

Review

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From marine predator to pharmacology: Conotoxin diversity, discovery, and therapeutic potential

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ABSTRACT

Cone snails (*Conus* spp.) biosynthesize a highly specialized venom comprising a vast arsenal of neurotoxic peptides, termed conotoxins. These peptides exhibit exceptional structural and functional diversity, characterized by unique disulfide-bond architectures, highly variable amino acid sequences, and precise interactions with defined molecular targets. This review presents a comprehensive synthesis of current knowledge on conotoxin biology, beginning with evolutionary origin and proceeding through the classification systems used to define their gene superfamilies and pharmacological families. Advances in discovery methodologies are also examined, with particular emphasis on the transformative role of high-throughput multi-omics in expanding conotoxin identification and characterization. Furthermore, this review analyzes the pharmacological properties of representative conotoxins acting on key ion channels and receptors and evaluates the structure-activity relationships that determine their potency, selectivity, and functional profile. Mechanistic insights derived from these studies have established conotoxins as powerful neuropharmacological tools and a rich reservoir for drug discovery. Their significant therapeutic potential is underscored by efficacy in chronic pain management, exemplified by the US FDA-approved drug ziconotide, and by growing evidence supporting applications in neuroprotection in disorders such as Alzheimer's disease and Parkinson's disease, as well as in selected cardiovascular conditions. Future perspectives are also discussed, with progress likely dependent on the

integration of computational design, peptide engineering, and bioengineering platforms to accelerate the translation of these marine peptides into next-generation clinical therapeutics.

Keywords: Conotoxin; Drug Discovery; Ion Channels; Neuropharmacology; Pain; Peptide Therapeutics; Venomics

INTRODUCTION

Cone snails (*Conus* spp.) rank among the most specialized and evolutionarily successful predators in marine ecosystems and represent a striking example of adaptive radiation. As members of the gastropod family Conidae, these mollusks have achieved broad ecological resilience and persistence across diverse tropical and subtropical aquatic ecosystems, with over 60% of known species distributed in the Indo-Pacific region (Figure 1A) (Fiorotti et al., 2023). Their geographic range extends from the coastal waters of the South China Sea, Africa, and the Red Sea to marine environments surrounding Japan and the Croatian coast, and further to tropical regions of the Americas, including the Caribbean Sea and Panamic Province (Guo et al., 2024; Puillandre et al., 2014). More than 800 extant species have been documented (Ahyong et al., 2024), underscoring the extraordinary evolutionary success of this lineage. Taxonomic resolution has been further supported by comprehensive reference resources such as the "Collection of Global Cone Snails" (Shi et al., 2018), which presents color images of 343 *Conus* species and provides an important framework for species identification and classification.

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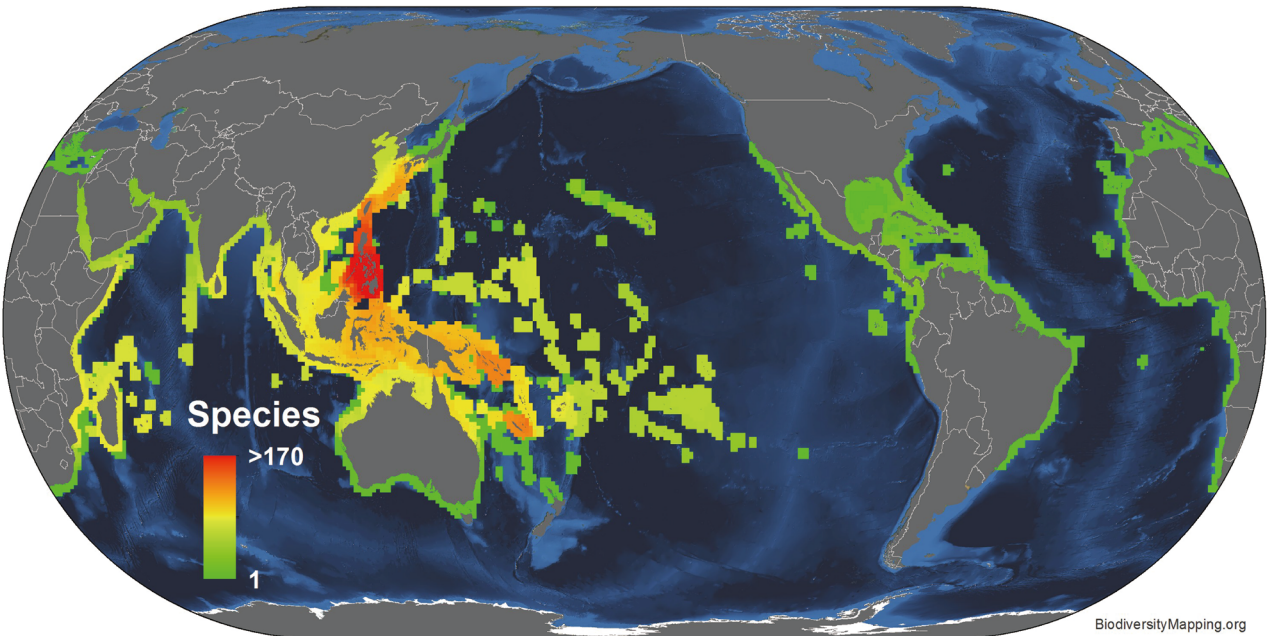
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A

Cone snail diversity



B



Figure 1 Global distribution and representative morphology of cone snails (*Conus* spp.)
A: Worldwide distribution and species richness of cone snails, with color intensity corresponding to number of species present in a given region. Highest diversity is concentrated in the Indo-Pacific coral reefs, known as the “Coral Triangle”. Map data were adapted from Jenkins & Van Houtan (2016). B: Collection of representative *Conus* species, showcasing morphological diversity in shell shape, size, and color patterns, which often correlate with their specific ecological niche and prey type. Images were adapted from Shi et al. (2018) with permission.

Although intricately patterned shells have long made cone snails highly desirable to collectors (Figure 1B), external appearance frequently obscures accurate species delimitation because shell morphology can vary extensively within species and across geographic regions (Gao et al., 2017, 2022; Olivera et al., 2014; Qasmi et al., 2024). This taxonomic complexity has driven broad adoption of integrative approaches that combine morphological assessment with molecular phylogenetics. Within this phylogenetic framework, feeding ecology remains an informative descriptor of biological diversification. Cone snails are generally classified into three trophic guilds (Figure 2A): piscivorous (fish-hunting), vermivorous (worm-hunting), and molluscivorous (mollusk-hunting), although these ecological categories do not strictly align with monophyletic lineages (Puillandre et al., 2014). Nevertheless, dietary specialization is commonly associated with pronounced divergence in venom system organization. Comparative transcriptomic and proteomic analyses further suggest that venom composition differs across these guilds in a manner consistent with prey-driven selective pressure. Piscivorous species are typically characterized by rapid-acting neurotoxins that efficiently disrupt neuromuscular signaling in fast-moving prey, whereas vermivorous and molluscivorous species often express prey-adapted repertoires shaped by differences in prey physiology and escape behavior (Koch et al., 2024). Parallel functional divergence is also evident in the radular tooth, the structure responsible for venom delivery, which exhibits remarkable morphological variation that correlates more closely with predation strategy than with a simple prey-type classification (Kohn et al., 1999). This functional specialization suggests strong evolutionary pressure for efficient prey capture. For instance, many piscivorous species employ a “taser-and-tether” strategy, in which a harpoon-like radular tooth rapidly immobilizes fast-moving fish (Figure 2B left), whereas others, including *Conus geographus*, utilize a “net-hunting” strategy that enables capture of multiple prey items simultaneously (Olivera et al., 2015). In contrast, vermivorous species typically possess slender, elongated radular teeth suited to worm capture (Figure 2B middle), while molluscivorous species often bear more robust teeth capable of penetrating the tough tissues of other gastropods (Figure 2B right) (Ratibou et al., 2024).

Mitochondrial phylogenetic analyses have provided compelling evidence that the remarkable diversity of cone snails likely emerged from a shared vermivorous ancestor, followed by incremental shifts toward molluscivory and piscivory (Espino et al., 2024; Gao et al., 2018, 2022; Lee & Park, 2022) (Figure 2A). This trophic diversification has been closely linked to, and mirrored by, progressive changes in venom composition at the molecular level (Chang et al., 2015; Koch et al., 2024; Rogalski et al., 2023). For example, proteomic evidence has suggested that defensive venom in ancestral worm-hunting lineages may have provided a functional molecular foundation that facilitated expansion toward more challenging prey types (Prashanth et al., 2022). In this context, venom composition can be viewed as a biochemical fingerprint of both evolutionary trajectory and ecological niche specialization in cone snails (Phuong et al., 2016). Persistent demands for rapid prey incapacitation and effective predator deterrence have imposed intense selective pressure across evolutionary time. Over millions of years, this process has shaped *Conus* venom into an exceptional repertoire of neuroactive compounds with extraordinary

functional breadth. Such evolutionary refinement has established conotoxins not only as a rich source of therapeutic candidates but also as highly informative pharmacological probes. Although ziconotide (Prialt®) remains the most prominent clinical success, the broader significance of these peptides often lies in their capacity to serve as lead scaffolds for target validation and for development of synthetic analogs.

Although numerous reviews have examined specific aspects of conotoxin research—ranging from taxonomy to ion channel pharmacology—an integrated perspective linking the evolutionary determinants of venom diversity with contemporary therapeutic discovery remains limited. This review addresses that need by establishing a unifying framework that bridges the biological demands shaping predator adaptation in *Conus* with the translation potential of the resulting toxin repertoire. Particular emphasis is placed on evolutionary mechanisms, including hypermutation and extensive post-translational modifications, that have generated an expansive and chemically sophisticated peptide library. This review further examines how emerging platforms, particularly multi-omics and artificial intelligence (AI)-assisted screening, are now enabling systematic access to this molecular diversity. By integrating zoological context with pharmaceutical and translational priorities, this work offers a comprehensive roadmap from marine venom evolution to the development of clinically relevant therapeutics.

CONOTOXIN DIVERSITY AND PHARMACOLOGICAL MECHANISMS: A TARGET-CENTRIC VIEW

Sustained evolutionary selection has transformed *Conus* venom into an extraordinary reservoir of neuropharmacologically active molecules. Rather than functioning as a simple toxic secretion, cone snail venom consists of a densely diversified cocktail of small disulfide-rich peptides, typically 10–45 amino acids in length, that selectively and potently modulate critical physiological targets, most notably voltage-gated sodium (Na_v), potassium (K_v), and calcium (Ca_v) channels, as well as nicotinic acetylcholine receptors (nAChRs) (Koch et al., 2024; Mir et al., 2016). Collectively, these targets regulate fundamental processes underlying membrane excitability and synaptic communication. Na_v channels drive initiation and propagation of action potentials, K_v channels govern repolarization dynamics and firing behavior, Ca_v channels couple membrane depolarization to neurotransmitter release, and nAChRs mediate rapid cholinergic signaling at neuromuscular junctions and within autonomic and central neural circuits. By exploiting this molecular architecture with extraordinary precision, conotoxins can rapidly disrupt sensorimotor function in prey, while also providing a mechanistic foundation for translational applications in areas such as analgesic development.

Conotoxin repertoires are also profoundly shaped by prey-driven selective pressure. Piscivorous *Conus* species rely on immediate neuromuscular incapacitation and therefore are commonly enriched in rapid-acting modulators of ion channels and receptors, particularly peptides directed against Na_v channels, Ca_v channels, and nAChRs. In contrast, vermivorous and molluscivorous lineages often deploy toxin mixtures more precisely tuned to the physiological traits and escape strategies of their respective prey. This tight association between prey type and venom composition provides an evolutionary rationale for the preferential expansion of distinct pharmacological modules within different

