# Conopeptides: From deadly venoms to novel therapeutics

Gregory S. Shen, Richard T. Layer and R. Tyler McCabe

Marine cone snails have developed many distinct venoms that contain biologically active peptides as part of an envenomation survival strategy for feeding and defense. These peptides, known as conopeptides, have been optimized through evolution to target specific ion channels and receptors with very high affinities and selectivities. Side effects of currently available therapies often arise from their lack of selectivity between pharmacologically relevant targets and targets that have a similar structure but different function. As conopeptides can be highly selective between closely related receptor subtypes, they could meet specific therapeutic needs with a reduced likelihood of side effects.

In the past 50 years, the understanding of the complex function of the CNS has increased dramatically together with an insight into the underlying etiology of CNS disorders and the subsequent rational selection of molecular targets for pharmacological intervention. For example, in the late 1960s, the primary neuropathological deficit in Parkinson's disease was shown to be a loss of dopamine neurons in the substantia nigra and a subsequent loss of the neurotransmitter dopamine within the striatum. This finding led to the use of L-dopa as a therapy to increase dopamine levels in Parkinson's disease.

This finding was followed by advances in molecular genetics, which have identified several hundred G-protein coupled receptors, 100 ion-channel subunits and several thousand other potential molecular targets[1]. In addition, several inherited neurological disorders (termed 'channelopathies'), including hyperkalaemic periodic paralysis[2], long-QT syndrome associated with deafness[3], familial hemiplegic migraine and possibly more common types of migraine[4], result from mutations in ion channels. Such mutant ion channels represent additional distinct therapeutic targets for drug discovery.

As the tools of molecular genetics become ever more powerful, it seems inevitable that the underlying etiology of sub-populations of complex CNS disorders such as schizophrenia, depression, epilepsy and neuropathic pain states will be elucidated. However, the lack of specificity of most currently available therapeutic drugs results in undesirable side effects that limit their usefulness in many CNS disorders. Therefore, the ability to quickly identify highly selective compounds that act at newly described targets will certainly provide an advantage.

Historically, natural products have been an excellent source of therapeutics (such as morphine derived from the opium poppy) or leads in structure–activity studies that can ultimately result in useful drugs. For example, epibatidine, a natural product found in the skin of the frog *Epipedobates tricolor*, has been used as a structural template for the synthesis of new chemical entities such as ABT594 (Ref. 5), and represent a novel, non-opiate approach to treating pain. As a result, scientists continue to look for novel natural sources of biologically active molecules.

Terrestrial sources of natural products have been studied for thousands of years. As early as the third century BCE (Before Common Era or BC), the ancient Greek philosopher Theophrastus described using poppy juice, the source of morphine. Compared with the study of terrestrial sources of natural products, the investigation of the vast biological diversity of marine natural products for potential leads and therapies is still in its infancy. Currently, over

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30 drugs harvested from the ocean are being clinically investigated by drug companies and universities. The didemnins, bryostatin 1 and dolastatin, isolated from invertebrate marine organisms, have been investigated for use as antitumor agents[6–8]. Ecteinascidin 743, isolated from the marine tunicate *Ecteinascidia turbinata*, is a structurally complex molecule with efficacy against several tumor lines in the low picomolar range and is currently being evaluated in clinical trials[9]. Tetrodotoxin, isolated from the reproductive organs of many species of *Tetrodontidae*, including the puffer fish, has been used extensively as a tool in the study of sodium channel pharmacology. The structural diversity displayed by these molecules is complex with motifs that include unnatural amino acids, macrocyclic structures and multiple ring systems.

## Cone snails and their venoms

Some of the most intriguing marine natural products are those derived from marine snails of the genus *Conus*. Cone snails are typically found on, or near, coral reefs in tropical waters throughout the world. As part of a defensive and feeding strategy, these unique molluscs use a complex venom delivered through a specialized radular tooth that serves both as a harpoon and as a disposable hypodermic needle to immobilize their prey.

