REVIEW

Cite this: *RSC Med. Chem.*, 2020, 11, 1235

Received 25th May 2020,
Accepted 11th September 2020

DOI: 10.1039/d0md00173b

rsc.li/medchem

Hormone-like conopeptides – new tools for pharmaceutical design

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Conopeptides are a diverse family of peptides found in the venoms of marine cone snails and are used in prey capture and host defence. Because of their potent activity on a range of mammalian targets they have attracted interest as leads in drug design. Until recently most focus had been on studying conopeptides having activity at ion channels and related neurological targets but, with recent discoveries that some conopeptides might play hormonal roles, a new area of conopeptide research has opened. In this article we first summarize the canonical pharmaceutical families of *Conus* venom peptides and then focus on new research relating to hormone-like conopeptides and their potential applications. Finally, we briefly examine methods of chemically stabilizing conopeptides to improve their pharmacological properties. A summary is presented of conopeptides in clinical trials and a call for future work on hormone-like conopeptides.

1. Introduction

Cone snails are carnivorous marine snails that hunt a variety of vertebrate and invertebrate prey using an envenomed harpoon.^{1,2} These snails at first appear to be unlikely candidates as a source of molecules for drug development, as they have been responsible for serious injuries and even

deaths upon human envenomation.³ Each cone snail species has a highly potent arsenal of diverse peptides active on various molecular targets of the nervous and endocrine systems to induce paralysis and respiratory arrest in prey (Fig. 1).^{4–6} Yet these same deadly compounds, the conopeptides, have been in the spotlight of drug discovery and development because of their potency and selectivity since the 1980s when the pharmaceutical potential of these natural peptides was discovered by Baldomero Olivera.^{1,7}

Conopeptides are typically rich in cysteine residues and their accompanying disulfide bonds, making their structures

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Fig. 1 Overview of the targets affected by conopeptides. The various mechanisms of action and targets with the names of the conopeptides that act on these targets are shown.

stable and relatively resistant to enzymatic degradation. Each of the *ca.* 1000 species of cone snail can produce up to a few hundred unique conopeptides; the diversity of conopeptides is therefore immense. The qualities of conopeptides – selectivity, potency, stability, and sheer variety – make them prime candidates for drug development research. Those conopeptides that are rich in disulfide bonds are often referred to as conotoxins and make up the majority of conopeptides. For the purposes of this review, the broader nomenclature will be used; conopeptides refer to all venom peptides produced by *Conus*. Conopeptide sequences and three-dimensional structures are catalogued in the database ConoServer (<http://conoserver.org>), which also provides

updated information on conopeptide classification as well as tools for analysing and discovering conopeptides.⁸

Extensive research over the last 40 years has been directed at characterizing the sequences, three-dimensional structures, frameworks, and biological activities of these highly diverse conopeptides.^{9–11} To date, most of the potential applications of conopeptides have been directed at developing new analgesics to at least partially replace opioid-based painkillers. Despite several clinical trials of a variety of conopeptides, so far only one conopeptide has been approved for use by the FDA, ω -conopeptide MVIIA from *Conus magus* (referred to by the generic name ziconotide and the trade name Prialt™, the latter an acronym for ‘primary alternative to morphine’).¹² Unlike morphine and other opioids, long-term treatment with ziconotide does not cause tolerance to the treatment; ziconotide does not produce addiction or induce withdrawal symptoms upon discontinuation.^{12,13} However, ziconotide needs to be injected intrathecally to localise at the central nervous system (CNS) site of action and consequently is mainly used in extreme cases such as palliative care for cancer or AIDS patients who no longer respond to opioid-based therapy.^{12,14} The success of ziconotide shows the potential of ion-modulating conopeptides as tools in drug discovery and development but also highlights the need for improved delivery methods for conopeptides and, indeed, for most peptide-based drug leads.

Considerable attention has been paid to the discovery and classification of these hyper diverse conopeptides as well as their applications in neuroscience and pharmacy.¹⁵ Since the first reports on conopeptides, approximately 1800 peer-reviewed articles have been published containing the keywords “conopeptide” or “conotoxin” according to a Scopus search. As illustrated in Fig. 2, the field continues to gather

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Fig. 2 Graphical representation of the number of peer-reviewed articles published containing the keywords “conopeptide” or “conotoxin” since the 1980s, as recorded by Scopus. The number of articles per year is shown in black bars, and the cumulative number of papers is shown by the grey line.

interest, with currently around 60 international publications each year appearing in the scientific literature (*i.e.* one per week). Approximately 70% of the published articles so far contain the keyword “structure function” and 60% contain the keyword “drug design” or “pharmaceutical application”. In contrast, only 3–4% of the peer reviewed literature on *Conus* contains the keywords “transcriptomics”, “proteomics”, or “venomics”. A Scopus search in May 2020 revealed that only 4% of the articles used keywords relating to the biology of *Conus* such as “evolution”, “diet”, or “*Conus* biology”. Reflecting this trend in the topic of original research papers, the majority of reviews on conopeptides deal with structure–function and pharmaceutical applications of these peptides. By contrast, over the past 10 years there has been relatively little literature reviewing the biology and venomics of *Conus*. In fact, only five major reviews have focused on these topics, as outlined in Table 1.

Recently, venomics studies of cone snails have revealed previously undescribed neurohormone-mimetic conopeptides.¹⁶ This discovery highlights the need for more foundational research to support even wider applications of conopeptides. The current review focuses on these neurohormone conopeptides, and reflects a biology-driven perspective on *Conus* as was described by early work of Olivera.¹⁷ We first provide some background on conopeptide classification to place the new developments in the field of hormonal conopeptides in context.

2. Conopeptides acting on ion channels and transporters of the nervous system

While we will focus here on hormone-like conopeptides that act on G-protein coupled receptors (GPCRs), we first provide an overview on conopeptides that act on ion channels and transporters of the nervous system. This section provides context, especially regarding the development of conopeptides for medical applications.