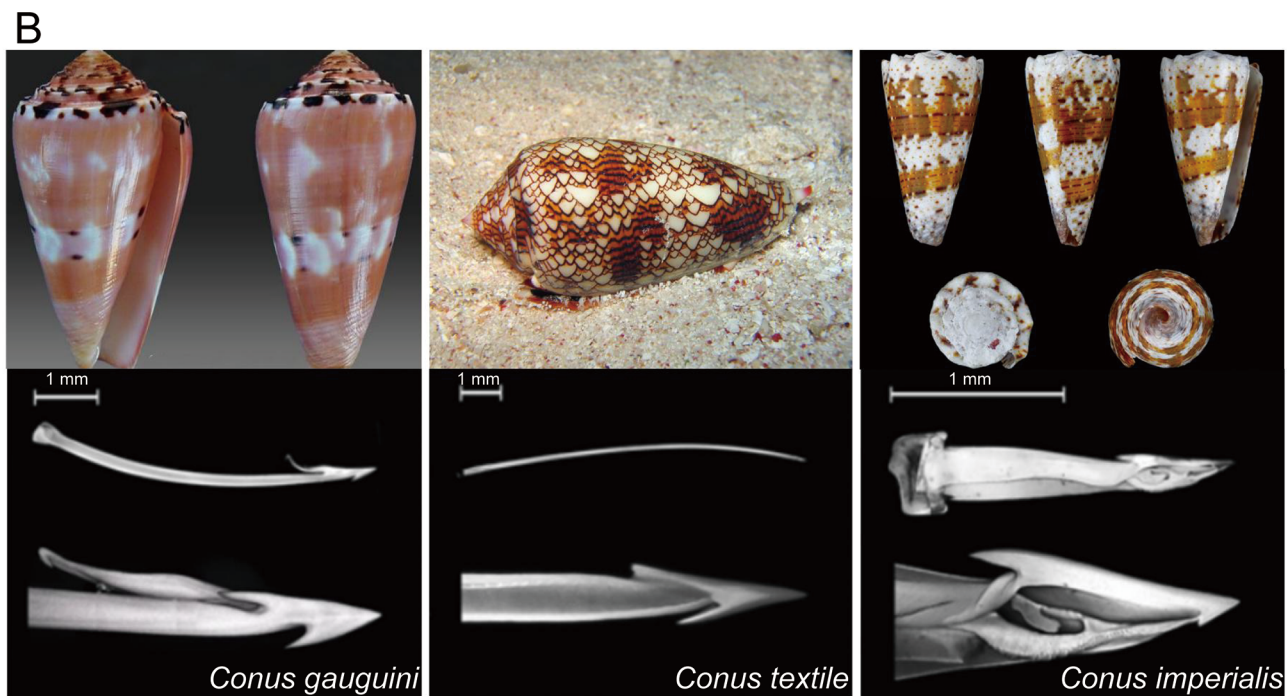
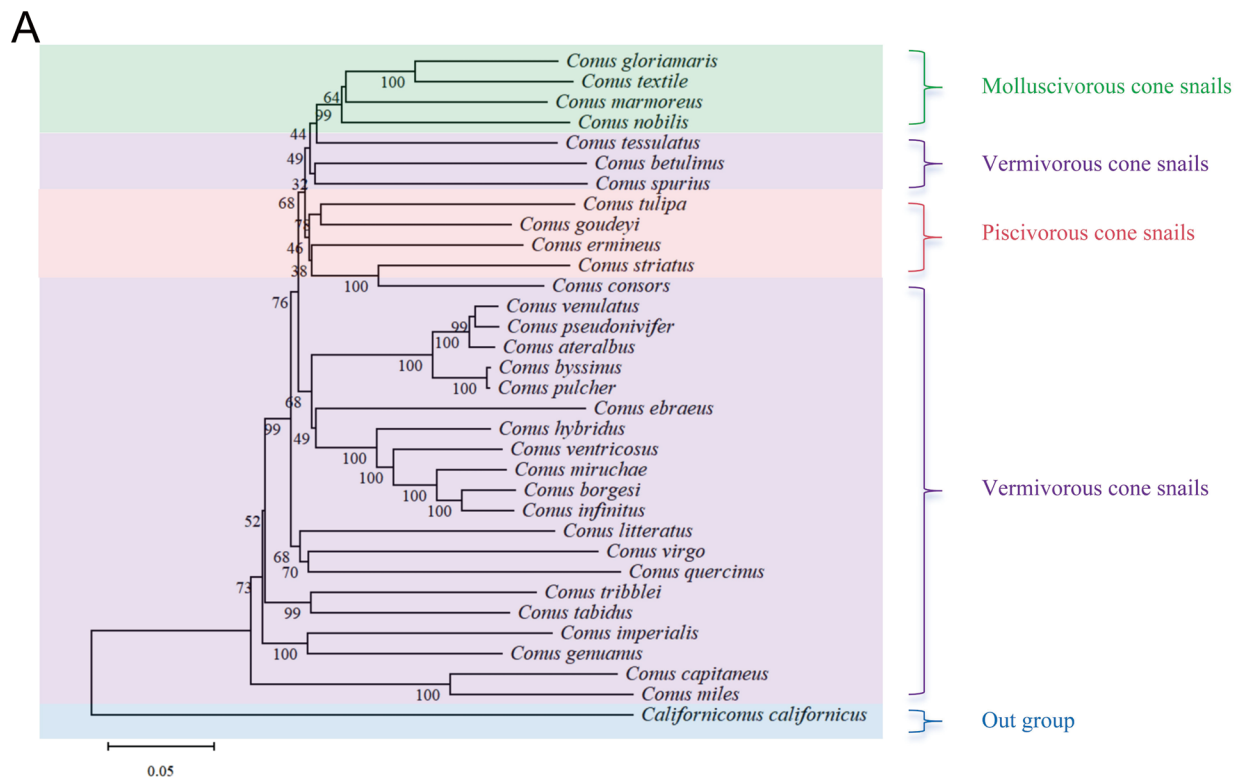


Figure 2 Dietary guilds, phylogeny, and radular tooth specializations in cone snails

A: Phylogeny inferred from complete mitochondrial genomes (see Supplementary Table S1 for full dataset) using maximum-likelihood implemented in MEGA v.11, with 1 000 bootstrap replicates. *Calorniconus californicus* was used as the outgroup. Numbers at nodes indicate bootstrap support (%), and scale bar indicates substitutions per site. B: Representative species from three ecological guilds (left to right): piscivorous (*Conus gauguini*), molluscivorous (*Conus textile*), and vermivorous (*Conus imperialis*), with corresponding radular tooth morphologies. Tooth scale bars: 1 mm. Images were obtained from Wikimedia Commons under CC BY-SA 3.0 licenses: *C. gauguini* (Almed2, <https://commons.wikimedia.org/w/index.php?curid=18174789>), *C. textile* (Richard Ling, <https://commons.wikimedia.org/w/index.php?curid=293495>), and *C. imperialis* (H. Zell, <https://commons.wikimedia.org/w/index.php?curid=23235747>). Radular tooth images were adapted from Dutertre et al. (2015).

trophic guilds. To interpret this extraordinary diversity, conotoxins are classified within a multilayered framework based on genetic origin, structural organization, and, most

importantly, pharmacological activity (Chang et al., 2014; Robinson & Norton, 2014; Sudewi et al., 2019).

At the structural level, most conotoxins are synthesized as

larger precursor proteins encoded by dedicated toxin genes. Although exceptions occur, including conopressins, most precursors conform to a conserved tripartite architecture comprising an N-terminal signal peptide that directs secretion, an intervening propeptide region removed during post-translational processing, and a C-terminal mature toxin region that gives rise to the final bioactive peptide (Robinson & Norton, 2014) (Figure 3A). This conserved precursor organization underpins the primary assignment of conotoxins into gene superfamilies, which are defined by strong sequence similarity within the signal peptide region (Wu et al., 2013). Within each superfamily, further subdivision is based on cysteine residue arrangement in the mature peptide, referred to as the cysteine framework, a structural feature that strongly influences three-dimensional (3D) folding and, in turn, pharmacological behavior (Mir et al., 2016) (Figure 3B). This hierarchical nomenclature, exemplified by combinations such as A-superfamily, framework I, and α -family, provides a powerful framework for organizing conotoxin diversity and for predicting likely biological targets from sequence and structural features (Gao et al., 2017).

This section applies these classification frameworks to the pharmacological activities of conotoxins by organizing discussion around their principal molecular targets (Figure 4). Examination of how specific structural frameworks have evolved to engage distinct ion channels and receptors reveals both the extraordinary functional breadth of this natural peptide library and its considerable potential for therapeutic development (Layer & McIntosh, 2006; Mir et al., 2016). Nevertheless, the vast diversity of conotoxin superfamilies, many of which remain functionally unresolved, precludes exhaustive treatment within a single review. Accordingly, the following subsections focus on the “Big Four” pharmacological families: μ -, κ -, ω -, and α -conotoxins. These families were prioritized as their molecular targets are defined most clearly, their mechanisms of action span distinct pharmacological modes ranging from pore blockade to allosteric modulation, and they have shown the greatest translational and clinical relevance to date.

Targeting Na_v channels

Na_v channels occupy a central position in electrical signaling and are therefore among the most compelling molecular targets in neuropharmacology. By controlling action potential initiation and propagation in excitable cells, these channels are essential for neuronal communication, sensory signaling, and neuromuscular function. Within the peripheral sensory system, Na_v1.7, Na_v1.8, and Na_v1.9 have attracted particular attention because of their established roles in nociception and their strong relevance to pain intervention strategies (Alsaloum et al., 2020; Goodwin & McMahon, 2021). Cone snail venom has evolved multiple peptide solutions to exploit this target class, with μ -conotoxins representing the best-defined Na_v-directed family. These peptides belong to the M-superfamily and are typically associated with cysteine framework III (CC-C-C-CC), a structural scaffold that supports highly efficient pore occlusion and potent suppression of channel conductance (Tosti et al., 2017).

Among Na_v-active conotoxins, μ -conotoxin KIIIA has emerged as a particularly informative model for understanding the molecular basis of channel inhibition. Detailed structure-activity studies have shown that inhibitory activity is mediated by a compact α -helical pharmacophore that displays key

residues, including Lys7, Trp8, Arg10, Asp11, His12, and Arg14, in an orientation that enables interaction with the channel pore (Khoo et al., 2009; Zhang et al., 2007). Functional investigation of this motif has identified Trp8 as a major determinant of activity and selectivity. Substitution at this position, including W8R and W8E variants, markedly shifts subtype preference between neuronal Na_v1.2 and skeletal muscle Na_v1.4 and also alters the reversibility of channel blockade (Van Der Haegen et al., 2011; Zhang et al., 2007). Furthermore, activity is shaped by disulfide architecture, with recent work demonstrating that specific non-native KIIIA disulfide isomers, including Isomer 1, can exceed the native peptide in potency against Na_v1.7 (Tran et al., 2022).

The pharmacological landscape of Na_v-targeting conotoxins extends beyond KIIIA alone. For example, μ O-conotoxin MrVIB targets Na_v1.8, showing promise as a candidate scaffold for analgesic development without the dependence-associated risks of opioid-based therapies (Ekberg et al., 2006). Additional conotoxin classes such as δ -conotoxins from the O-superfamily (e.g., TxVIA) engage Na_v channels through mechanistically distinct routes rather than pore blockade, thereby providing a fundamentally different experimental framework for studying conditions such as neuropathy and pruritus (Wang et al., 2020). Although precise subtype selectivity remains a considerable challenge across Na_v-directed conotoxins, accumulated mechanistic insight, particularly from peptides such as KIIIA, has established these molecules as excellent scaffolds for engineering next-generation analgesics (Kuang et al., 2013; McMahon et al., 2022; Pei et al., 2024; West et al., 2002) (Table 1).

Targeting K_v channels

K_v channels represent the most structurally and functionally diverse class of ion channels and are critical regulators of membrane excitability, cardiac rhythmicity, and immune signaling (Choe, 2002). Perturbation of K_v channel activity has been implicated in a broad spectrum of pathological states, including epilepsy, cardiac arrhythmias, and tumor progression (Huang & Jan, 2014; Snyders, 1999).

Multiple conotoxin families, most prominently the κ - and κ M-conotoxins, have evolved to specifically target K_v channels through distinct structural strategies (Table 2). κ -Conotoxin PVIIA of the O-superfamily, the first K_v channel blocker identified from cone snail venom, inhibits Shaker K⁺ channels through interaction with the outer vestibule. This activity conforms to the classical functional dyad model, in which a key lysine residue acts in concert with a hydrophobic partner, and is highly influenced by the pore residue T449 (Shon et al., 1998). In a striking evolutionary divergence, the κ M-conotoxin RIIIK of the M-superfamily targets similar channels (e.g., K_v1.2) but lacks a functional dyad. Instead, channel blockade is driven by a peripheral cluster of positively charged residues, including Leu1, Arg10, Lys18, and Arg19, that positions the toxin over the pore in a lid-like configuration (Al-Sabi et al., 2004).

Expanding this pharmacological complexity, κ M-conotoxin RIIIJ demonstrates striking selectivity for asymmetric heteromeric K_v channel assemblies and preferentially inhibits channels containing K_v1.2/K_v1.1 or K_v1.6 subunits relative to homomeric complexes (Cordeiro et al., 2019). Although RIIIJ shows greater blocking potency in these contexts, the related analog RIIIK has shown selective cardioprotective activity in ischemia/reperfusion models (Chen et al., 2010; Ferber et al.,

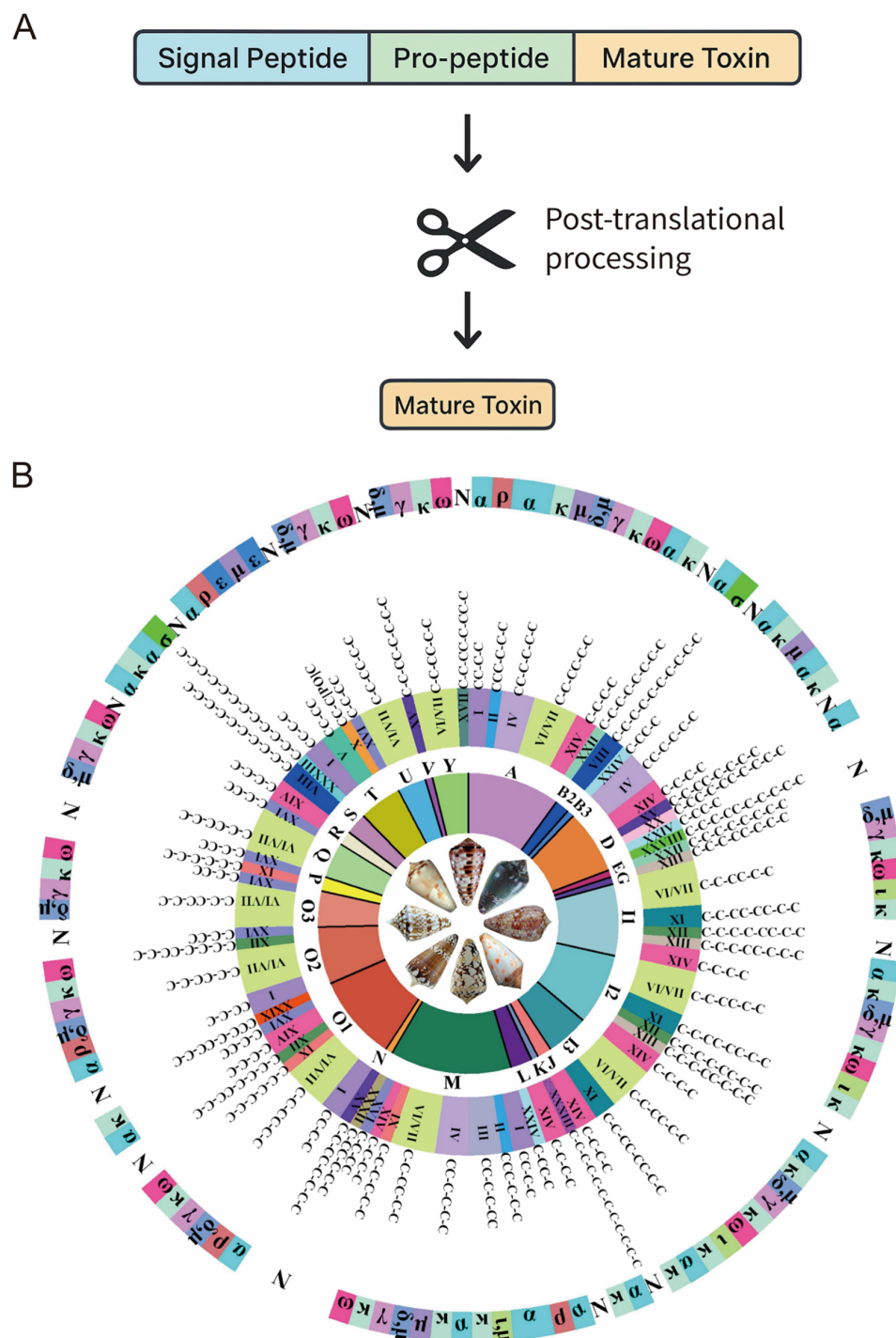


Figure 3 Conotoxin precursor architecture and hierarchical classification

A: Schematic representation of conotoxin precursor maturation. Precursor consists of three domains: A conserved N-terminal signal peptide, a variable pro-peptide, and the C-terminal mature toxin region. Post-translational processing cleaves the precursor to generate the biologically active mature peptide. B: Circular schematic illustrating the hierarchical classification system of various conotoxins. From the center outward, rings depict: (i) representative *Conus* snail species; (ii) major gene superfamilies (e.g., A, M, O), defined by signal peptide homology; (iii) corresponding cysteine frameworks (e.g., Framework I, IV, VI/VII) together with their characteristic C-C patterns, which determine peptide folding; and (iv) resulting pharmacological families (e.g., α , μ , ω), which are associated with the principal molecular targets of the conotoxins. 'N' denotes an unspecified pharmacological family. Panel B was adapted from Gao et al. (2017).