Since the relatively recent appearance of *Conus* as a distinct genus approximately 50 million years ago[10], cone snails have developed into one of the most diverse marine groups with over 500 estimated living species. While this degree of diversity is impressive, cone snails can be placed into three subgroups according to their prey preference. One group feeds on marine worms including polychaetes, echiuroids and hemichordates, another group primarily hunts molluscs, and the third group primarily eats fish. *Conus* venoms are principally composed of small peptides, named conopeptides, which typically vary in length from 8 to 31 amino acids and target ion channels or G-protein coupled receptors. In addition to linear peptides, several classes of conopeptides containing constrained cysteine bridges have been identified, some as short as 8 amino acids.

The development of such potent and chemically diverse venom, which simultaneously acts on multiple components of the nervous system in their prey, is probably caused by the physical limitations of cone snails relative to other more mobile predators. For example, conopeptides found in the venom of fish-hunting cones have been optimized through millions of years of evolutionary pressure on slow-moving snails that must immobilize and ingest fast-moving fish for survival in an environment rich in potential competitors. Consequently, these species have evolved a distinct complement of conopeptides. All known conopeptides are derived from peptide precursors of 70–80 amino acids in length, where the N-terminal prepropeptide sequence within a given conopeptide family remains relatively conserved. The C-terminus, which contains the mature conopeptide, represents a hypervariable region that is readily mutated. Mutation frequencies vary by more than one order of magnitude across these precursor sections, with the mature toxin region undergoing the highest mutation rate[11]. The rate of conopeptide evolution is higher than that of most other known proteins[12].

Hence, by retaining a given disulfide framework or backbone structure, and mutating loop regions between cysteine groups or specific amino acids in a mature peptide, slight species variation in the amino acid sequence results in distinct pharmacological activities. As new pharmacological targets have evolved in prey species, the high rate of mutation in the mature peptide has enabled the snails to optimize venom components for new receptor subtypes. By contrast, peptides have evolved that act on common targets. For example, α-conopeptides inhibit neuromuscular nicotinic receptors through competitive inhibition at acetylcholine binding sites[13], while the unrelated ψ-conopeptides inhibit the same receptors through a non-competitive mechanism[14]. As venom from a single cone snail can contain up to 200 biologically active components, the medicinal chemist could assemble a library of biologically distinct active molecules.

## Classification of conopeptides

Conopeptides are typically classified into categories based on their amino acid sequence and disulfide-bridge framework. The vast diversity of the conopeptides can be illustrated by considering a few specific examples (Fig. 1). The conantokins are a specific class of linear peptides of 17–27 amino acids in length, which inhibit the *N*-methyl-D-aspartate (NMDA) receptors[15,16]. Contryphans are the smallest-bridged conopeptides identified so far, containing only one disulfide bridge[17]. The α-conopeptides contain two disulfide bridges forming two loops of variable size and specifically inhibit muscular or neuronal nicotinic acetylcholine (nACh) receptors[18,19]. Meanwhile, the ω-conopeptides contain three disulfide bridges and selectively inhibit certain classes of calcium channels[20]. The μ-conopeptides also contain three disulfide bridges but have a different bridging pattern from the ω-conopeptides[21] and selectively block sodium channel subtypes. These differences in bridging structure and loop variability contribute to the selectivity of the conopeptides for a range of receptors and ion channels.