2.1 Conopeptide classifications

The diversity of conopeptides has necessitated a complex classification system based on primary structure, genetic origin or pharmacology. Conopeptide classifications have been extensively reviewed, for example recently by Jin *et al.*⁹ or Mir *et al.*,¹⁸ and updated information can be found on ConoServer. Briefly, at a structural level, conopeptides are described by the number and placement of cysteine residues (‘Cysteine Framework’ classification).^{15,19} In terms of genetic origin, conopeptides are expressed as precursor proteins, which undergo post-translational modifications to lead to mature peptide toxins.⁸ The higher sequence conservation of the signal peptide compared to the mature peptide region in conopeptide precursors has prompted the classification of conopeptides into gene superfamilies according to signal peptide similarity. At least 26 gene superfamilies have been described, *i.e.*, A, B1, B2, B3, C, D, E, F, G, H, I1, I2, I3, J, K, L, M, N, O1, O2, O3, P, S, T, V, and Y.^{19,20} Conopeptides acting on the nervous system are also classified according to their pharmacological activity, forming 11 canonical pharmacological families.^{19,20}

2.2 Examples of conopeptide canonical pharmacological families

Conopeptides act on a sweeping variety of ligand-gated and voltage-gated ion channels and transporters. The pharmacological family classification scheme groups peptides by the type of activity on these targets, with the canonical pharmacological classes named using Greek letters. Only a selection of pharmacological families, especially those that are targets for drug discovery, will be mentioned here. A number of reviews of these families have been published, as listed in Table 1, and information on them and their members can also be found in ConoServer. Some examples of conopeptides belonging to the pharmacological families discussed here are shown in Fig. 3.

The ω -conopeptides block voltage-gated calcium channels,²⁹ and have been extensively studied. GVIA and MVIIA are amongst the earliest and best known of this family and are selective for N-type voltage-gated calcium channels.³² GVIA has been used as a molecular probe of this ion channel in a large number of physiological studies. MVIIA, which was mentioned above as ziconotide (Prialt™), remains the only conopeptide on the market for treatment of chronic pain.¹⁴ This family constitutes an exciting resource for the discovery of new analgesics because their mechanism of action is independent of the opioid receptor pathway.²⁹ The closely related conopeptide CVID from *Conus catus* was also investigated as a potential new analgesic because of its selective binding to calcium channel subtype 2.2 but was abandoned after preclinical trials due to potential cytotoxicity concerns.^{21,40} As for other peptide-based drugs, limited uptake through the blood–brain barrier is a constraining factor in applications of these conopeptides,²⁹ with the requirement for intrathecal delivery *via* a surgically

Table 1 Reviews on conopeptides over the last 10 years categorised by subject focus

Title and year	Main topic and focus of review	Structure	Pharma	Biology	Venomics	Ref.
Conotoxins: Chemistry and Biology (2019)	Comprehensive review summarizing novel conotoxin discoveries since 2014	•	•	•	•	9
<i>Conus</i> Envenomation of Humans: In Fact & Fiction (2019)	An introduction to <i>Conus</i> diet, venom, and interactions with humans			•		3
Pain therapeutics from cone snail venoms: From ziconotide to novel non-opioid pathways (2019)	A detailed review on conopeptides that have potential for therapeutic development, with a focus on ziconotide and relevant discussion of analgesic neuropharmacology	•	•			13
Ziconotide Monotherapy: Systematic Review of Randomised Controlled Trials (2017)	A focused review on outcomes for patients treated with ziconotide		•			14
Cone Snails: A Big Store of Conotoxins for Novel Drug Discovery (2017)	A review with wide scope, covering cone snail biology, conopeptide classification, venomics discovery, and structure–function studies	•	•	•	•	21
Small Packages, Big Returns: Uncovering the Venom Diversity of Small Invertebrate Conoidean Snails (2016)	A historical perspective on venomics techniques and discovery of conopeptides with sections on specific applications		•		•	22
Conotoxins: Structure, Therapeutic Potential and Pharmacological Applications (2016)	An overview of gene superfamily classification and nomenclature, structural studies, and pharmacological applications	•	•			18
Cyclization of Disulfide-Rich Conotoxins in Drug Design Applications (2016)	A review of conotoxins for synthetic chemists, emphasizing modified peptides, chemically stabilized structures and their applications	•	•			23
Bioactive Mimetics of Conotoxins and other Venom Peptides (2015)	A review of small molecule drug leads designed to imitate the potency and selectivity of conopeptides	•	•			24
Therapeutic conotoxins: a US patent literature survey (2015)	An analysis of conopeptides submitted for patents and their therapeutic applications	•	•			25
A perspective on toxicology of <i>Conus</i> venom peptides (2015)	A review covering molecular taxonomy, venomics, and framework classification with a specific emphasis on Indian cone snail species	•		•		26
Prey-Capture Strategies of Fish-Hunting Cone Snails: Behaviour, Neurobiology and Evolution (2015)	A biology-focused review on the evolution of fish-hunting <i>Conus</i> clades, with perspectives on the evolution of prey capture strategies and behavioural traits			•		27
Discovery, synthesis, and structure–activity relationships of conotoxins (2014)	Review covering vast genetic and pharmacological diversity of conopeptides, detailed folding and structural characteristics, as well as chemical synthesis strategies for modified conopeptides	•	•			15
The Cone Snails on medicines (2014)	A brief review of conopeptides in pharmacological applications, published in French		•			4
Conotoxin Gene Superfamilies (2014)	A practical guide for classification of putative conopeptides using high-throughput genomic techniques and a review of gene superfamily classifications of conopeptides	•				28
Omega-Conotoxins as Experimental Tools and Therapeutics in Pain Management (2013)	A focused review of ω -conopeptides as applied to pain management, with a review of relevant neuropharmacology in chronic and neuropathic pain		•			29
Diversity of <i>Conus</i> peptides that target the nicotinic acetylcholine receptors (2013)	A review centred on conopeptide pharmacological families that interact with nicotinic acetylcholine receptors	•	•			30
Cone Snail Venom Peptides: Biological and Neuropharmacological Perspectives (2013)	A brief introduction to conopeptides and their applications in pharmacy		•	•		31
Analgesic conotoxins: block and GPCR modulation of N-type calcium channels (2012)	A spotlight review on omega and alpha pharmacological families with an emphasis on conopeptides that entered clinical studies	•	•			32
Cyclization of conotoxins to improve their biopharmaceutical properties (2012)	A review of synthetic modifications of conopeptides with special attention to alpha and chi superfamilies	•	•			33
Conotoxins that Confer Therapeutic Possibilities (2012)	An extensive analysis of the possible applications of conopeptides, organized by pharmacological target and applications	•	•			34
<i>Conus</i> Venom Peptide Pharmacology (2012)	A broad analysis of conopeptides and their pharmacological modes of action, with emphasis on their applications	•	•			6

Table 1 (continued)