2004). Together, these observations highlight how subtle structural variation, by directing recognition toward homomeric pores or heteromeric channel assemblies, can generate distinct pharmacological and therapeutic profiles. As a result, K_v -targeting conotoxins have become powerful molecular tools

for resolving channel-specific physiological functions and for informing translational design.

Targeting Ca_v channels

Ca_v channels translate membrane depolarization into

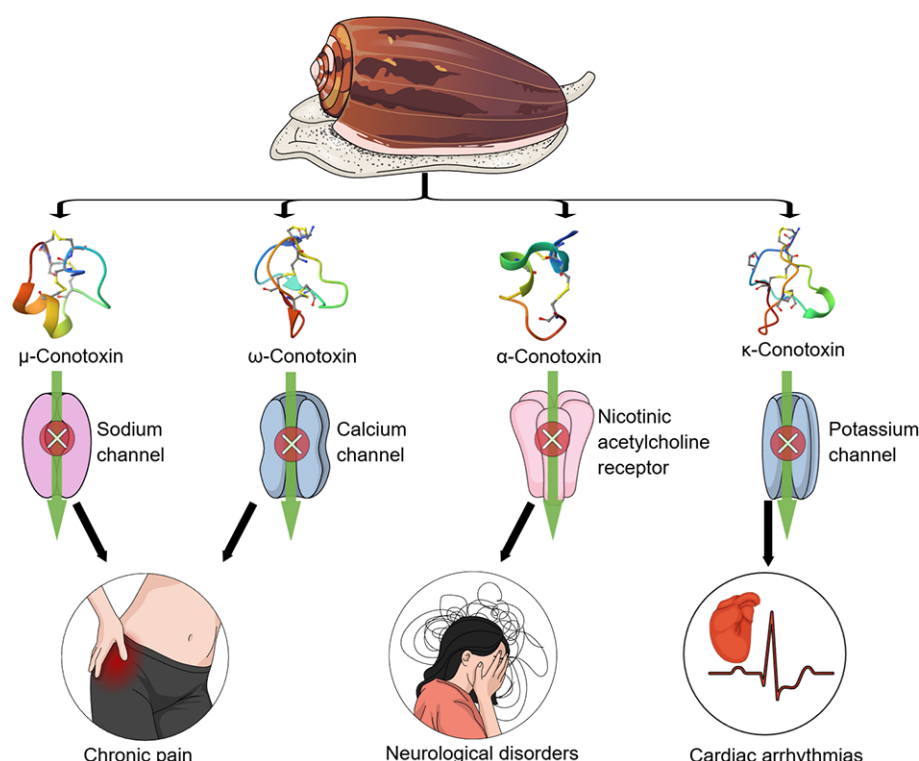


Figure 4 Primary molecular targets of various conotoxins

Major classes of conotoxins (μ , κ , ω , and α) selectively block or modulate their respective ion channel and receptor targets (such as Na_v , K_v , Ca_v , and nAChR), leading to therapeutic applications in pain, neurological disorders, and cardiovascular disease.

Table 1 Representative Na_v channel-targeting conotoxins

Conotoxin	Target	Mechanism of action	Therapeutic potential/use	Species	References
μ -Conotoxin GlIIA	$\text{Na}_v1.4$ (skeletal muscle)	Pore-blocking inhibitor, preventing ion conduction	Research tool for studying skeletal muscle excitability	<i>Conus geographus</i>	Wilson et al., 2011
μ O-Conotoxin MrVIB	$\text{Na}_v1.8$ (peripheral neurons)	Pore-blocking inhibitor of $\text{Na}_v1.8$, selectively targeting peripheral pain pathways	Development of novel analgesics for chronic pain	<i>Conus marmoreus</i>	Ekberg et al., 2006
δ -Conotoxin EVIA	$\text{Na}_v1.2$, $\text{Na}_v1.3$, $\text{Na}_v1.6$ (neuronal)	Slows/inhibits fast inactivation of Na_v channels, prolonging channel opening	Potential applications in pain modulation and epilepsy research	<i>Conus ermineus</i>	Barbier et al., 2004
μ -Conotoxin TIIIA	$\text{Na}_v1.4$ (skeletal muscle)	Pore-blocking inhibitor of Na_v channels	Potential analgesic; research tool for neuronal excitability	<i>Conus tulipa</i>	Lewis et al., 2007
μ -Conotoxin KIIIA	$\text{Na}_v1.2$, $\text{Na}_v1.4$, $\text{Na}_v1.7$	Pore-blocking inhibitor of multiple Na_v subtypes	Potential analgesic; research tool for neuronal excitability	<i>Conus kinoshitai</i>	Pan et al., 2019
μ -Conotoxin SxIIIC	$\text{Na}_v1.7$	Potent pore-blocking inhibitor of human $\text{Na}_v1.7$ channels	Promising candidate for analgesic development	<i>Conus striolatus</i>	McMahon et al., 2022
μ -Conotoxin SmIIIA	$\text{Na}_v1.2$, $\text{Na}_v1.4$, $\text{Na}_v1.7$	Potent pore-blocking inhibitor	Potential therapeutic for epilepsy; research tool	<i>Conus stercusmuscarum</i>	Zhang et al., 2013
μ -Conotoxin PIIIA	$\text{Na}_v1.1$, $\text{Na}_v1.4$, $\text{Na}_v1.6$	Pore-blocking inhibitor of skeletal muscle Na_v channels	Research tool for muscle physiology studies	<i>Conus purpurascens</i>	Safo et al., 2000; Wilson et al., 2011
μ -Conotoxin CnIIIC	$\text{Na}_v1.2$, $\text{Na}_v1.4$, $\text{Na}_v1.7$	Potent pore-blocking inhibitor of multiple Na_v subtypes	Potential analgesic; research tool for neuronal excitability	<i>Conus consors</i>	Favreau et al., 2012
μ -Conotoxin BuIIIB	$\text{Na}_v1.3$, $\text{Na}_v1.4$	Potent pore-blocking inhibitor of neuronal and muscle Na_v subtypes	Research tool for studying Na_v channel function	<i>Conus bullatus</i>	Kuang et al., 2013

Na_v , voltage-gated sodium channel.

intracellular signaling events and thereby regulate critical physiological processes, including neurotransmitter release, muscle contraction, and gene expression (Miller, 1987). Among these channels, the N-type $\text{Ca}_v2.2$ channel occupies an important position in spinal pain transmission and has therefore emerged as a major therapeutic target for analgesic intervention.

ω -Conotoxins, defined by the six-cysteine framework IV (C-C-C-C-C-C) and the inhibitory cystine knot (ICK) motif, represent one of the most selective inhibitors of neuronal Ca_v

channels (Lewis et al., 2012; Ramírez et al., 2017) (Table 3). A prominent representative is ω -conotoxin MVIIA, developed clinically as the FDA-approved drug ziconotide for the treatment of severe chronic pain (Miljanich, 2004; Olivera et al., 1987).

Detailed structure-activity studies combining nuclear magnetic resonance (NMR) spectroscopy with targeted mutagenesis have clarified the precise molecular basis underlying the potency and subtype selectivity of these peptides. In canonical ω -conotoxins such as MVIIA and GVIA,

Table 2 Representative K_v channel-targeting conotoxins

Conotoxin	Target	Mechanism of action	Therapeutic potential/use	Species	References
κ-Conotoxin PVIIA	<i>Drosophila</i> Shaker K ⁺ channel	Selectively inhibits K _v channel as a pore blocker by interacting with S5–S6 pore-forming region.	Molecular probe for studying K _v channel structure-function and differentiating between K _v subtypes.	<i>Conus purpurascens</i>	Shon et al., 1998
κM-Conotoxin RIIIK	K _v 1.2	Potent and selective pore-blocking inhibitor of K _v 1.2	Therapeutic potential in neurological disorders	<i>Conus radiatus</i>	Al-Sabi et al., 2004
κM-Conotoxin RIIIJ	K _v 1.6	Potent and selective pore-blocking inhibitor of K _v 1.6	Research tool for pain and ion channel pharmacology	<i>Conus marmoreus</i>	Chen et al., 2010

K_v, voltage-gated potassium channel.

Table 3 Representative Ca_v channel-targeting conotoxins

Conotoxin	Target	Mechanism of action	Therapeutic potential/use	Species	References
ω-Conotoxin GVIA	N-type (Ca _v 2.2)	Irreversible pore-blocking inhibitor of N-type Ca _v channels	Gold-standard research tool for studying N-type channels; not for therapeutic use due to irreversibility	<i>Conus geographus</i>	Jones & Bulaj, 2000
ω-Conotoxin MVIIA (Ziconotide)	N-type (Ca _v 2.2)	Reversible, high-affinity pore-blocking inhibitor of N-type channels	FDA-approved analgesic (Prialt®) for severe, chronic pain	<i>Conus magus</i>	Jones & Bulaj, 2000
ω-Conotoxin CVID	N-type (Ca _v 2.2)	Potent and selective pore-blocking inhibitor of N-type channels	Investigated as a potential analgesic with a better therapeutic window than Ziconotide	<i>Conus catus</i>	Schroeder & Lewis, 2006
ω-Conotoxin MVIIC	N-type (Ca _v 2.2) & P/Q-type (Ca _v 2.1)	Blocks both N-type and P/Q-type Ca _v channels	Investigated for chronic pain, but clinical development was halted	<i>Conus magus</i>	Jones & Bulaj, 2000
ω-Conotoxin SVIB	N-type (Ca _v 2.2) & P/Q-type (Ca _v 2.1)	Broad-spectrum inhibitor of N-type and P/Q-type channels	Research tool for studying diverse roles of Ca _v channels in neurotransmission	<i>Conus striatus</i>	Schroeder & Lewis, 2006

Ca_v, voltage-gated calcium channel; FDA, US Food and Drug Administration.

the principal binding surface is organized around a pharmacophore centered on Lys2 and Tyr13. The hydroxyl group of Tyr13, together with its precise spatial positioning within Loop 2, has been identified as the primary determinant of high-affinity binding to N-type channels (Nielsen et al., 1999b, 2000). Furthermore, domain-exchange studies involving MVIIA, which preferentially targets N-type channels, and MVIIC, which shows selectivity toward P/Q-type channels, have revealed that subtype discrimination does not arise from any single residue in isolation but from coordinated contributions of Loop 2 and Loop 4. Homogeneous loop combinations, such as Loop 2/4 derived from MVIIA, confer high selectivity, whereas heterogeneous loop combinations reduce discrimination between N-type and P/Q-type channels (Nielsen et al., 1999a).

Further evolutionary refinement is evident in ω-conotoxin CVID, which exhibits significantly greater selectivity for N-type relative to P/Q-type channels than MVIIA. Structural analysis has shown that CVID possesses a unique hydrogen-bond network that anchors Loop 4 in close proximity to Loop 2, thereby generating a more rigid globular binding interface. This conformational constraint reduces entropy loss during target engagement and, in turn, enhances both selectivity and potency (Lewis et al., 2000). These findings illustrate how subtle evolutionary modification of loop architecture within the cystine scaffold can produce pronounced differences in pharmacological outcomes.

Targeting nAChRs

nAChRs are essential ligand-gated ion channels that mediate rapid synaptic transmission throughout the central and peripheral nervous systems (Dani, 2001). Extensive subtype heterogeneity, generated through diverse combinations of 17 receptor subunits, makes these receptors both pharmacologically complex and therapeutically important in conditions such as addiction, cognitive impairment, and pain (Dani & Bertrand, 2007).

Among conotoxins, α-conotoxins are the most extensively

studied antagonists of nAChRs and are typically defined by framework I (CC-Xm-C-Xn-C), a scaffold that supports remarkable subtype selectivity (Azam & McIntosh, 2009) (Table 4). Structure-activity analyses have shown that this selectivity arises from highly specific interactions between the toxin loop regions and the agonist-binding pocket of the receptor. In α-conotoxin LvlA, for example, extensive mutagenesis and structural investigation identified Asn9 as a major determinant of subtype discrimination. Substitution of this residue with alanine ([N9A]LvlA) produced a greater than 2 000-fold increase in selectivity for α3β2 relative to α3β4 nAChRs. Crystal structure analysis further revealed that this selectivity is driven by the interaction of LvlA with specific residues on the complementary β2 subunit, particularly Phe106, Ser108, and Ser168 (Xu et al., 2017; Zhu et al., 2020).