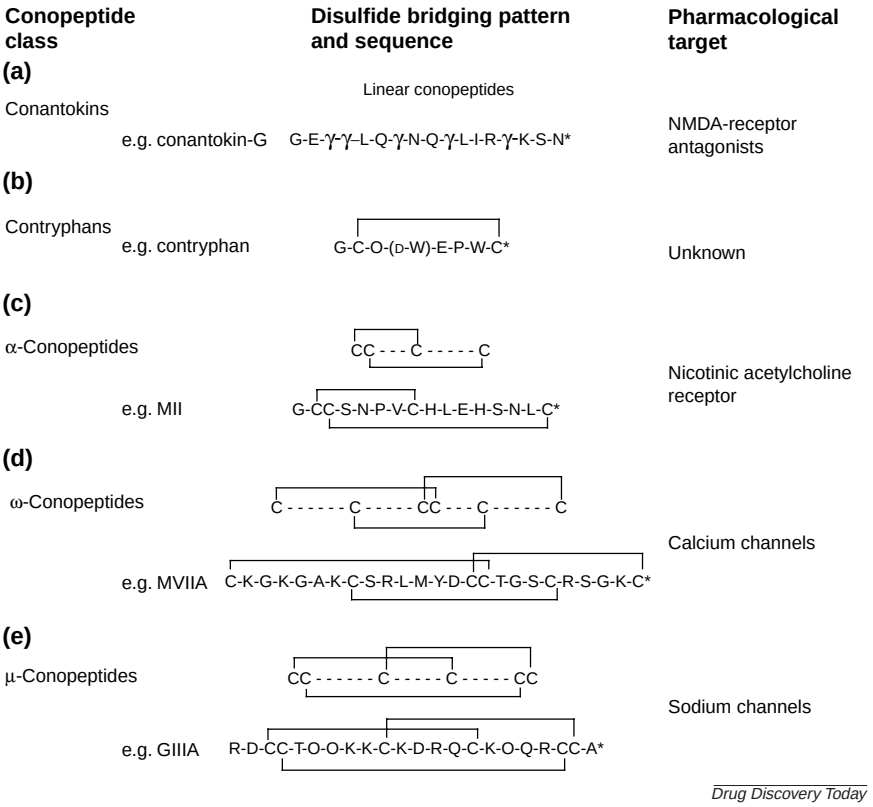


***Figure 1.*** *Disulfide bridging patterns and amino acid sequences of some common conopeptides. Abbreviations: *, amidated C-terminus;* D*-W,* D*-tryptophan; γ, γ-carboxyglutamic acid; O, 4-*trans*-hydroxyproline.*

## Structure–activity relationships. The α-conopeptide, ImI, as an example

The unique selectivity of the conopeptides for different receptors and ion channels can be directly attributed to the spatial arrangement of different regions and functional groups of the peptide in three-dimensional space. Modification of the loop regions are particularly important in defining selectivity of the peptides for different receptor subtypes, and the structural determination of these highly subtype-selective molecules has recently increased interest in correlating structure to function.

Conopeptide structures have been determined using both NMR and X-ray crystallography[22–24]. The class of conopeptides best characterized structurally are the α-conopeptides. These bind at the interface of the distinct subunits comprising nACh receptors. Their structures provide much information for elucidating ligand–receptor interactions at, or near, the binding sites of acetylcholine at various nACh receptor subtypes. The smallest known α-conopeptide isolated so far is ImI, which contains 12 amino acids and is selective for neuronal nACh receptors comprised of α7 subunits[25]. Generally, the α-conopeptides that contain three amino acids in the first loop and five amino acids in the second loop (3,5 type) are specific for nACh receptors of the neuromuscular junction, while those that contain four and seven amino acids in the two loops (4,7 type), respectively, are specific for various neuronal nACh receptors. ImI is an exception to this generalization, defined by a 4,3 loop structure. Extensive structure–activity studies of both ImI and the α7 subtype of nACh receptors have identified Asp5, Pro6, Arg7 and Trp10 to be crucial groups in the conopeptide, both for biological activity and for forming a binding face on one side of the molecule[26–28].

Comparing the four different α-conopeptides ImI, MII, PnIA and PnIB provides information for determining the useful important structural features for subtype selectivity. All of these conopeptides contain two α-helical regions in the first loop caused by the highly conserved amino acid sequence, and the structural similarities of this loop can be seen in Fig. 2. Differences in the second loop are more pronounced. The solvent-accessible areas of the α-conopeptides appear to be important for binding. Interestingly, Arg7 and Trp10 of ImI and residues 10 and 11 of PnIA and PnIB are solvent-exposed and might contribute to the noted differences in selectivity. It appears that these flexible side chains determine the biological selectivity, and the presence of a negative charge, a basic nitrogen and an aromatic ring are requisite for neuronal α7 nACh receptor activity[25].