Title and year	Main topic and focus of review	Structure	Pharma	Biology	Venomics	Ref.
Towards an integrated venomomics approach for accelerated conopeptide discovery (2012)	A review focusing on bioassay-guided approaches compared with integrated-omics approaches as applied in <i>Conus</i>				•	35
Therapeutic Potential of Conopeptides (2012)	A forward-looking review of potential applications of conopeptides in pharmacy with a look at the development of ziconotide		•			36
Therapeutic Potential of Cone Snail Venom Peptides (Conopeptides) (2012)	A review of relevant neuropharmacological targets modified by conopeptides including an overview of ziconotide and Xen2174. Emphasis on applications of conopeptides in neurobiology	•	•			11, 37
Engineering of Conotoxins for the Treatment of Pain (2011)	A review on synthetic modification of conopeptides to improve their <i>in vivo</i> efficacy, with special attention to the α -conotoxin family	•	•			37
Novel Pharmacological Targets from Indian Cone Snails (2011)	A brief review with a focus on Indian species of <i>Conus</i> and the pharmacological families found in these species	•				38
Conotoxins: natural product drug leads (2009)	A broad overview of conopeptides covering nomenclature, structures, tools for structure determination and synthetic derivatives of conopeptides; emphasis on α -conopeptides	•	•			39

Name	Species	Sequence	3D Structure	Pharmacological Family
Vc1.1	<i>C. victoriae</i>	<p>GCCSDPRCNYDHPEIC# Cysteine Framework I (CC-C-C)</p>	<p>(2h8s)</p>	α -conopeptide (nAChR inhibitor) N-type calcium channel via GABA _B pathway
MrlA	<i>C. marmoreus</i>	<p>NGVC^γCGYKLCHOC Cysteine Framework X (CC-C-OC)</p>	<p>(2ew4)</p>	χ -conopeptide (norepinephrine transporter inhibition)
CnIIIC	<i>C. consors</i>	<p>ZGCCNGPKG^γSSKWC^γRDHARCC# Cysteine Framework III (CC-C-C-CC)</p>	<p>(2yen)</p>	μ -conopeptide (voltage-gated sodium channel blocker)
MVIIA	<i>C. magus</i>	<p>CKGKGAK^γCSRLMYD^γCCTGSCRS^γGKC# Cysteine Framework VI/VII (C-C-CC-C-C)</p>	<p>(1mvi)</p>	ω -conopeptide (voltage-gated calcium channel blocker)
GVIA	<i>C. geographus</i>	<p>CKSOGSS^γC^γOTSYN^γCCRSC^γNOYTKRCY# Cysteine Framework VI/VII (C-C-CC-C-C)</p>	<p>(2cco)</p>	ω -conopeptide (voltage-gated calcium channel blocker)
conantokin-G	<i>C. geographus</i>	<p>GE^γYLQYNQYLIRYKSN# Cysteine Framework VI/VII (C-C-CC-C-C)</p>	<p>(1onu)</p>	NMDA receptor antagonist

Fig. 3 Amino acid sequence, three-dimensional (3D) structure and biological activity of a selection of conopeptides acting on ion channels and transporters of the nervous system. The *Conus* genus is abbreviated as "C." in the second column. The disulfide bond connectivity is shown by orange lines on the sequence. Other post-translational modifications are shown in blue: # = C-terminal amidation, O = 4-hydroxyproline, Z = pyroglutamic acid, γ = γ -carboxyglutamic acid. The regular secondary structure elements are shown under the sequence: α -helices as red zigzag and β -strands as blue arrows. The cysteine framework classification is given under each sequence and the pattern that defines it is provided (e.g. "CC-C-C" indicates two adjacent Cys residues followed by two Cys residues that are each separated by a stretch of sequence). The 3D structures are all NMR solution structures and the corresponding PDB identifier is given in parentheses. The definition and target of the pharmacological family is given in parentheses in the last column.

implanted pump being a significant complicating factor in their commercial development.

One of the first pharmacological families to be discovered is the α -conopeptides, which are inhibitors of nicotinic acetylcholine receptors (nAChRs). nAChRs are ligand-gated ion channels that exist as numerous subtypes in the nervous system as well as in several other non-neuronal cell types.⁴¹ For example, *Conus regius* RgIA is a low nanomolar selective inhibitor of the $\alpha 9\alpha 10$ nAChR,⁴² and it was shown to have potential applications as an analgesic and anti-inflammatory agent.^{43,44} Another peptide with analgesic properties from this pharmacological family, *Conus victoriae* Vc1.1, is also selective for the same nAChR subtype,^{45,46} but it has more potent activity through inhibition of N-type calcium channels *via* the GABA_B receptor (GPCR) dependent pathway,⁴⁷ which was suggested to be the preponderant analgesic target for this peptide.⁴⁸ Vc1.1 entered clinical trials as an analgesic replacement for morphine but was withdrawn reportedly because of ambiguities about the analgesic molecular target and presumably other priorities of the funding company. This peptide was later improved *via* the development of a backbone cyclic variant that has oral activity in animal models of chronic pain.^{49,50}

Conopeptides of the χ pharmacological family act as inhibitors of the neuronal norepinephrine (noradrenaline) transporter. This family is relatively small in size, with only four conopeptides belonging to it identified so far. These conopeptides all display the cysteine framework X and are smaller than 1400 kDa in molecular weight.^{19,40} Targeting the norepinephrine signalling system has been the goal of both research on antidepressants and on the treatment of chronic neuropathic pain.^{40,51} MrIA, a χ -conopeptide from *Conus marmoreus* went through FDA phase II clinical trials.^{34,40,52} Instability in the native conopeptide structure was addressed by chemical modification in preclinical studies, highlighting that, as in small molecule drug development, there is a need for medicinal chemistry optimization prior to candidate development.

Three pharmacological families of conopeptides affect voltage-gated sodium (Na_v) channels. The μ -conopeptides are channel blockers, the δ -conopeptides delay the inactivation of the channel,^{53–55} and the ι -conopeptide are also activators but do not delay inactivation.⁵⁶ The μ -conopeptides have been more widely studied and are invaluable tools in neuroscience, especially for the ability of some of them to selectively inhibit the mammalian muscle subtype Na_v1.4.⁵⁷ The μ -conopeptide CnIIIC was advocated as an agent to smooth facial lines when delivered in a cosmetic cream and was commercialized as a cosmetic under the names XEP-018 and XEP-30.^{9,58}

Conantokins are a group of similar conopeptides, which act as N-methyl-D-aspartate (NMDA) receptor antagonists.⁵⁹ These peptides display a number of γ -carboxyglutamate post-translational modifications and do not contain disulfide linkages.⁵⁹ As NMDA antagonists, this group could have applications in understanding diseases such as epilepsy,

Alzheimer's disease and ischemic damage in stroke. NMDA receptor overactivity is a contributing factor to both ischemic damage and seizures.⁶⁰ In a model of ischemic damage in stroke, conantokin-G mediated NMDA receptor activity, and provided a neuroprotective effect against neuronal structural damage and Ca²⁺ overload.⁶¹ Although this compound did not remediate all effects of ischemic damage, it did reduce neural degradation in this model.⁶¹

2.3 Engineering of conopeptides for proteolytic and conformational stability

Conopeptides typically display potent and selective activity at their molecular targets but, like many peptides, they can have low conformational stability as well as high susceptibility to degradation by proteases in a biological context, decreasing their therapeutic potential. Several engineering strategies have been developed to increase the stability of peptides and some have been applied to conopeptides acting on ion channels.^{37,62} In this section we provide a few examples of chemical modifications of conopeptides that could also be applied to engineer hormone-like conopeptides to improve their pharmaceutical potential. An illustration of two basic methods of chemical stabilization is shown in Fig. 4.