Similar principles have emerged from work on α-conotoxin Vc1.1, which targets α9α10 nAChRs. Notably, scanning mutagenesis demonstrated substantial functional plasticity across the peptide surface, with substitutions at positions Ser4 and Asn9 (e.g., S4K and N9A) markedly enhancing potency and residues located within the hydrophobic core showing limited tolerance to modification (Halai et al., 2009). Recent studies on α-conotoxin TxIB have extended this strategy beyond side-chain substitution to backbone engineering. In that context, the [2,4] disulfide bond was identified as a chemically permissive site for modification, and insertion of rigid linkers such as xylene at this position improved serum stability while preserving potent inhibition of α6/α3β2β3 nAChRs (Zhang et al., 2021a). Together, these mechanistic insights have elevated conotoxins from mere receptor antagonists to sophisticated molecular templates for rational drug design (Luo et al., 2014; Xu et al., 2025; You et al., 2019).

Targeting other receptors and transporters

The pharmacological scope of conotoxins extends well beyond the major classes of voltage-gated ion channels and

Table 4 Representative nAChR-targeting conotoxins

Conotoxin	Target	Mechanism of action	Therapeutic potential/use	Species	References
α -Conotoxin ARIIB	$\alpha 7$, $\alpha 6\beta 2^*$, $\alpha 3\beta 2$ nAChRs	Competitive antagonist	Pharmacological tool for studying nAChR subtypes; nicotine addiction research	<i>Conus arenatus</i>	Azam & McIntosh, 2009; Michael et al., 2004
α -Conotoxin Iml	$\alpha 7$, $\alpha 3\beta 2$ nAChRs	Competitive antagonist	Classic pharmacological tool for chronic pain and neurological research	<i>Conus imperialis</i>	Azam & McIntosh, 2009; Michael et al., 2004
α -Conotoxin MII	$\alpha 3\beta 2^-$, $\alpha 6$ -containing nAChRs	Potent competitive antagonist	Investigated as a drug lead for severe neuropathic pain	<i>Conus magus</i>	Michael et al., 2004
α -Conotoxin PIA	$\alpha 3\beta 2^-$, $\alpha 6$ -containing nAChRs	Competitive antagonist	Pharmacological tool for targeting pain and neuropsychiatric pathways	<i>Conus purpurascens</i>	Azam & McIntosh, 2009
α -Conotoxin Vc1.1	GABA-B Receptor (indirectly $\text{Ca}_v 2.2$)	Allosteric modulator of GABAB receptors, leading to inhibition of N-type Ca_v channels	Drug candidate for neuropathic pain (reached clinical trials)	<i>Conus victoriae</i>	Azam & McIntosh, 2009
α -Conotoxin GI	Muscle-type ($\alpha 1\beta 1\delta \gamma$) nAChR	Classic competitive antagonist of neuromuscular junction	Gold-standard research tool for studying muscle nAChRs; not for pain	<i>Conus geographus</i>	Azam & McIntosh, 2009
α -Conotoxin MriC	$\alpha 7$ nAChR	Allosteric modulator/antagonist with complex pharmacology	Research tool for studying $\alpha 7$ nAChR function and modulation	<i>Conus marmoreus</i>	Azam & McIntosh, 2009
α -Conotoxin LsIA	$\alpha 3\beta 2$, $\alpha 7$ nAChRs	Competitive antagonist	Research tool for pain, anxiety, and depression models	<i>Conus limpusi</i>	Azam & McIntosh, 2009
α -Conotoxin OmiA	$\alpha 7$ nAChR	Competitive antagonist	Research tool for neurological diseases and pain pathways	<i>Conus omaria</i>	Azam & McIntosh, 2009
α -Conotoxin TxIB	$\alpha 6/\alpha 3\beta 2\beta 3$ nAChR	Selective competitive antagonist	Drug lead for anti-addiction therapies (nicotine dependence)	<i>Conus textile</i>	Li et al., 2020
α O-Conotoxin GeXIVA	$\alpha 9\alpha 10$ nAChR	Potent competitive antagonist	Drug lead for inflammatory and neuropathic pain	<i>Conus geographus</i>	Shelukhina et al., 2023
α -Conotoxin LvIA	$\alpha 3\beta 2$, $\alpha 3\beta 4$ nAChR	Competitive antagonist	Research tool for neuroinflammation and cognitive disorders	<i>Conus lividus</i>	Xu et al., 2017
α -Conotoxin TxID	$\alpha 3\beta 4$ nAChR	Selective competitive antagonist	Pharmacological tool for pain pathways involving $\alpha 3\beta 4$ nAChR	<i>Conus imperialis</i>	Cuny et al., 2016

nAChR, nicotinic acetylcholine receptor; GABA-B, gamma-aminobutyric acid type B receptor; Ca_v , voltage-gated calcium channel.

nicotinic receptors (Layer & McIntosh, 2006). A substantial subset of these peptides acts on less conventional targets that govern synaptic integration, neuromodulatory signaling, and transmitter homeostasis, including NMDA receptors, 5-HT₃ receptors, and monoamine transporters such as the norepinephrine transporter (NET). By engaging these systems, conotoxins can reshape circuit excitability, influence affective and sensory processing, and modulate pain pathways without necessarily producing the rapid neuromuscular paralysis classically associated with cone snail venom. Among these non-canonical effectors, conantokins represent a distinctive class of linear peptides enriched in γ -carboxyglutamic acid and are known as selective antagonists of NMDA receptors, particularly receptor assemblies containing the NR2B subunit. Because NMDA receptors are glutamate-gated channels that play central roles in excitatory neurotransmission and synaptic plasticity, and because excessive NMDA signaling contributes to hyperexcitability and excitotoxic damage, conantokins have attracted considerable interest as potential candidates for treating epilepsy and ischemic injury (Hansen et al., 2017; Layer et al., 2004). Other conotoxins engage entirely different receptor systems. For example, σ -conotoxin GVIIIA antagonizes the 5-HT₃ serotonin receptor, while ρ - and χ -conopeptides selectively inhibit $\alpha 1$ -adrenoceptors and NET, respectively (England et al., 1998; Sharpe et al., 2001). These targets regulate distinct but therapeutically important physiological processes: 5-HT₃ functions as a ligand-gated cation channel involved in fast serotonergic signaling and contributes to nausea, visceral pain, and sensory processing; $\alpha 1$ -adrenoceptors regulate vascular tone and sympathetic neurotransmission, making them relevant to both cardiovascular and nociceptive control; and NET determines the magnitude and duration of

noradrenergic signaling by clearing norepinephrine from the synaptic space, thereby influencing arousal and descending pain modulation.

Additional discoveries have further expanded the therapeutic landscape of cone snail peptides. For example, con-insulins, such as Con-Ins G1, mimic human insulin and can rapidly induce hypoglycemia (Safavi-Hemami et al., 2015), while conutalakin-G, a neurotensin receptor agonist, has shown potent analgesic activity in clinical trials (Lee et al., 2015). Functional convergence is also evident in certain α -conotoxins, including Vc1.1 and RgIA, which inhibit N-type Ca_v channel signaling indirectly through activation of GABA_B receptors rather than through direct pore interaction (Callaghan et al., 2008). This mechanism reveals an additional route to analgesia, as GABA_B activation reduces presynaptic $\text{Ca}_v 2.2$ -dependent transmitter release and thereby dampens nociceptive neurotransmission.

DISCOVERY APPROACHES OF NOVEL CONOTOXINS

The extraordinary breadth of natural conotoxin diversity, estimated to encompass millions of unique peptides distributed across approximately 800 *Conus* species, represents both a major opportunity for bioactive peptide discovery and a significant analytical challenge (Wiene et al., 2022). Fewer than 3% of these molecules have been structurally and functionally characterized, and substantial venom heterogeneity can occur even within a single species, underscoring the need for highly sensitive, scalable, and high-throughput discovery strategies (Dutertre et al., 2014; Fouda et al., 2021; Phuong et al., 2016). Efforts to uncover this molecular diversity have evolved through three broad phases: traditional discovery, bioengineering-driven exploration, and modern venomomics (Figure 5A).

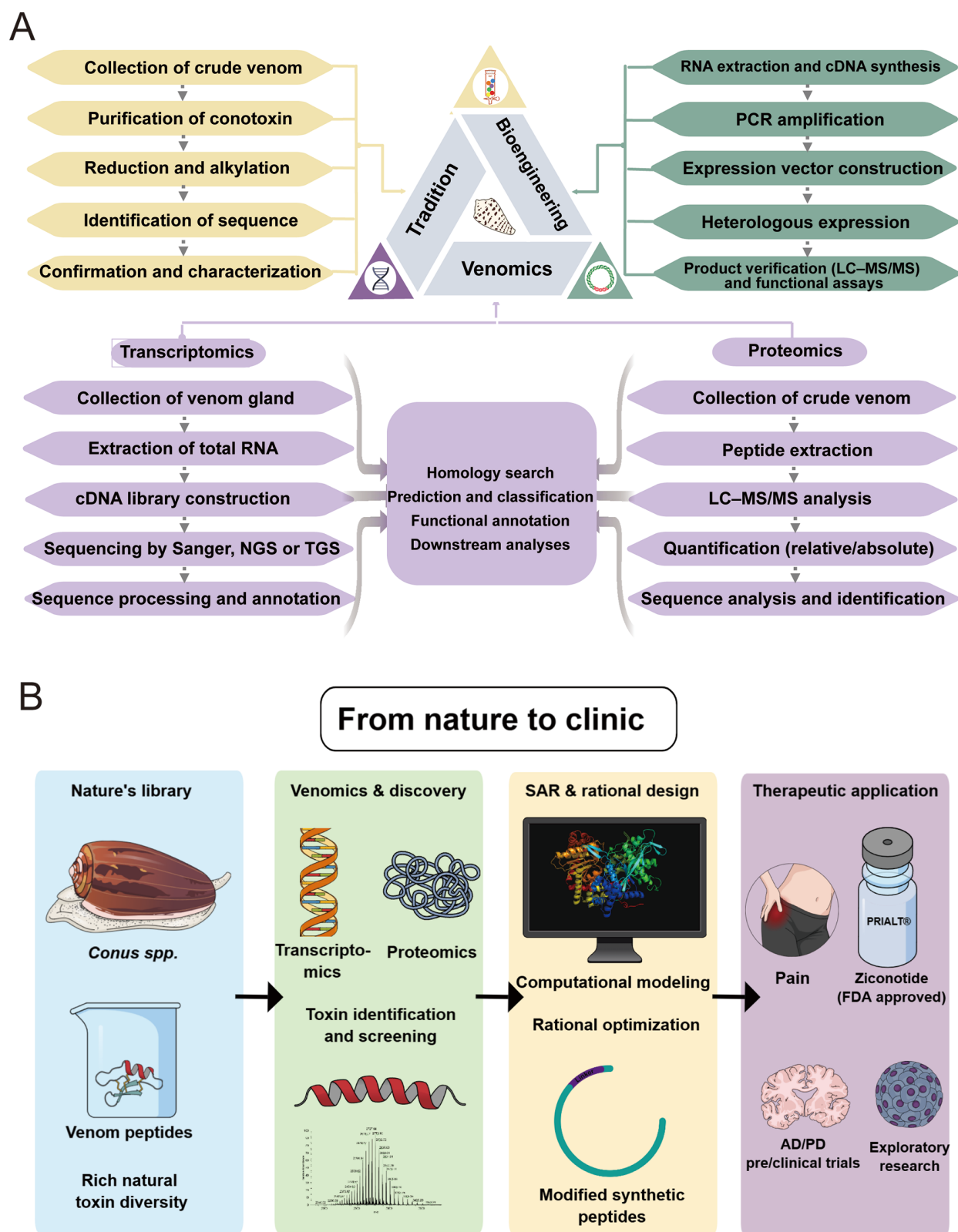


Figure 5 Integrated workflow for conotoxin discovery and translational development

A: Major methodological frameworks used for conotoxin discovery. Traditional strategy (left) relies on purification and characterization of peptides directly from crude venom. Bioengineering strategy (right) applies recombinant DNA technology for heterologous expression and subsequent product verification/functional testing. Venomics strategy (bottom) integrates high-throughput transcriptomics and proteomics with bioinformatic analyses to identify novel toxin sequences. B: Schematic overview of the conotoxin drug discovery pipeline from natural venom to clinical application, illustrating progression from cone snail biodiversity and venom peptide libraries through venomics-based lead identification, SAR-guided optimization, including computational modeling and chemical modification, and subsequent translational development, exemplified by the FDA-approved analgesic ziconotide and additional candidates in preclinical and clinical evaluation. NGS, next-generation sequencing; TGS, third-generation sequencing; SAR, structure-activity relationship; AD/PD, Alzheimer's disease/Parkinson's disease.

In comparison with many other venomous animals, including snakes, frogs, and arthropods, cone snail venom displays a distinctive molecular architecture dominated by short disulfide-rich peptides derived from secreted precursors and further diversified through extensive sequence variation and complex post-translational processing (Craig et al., 1999a). This biochemical organization is closely aligned with conotoxin function, as many of these peptides exhibit exceptional potency and subtype selectivity toward neuronal ion channels and receptors, features consistent with the demands of rapid prey immobilization (Olivera & Teichert, 2007). In contrast, snake venom typically contains a substantial fraction of high-molecular-weight proteins and enzymatic components, including proteases and phospholipases, that drive hemotoxic and cytolytic pathologies (Ferraz et al., 2019), whereas amphibian skin secretions are often enriched in linear host-defense peptides with broader membrane-directed activities (Patocka et al., 2019). These compositional and functional differences help explain why conotoxin discovery is particularly dependent on peptidogenomic strategies and on transcriptomic and proteomic approaches capable of resolving post-translational modifications.