## Promising new therapies

### *Conantokins as therapies*

Conantokin G (con-G) was originally isolated from the venom of the fish-hunting snail, *C. geographus*[15,16] and is a 17 amino acid linear peptide that adopts an α-helical conformation in solution and contains post-translational modifications[24,29]. These modifications include five carboxyglutamic acid (Gla) residues and an amidated C-terminal. The conantokins are characterized by the presence of a

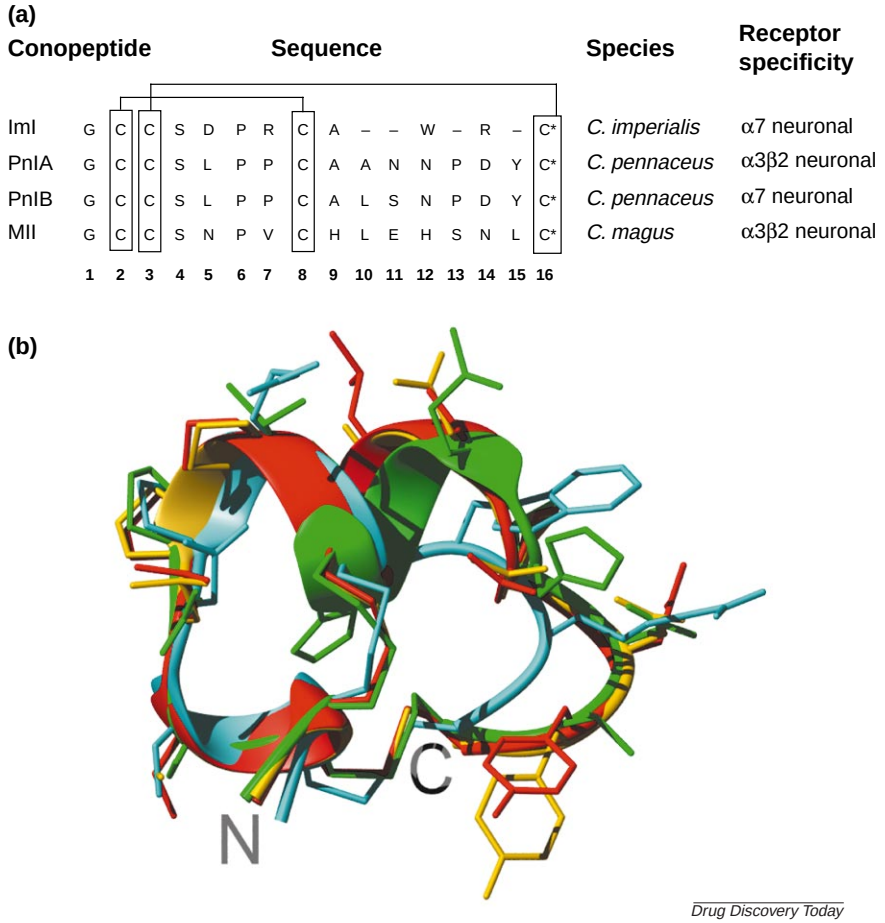


***Figure 2.*** *(a) The sequence assignment of the α-conopeptides ImI, PnIA, PnIB and MII and (b) the three-dimensional NMR or X-ray structures. The disulfide bridging patterns are outlined in boxes. The backbone atoms N, $C^{\alpha}$, and C′ of ImI residues 1–9, 11 and 12 (blue) are overlapped with residues 1–9, 14 and 16 in PnIA (yellow), PnIB (red) and MII (green). Differences in specificity might be caused by side chain positioning. Abbreviations: *, amidated C-terminus. Reprinted with permission from the American Chemical Society.*

Gly-Glu-Gla-Gla N-terminus and all known conantokins are linear except for the conantokin isolated from *C. radiatus,* which has a three amino acid disulfide-bridged loop near the C-terminus.