A simple method to decrease susceptibility to proteases is to introduce non-canonical amino acid residues such as D-amino acids.⁶² For example, the substitutions of the arginine residues of RgIA by D-arginines resulted in an increase in proteolytic resistance and, serendipitously, in an increase in activity at the human $\alpha 9\alpha 10$ nAChR.⁶³

Creating cross-links between side chains can also be used to stabilise the conformation of proteins and peptides, which could help reduce proteolytic degradation as well as increase activity.⁶⁴ This strategy has been used to stabilise the disulfide poor conantokin-G, resulting in the conformational stabilization of this single α -helix peptide.⁶⁵ Remarkably, this stapled peptide was more selective for the NMDA receptor NR2B than conantokin-G and had similar anticonvulsant activity in a mouse model of epilepsy, but without any of the motor impairment behaviour displayed by the native peptide.⁶⁵

Fig. 4 Schematic representation of methods for biosynthetically (by a cone snail) or synthetically (in the laboratory) modifying linear peptides to improve stability and bioavailability. Cone snails produce a variety of linear peptides *via* ribosomal biosynthesis and have naturally evolved disulfide bond formation as an efficient endogenous mechanism for stabilising them. Human-designed stabilization approaches include stapling and cyclization.

Disulfide bonds created by oxidation of pairs of cysteine side chains form natural cross-links that stabilize conotoxins. Nevertheless, disulfide bonds can be reduced in certain biological conditions, such as occurs in the gut. Replacement of disulfide bonds by non-natural bridges, such as dicarba or diselenide bridges, was attempted for a number of conotoxins.^{66–71} Diselenide bridges have similar conformations to disulfide bridges, and this replacement typically does not affect activity.⁶⁸ The preferential pairing of selenocysteines also was used for regioselective formation of cross-links towards a target connectivity.⁷² This strategy was used in producing BuIIIB, which is one of the only compounds selective for Na_v 1.3,⁶⁶ and MrVIB, a selective inhibitor of the pain target Na_v1.8.⁶⁷ By contrast, dicarba bridges induce small modifications to backbone conformations compared to the native peptide; in some instances these changes result in a change of activity. For example, 2,8-dicarba Vc1.1 and 2,8-dicarba RgIA are only active *via* the GABA_B receptor dependent pathway whereas 3,16-dicarba Vc1.1 and 3,12-dicarba RgIA block the α 9 α 10 nAChR without activity *via* the GABA_B receptor dependent pathway.^{69,70}

Backbone cyclization is a common method in peptide engineering to prevent degradation by exoproteases, and this has been applied to a number of conotoxins.^{23,33} Synthetic cyclization involves extending and connecting the N- and C-termini to form a loop, and generally leaving the rest of the peptide in its natural fold. This can be done by linkers of various lengths to preserve the natural structure of the bioactive peptide. For example, a backbone cyclic variant of α -conotoxin MII increased proteolytic resistance without affecting its activity at nAChRs.⁷³ By contrast, if the termini are involved at the interface with the receptor, this could lead to a drop in activity, which occurred after backbone cyclization of κ -conopeptide PVIIA, which is a voltage-gated potassium channel blocker.⁷⁴ Backbone cyclization also removes the charges from the termini, which could help increase permeability through membranes and hence increase oral bioavailability.⁷⁵ As noted earlier, a backbone cyclic analogue of α -conotoxin Vc1.1 was shown to be orally active in a mouse model of neuropathic pain, creating the first conopeptide-based lead compound with oral activity.⁴⁹

Natural head-to-tail cyclic peptides exist in nature and demonstrate superior stability to linear counterparts under a multitude of conditions.^{33,73} For example, many plants produce natively ligated head-to-tail cyclic peptides.^{76–78} These disulfide rich cyclic peptides are naturally produced in multiple plant tissues and consistently demonstrate high stability under a multitude of conditions, as would be expected from their structural constraints.^{76–78} Since naturally occurring cyclic peptides occur in plants, it may be of interest for future researchers to investigate whether cone snails are capable of natively cyclizing their venom peptides.

In summary, conopeptides acting on ion channels and transporters of the nervous system have diverse potential applications in the biomedical field and are amenable to chemical re-engineering to optimise this potential. The

remainder of this article aims to shine light on the more recently described hormone-like conopeptides that show promise for therapeutic development.

3. Linear hormone-like conopeptides

Several studies have reported a variety of hormone-like peptides in *Conus* venom, but so far no reviews have appeared about these peptides, prompting the following summary and analysis. Interestingly, these hormone-mimetic peptides have been confirmed to have a venom function, despite homology to endogenous signalling peptides from both invertebrates and vertebrates. These molecules provide a fascinating evolutionary and structural connection between widely diverse marine and terrestrial animals. It is instructive to group them into classes of increasing complexity of disulfide content, and we will start with the hormone-like conopeptides that have no disulfide bonds.

3.1 Contulakins – neurotensin receptor agonists

The first discovered contulakin, contulakin-G, was originally isolated from the venom of *C. geographus*, and was shown to have similar sequence and activity to neurotensin.⁷⁹ Two other contulakins were recently identified at the transcriptomic level from *C. generalis* (contulakin-Ge) and *C. characteristicus* (contulakin-Ca).⁸⁰ Contulakin-G displays two post-translational modifications, *i.e.*, a pyroglutamic acid at its N-terminus and a glycosylated threonine. We note that contulakin-Ge and contulakin-Ca cannot display these modifications according to their sequence. The three contulakins mentioned above resemble neurotensin at the C-terminus but have no disulfide bond, even though neurotensin contains one disulfide bond (Fig. 5A).