Traditional approach: From crude venom to pure peptide

Beginning in the 1970s, conotoxin discovery was primarily driven by bioassay-guided fractionation of crude venom extracts (Cruz et al., 1978). In this classical workflow, snail venom is obtained either through dissection of the venom duct followed by solvent extraction or through collection of secreted venom from live snails after stimulation (e.g., prey exposure), with the latter providing a reproducible means of sampling venom as delivered during predation (Fu et al., 2018). The crude extract is subsequently subjected to fractionation and chromatographic separation, commonly through size-exclusion procedures followed by C18 reverse-phase high-performance liquid chromatography (RP-HPLC), while liquid chromatography-mass spectrometry (LC-MS) is used to monitor fraction composition and guide iterative purification (Fu et al., 2018; Jakubowski & Sweedler, 2004). Structural characterization of purified peptides then depends on accurate mass measurement together with *de novo* sequencing using Edman degradation, tandem mass spectrometry (MS/MS), or both, while discrimination among closely related chemical variants often requires specialized MS strategies (Fu et al., 2018). A major challenge in this process is the high prevalence of post-translational modifications, including proline hydroxylation, glutamate γ -carboxylation, tryptophan bromination, pyroglutamate formation, and C-terminal amidation, all of which can complicate sequencing and hinder confident identification of mature proteoforms (Craig, 2000; Fu et al., 2018; Jimenez, 2021). Consequently, although traditional bioassay-guided isolation remains essential for definitive structure-function validation of mature toxins, this strategy is labor-intensive and inherently difficult to scale, motivating the increasing adoption of omics-enabled discovery pipelines (Fu et al., 2018). Even so, purification of native peptides remains essential for unambiguous definition of mature toxin proteoforms and associated post-translational modification patterns, which often cannot be reliably inferred or faithfully reproduced from sequence data alone.

Bioengineering approach: Cloning and recombinant expression

By the late 20th century, gene cloning had emerged as a more

economical and less material-intensive route for conotoxin discovery and production (Shon et al., 1995). Using sequence-derived primers designed from previously identified conotoxins, target genes could be amplified from small amounts of genomic DNA or cDNA and subsequently expressed in recombinant systems such as *Escherichia coli* or yeast (Zhan et al., 2003). Multiple recombinant production formats have since been developed, among which periplasmic expression in *E. coli* has become a well-established strategy to promote disulfide formation (Klint et al., 2013). In many cases, conotoxins are produced as fusion proteins incorporating solubility and folding partners such as thioredoxin (Pi et al., 2007) and SUMO (Hernandez-Cuevas & White, 2012), thereby facilitating peptide recovery and controlled oxidative folding following tag removal. Fusion to DsbC has also proven effective, particularly in periplasm-directed systems (Sequeira et al., 2017).

Even with these advances, accurate reconstruction of native bioactivity remains highly dependent on correct disulfide connectivity, presenting a major challenge as differences between *in vivo* and *in vitro* oxidative folding have become increasingly evident (Bulaj & Olivera, 2008). In cone snails, folding and maturation occur within a highly specialized venom-duct environment containing protein disulfide isomerases and enzymatic machinery capable of introducing modifications such as γ -carboxylation, features that are difficult to recapitulate in standard expression hosts (Bulaj et al., 2003). As a result, recombinant production is frequently coupled to carefully optimized *in vitro* oxidative folding protocols (Banerjee et al., 2013; Gyanda et al., 2013). Despite these limitations, *E. coli*-based strategies remain valuable for rapid generation of sufficient peptide material for pharmacological evaluation, mutational analysis, and structure-activity investigation (Saez et al., 2014; Sequeira et al., 2017), especially when native venom is scarce. This approach has enabled production of numerous newly identified conotoxins, including the κ -superfamily peptide Im23a and the insecticidal peptide Vn2 (Ho et al., 2025; Kancherla et al., 2015; Spiezia et al., 2012; Wang et al., 2008; Ye et al., 2012). More recently, the O1-conotoxin Tx6.7, a potent Ca_v channel inhibitor, was cloned from the genomic DNA of *Conus textile* (Zhou et al., 2023).

Despite these advantages, the bioengineering approach remains constrained by significant limitations. Dependence on previously known sequences restricts the ability to discover entirely novel conotoxin families. Furthermore, recombinant expression systems do not consistently yield peptides with native folding states and full functional activity. For instance, recombinant TxIA, LvIA, and TxIB showed approximately 30-fold lower potency or complete loss of activity relative to their corresponding native counterparts (El Hamdaoui et al., 2019; Yu et al., 2018; Zhu et al., 2016).

Venomics: A high-throughput revolution

The current era of conotoxin discovery is dominated by venomics, a powerful strategy that integrates high-throughput omics technologies, such as genomics, transcriptomics, proteomics, and peptidomics, with increasingly sophisticated bioinformatic analysis (Moutinho Cabral et al., 2024). This transition has dramatically accelerated peptide discovery while simultaneously transforming understanding of conotoxin diversity, maturation, and evolutionary diversification (Dutertre et al., 2013) (Table 5).

Table 5 Representative conotoxins discovered by different approaches in the last decade

Approach	Name	Species	Sequence	Target/activity	References
Transcriptomics	Mr1.1	<i>Conus marmoreus</i>	GCCSHPACSVNNPDIC*	nAChR	Dutertre et al., 2013
	Conomorphin-M		DWEYHAHPKONSEWT	N/A	Dutertre et al., 2013
	MrIIIF		VCCPFGGCHELCYCCD*	Analgesic	Dutertre et al., 2013
	N/A	<i>Conus betulinus</i>	CNLPKIVGPCKAYMPSFFYNTGTGQCERFVYGGCGGNAN	N/A	Yao et al., 2019
		<i>Conus characteristicus</i>	RFETKQECEGKCQR		Yao et al., 2019
		<i>Conus generalis</i>	CQQPKAPGRCMAYMERYFFNSEKGACEQFIYGGCEGNE		Yao et al., 2019
		<i>Conus quercinus</i>	NNFETLEACQTAC		Yao et al., 2019
Proteomics	Eb3-H0	<i>Conus ebraeus</i>	CNLPKIVGPCKAYMPSFFYNTGTGQCERFVYGGCGGNAN		Yao et al., 2019
			RFETKQECEGQCQR		
	Vr3-DPP03	<i>Conus varius</i>	QDISPNERKR		
	Bt2	<i>Conus betulinus</i>	VDGLNHPEPSFGED		
	PnIVB	<i>Conus pennaceus</i>	YGWTCWLGCSPCGC		
	N/A	<i>Conus eburneus</i>	LTYHAGCPVLMGNKWIANKWIWHYGNMFR		
			LATSLGLDLR		
		<i>Conus quercinus</i>	SSEEREHAEKLMTFQNQR		
		<i>Conus radiatus</i>	SSARSTDDNGNDR		
		<i>Conus ochroleucus</i>	AMAELEAKKAQEALK		
Multi-omics	Conantokin-L2.2	<i>Conus literatus</i>	TFEDVEELGKELDANLTK		
	A_1	<i>Conus imperialis</i>	DYCHKYGYTIG	N/A	Jin et al., 2019a
	A_2		WCHPNPYTVG		Jin et al., 2019a
	A_3_Bn1.3		DYCCHRGPCMVWCG		Jin et al., 2019a
	M_1		CCMRPICMCP-CCVNG		Jin et al., 2019a
	M_2_Eu3.3		CCQAACSPWLCLPCC		Jin et al., 2019a

nAChR, nicotinic acetylcholine receptor; NMDA, N-methyl-D-aspartate receptor; N/A: Not applicable. Entries marked "N/A" represent predicted mature toxins identified via high-throughput omics (transcriptomics/proteomics) for which functional targets have not yet been experimentally validated; they are included here as representative because they highlight structurally and/or lineage-distinct conotoxin diversity (e.g., characteristic precursor features or cysteine frameworks) revealed by omics despite missing functional annotation.

Venomics has evolved well beyond early efforts focused solely on cataloguing venom components and now functions as a multidimensional framework that also incorporates immunological and functional perspectives, including antivenomics and toxicovenomics, thereby enabling a more systems-level view of venom complexity (Lomonte & Calvete, 2017). A major advance has been the emergence of post-translational modification-aware peptidomic and proteomic pipelines capable of resolving mature toxin proteoforms and biosynthetic intermediates that cannot be reliably inferred from precursor sequences alone. Comprehensive characterization therefore requires explicit consideration of the broad dynamic range of venom proteins, prevalence of post-translational modifications, and potential for experimental artifacts introduced during sample collection and processing (Zelanis & Tashima, 2014). Integrative multi-omics analyses have further shown that venom ducts can exhibit extensive mature peptide diversification together with strong regional compartmentalization, such that distinct duct segments

contain highly specific peptide and protein repertoires (Biass et al., 2015). Beyond descriptive profiling, comparative venomics is increasingly being applied in evolutionary and ecological contexts to evaluate how venom composition shifts across lineages, habitats, and ecological strategies (Sunagar et al., 2016). In parallel, quantitative venomics, including proteoform-resolved and absolute quantification strategies, has become essential for capturing spatiotemporal variation and for defining the adaptive landscape underlying intraspecific venom evolution (Calvete, 2018). Despite this progress, critical bottlenecks remain. For instance, mature-toxin MS sequencing is still hindered by frequent post-translational modification and functional deorphanization, namely rapid assignment of biological activity and molecular targets, continues to limit throughput (Kaas & Craik, 2015). Consequently, the field is increasingly converging on standardized integrative proteotranscriptomic workflows coupled with more scalable target-identification strategies to connect newly discovered sequences with experimentally

validated biological functions (Robinson et al., 2017).

Transcriptomics: Unveiling the genetic framework

Transcriptomics has become a central pillar of modern venomomics by providing a comprehensive view of gene expression within the venom duct and revealing the genetic architecture that underlies conotoxin biosynthesis. Progress in this area has been driven by rapid advances in sequencing technology (Heather & Chain, 2016). Although third-generation platforms such as Oxford Nanopore and PacBio HiFi are increasingly being adopted because of their capacity for long-read sequencing and improved assembly of complex genomic regions (De Vivo et al., 2022; Herráez-Pérez et al., 2023; Liao et al., 2022), next-generation sequencing (NGS) remains the principal engine of high-throughput conotoxin discovery owing to its strong balance of accuracy, scalability, and cost-efficiency.

A standard transcriptomic workflow typically begins with RNA isolation from dissected venom duct tissue, followed by cDNA library construction, sequencing, and bioinformatic analysis for prediction and annotation of conotoxin precursor transcripts. Successful application of this strategy across numerous *Conus* species has driven a dramatic expansion in the number of reported conotoxin sequences (Himaya & Lewis, 2018). The scale of this discovery power is illustrated by a landmark study on a single *Conus episcopatus* specimen, which yielded more than 3 000 unique conotoxin sequences (Lavergne et al., 2015), although this number likely included transcript variants and partial sequences rather than exclusively distinct mature toxin entities. Transcriptomics has also been instrumental in identifying entirely novel peptide families with therapeutic relevance, as exemplified by the identification of venom-derived insulins across multiple *Conus* species (Guo et al., 2024; Safavi-Hemami et al., 2015). However, this vast transcriptomic landscape requires validation at the protein level to confirm which of these predicted peptides are ultimately translated, processed, and secreted into venom.

Proteomics: Profiling the expressed arsenal

While transcriptomics defines the biosynthetic potential of the venom duct, proteomics reveals the peptide repertoire that is actually produced and present within the venom matrix. Through advanced MS analysis, proteomics enables direct characterization of mature and biologically active peptides (Ebou et al., 2021). In many cases, this level of analysis exposes a degree of molecular complexity far greater than predicted by transcriptomics alone. For instance, proteomic analysis of *Conus marmoreus* venom detected approximately 8 000 peptide components, in contrast to the 105 precursor sequences identified at the transcriptomic level. Although this discrepancy is consistent with extensive post-translational modification, a substantial fraction of the detected masses likely corresponds to processing intermediates and fragmentation products rather than distinct functional toxins (Dutertre et al., 2013). Proteomics has also been instrumental in defining venom composition in endemic species such as *Conus taeniatus* and in resolving dynamic variation in venom composition during delivery (Fouda et al., 2021; Safavi-Hemami et al., 2010).

Multi-omics: An integrated strategy

Among currently available discovery approaches, multi-omics provides the most comprehensive and analytically powerful

strategy by combining sequencing data from two or more omics platforms. While traditional isolation methods remain indispensable for definitive functional verification, they are often too labor-intensive, slow, and costly for large-scale screening. In contrast, multi-omics strategies enable substantially higher throughput and lower per-sequence cost, making them especially well-suited for rapid and systematic bioprospecting. The combination of transcriptomics and proteomics, often implemented as proteotranscriptomics, has proven particularly effective. Through this integration, peptide signals detected at the protein level can be cross-validated with their corresponding gene transcripts, thereby reducing false-positive assignments and strengthening sequence confidence (Jin et al., 2019a). This integrated strategy has enabled the discovery of a novel cysteine framework together with the potent peptide Que-1.0 in *Conus quercinus* (Zhang et al., 2021b). More recently, combined analysis of genomic, transcriptomic, and peptidomic data in *Conus betulinus* has provided the first holistic view of conopeptide biosynthesis from gene to mature peptide and enabled prediction of previously unrecognized analgesic and insecticidal candidates (Peng et al., 2021). This integrative framework is being further strengthened by emerging computational tools that leverage machine-learning approaches to mine omics data for novel candidates with increasing efficiency and predictive power (Li et al., 2018; Wu et al., 2024a).

Taken together, the transition from classical fractionation-based workflows to integrated multi-omics has reshaped the discovery landscape of conotoxin research. These powerful platforms have successfully uncovered an expanding repertoire of novel peptides, and the next major challenge lies in systematic characterization of their pharmacological activities across diverse biological targets.

