introduction of lactam bridges and structure-inducing probe (SIP)-tail into the peptide structure as well as cyclization of the peptide enhance the secondary structure of the peptide thus protecting it from enzymatic cleavage [66].

Conjugation of drug peptides with small molecules and macromolecules like polyethylene glycol (PEG), albumin, oligoribonucleotides, or antibodies, can also be a means of selectively targeting the peptide to the required site of action, extending the circulating half-life of the peptide, improving its stability as well as reducing renal clearance thus leading to therapeutics that have to be administered less frequently [55,66]. Conjugation of a bioactive peptide to an antibody, for example, ensures that the peptide molecule is targeted directly to its site of action by the antibody [39,65,66] and covalently attaching albumin-binding small molecules to peptides has been shown to reduce glomerular filtration, improve proteolytic stability, and prolong half-life [65]. Conjugation of a peptide molecule to polyethylene glycol (PEG) has also been used to limit globular filtration and increase circulating plasma half-life of the peptides. However, there are safety concerns related to the use of PEG in injectable therapeutics, thus limiting its use [66].

A large number of permeability enhancers have been reported to increase intestinal absorption of peptides, such as surfactants, bile salts, phospholipids, fatty acids, and glycerides [65,66]. For example, incorporating peptide drugs in Liposomes would facilitate their passage through the phospholipid bilayer of the cell membrane. The enhanced plasma half-life of a peptide as well as chemical stability can also be achieved by means of formulations. Most peptide drugs are formulated as injectibles and recently, as an implantable device that delivers the drug molecule directly into the blood stream [66].

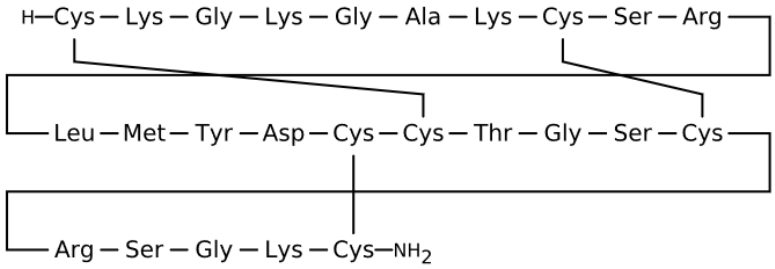

Fig. 5. Ziconotide
(Source: PubChem; https://pubchem.ncbi.nlm.nih.gov)

5. CHALLENGES AND FUTURE PERSPECTIVES IN BIOPROSPECTING PEPTIDES FROM MARINE MOLLUSCS

In general, only about 1% of the population of molluscan species has been investigated for bioactive secondary metabolites. This could be as a result of the vast number of species in the phylum, the diversity in the habitats and ecological niches of species in the phylum and our relatively poor knowledge of the biology of the different taxa [1]. There is little ethnomedical history of marine molluscs [1]. This means that most studies on these organisms have to undergo screening from scratch and experience quite a bit of trial and errors. Where ethnomedical accounts do exist, the active ingredients contributed by the mollusca are typically unknown, as very few scientific researches have been undertaken to authenticate the health benefits of molluscs, as attention is usually given to the bioaction of plant species [18,21].

The isolation and purification as well as screening processes involved in obtaining bioactive peptides are delicate, time consuming and tedious processes especially when the peptides of interest are not simple or easy to purify or are present in only trace amounts in the marine organism thus resulting in low yields [67]. This hindrance often discourages investigators.

A sustainable supply of original marine source is of utmost importance and could also present a major obstacle especially in the collection of deep sea marine organisms. This typically requires specialized skills and equipment. Hence, bioprospecting marine studies were usually conducted in shallow coastal waters. General scuba divers can only access habitats at a depth of around 50 m [32] and more specialized diving skills are required to explore deeper environments and caves. Hence, a large portion of the marine environment and the marine organisms living in these inaccessible portions are left unexplored, resulting in a lack of knowledge in the biology and chemistry of quite a number of marine wildlife.

However, in recent times this problem is being solved by the growing efforts to explore the deep seas using sophisticated methods and equipments. With advances and improvements in Scuba diving gears, the use of submarines or remotely operated vehicles and having mobile labs on boats to facilitate quick analysis, it is possible to start bioprospecting from several unique marine ecosystems thus opening up new frontiers for the discovery of new bioactive marine compounds in general. However, this greatly increases the research costs into bioprospecting, especially in resource poor countries with coastal waters. Nevertheless, in light of the broad spectrum of bioactivities and potential applications of marine peptides and their economic importance, the cost may well be worth it and can be alleviated by collaborations between pharmaceutical companies, biotechnology companies, Research institutes, governments and other stakeholders.

With the ability to access hitherto inaccessible marine environments, concerns about human interference in delicate ecosystems and conservation concerns, come into play and these concerns must be fully and carefully addressed before any exploration efforts. For example, Mariculture or aquaculture (i.e. cultivation of marine organisms in the open ocean versus cultivation of marine organisms in artificial conditions), have been proposed as methods of circumventing the problem of harvesting large numbers of marine species from the wild to satisfy research and commercial demands or applications [67,68]. A major drawback to this

idea is the possibility of ecophysiological diversity (i.e. differences between members of the same species due to differences in environmental factors and interactions), which can interfere with the production of bioactive peptides [68] especially in situations in which the primary producer of the bioactive component is an associated symbiotic microorganisms [45,46,68]. Generally these symbiotic microorganisms cannot be cultured using classical methodologies. However, since most bioactive peptides are short-chained, containing only a few amino acids, after initial isolation and structure elucidation, they can then be produced, theoretically, by chemical peptide synthesis or by heterologous peptide expression in cultured microorganisms in combination with fermentation techniques [67].

6. CONCLUSION

The Phylum mollusca are promising sources of marine bioactive peptides of clinical and scientific importance, with a few peptide products currently on the market for use as drugs and supplements and several more still in the clinical trials and in clinical development. The intense pressure to find and develop active novel peptide molecules, and indeed other bioactive molecules, for use as drugs will continue to drive the bioprospecting of marine molluscs and other marine species especially in the wake of the growing exploration of previously unexplored marine habitats and in view of the biological activity of marine peptides with biotechnological and medical applications that have been discovered thus far.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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