Neurotensins are signalling peptides commonly found in the central nervous system and the digestive system of animals where they modulate dopamine signalling and regulate a wide variety of physiological processes.⁸¹ Inflammation is one important function that is influenced by neurotensin interactions, including modulating macrophages and slowing the production of cytokines.⁸¹ Contulakin-G was the first neurotensin isolated from an invertebrate.⁷⁹ Contulakin-G activates the rat neurotensin receptor 1 (NTR1) with an EC₅₀ of 20–30 nM whereas neurotensin is one order of magnitude more potent at this receptor.⁷⁹ Contulakin-G was shown to be selective for NTR1 as it could not activate NTR2.⁷⁹ Despite its lower potency and displaying only half the efficacy of neurotensin, contulakin-G is a more potent analgesic in several animal models of pain.^{13,82} It was reported to have an analgesic effect in animal trials^{79,83} and was investigated under the name CGX-1160 as an intrathecal painkiller acting independently from the opioid pathway.^{83,84}

3.2 Conorfamide and conoNPY conopeptides

RFamide peptides are a large group of neuropeptides found across the animal kingdom.⁸⁵ In molluscs, there

Fig. 5 Amino acid sequence alignments between hormone-like conopeptides without disulfide bonds and their corresponding endogenous hormones: A) contulakins aligned with two vertebrate neurotensins, B) conorfamides aligned with FMRFamides from a marine annelid polychaete worm (*Platynereis dumerilii*) and the mollusc *Aplysia californica*, C) neuropeptides encoded by prohormone-4 precursors from *Conus* species compared to those of honeybee and the pacific oyster (*Crassostrea gigas*), D) conomap-Vt aligned with two myoactive tetradecapeptides from a marine worm and the mollusc *Lymnaea stagnalis*, E) comparison between conoNPYs and neuropeptide Y/F from humans, a mollusc, and a marine worm. The legend provides the definition of amino acid residues resulting from post-translational modification (PTM). The names of the endogenous peptides are on a grey background. Amino acids displaying similar physico-chemical properties are represented on a background of similar color. An “^a” was appended to the names of the peptide sequences which were predicted from a nucleic acid sequence.

are five families of RFamides, *i.e.*, FRMFamide-related peptides (FaRP), LRFamide peptides, Luqin peptides, neuropeptide F peptides and cholecystokinin/sulfakinin peptides.⁸⁶ Peptides belonging to FaRP and neuropeptide F were identified in the venom of cone snails, as detailed below.

Conorfamides are RFamides peptides that belong to the FaRP family and are similar to the FMRFa peptides from other molluscs and worms, as shown in Fig. 5B.⁸⁶ The first conorfamide, conorfamide-Sr1, was isolated from *Conus spurius*.⁸⁷ A homologous peptide, conorfamide-Sr2, also from *Conus spurius*, was subsequently shown to cause paralysis in invertebrates.⁸⁸ However, the activity of the conorfamides is not limited to invertebrates, as conorfamide-Sr1 caused hyperactivity in mice after intra cranial injection at 1.0–4.0 nmol g⁻¹.⁸⁷ A similar experiment done with *Conus victoriae* conorfamide-Vc1 at a higher rate of 10–20 nmol g⁻¹ resulted in complete incapacitation of injected mice, whereas at lower doses hyperactivity was also observed.⁸⁹ It was noted during that experiment that conorfamide-Vc1 caused muscle twitching.⁸⁹ It was later shown that conorfamide-Vc1, as well as other conorfamides, can activate human and mouse itch

receptors, which are Mas related GPCRs (Mrgpr), with an EC₅₀s of 0.5–1.8 μM.⁹⁰

The neuropeptide F family found in cone snail venom are called conoNPY peptides (Fig. 5E). Its first two members were identified from *Conus betulinus* venom, and the injection of one into mice was reported to cause brief hyperactivity.⁹¹ Other conoNPY peptides were subsequently discovered in *Conus praecellens* and *Conus gloriamaris*.^{92,93} Human neuropeptide Y, which is the equivalent to mollusc neuropeptide F, is a neural signalling peptide controlling multiple biological functions such as food intake, storage of energy, pain, vasoconstriction and involved in numerous disorders such as anxiety, depression, obesity, cardiovascular disease, addiction, and epilepsy.⁹⁴ Therefore, the discovery of a homologous version in *Conus* venom which is active in mammals could provide a novel tool for neuroscience research.⁹¹

Three truncated neuropeptide F/Ys were also discovered in *Conus textile* venom and, when applied at 50 μM, were reported to slow the desensitization of the acid-sensing ion channel ASIC3, which is a nociceptor involved in pain.⁹⁵ The four residue peptide RPRFamide (ConoNPY-Tx1.1) has an

EC₅₀ of 3–4 μ M, making it one of the most potent RF-amide modulators of ASIC channels.⁹⁵

3.3 Conomap conopeptides

The first conomap peptide, conomap-Vt, was discovered in *Conus vitulinus*.⁹⁶ Unusually for any conopeptide, conomap-Vt has a D-phenylalanine, which is located at position 2 (Fig. 5D).⁹⁶ This peptide had no homology to other conopeptides known at the time but shares 65% sequence identity with myoactive tetradecapeptides (MATPs),⁹⁶ which are expressed endogenously by molluscs, annelids and insects (where they are named allatotropins) to induce excitation in smooth muscles.^{97,98} The precise mode-of-action of endogenous MATPs has not yet been determined, and so determining the mode-of-action of conomap peptides has also been challenging.⁹⁶ It was nevertheless found that conomap-Vt has an excitatory effect in mollusc tissue preparation and that this effect is unrelated to cholinergic receptors.⁹⁶ The muscle contraction activity was very potent, with an EC₅₀ of 8 nM, and dependent on the presence of the D-phenylalanine.

3.4 Prohormone-4 neuroactive peptide

Prohormone-4 is a protein precursor discovered in *Apis mellifera* (honeybees) that encodes a short peptide without disulfide bonds. The expression of this endogenous peptide in bee brain was found to be upregulated during pollen foraging.⁹⁹ This potentially neuroactive peptide is derived from the precursor prohormone-4 after cleavage of a signal peptide at its N-terminus and at a dibasic furin-type cleavage site at its C-terminus. This prohormone-4 (precursor) contains a disulfide rich amino acid sequence with homology to the LDL-A domain following the C-terminal furin-type cleavage site. Protein precursors displaying homology to the honeybee prohormone-4 were identified in *C. geographus*, *C. gloriamaris*, *C. tessulatus*, *C. varius*, *C. victoriae*, and *C. virgo* (Fig. 5C).^{16,93} Some of these cone snail peptides have been identified at the protein level in the venom indicating they are injected in the prey.¹⁶ The target(s) of these potentially neuroactive peptides are unknown and further work needs to be done to determine their function. An additional class of prohormone-4-like precursors has also been found in several