DISSECTING AND DESIGNING CONOTOXINS: A GUIDE TO STRUCTURE-ACTIVITY RELATIONSHIPS

Structure-activity relationships in conotoxins provide the essential framework that connects natural venom peptides with rational therapeutic development. This framework explains the exceptional potency and selectivity of conotoxins and, more importantly, defines the principles required to redesign these molecules into safer and more effective pharmacological agents. This section first examines the core structural elements that shape the conotoxin framework, spanning primary amino acid sequence to stabilized 3D scaffold. It then addresses how these features govern molecular recognition at the target interface, highlighting representative examples that illustrate the structural basis of high-affinity and subtype-selective binding. Finally, this section examines how these principles are being applied through chemical synthesis and computational design to develop next-generation peptide therapeutics, and it concludes by considering the major challenges and future directions in this area. At a mechanistic level, conotoxin structure-activity relationships provide a powerful framework for linking specific structural determinants, including loop composition, charge distribution, disulfide connectivity, and post-translational modification, to target-binding sites, dominant interaction modes such as electrostatic steering, hydrogen bonding, and hydrophobic packing, and downstream functional outcomes, including pore occlusion, gating modulation, subtype selectivity, and binding kinetics (Association rate constant/ Dissociation rate constant).

Conotoxin architecture: From amino acid sequence to hyperstable scaffold

Biological activity in conotoxins is rooted in a compact primary sequence, most often comprising approximately 10–30 amino acid residues, yet the functional sophistication of these peptides far exceeds their small size. A defining feature of most conotoxins is the conserved arrangement of cysteine residues, which establishes the structural basis for oxidative folding into a tightly constrained disulfide-bonded scaffold. These disulfide networks generate hyperstable 3D architectures with marked resistance to thermal and chemical denaturation (Craig & Adams, 2007; Jones & Bulaj, 2000). Although certain linear conotoxins, such as conantokins, derive structural stability through alternative mechanisms such as metal binding, this rigid disulfide-stabilized scaffold remains the dominant architecture across most conotoxin families and is essential for precise spatial presentation of functional residues to the biological target.

Within these constrained scaffolds, conotoxins adopt well-defined secondary and tertiary structures that commonly include α -helices, β -sheets, and connecting flexible loops (Muttenthaler et al., 2011; Sana et al., 2022). The pattern of cysteine spacing and connectivity, conventionally described as the cysteine framework, often correlates with specific pharmacological activities and target preferences. For instance, a triple-stranded β -sheet motif stabilized by three disulfide bonds is characteristic of many ω -conotoxins directed against Ca_v channels (Yamamoto & Takahara, 2009), whereas the compact two-loop architecture is a defining feature of α -conotoxins that target nAChRs (Millard et al., 2004) (see Figure 6). Beyond conferring global stability, disulfide-bonded scaffolds determine the 3D display of functionally critical residues (Millard et al., 2004). In many conotoxins, intercysteine loops act as modular pharmacophore-bearing elements, such that even minor amino acid substitutions can remodel local electrostatic potential and molecular surface geometry without disrupting the overall fold, thereby producing pronounced shifts in potency and subtype selectivity (Kang et al., 2007). Disulfide connectivity and loop length also shape the accessible conformational ensemble (Jiang & Ma, 2010; Jin et al., 2007), affecting the degree of preorganization of the bioactive state and thus the entropic cost of target engagement (Paul George et al., 2019), an effect that may be reflected more strongly in association and dissociation kinetics than in equilibrium affinity alone (Kang et al., 2007).

This structural foundation is further diversified through post-translational modification, which adds an additional level of chemical and functional diversity. Modifications such as C-terminal amidation can enhance resistance to degradation, whereas proline hydroxylation can introduce new hydrogen-bonding capacity and thereby modulate scaffold conformation and binding affinity (Bulaj & Olivera, 2008; Gerwig et al., 2013). Mechanistically, such modifications can influence both protease resistance and target recognition by reshaping local hydrogen-bonding networks, altering side-chain pK_a and charge distribution, and stabilizing conformations that favor productive binding. The presence of unique residues, such as γ -carboxyglutamate and bromotryptophan, can further alter pharmacological behavior in substantial ways (Gao et al., 2022; Ruffner et al., 2015). The mature bioactive conotoxin should therefore be viewed not as a simple peptide sequence, but as an integrated chemical entity whose activity emerges

from the combined effects of amino acid composition, disulfide architecture, conformational organization, and specific chemical modification.

Structure-target interface: Mechanisms of molecular recognition

The precisely folded conotoxin scaffold generates a distinctive 3D surface topology that enables selective engagement of defined binding sites (“locks”) on ion channels and receptors. Within this framework, mechanistic structure-activity analysis moves beyond simple description of peptide fold to map how individual residues and broader surface features, including charge distribution, hydrophobic patterning, and loop geometry, govern target recognition and functional response. This analysis links specific structural determinants to the binding site engaged, the dominant intermolecular forces involved, such as electrostatic steering, hydrogen bonding, cation- π interactions, and hydrophobic packing, and the resulting pharmacological consequences, including pore occlusion, gating modulation, subtype selectivity, and binding kinetics (Figure 7).

Pore blockade: Vestibule recognition, electrostatics, and occlusion

In pore-blocking conotoxins, structure-activity relationships are dictated primarily by the manner in which charged and aromatic residues interact with the extracellular vestibule and the outer pore or selectivity filter region of the channel. Positively charged side chains can be electrostatically steered toward acidic vestibular residues, increasing the Association rate constant (k_{on}), whereas a subset of basic residues may project into the permeation pathway and impose direct steric and electrostatic occlusion. The extent of channel inhibition, including whether blockade is complete or partial and whether it displays voltage dependence, is strongly influenced by binding pose and by the depth to which critical side chains penetrate the outer pore. A well-established example is the FDA-approved drug ziconotide (ω -conotoxin MVIIA), which binds the outer vestibule of N-type $\text{Ca}_{v2.2}$ channels and prevents Ca^{2+} influx, thereby interrupting nociceptive transmission (Figure 7C) (Dong et al., 2021). Comparable principles govern μ -conotoxins such as GIIIA and KIIIA, which potently block Na_v channels by positioning key basic residues, particularly Arg and Lys, near or within the outer pore, where they form strong electrostatic interactions that stabilize an occluding configuration (Figure 7B) (Pan et al., 2019).

Orthosteric versus allosteric mechanisms: Reshaping receptor activation and subtype selectivity

In ligand-gated ion channels such as nAChRs, many α -conotoxins engage subunit interfaces that overlap with, or lie immediately adjacent to, the endogenous agonist-binding region and therefore function as competitive antagonists. In these interactions, structure-activity relationships are determined by shape and electrostatic complementarity between the toxin surface and the aromatic-rich binding pocket, together with the contribution of loop residues that contact subtype-specific positions at the receptor interface. Notably, even in cases categorized as competitive binding, α -conotoxins can influence receptor behavior more broadly by stabilizing particular conformational states, thereby altering gating efficacy and desensitization kinetics in ways that extend beyond simple site occupancy (Figure 7A) (Xu et al., 2017). Consequently, subtype selectivity often depends on a limited

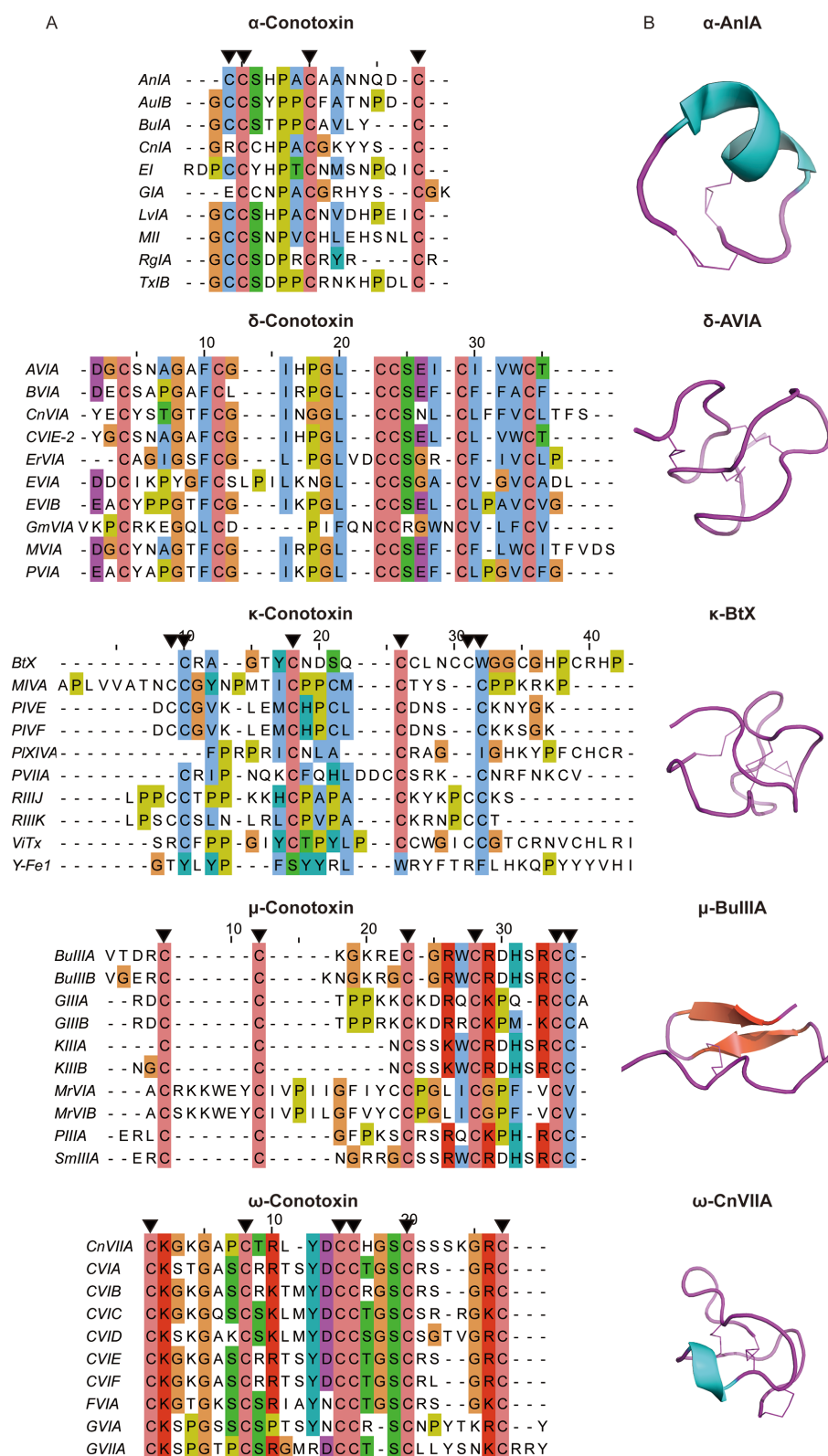


Figure 6 Sequence alignment and representative structures of five conotoxin superfamilies

A: Sequence alignment of 10 representative conotoxins from each superfamily. Conserved residues are highlighted, with particular emphasis on cysteine residues involved in disulfide-bond formation. Alignment shows extensive sequence diversity within each conotoxin type while preserving key structural motifs required for biological activity. B: Representative 3D structures of the first conotoxin shown for each type, predicted using AlphaFold2 and visualized in PyMOL (Jumper et al., 2021). These models illustrate characteristic folding patterns and disulfide connectivities associated with each conotoxin type, including α -AnIA, δ -AVIA, κ -BtX, μ -BullIA, and ω -CnVIIA (from top to bottom). The structures provide insights into the structural and functional diversity that underpins potential pharmacological specialization among representative conotoxins.

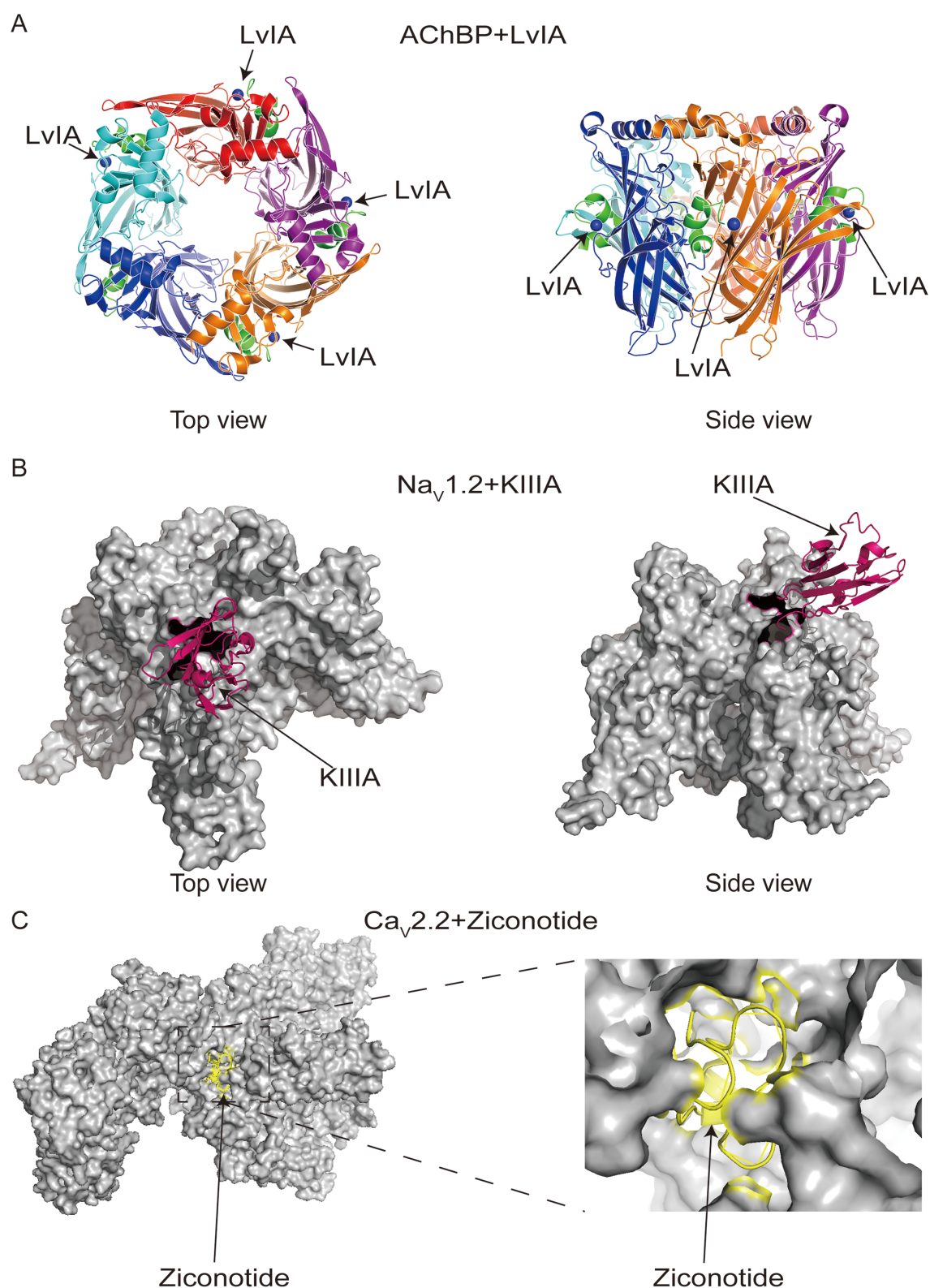


Figure 7 Diverse structural mechanisms underlying conotoxin interactions with ion channels and binding proteins