Much evidence indicates that conantokins produce their effects *in vivo* through functional inhibition of the NMDA-receptor complex and, hence, con-G and related peptides might have evolved to impair sensory organs such as the lateral line system of fish that detects pressure changes. Firstly, in cerebellar cells, con-G inhibits glutamate-induced neurotoxicity[30], NMDA-induced increase in intracellular calcium[31], and NMDA-induced, but not kainic acid-induced, increases in cGMP accumulation[32]. Secondly, con-G and con-T inhibit NMDA-evoked currents in rat hippocampal slices[33] and *Xenopus oocytes* injected with mouse brain mRNA[34]. Finally, con-G non-competitively inhibits polyamine-stimulated [$^3$H]MK801 binding[32]. Most importantly, the action of conantokins on NMDA receptors appears to be subunit-specific. This specificity is suggested by many findings, including that con-G inhibits only 70% of the NMDA-evoked increase in intracellular free calcium in cerebellar granule cells[31], and that conantokins might interact through a polyamine-dependent mechanism that is known to be subunit-specific[32,35]. Whether this selectivity is caused by an action on distinct subunit combinations or on the activation state of the NMDA receptor, is currently under investigation.

In mammals, activation of the NMDA receptor plays a role in the pathophysiology of CNS disorders. Because of a fortunate similarity between the targets at which conantokins act in fish and the mammalian NMDA receptor, conantokin peptides are being considered as potential therapies for CNS disorders. While other classes of NMDA-receptor antagonists including competitive antagonists and voltage-dependent ion-channel blockers are relatively effective in animal models of disease, unfavorable side effect profiles and a narrow therapeutic window limit their usefulness. Conantokins appear to act through a novel mechanism, are metabolically stable, have a high protective index [the ratio of toxic $dose_{50}$ (the dose at which 50% of the tested group displays toxicity) to effective $dose_{50}$] in preliminary animal studies and are selective for a subset of NMDA receptors. Hence, they might not share the side effects normally associated with other types of NMDA-receptor antagonists.

Conantokins have been assessed in animal seizure models and found to potently block seizures in a dose-dependent manner (McCabe, R. *et al.* (1996) *In vivo* antiseizure activity of conantokin–R and analogs isolated from *Conus*

snails. *American College of Neuropsychopharmacology (ACNP) Conference,* 10 December 1996, San Juan, Puerto Rico). The large difference between the effective dose and the behavioral toxic dose is striking when compared with other anticonvulsant compounds in this model. These findings indicate that the conantokins represent a novel class of NMDA-receptor antagonists that can modulate CNS excitability. Given their large therapeutic window and adequate CNS penetration (or a suitable method of delivery), the conantokins offer a unique approach to the treatment of seizure disorders.

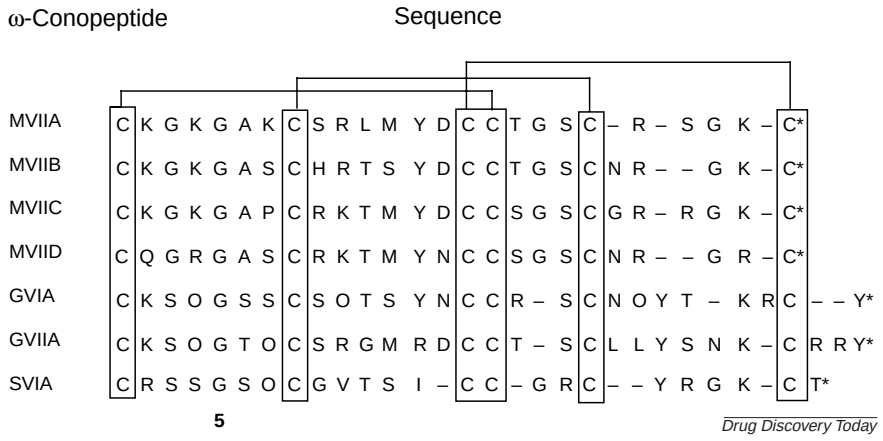


***Figure 3.*** *Sequence assignment of ω-conopeptides isolated from* Conus magus *(M),* Conus geographus *(G) and* Conus striatus *(S). The disulfide bridging patterns are outlined in boxes. Abbreviations: *, amidated C-terminus; O, 4-*trans*-hydroxyproline.*