Fig. 6 Amino acid sequence alignments between hormone-like conopeptides with a single disulfide bond and their corresponding endogenous hormones: A) conopressins aligned with four vertebrate vasopressins and oxytocins, B) NMR solution structure of conopressin-T (BMRB identifier 20007), C) conoCAPs compared to crustacean cardioactive peptides (CCAP) from a marine worm (*Capitella teleta*) and mollusc (*Mizuhopecton yessoensis*), and D) alignment of elevenins found in cone snail venom and those of the sea snail *Lottia gigantea* and a marine worm. The definition of amino acids resulting from post-translational modifications can be found in Fig. 5. The names of the endogenous peptides are on a grey background. Amino acids displaying similar physico-chemical properties are represented on a background of similar color. An "a" was appended to the names of the peptides predicted from a nucleic acid sequence. The disulfide bonds are indicated with an orange line.

other cone snails but it is unclear if they encode neuroactive peptides.^{16,92,100}

4. Single disulfide hormone-like conopeptides

4.1 Conopressin conopeptides – vasopressin/oxytocin receptor agonists or antagonists

Conopressins are members of the vasopressin–oxytocin family because of their homology to the human vertebrate oxytocins and vasopressins,¹⁰¹ as shown in Fig. 6A. Oxytocin is ubiquitous and highly conserved in the animal kingdom and is among the most ancient neuropeptide signalling systems.¹⁰² Interestingly, piscivorous cone snails (e.g. *C. tulipa* and *C. consors*) express conopressins that are more similar to fish and other human vasopressin/oxytocins than other discovered sequences. By contrast, the sequences of the conopressins from a wide variety of other cone snails seem to be more similar to the annelid and mollusc vasopressins (called vasotocin and conopressin, respectively).

Oxytocin acts on the central nervous system as well as muscle cells, generally affecting reproduction, water retention, and feeding behaviour.^{102,103} Accordingly, the GPCR targeted by conopressin G (also called Lys-conopressin) in *L. stagnalis* was found to be expressed in neurons and muscles.¹⁰⁴ Conopressin G was initially identified in *C. geographus*,¹⁰¹ and identical peptides were also expressed in a range of other cone snail species, including *C. araneosus*, *C. lividus*, *C. lorioisii* and *C. imperialis*.¹⁰⁵ Different cone snail species generally express distinct conopeptides in their venom,¹⁹ and the discovery that conopressin G is present in several species suggests it plays an important role in the envenomation strategy. Intra-cranial injection of conopressin G and conopressin S in mice showed similar activity to injected oxytocin.¹⁰¹ Nevertheless, cone snail venom duct conopressins display interesting sequence variations compared to mammalian oxytocins and vasopressins, especially regarding the overall charge of the peptide ranging from −1 (*C. villepinii* conopressin Vil)¹⁰⁶ to +3 (conopressin G and conopressin S).¹⁰¹

Conopressins have varying selectivities between the human oxytocin receptor and the three human vasopressin receptor subtypes V_{1A}, V_{1B} and V₂; this could be exploited to create selective ligands for medical applications or physiological studies. *C. miliaris* conopressin-M1 was shown to have partial agonist activity at the human and zebra fish V_{1A} and V_{1B} receptors but not at the V₂ receptor, whereas conopressin M2 was a selective full agonist of the zebra fish V₂ receptor.¹⁰⁷ The selectivity for receptor isoforms could be further engineered from these peptides to create selective agonists, albeit we note that they are only weakly active with EC₅₀ of 1–40 μM (vasopressin has picomolar to low nanomolar activity at the human receptors). Conopressins can also display antagonistic activity; conopressin T was indeed reported to be an antagonist active at 60 nM on the human V_{1A} receptor.¹⁰⁸ The nature of the amino acid at

position 9 was shown to control the activity between agonism and antagonism at the V_{1A} receptor.¹⁰⁸

4.2 ConoCAP conopeptides

Three conoCAP peptides were identified in *Conus villepinii* venom glands, including one at the protein level and two predicted from the transcriptome. They were identified based on their homology to crustacean cardioactive peptides (CCAP).¹⁰⁹ A unique conoCAP protein precursor encoding the three peptides was identified in that study, and a similar conoCAP precursor transcript was subsequently found in *Conus gloriamaris*.⁹³ As shown in Fig. 6C, conoCAP-Vila has more similarity to the annelid *Capitella teleta* CCAP than the two other conoCAP peptides from the same species. Conversely conoCAP-Vilb and conoCAP-Vilc are more similar to the mollusc CCAP than conoCAP-Vila, suggesting that they are optimized for different prey receptors. CCAPs are found in invertebrates and are involved in several neurohormone signalling pathways.¹⁰⁹ The main role of CCAPs is to induce contractions in heart muscle^{110,111} but they also control muscles in the gut¹¹² and oviduct.¹¹³ CCAPs also take part in moulting behaviour regulation¹¹⁴ and in immune responses.¹¹⁵ CCAPs are nanomolar ligands of CCAP receptors, which are GPCRs identified in insects^{116,117–119} and crustaceans.^{115,120} These receptors have high sequence similarity to mammalian neuropeptide S receptors and vasopressin–oxytocin receptors.¹²⁰ In contrast to CCAPs, conoCAP-a decreases the rate of contraction in heart muscle and has been reported to produce arrhythmia.¹⁰⁹ Based on the opposite effects of conoCAP to the endogenous ligand activity, conoCAPs were proposed to have a different molecular target to other CCAPs¹⁰⁹ but we note that similar ligands of the same GPCR can have opposite types of activity.

4.3 Elevenin conopeptide

Elevenin-Vc1 is a conopeptide from *Conus victoriae* with homology to elevenins from other invertebrates, as shown in Fig. 6D.¹⁶ The endogenous elevenin in *A. californica* influences locomotion, although the mechanism of action is not clear.¹⁶ Elevenin-Vc1 as well other elevenin-like peptides from *C. marmoreus*, *C. tessulatus*, *C. varius* and *C. virgo*, mimics this endogenous signalling peptide, but further work needs to be done to determine their physiological role.¹⁶

5. Disulfide-rich hormone-like conopeptides

5.1 Coninsulin conopeptides

Coninsulins are the smallest known functional insulin analogues discovered to date, mimicking the two chain, three disulfide structure of mammalian insulins.^{121,122} At least 25 species of cone snails have been found to produce coninsulins in their venom with some species producing several isoforms.^{16,121,123–126} When injected in fish, coninsulins G1, G3, T1A, T2, K1, K2 lower blood glucose

levels with similar potency as human insulin.^{121,123} Releasing coninsulin G1 in a fish tank resulted in the reduction of fish locomotor activity,¹²¹ indicating that the coninsulins are part of the “nirvana cabal”,¹²¹ a group of cone snail toxins causing general hypoactivity.¹⁷

Coninsulin and human insulin have similar disulfide connectivity but share little sequence identity. A comparison between the amino acid sequences and disulfide-connectivity of chains A and B of human insulin and those of a coninsulin is shown in Fig. 7A. Coninsulin G1 displays two post-translational modifications that are not displayed by endogenous insulins, including modification of two glutamate residues into gamma-carboxyglutamate, one proline residue into hydroxyproline and C-terminal amidation.¹²¹ It was shown that the coninsulins of fish-, worm-, and mollusc-hunting cone snails have higher homology to their prey than the respective endogenous insulin. This suggests that coninsulins have a fine-tuned activity to the insulin receptors of prey and possibly the receptors of predators and competitors.¹²⁶ For example, the amino acid sequence of *C. tulipa* and *C. geographus*, two fish-hunting species, are compared to that of the zebrafish in Fig. 7A.