A: Allosteric modulation of an acetylcholine-binding protein (AChBP): Top (left) and side (right) views of α -conotoxin LvIA (cartoon representation, colored by chain) bound to the pentameric *Aplysia californica* AChBP (cartoon representation, colored by chain). LvIA binds at subunit interfaces, away from the central pore, demonstrating an allosteric mechanism relevant to nAChR modulation (PDB ID: 5XGL). B: Pore blockade of a voltage-gated sodium channel: Top (left) and side (right) views of μ -conotoxin KIIIA (pink cartoon) physically occluding the ion conduction pathway of the human Na_v1.2 channel (gray surface) (PDB ID: 6J8E). C: Pore blockade of a voltage-gated calcium channel by a clinically approved peptide: Overall view (left) of human N-type Ca_v2.2 channel (gray surface) complexed with ω -conotoxin MVIIA (ziconotide, yellow cartoon). Right panel shows a magnified view of toxin binding site (indicated by dashed box within left panel), highlighting occlusion of the ion-conduction pathway by ziconotide. This interaction provides the structural basis for analgesic activity (PDB ID: 7VFU).

number of loop residues that fine-tune local intermolecular interactions, including hydrogen bonding, cation- π interactions, and hydrophobic packing, at the subunit interface. This principle is well illustrated by α -conotoxins such as MII and Vc1.1, in which subtle sequence variation within the contact loops is sufficient to remodel the binding interface and produce distinct recognition profiles across nAChR subtypes (Wakamatsu et al., 1992; Xu et al., 2017).

Kinetic structure-activity relationships: Why Dissociation rate constant can matter as much as affinity

Mechanistic evaluation of conotoxin structure-activity relationships must distinguish equilibrium potency, such as Half-Maximal Inhibitory Concentration (IC_{50}) or Equilibrium Dissociation Constant (K_d), from the kinetic processes that govern target engagement and release (Bernetti et al., 2017). Conotoxin analogs with comparable equilibrium affinities can nevertheless exhibit striking differences in duration of action because individual substitutions may exert disproportionate effects on Dissociation rate constant (k_{off}) by stabilizing or destabilizing a specific bound orientation or receptor conformational state (Bernetti et al., 2017). This distinction carries particular importance in therapeutic development, where prolonged target residence time can enhance efficacy but may also intensify mechanism-based adverse effects at the intended target (Copeland et al., 2006). Accordingly, electrophysiological measurements that resolve kinetic behavior, including block and unblock time constants and recovery profiles, provide essential mechanistic information that cannot be captured by endpoint IC_{50} values alone (Ferruz & De Fabritiis, 2016).

Engineering conotoxins: From rational design to therapeutic leads

A mechanistic understanding of structure-activity relationships makes it possible to move beyond native peptides and rationally engineer analogs with improved pharmacological and translational profiles. In practice, conotoxin optimization is most effective when organized around the property targeted for improvement, including potency and subtype selectivity, conformational control and stability, or developability and pharmacokinetic performance, because each objective is driven by distinct structural and biophysical determinants.

Designing for potency and subtype selectivity: Mapping the pharmacophore

Mechanistically guided optimization typically begins with pharmacophore mapping to distinguish residues that primarily

determine binding affinity from those encoding subtype selectivity. This is often accomplished through alanine-scanning strategies, charge-reversal substitutions (Halai et al., 2009; Jacobsen et al., 1999), and, where experimentally feasible, double-mutant cycle analysis to define specific pairwise interactions at the toxin-target interface. Such residue-level maps provide a clear framework for introducing minimal sequence modifications that preserve the native fold while reshaping electrostatic complementarity and surface geometry at the binding interface (Luo et al., 1999).

Within this framework, site-directed mutagenesis remains a foundational tool for conotoxin engineering (Turner et al., 2019). For example, mutational analysis of α -conotoxin Vc1.1 identified residues essential for analgesic activity and thereby established a rational basis for subsequent optimization (Halai et al., 2009). Once these functional hotspots are defined, focused analog series can be constructed to refine potency and subtype selectivity while retaining the native disulfide-stabilized scaffold (Armishaw et al., 2010).

Designing for conformational control and stability: Preorganization and protease resistance

Once functionally critical residues have been defined, engineering efforts often shift toward reinforcement of conformational integrity and biochemical stability while preserving the target-binding surface. In this context, chemical synthesis and scaffold remodeling provide particularly effective routes for optimization. Backbone cyclization, achieved by covalently linking the N- and C-termini, can impose substantial structural constraint and markedly enhance resistance to proteolytic degradation (Clark et al., 2005). Beyond improving stability, cyclization and related conformational restriction strategies can favor preorganization of the binding-competent conformation, thereby lowering the entropic cost associated with target engagement and, in some cases, reducing the Dissociation rate constant (k_{off}) and extending functional duration (Figure 8) (Piserchio et al., 2004).

A complementary strategy involves incorporation of noncanonical amino acids, which can introduce novel chemical functionalities unavailable within the standard amino acid repertoire. Such substitutions can strengthen specific interactions, including hydrophobic packing or hydrogen bonding, reduce chemical liabilities, and improve metabolic stability (Werner et al., 2016). They also provide expanded control over local charge distribution and side-chain geometry, enabling fine structural adjustments not accessible through

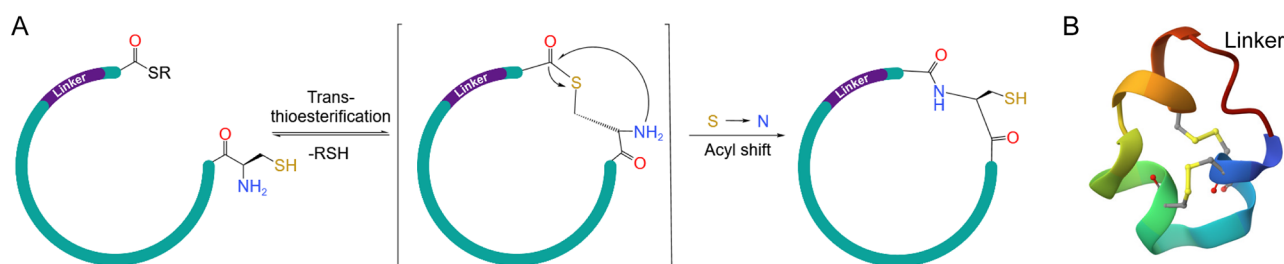


Figure 8 Intramolecular native chemical ligation for generation of N-C backbone-cyclized conotoxins (Jin et al., 2019b)

A: Schematic of backbone cyclization by intramolecular native chemical ligation. Sequence is assembled by solid-phase peptide synthesis with inclusion of a short linker region (purple) that spans the distance between the N- and C-termini. Cyclization requires a C-terminal thioester and an N-terminal cysteine. The reaction proceeds through initial transesterification, followed by an S-to-N acyl shift, ultimately generating backbone cyclization through formation of a peptide bond. B: Representative 3D NMR structure of a cyclized α -conotoxin, cyclic-MII, containing a six-residue linker (PDB ID: 2AJW).

canonical residue substitution alone. Importantly, evaluation of these modifications must extend beyond confirmation of fold retention and should include direct assessment of mechanistic integrity, for example whether the engineered peptide retains pore blockade or gating modulation as the dominant mode of action, using appropriate functional assays (Jwad et al., 2020).

Designing for developability and pharmacokinetics: Translating structure-activity relationships to *in vivo* performance

Enhancement of potency through structure-activity optimization does not automatically translate into improved performance *in vivo*, because solubility, serum stability, and tissue distribution frequently determine effective exposure at the site of action (Atangcho et al., 2019). For this reason, developability-oriented optimization must proceed in parallel with mechanism-preserving functional evaluation to ensure that improvements in stability are not made at the expense of target engagement (Furman et al., 2015). In practice, this requires integration of biochemical stability assays, such as serum and protease resistance testing, with biophysical characterization of aggregation propensity and solubility, together with cellular or tissue-based assays that report target-relevant function and kinetic behavior (Evers et al., 2022).

Significant advances are also emerging from the convergence of these experimental strategies with computational design. Molecular dynamics (MD) simulations can predict how sequence modification reshapes peptide structure, conformational flexibility, and preorganization, whereas docking approaches can suggest plausible binding poses and help prioritize analogs for synthesis (Kazmirchuk et al., 2023). AI-driven structure prediction, including platforms such as AlphaFold, is beginning to generate informative models for previously uncharacterized conotoxins and thereby accelerate hypothesis generation for structure-activity studies (Jumper et al., 2021). When combined with iterative experimental validation, this “*in silico* design-build-test” cycle has the potential to reduce screening time and cost while enabling more systematic, property-driven engineering of peptide therapeutics (Guo et al., 2025; McCarthy & Gonen, 2024).

Challenges and future directions in conotoxin structure-activity research

Despite remarkable progress, conotoxin structure-activity research is still constrained by several unresolved problems that continue to limit both mechanistic interpretation and translational progress. A large proportion of conotoxin chemical diversity remains uncharacterized, and many newly discovered peptides still lack definitive target assignment, making mechanistic interpretation of structure-activity relationships inherently incomplete. High-resolution structural analysis also remains a central bottleneck, particularly for conotoxins in complex with their membrane-embedded targets, where multiple receptor conformations and alternative binding poses may contribute to functional output. In parallel, reliable prediction of how sequence variation and chemical modification influence not only binding affinity and efficacy, but also solubility, serum stability, and tissue exposure, remains a highly multidimensional challenge spanning both pharmacodynamic and pharmacokinetic behavior (Halai et al., 2009; McCarthy & Gonen, 2024).

Two additional factors frequently complicate mechanistic interpretation. The first is strong dependence on membrane

context. For membrane proteins, conotoxin activity can shift substantially with lipid composition, membrane potential, and extracellular ionic strength, each of which can reshape toxin orientation, binding pose, and apparent kinetic behavior (Jung et al., 2010). Consequently, structure-activity patterns derived from simplified experimental systems, such as detergent-solubilized targets or heterologous non-native expression platforms, may not fully reproduce the interactions that occur in native or near-native membrane environments (Hung et al., 2017). A second complication arises from proteoform diversity driven by post-translational modifications. Peptides derived from the same precursor can differ in hydroxylation, bromination, amidation, pyroglutamylation, glycosylation, and other mature modifications, with resulting differences in binding energetics, residence time, and pharmacological effect. This complexity underscores the importance of linking structure-activity analysis to venomomics-based characterization of mature peptide forms rather than inferring mechanism from precursor sequence alone.

Future progress in conotoxin structure-activity research will likely depend on convergence of emerging technologies with standardized, mechanism-resolved datasets. Integration of AI and machine learning with large-scale experimental data, including high-throughput mutagenesis, electrophysiology-derived kinetic measurements, and proteomic and venomomic profiling, should support development of predictive models that capture not only equilibrium potency but also kinetic behavior and context dependence (Li et al., 2024). In parallel, continued advances in cryo-electron microscopy are expected to yield increasingly detailed views of toxin-channel and toxin-receptor complexes and may help resolve alternative binding poses and state-dependent recognition. Ultimately, the long-term objective is to construct a more complete roadmap that links sequence, structure, mechanism, and functional outcome with sufficient resolution to enable *de novo* design of conotoxin-inspired therapeutics tailored to specific disease contexts (Li et al., 2025). The following section considers how such engineered molecules perform in preclinical and clinical settings and highlights the translational factors that shape progression from optimized peptides to potential therapeutic agents.

THERAPEUTIC APPLICATIONS OF CONOTOXINS: FROM CLINICAL PAIN MANAGEMENT TO EMERGING FRONTIERS

After examination of the structural basis and molecular mechanisms that govern representative conotoxins, attention has shifted to therapeutic translational applications and the challenge of converting these marine neuropeptides into interventions for human disease. This transition has revealed both immense promise and formidable barriers. Ziconotide remains the only conotoxin-based drug with FDA approval, underscoring how rarely potent venom peptides successfully advance through the full clinical development pipeline. Other candidates, such as the norepinephrine transporter inhibitor Xen2174, demonstrated therapeutic activity but encountered major obstacles during clinical evaluation, including limitations in safety margins and dosage practicality (Okkerse et al., 2017). For this reason, conotoxins are often most appropriately viewed not as ready-made drugs, but as highly precise molecular probes that establish the therapeutic validity of specific ion channels, receptors, and transporters. Renewed momentum in this field has emerged from progress in peptide

engineering and delivery technology, which is beginning to convert potent but unstable venom peptides into more viable clinical candidates (Figure 5B). Beyond the landmark example of ziconotide, an expanding group of conotoxin-derived peptides and conotoxin-inspired analogs has entered preclinical development and, in some cases, early-stage clinical evaluation. Most remain developmental leads rather than approved therapeutics, yet their value often lies in the degree of target selectivity they provide, including selective engagement of defined nAChRs, NETs, and ion-channel isoforms, a level of precision that is often difficult to achieve with small-molecule compounds. To clarify this translational landscape, the following discussion highlights representative examples and distinguishes explicitly among approved drugs, clinically evaluated candidates, and preclinical lead scaffolds. Within this framework, ziconotide is considered as an approved therapeutic, Xen2174 as a clinically evaluated candidate that encountered developmental setbacks, and several nAChR- and Na_v-modulating conotoxins, including TxIB, Vc1.1, and MII-derived probes, as representative preclinical leads and target-validation scaffolds.