### *MVIIA: A potent analgesic drug*

The ω-conopeptides are small, constrained peptides of 24–31 amino acids in length, and target voltage-sensitive calcium channels (VSCC)[36]. The ω-conopeptides contain six cysteine molecules that form three disulfide bridges that constrain the peptide into a relatively rigid conformation. The regions between the cysteine groups are exceptionally variable and define the VSCC subtype-selectivity. The inclusion of 4–6 basic amino acids and the placement of a glycine at position 5 appears to be highly conserved in all the ω-conopeptides (Fig. 3)[37,38].

Conopeptide MVIIA, isolated from *C. magus*, is an ω-conotoxin of 25 amino acids containing three disulfide bridges that selectively blocks N-type calcium channels. These channels are involved in neurotransmitter release[39] and are crucial components of the neural pathways that mediate pain. Of the four different ω-conopeptides isolated from *C. magus* (MVIIA, B, C and D), MVIIA has the highest selectivity for the N-type calcium channel[36]. The high affinity and selectivity of MVIIA for the N-type VSCC and its potential antinociceptive effects led to consideration of this peptide as a therapeutic agent, and it is currently undergoing clinical development for use in the treatment of chronic pain by Neurex (now a part of Elan Pharmaceuticals, Dublin, Ireland). The clinical use of MVIIA is supported by preclinical studies showing that acute and continuous intrathecal infusion of MVIIA reduces nociceptive responses and behaviors in the formalin test in rats[40].

The antinociceptive effects of MVIIA were also assessed in several chronic pain models that measure increased sensitivity to either a thermal or mechanical stimulus following nerve or tissue damage. Intrathecal administration of MVIIA dose-dependently reduced the persistent tactile allodynia (pain caused by a stimulus that does not normally provoke pain) that follows nerve injury induced by tight ligation of lumbar spinal nerves[41]. Heat-hyperalgesia (an increased response to a stimulus that is normally painful) and mechano-allodynia also are reduced by MVIIA in other models of neuropathic pain[42-45]. Neuropathic pain was reduced following subacute administration by continuous intrathecal infusion of MVIIA without the development of tolerance[42].

Inflammatory pain can be induced in animals by injection of kaolin and carrageenan into the knee joint, which increases joint circumference, guarding of the limb, and decreases the latency to escape a radiant heat source. The intrathecal administration of MVIIA prevents heat hyperalgesia or sensitivity when the drug is given before the induction of inflammation, and also reverses heat hyperalgesia when given four hours after induction of inflammation[46].

Clinical trials for MVIIA in the treatment of neuropathic pain have been promising. In general, following intrathecal administration of MVIIA, a significant reduction in pain was shown in different patient populations. In trials with HIV patients and terminally ill cancer patients who responded poorly to opioids, MVIIA reduced pain with no development of tolerance[47]. The successful development of MVIIA demonstrates the utility of conopeptides as therapies.

## Delivery of conopeptides

Most peptides with therapeutic potential, such as the analgesic enkephalins, act as neurotransmitters or

neuromodulators in the nervous systems and have specific peptidases that then terminate their action. While this is useful physiologically, it provides an obstacle for drug development. By contrast, conopeptides have not evolved as neurotransmitters but as venom components. Thus, evolution has post-translationally modified many conopeptides to give them unique properties compared with the mature peptides. For example, many conopeptides contain an amidated carboxy terminus while conantokins are enriched in γ-carboxyglutamate residues, a post-translational modification of glutamate[16,48]. Contulakin-G (Ref. 49) and κA-conopeptide SIVA (Ref. 50) contain glycosylated threonine and serine residues, respectively, and contryphan-R contains a D-tryptophan residue[17]. Several conopeptides, including bromocontryphan and the 'bromosleeper' peptide from *C. radiatus*, contain a brominated tryptophan residue[51,52] The mature form of bromocontryphan, an eight amino acid peptide from *C. radiatus*, has one disulfide bridge, hydroxylation of Pro4, epimerization of Trp4 to D-Trp, bromination of Trp7 and C-terminal amidation[49]. Conopeptide ϵ-TxIX, a 13 amino acid peptide from *C. textile*, contains two disulfide bridges, bromination of Trp7, hydroxylation of Pro13, glycosylation of Thr10, and γ-carboxylation of Glu1 and Glu4 (Fig. 4)[53].