The chain B of coninsulins is shorter than that of human insulin. Artificial truncation of chain B of human insulin to mimic that of coninsulins resulted in loss of activity at the human insulin receptor,¹²² which is not surprising considering that the truncated segment interacts with the receptor.¹²⁷ Surprisingly, despite the truncation of the B chain compared to human insulin, the coninsulins are active at the human insulin receptor, displaying only a 8 to 20 fold decrease in activity (EC_{50} s of 12 to 30 nM).¹²³ Molecular models of the interaction of coninsulins G1, T1A and K1 in complex with the human insulin receptor suggest that position 15 of the coninsulins replaces Tyr^{B24} found in human insulin, which is at the interface and located in the “truncated” chain B segment.^{122,123} These predictions were very recently confirmed through X-ray crystallography of the three-dimensional complex between coninsulin G1 and the human insulin receptor, as shown on Fig. 7B.¹²⁸

Insulins typically form aggregates at high concentration because of inter-molecular interactions with the C-terminal tail of the B chain.¹²⁹ With shorter B chains, the coninsulins are not subject to similar aggregation and were also shown to reduce the blood glucose level in a mouse model of diabetes, suggesting potential medical applications to treat diabetes.^{123,129} Based on

Fig. 7 Comparison between the hormone-like conopeptide coninsulins and granulin-like conopeptides with their corresponding endogenous hormones: A) alignment between the amino acid sequences of vertebrate insulins with those of several cone snail coninsulins, B) crystallographic structure of coninsulin-G1 bound to the human insulin receptor (PDB identifier 6veq), and C) sequence and three-dimensional structure comparison between human granulin A and conotoxin MiXXVIIA. The definition of amino acids resulting from post-translational modifications can be found in Fig. 5. The names of the endogenous peptides are on a grey background. Amino acids displaying similar physico-chemical properties are represented on a background of similar color. An “^a” was appended to the names of the peptides predicted from a nucleic acid sequence. The disulfide bonds are indicated with an orange line. On panel A, a potential additional disulfide bond is indicated with a dashed line.

coninsulin G1, a minimised human insulin variant was recently designed and found equipotent to insulin lispro,¹²⁸ the most commonly prescribed insulin derivative.

5.2 Thyrostimulin conopeptides

Another hormone-like conopeptide, thyrostimulin, was identified in the venom of several cone snails.¹⁶ Thyrostimulin is expressed by a wide range of organisms, including nematodes, insects, molluscs and humans, although some species such as hymenopterans (e.g. bees, ants) do not seem to make them.¹³⁰ In humans, thyrostimulin acts at the thyroid-stimulating hormone receptor, which is a GPCR influencing metabolism and is involved in a range of medically relevant processes, from weight loss *via* increasing metabolic rate to progression of cancer in human ovaries.¹³⁰ This receptor is also an optimal target for treating osteoporosis; two peptides (teriparatide and abaloparatide) target this receptor and are currently available on the market,¹⁰² suggesting that derivatives from the cone snail thyrostimulin might also find medical applications. Thyrostimulin is made of the non-covalent association of two chains, glycoprotein $\alpha 2$ and glycoprotein $\beta 5$.¹³¹ Both chains have been found at the transcript level in cone snails (*C. victoriae*, *C. virgo*, *C. tessulatus* and *C. magus*),^{16,132} and some tryptic fragments for the $\alpha 2$ chain were found at the protein level in *C. victoriae* venom.¹⁶

5.3 Conorphins – opioid receptor agonists

The only member of this class, conorphin T, has been shown to activate the κ opioid receptor, which is similar to the endogenous agonist dynorphin A.¹³³ It was discovered from a fractionation-based screening of the venom of *C. textile*, but it displays no sequence homology to dynorphin A. It has a short sequence, NCCRRQICC(NH₂), with the four cysteine residues forming two disulfide bonds. The three disulfide isomers were synthesised and the bead connectivity had the highest affinity and potency, with a K_i of 80 nM but an EC₅₀ of only 10 μ M. Through an impressive series of modifications, the activity of a synthetic variant of conorphin T displayed a K_i of 250 pM and an EC₅₀ of 360 pM. More importantly, this compound displays >125 000-fold selectivity for the κ opioid receptor over the δ and μ opioid receptors, providing obvious opportunity for development of a selective analgesic.

5.4 Granulin-like conopeptides

Granulin-like conopeptides have four disulfide bonds and display two β -hairpin turns that are structurally homologous to those of the human granulin A.¹³⁴ The granulin-like conopeptide MiXXVIIA was first identified from *Conus miles* venom, and 17 transcripts encoding for similar cysteine framework XXVII conopeptides were discovered in 10 cone snail species.¹³⁴ In a rare sequence motif, this cysteine framework contains a cysteine triplet (CCC). As shown in Fig. 7C, the three-dimensional structure of the N-terminal β -sandwich domain of granulin A¹³⁵ superimposes well with

that of the granulin-like conopeptide MiXXVIIA, including the position of two disulfide bridges.¹³⁴ The sequence alignment based on structural overlay shows that there is no sequence identity besides the eight cysteine residues (Fig. 7C).

Granulin A is proteolytically released from a large precursor called progranulin, which is involved in development, adult tissue regeneration and inflammation.¹³⁶ Progranulin is also associated with a wide range of diseases and disorders, such as cancer, Alzheimer's and Parkinson's diseases.¹³⁶ In neurodegenerative diseases, a shortage of progranulin causes frontotemporal dementia due to neuron loss,¹³⁷ and conversely supplementing progranulin in mouse models of Parkinson's and Alzheimer's diseases was neuroprotective.^{138,139} *Conus* granulin-like peptides are reported to have proliferative activity with EC₅₀ of 17 μ M,¹³⁴ whereas granulin A was shown to be antiproliferative at nanomolar concentration.¹³⁵ By contrast, a worm granulin used in the conopeptide granulin-like study was also proliferative, albeit at 1000 fold higher potency with EC₅₀ of 11 nM.¹³⁴ It is worth noting that human granulin F is also proliferative, contrary to granulin A. The structurally similar but sequentially different MiXXVIIA and granulins seem therefore to have similar bioactivity, albeit it remains to be confirmed that they have similar molecular targets. Research may discover uses for *Conus* granulin-like peptides, potentially after modifications of the surface residues as diagnostic tools, exploring the role of these peptides in neurodegeneration and tumour progression.