Improving the delivery of therapeutic peptides through controlled release technologies such as mechanical pumps, polymeric devices and electrophoretic patches is ongoing. Delivery of a conopeptide into the CNS using a mechanical device has already been accomplished. Neurex has filed a new drug application (NDA) involving delivery of the analgesic conopeptide, MVIIA, intrathecally using the Medtronic Synchromed® pump[47]. This infusion system is an implanted and programmable device that provides reliable, convenient, site-specific, rate- and pattern-controlled drug (solution) delivery for chronic treatment regimens. Interim analysis revealed substantial efficacy and no device-related problems. The success of this approach indicates that delivery of conopeptides into the CNS is a feasible strategy, particularly in patients with poorly controlled disorders such as intractable pain.

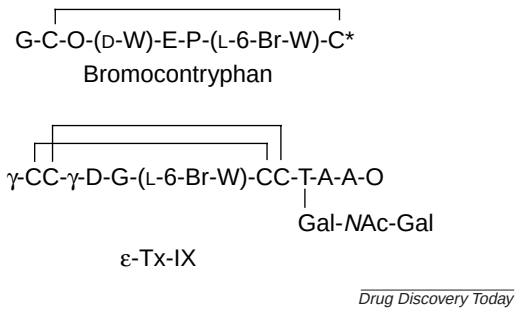


***Figure 4.*** *Conopeptides with a high degree of post-translational modifications. Abbreviations: *, amidated C-terminus; D-W, D-tryptophan; Gal-NAc-Gal, disaccharide moiety; γ, γ-carboxyglutamic acid; L-6-Br-W, L -6-bromotryptophan; O, 4-*trans*-hydroxyproline.*

Conopeptides meet many of the requirements for device-based delivery. The ideal drug for such delivery would meet the following criteria:

- The drug must be highly water soluble to be delivered in a physiologically compatible vehicle such as artificial cerebrospinal fluid.
- The drug must be stable in this vehicle for at least several weeks to minimize pump loading procedures and be potent and soluble to such an extent that it can be concentrated sufficiently to minimize the size of the reservoir of the pump.
- The drug should have a broad spectrum of activity and a rapid time-to-onset-of-action at non-toxic doses. The criteria for an ideal compound being considered for device-based delivery are those required for a venom component delivered through injectible harpoon, and conopeptides have evolved to overcome such obstacles through millions of years of evolution.

## Future directions

The potential of conopeptides for use not only as novel probes for specific receptor subtypes, but also as therapeutics for disease is well documented. Their high specificity and affinity make conopeptides ideal candidates for drug development for the treatment of specific disorders with identified pharmacological targets. In addition to the indications already mentioned, other therapeutic indications are being explored (Table 1). The μ-conopeptides, which target neuromuscular sodium channels[54], have the potential to treat neuromuscular disorders. α-Conopeptides, which are highly selective for nACh receptors that discriminate between closely related subtypes[18,55], have many potential applications in anxiety, Parkinson's disease, pain, as muscle relaxants and as antihypertensive agents. This class of conopeptides might also be useful for the treatment of human small-cell lung carcinoma, a highly aggressive tumor composed of neuroendocrine secretory cells that express neuronal-type nACh receptors. Activation of these receptors causes secretion of mitogenic hormones and stimulates cell proliferation. As α-conopeptide ImI inhibits these effects, it might be useful for

**Table 1. Conopeptide classes, targets and potential therapeutic indications**

| Peptide class | Mechanism of action | Potential therapeutic indication |
|---|---|---|
| Conantokins | NMDA receptors | Epilepsy, pain, stroke, Parkinson's disease |
| α-Conopeptides | Antagonists of neuronal and skeletal muscle nAChR | Anxiety, Parkinson's disease, pain, muscle relaxants, antihypertensive agents, cancer[61] |
| μ-Conopeptides | Skeletal muscle sodium channels | Neuromuscular block |
| ω-Conopeptides | Calcium channels | Stroke, pain |
| κ-Conopeptides | Potassium channels | Hypertension, arrhythmia, asthma |
| Conopressin | Vasopressin receptors | Blood pressure regulation |
| Contulakin-G | Neurotensin receptors | Pain and CNS disorders |

Abbreviations: nAChR, nicotinic acetycholine receptor; NMDA, *N*-methyl-D-aspartate

treating this form of cancer[56]. μO-conopeptides inhibit neuronal sodium channels[57], while κ-conopeptides interact with potassium channels[58] and might have a wide therapeutic potential. Other families of conopeptides target G-protein coupled receptors, such as the conopressins that are active at vasopressin receptors[59]. Moreover, contulakin-G, a conopeptide with C-terminal homology to neurotensin that was recently isolated from the venom of *C. geographus*, binds to cloned neurotensin receptors[49].

There is a vast potential for conopeptides that remain relatively unknown and discovery of conopeptides with selectivity towards other ion channels and G-protein coupled receptors will undoubtedly follow. Conopeptides have been optimized through evolutionary selective pressures to function as venom components. Often, slight differences in conopeptide sequence result in dramatic changes in specificity. Alternatively, conopeptides with relatively little homology but typically with a common cysteine bridging pattern, act at common targets.

As some targets for which the discovery of novel ligands would be attractive to the pharmaceutical industry are unlikely to be found in prey species of *Conus*, such as mutant ion channels underlying human 'channelopathies', it is unlikely that a venom component will ever be found that is selective for such a target. However, cone snails have developed scaffolds that are optimal for presenting crucial residues within a pharmacophore. For example, conopeptides that target calcium, sodium and potassium channels all contain a common structural motif termed a cystine knot[60], which is comprised of the knot and a triple-stranded β-sheet. The knot is defined by a ring formed by disulfide bridges. Hence, conopeptides are also valuable for the unique structural information contained within their constrained peptide scaffolds.

The extensive structure–activity information that can be obtained through relatively simple amino acid modifications makes the conopeptides extremely useful as 'scaffolds' for potential new therapies. Combinatorial peptide approaches using time-tested conopeptide scaffolds might result in drugs with selectivity profiles that nature never had a reason to develop. Alternatively, relatively simple peptide modifications, followed by more substantial changes, could lead medicinal chemists to develop, not only more effective peptides for a given therapeutic application, but also provide information leading to the development of small organic molecules with similar profiles.

## Conclusions

Apart from the advances made in the use of peptides as potential therapies, delivery of peptides across biological barriers is a continual obstacle. The delivery of therapeutic peptides through controlled release devices such as mechanical pumps, polymeric devices, electrophoretic patches and cell encapsulation systems coupled with the modification of peptides into new entities and conjugates is an ongoing effort. Unique and effective methodologies are continually being added to the repertoire of effective peptide delivery strategies. As new delivery methodologies and controlled release systems are developed, the feasibility of delivering peptides into the CNS becomes more promising. Because of their small size, high specificity and potency, conopeptides make these delivery strategies more attractive.

As conantokin-G and MVIIA move through the various stages of drug development, new conopeptides with interesting potential applications will undoubtedly be discovered. While both of these peptides might require ancillary devices for delivery, they provide effective treatments where none exist now. Moreover, given appropriate delivery strategies, the therapeutic potential of the conopeptides for CNS disorders is remarkable. These authors are aware of no other source of natural products, marine or terrestrial, that more efficiently package such a diversity of receptor-subtype selectivity and potency as the peptides contained within cone snail venoms.

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