In summary, *Conus* venom has a great diversity of peptidic components acting on many different elements of the nervous and endocrine systems of prey species. Hormone-like conopeptides might currently be the least understood elements of *Conus* venom, but they provide unique opportunities for the development of novel therapeutics that have not been explored.

6. Clinical development of hormone-like toxins

6.1 Conopeptides

As noted earlier in this article, several conopeptides and analogues have entered clinical trials over the last 30 years, but ziconotide (PrialtTM) remains the sole conopeptide drug to successfully reach the market. Fig. 8 gives a timeline of the various conopeptide-based drug candidates that have entered clinical trials. Contulakin-G (CGX-1160) is the only hormone-like conopeptide to have entered clinical trials, and despite its classification as an orphan drug by the FDA and encouraging results in a small scale trial,⁸⁴ its development was halted when the company that had the license, Cognetix, Inc., bankrupted in 2005.¹³

6.2 Protein and peptide hormones

The majority of peptide therapeutics approved for use in the clinic are hormones or act as hormones in the endocrine

Fig. 8 Timeline showing conopeptides that have entered clinical trials over the past 30 years. Names of conopeptides are given with the years clinical investigations began and ended.

system. A total of 63 peptides have been approved by FDA and EMA, and 90% of them, *i.e.* 57 peptides, act on the endocrine system.^{140,141} Twelve of the peptide hormone drugs are native peptides, including ten human hormones (insulin, corticotropin, vasopressin, oxytocin, somatostatin, calcitonin, glucagon, gonadorelin, carperitide, nesiritide), one peptide (eledoisin) from the salivary gland of the octopus *Eledone moschata* and one peptide (exenatide) from the salivary gland of Gila monster *Heloderma suspectum*. Besides these native peptides, 80% of the peptide drugs acting on the endocrine system are analogues of endogenous hormones. As seen with eledoisin and exenatide, the conservation of the endocrine system throughout the animal kingdom offers an opportunity to use orthologous hormones as natural variants of human hormones.¹⁰² Such hormone orthologues can potentially display advantageous pharmacokinetics, selectivity or potency. For example, octopus eledoisin displays slightly increased activity compared to human substance P^{142,143} and is used in a topical application in ophthalmology for primary open-angle glaucoma and iatrogenic dry eye disease.¹⁴⁴ This peptide exemplifies that hormones from molluscs can indeed be used as human therapeutics. With this in mind, peptides with hormone activity found in cone snail venoms are especially promising because venom peptides have typically evolved properties desirable for therapeutics: potency, resistance to degradation, and speed of effect.^{145,146} For example, exenatide has similar activity to glucagon-like peptide 1 and displays a 72 fold longer half-life *in vivo*, *i.e.* 2.4 h vs. 2 min, than GLP-1.¹⁴⁷ In 2017, exenatide was the 260th most commonly prescribed drug in the United States – with 1.6 million prescriptions – <https://clincalc.com/DrugStats/Top300Drugs.aspx>.

The success of exenatide supports the notion that hormone peptides found in venoms (or salivary glands) can be used as drug leads and indeed in some cases developed into drugs. Perhaps surprisingly, this is still possible in cases

where the contribution of the natural peptide to the overall envenomation of prey is poorly understood, as is the case for exenatide. In other approaches to drug development the activity of natural peptides has been mimicked to great effect using rational design, including for peptides found in venoms or molecules inspired from these peptides.¹⁴⁸ Linaclotide (14 amino acids; two disulfide bonds; approved in 2012) is an analogue of a gut bacterial enterotoxin. It is used in the clinic for its ability to mimic the activity of the hormone uroguanylin to treat chronic idiopathic constipation and irritable bowel syndrome with constipation.¹⁴⁹ Echistatin is a 49 amino acid disulfide-rich peptide isolated from elapid snake venom with potent antiplatelet aggregation properties.¹⁵⁰ It was used as a template to develop two marketed anticoagulant drugs: the seven-amino acid peptide eptifibatide and the small molecule peptidomimetic tirofiban, both approved in 1998. Two other anticoagulant peptide drugs, lepirudin and bivalirudin, were developed as analogues of the 65-amino acid protein hirudin, a potent inhibitor of thrombin found in leech saliva.^{148,151,152} Lepirudin only differs from hirudin by two substitutions whereas bivalirudin is a 20-amino acid linear peptide, which was designed from the C-terminus of hirudin.¹⁴⁸ Finally, the small molecule peptidomimetic captopril is an angiotensin converting enzyme inhibitor designed from a five-amino acid peptide QKWAP discovered in the venom of the pit viper *Bothrops jararaca*.¹⁵³ Captopril was the first angiotensin-converting enzyme (ACE) inhibitor on the market to treat hypertension, becoming a blockbuster drug amassing hundreds of millions of dollars annual sales until it became a generic medicine in 1996.

Conclusions and outlook

Conopeptides remain a highly active area of research and recent discoveries have uncovered new pharmacological roles for some of these fascinating marine biomolecules. With only a small fraction of the estimated total number of conopeptides so far examined, a wealth of these peptides and their associated applications remains to be discovered. But even extensively researched conopeptides are still presenting researchers with new information and potential new applications, as demonstrated by a recent study on ziconotide suggesting possible roles in the treatment of depression.¹⁵⁴

Hormone-like conopeptides, despite receiving less attention than other pharmacological families so far, largely because of their recent discovery, present real opportunities to expand the field of conopeptide-based pharmaceuticals. Chemical design and re-engineering techniques have already given researchers the tools to stabilize these hormone-like conopeptides for applications in medicine. We anticipate that applying these tools to hormone-related conopeptides and to the extensive range of currently known conopeptides will be a highly active area of research in the coming five years. Furthermore, we are optimistic that future research will continue to uncover new drug leads from the extensive arsenal of *Conus* venom peptides

and that more conopeptides will join ziconotide in the human pharmaceutical armamentarium. A combined peptide and omics-based approach presents great opportunities to achieve this outcome.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

DJC is an ARC Australian Laureate Fellow (FL150100146). Work in our laboratory on conotoxins is supported by a grant from the Australian Research Council (DP150103990).

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