This section explores the therapeutic landscape of conotoxins through a series of translational domains. It begins with the most established achievement in the field, chronic pain management, then discusses the growing interest in neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD), as well as emerging frontiers, including cardiovascular disease, oncology, and nicotine addiction. Together, these applications illustrate the unusual versatility of conotoxins as a source of both mechanistically informative pharmacology and next-generation therapeutic design.

Landmark success: Conotoxins in pain management

The most compelling clinical translation of conotoxin research has emerged in analgesia. Chronic pain, particularly neuropathic pain, remains a persistent and often treatment-resistant condition, with limited efficacy and substantial adverse effects associated with conventional therapies such as opioids. Conotoxins have introduced a fundamentally different therapeutic paradigm by enabling highly target-selective and non-opioid modulation of neuronal signaling pathways involved in pain transmission.

The flagship example is ω -conotoxin MVIIA, marketed as ziconotide, which received approval from the US FDA for management of severe, intractable chronic pain in patients intolerant of, or refractory to, existing treatments, including intrathecal morphine (Staats et al., 2004). Ziconotide exerts a potent analgesic effect through selective inhibition of N-type Ca_v2.2 channels expressed on spinal cord neurons, thereby suppressing the release of pronociceptive neurotransmitters at synaptic terminals (Dong et al., 2021). Despite its high efficacy, clinical use remains constrained by the requirement for intrathecal administration, which has stimulated continued efforts to develop analogs with improved delivery profiles or alternative administration strategies.

Beyond this established example, a broad range of conotoxin-derived peptides continues to inform analgesic discovery. For example, μ -conotoxins that inhibit Na_v channels serve as important lead scaffolds and pharmacological probes for pain-associated Na_v subtypes, including μ -conotoxin KIIIA (Zhang et al., 2007). Aberrant activity of specific Na_v channel subtypes, such as Na_v1.7 and Na_v1.8, in primary sensory

neurons represents a key driver of neuropathic pain pathophysiology. Selective inhibition of these channels can prevent the generation and propagation of nociceptive signaling, offering a mechanistically targeted approach to pain control (Tosti et al., 2017). Continued investigation in this area highlights the immense value of conotoxins as precision pharmacological tools and as foundational scaffolds for development of next-generation analgesic targets.

Emerging insights into neurodegenerative disease

Neurodegenerative disorders such as AD and PD are defined by progressive, cell type-specific neuronal degeneration and currently lack effective disease-modifying therapies. Conotoxins have gained increasing attention as highly selective modulators of neural circuitry, offering a precise means to investigate and potentially correct the dysfunctional signaling pathways implicated in these diseases.

In AD, impairment of cholinergic neurotransmission represents a central feature of cognitive decline. Within this context, several α -conotoxins that target nAChRs have shown therapeutic promise. Selective modulation of receptor subtypes, such as $\alpha 7$ nAChR, can enhance cholinergic signaling and is associated with improved cognitive performance in preclinical models (Medeiros et al., 2014).

In PD, the principal value of conotoxins has resided in their capacity to resolve the receptor-level mechanisms governing dopaminergic neurotransmission rather than in direct blockade of excitotoxic injury. Particular attention has focused on $\alpha 6\beta 2^*$ nAChR subtypes, which are localized on presynaptic striatal catecholaminergic nerve terminals and play a key role in regulation of dopamine release. Selective antagonists such as α -conotoxin MII and its rationally designed analog α -conotoxin MII[E11A] have provided precise tools for investigating these receptor populations. Using these probes, studies have demonstrated that $\alpha 6$ -containing nAChRs are selectively lost in the striatum of MPTP-treated primates (Bordia et al., 2007) and in human PD (Quik et al., 2007). High-affinity interactions with α -conotoxin MII[E11A] have also revealed functional heterogeneity among striatal dopaminergic fibers, distinguishing populations with lower and higher action potential thresholds for dopamine release (Turner et al., 2009). Collectively, these findings establish conotoxins as powerful molecular probes for elucidating the structural determinants of nAChRs that underlie PD pathophysiology. At present, compounds in this area, including α -conotoxin MII and engineered analogs such as MII[E11A], are best regarded as preclinical lead scaffolds and target-validation tools that enable exceptionally selective analysis of disease-relevant cholinergic-dopaminergic circuitry.

Investigating systemic applications: Cardiovascular and oncological targets

The pharmacological relevance of conotoxins extends beyond central nervous system modulation, with growing interest in systemic contexts, particularly in cardiovascular disease and cancers, where ion channel dysregulation plays a central role in disease progression.

Cardiovascular diseases: Ion channels govern fundamental aspects of cardiovascular function, including regulation of vascular tone and control of cardiac rhythm. Targeting these pathways with conotoxins offers a mechanistically precise approach to modulate pathological signaling. Certain ω -conotoxins, through blockade of Ca_v channels located on sympathetic nerve terminals, suppress norepinephrine release

and thereby promote vasodilation, suggesting a potential strategy for treating hypertension (Clasbrummel et al., 1989). In parallel, conotoxins that target specific K_v channel subtypes in cardiomyocytes have attracted interest as modulators of cardiac arrhythmias. By influencing the repolarization phase of the cardiac action potential, these peptides have demonstrated the capacity to restore electrical stability and correct arrhythmic activity in preclinical systems (Terlau & Olivera, 2004).

Cancers: Ion channels have emerged as important regulators of cellular processes that underpin tumor development, including proliferation, migration, and apoptosis. Within this framework, conotoxins have shown preliminary anti-tumor activity by perturbing channel-dependent signaling in malignant cells. Several conotoxins that inhibit specific Ca_v channels, which are frequently up-regulated in cancer cells, have been shown to disrupt the cell cycle and induce apoptosis, suggesting a potential avenue for further mechanistic investigation rather than immediate clinical translation (Díaz-García & Varela, 2020).

Modulating complex behaviors: Representative roles in nicotine addiction treatment

Drug addiction represents a complex neurobiological disorder involving neural circuits that govern reward, reinforcement, and behavioral adaptation. Within this framework, nAChRs represent central molecular determinants of nicotine addiction, particularly through subtype-specific control of dopaminergic signaling in reward-related brain regions. Conotoxins have emerged as uniquely selective modulators of these receptor systems, enabling targeted interrogation and manipulation of addiction-relevant pathways.

Accumulating preclinical evidence further suggests that highly subtype-selective α -conotoxins may provide non-addictive strategies for modulating cholinergic signaling associated with nicotine dependence. For example, α -conotoxin TxIB, a selective antagonist of $\alpha 6/\alpha 3\beta 2\beta 3$ -containing nAChRs, has shown efficacy in reducing nicotine-associated behavioral responses in animal models, consistent with the prominent role of $\alpha 6$ -containing nAChR populations in reward circuitry and addiction-related brain regions (Huang et al., 2022; Xu et al., 2025). In addition, α -conotoxin Vc1.1 and related analogs have been widely explored as analgesic and neuromodulatory leads and continue to serve as important preclinical scaffolds for refinement of potency, receptor selectivity, and pharmacological properties.

Although these conotoxin-based candidates remain largely within the preclinical domain, their capacity for precise receptor subtype selectivity highlights their value as lead structures for development of next-generation therapeutics targeting nicotine addiction and related substance use disorders.

CONCLUSIONS AND FUTURE PERSPECTIVES

Cone snail venom exemplifies an advanced evolutionary adaptation in which sustained predatory pressure has driven the emergence of a highly diverse and functionally specialized repertoire of neuroactive peptides. This review has highlighted the breadth of conotoxin diversity and the molecular principles underlying biological activity, showing how these peptides achieve exceptional potency and target specificity through precise interactions with ion channels and receptors. High-throughput omics has transformed the field by rapidly

expanding sequence and functional knowledge. Detailed characterization of these interactions has provided powerful tools for neuroscience and has supported clinical translation, most notably through the US FDA-approved analgesic ziconotide. In parallel, advances in structure-activity analysis now support rational optimization of native scaffolds for improved therapeutic performance.

Despite these remarkable advances, the full potential of this evolutionary innovation remains to be unlocked. Translation from venom-derived peptide to clinically viable therapeutic continues to present significant challenges, including limitations in delivery, stability, and predictability of *in vivo* behavior. Continued progress will depend on integration of emerging technologies that enable more precise mapping of sequence, structure, and function across diverse biological contexts. The next frontier in conotoxin research will therefore focus on the following key areas.

Deciphering “venom dark matter”

High-throughput omics approaches have uncovered an expansive repertoire of putative conotoxin sequences, although a significant portion remains functionally uncharacterized. Bridging this discovery-to-function gap represents a central challenge for the field. Future efforts will require the large-scale integration of high-throughput functional screening platforms, such as automated patch-clamp electrophysiology and high-content imaging, to enable rapid assignment of biological function to this vast peptide library and identify novel leads with unique mechanisms of action.

Precision bioengineering and AI-driven design

Future development of conotoxin-based therapeutics will increasingly rely on precision engineering strategies that combine advanced experimental and computational approaches. Recent developments in structural prediction and machine learning now enable systematic *in silico* evaluation of sequence-structure-function relationships, with platforms such as AlphaFold2 and RoseTTAFold (Baek et al., 2021; Jumper et al., 2021) providing predictive frameworks for assessing the impact of amino acid substitutions on peptide conformation and target engagement. These capabilities facilitate rational design of peptides with predefined pharmacological properties, such as enhanced subtype selectivity, reduced off-target effects, and improved resistance to proteolytic degradation. In parallel, chemical modification strategies, including backbone cyclization, peptide stapling, and incorporation of noncanonical amino acids, will provide robust approaches for generating next-generation drug candidates. Indeed, the effectiveness of these strategies is already evident across the broader field of peptide therapeutics, with demonstrated applications in tissue regeneration (Ru et al., 2025; Wu et al., 2024b), wound repair (Wang et al., 2025), and neuroprotection (Ye et al., 2025). These encouraging advances provide a compelling roadmap for the future development of engineered conotoxins.

Expanding the therapeutic target landscape

Therapeutic exploration of conotoxins has historically centered on voltage-gated ion channels and nicotinic acetylcholine receptors; however, increasing evidence indicates that this scope represents only a fraction of the accessible target space. Conotoxins capable of engaging alternative receptor classes, including G protein-coupled receptors, membrane

transporters, and potentially intracellular signaling components, reveal a broader pharmacological potential than previously appreciated. Representative examples include conotakin-G, which targets neurotensin receptors and exhibits analgesic activity (Craig et al., 1999b), and conopressins, which modulate vasopressin and oxytocin receptor systems (Dutertre et al., 2008). Systematic expansion of these studies is expected to identify valuable peptides with novel target specificities and may open new avenues for intervention in metabolic, psychiatric, and autoimmune disorders.

Sustainable production and biomanufacturing

Advancement of conotoxin-based therapeutics requires scalable and economically viable production strategies, as reliance on natural venom extraction is not compatible with clinical demand. Solid-phase peptide synthesis remains the primary approach for laboratory-scale production; however, synthesis costs increase substantially with peptide length, complex disulfide architectures, and requirements for post-translational modification, creating a significant economic bottleneck for large-scale translation. To address these limitations, increasing emphasis has been placed on synthetic biology and recombinant expression systems. Engineering of microbial hosts, such as periplasmic expression in *E. coli*, has enabled more efficient formation of disulfide bonds and improved peptide yield (Klint et al., 2013; Turchetto et al., 2017). However, optimizing systems for scalable production of peptides with complex post-translational modification remains a critical challenge. Continued development of integrated biomanufacturing platforms will be essential to achieve cost-effective production and to support broader clinical translation of conotoxin-derived therapeutics.

Beyond the genus *Conus*: Untapped potential of Conoidea

While the genus *Conus* has long dominated venom-based research, recent evolutionary analyses and multi-omics studies highlight the immense potential of the broader superfamily Conoidea, particularly within the Turridae and Terebridae families. Transcriptomic analyses have revealed extensive venom diversity in both groups. Within Turridae, for example, 41–74 putative toxin genes per species have been identified, many of which share similarity with established conotoxin superfamilies (Gonzales & Saloma, 2014). Within Terebridae, 139 putative teretoxins have been discovered and classified into 14 gene superfamilies, 13 of which appear restricted to this lineage (Gorson et al., 2015). Despite structural parallels with conotoxins, these venoms exhibit distinct biochemical signatures. For example, turrid toxins are characterized by enrichment of methionine, tyrosine, and arginine, yet contain comparatively few cysteine residues (López-Vera et al., 2004). Such findings support the view that each major toxoglossan lineage has independently evolved a distinct set of venom gene superfamilies (Olivera et al., 2012). Consequently, limiting the scope of research to *Conus* alone risks overlooking a vast and potentially valuable diversity of neuroactive peptides with potential therapeutic relevance.

In conclusion, cone snail venom should be regarded not as a simple toxin cocktail, but as a dynamic and evolving library of molecular solutions refined over millions of years. Integration of evolutionary insight with modern biotechnology is poised to unlock the full potential of this extraordinary resource, heralding a new wave of highly specific and potent therapeutics to address some of humanity's most pressing medical needs.

SUPPLEMENTARY DATA

Supplementary data to this article can be found online.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

K.L.M.: Writing - Original draft, Funding acquisition. J.X.F.: Writing - Original draft. J.C.: Writing - Original draft. Y.L.L.: Writing - Original draft. M.L.H.: Writing - Original draft. Z.C.: Writing - Revising & Editing. L.M.L.: Writing - Revising & Editing. Q.S.: Conceptualization, Funding acquisition, Writing - Revising & Editing. B.M.G.: Conceptualization, Funding acquisition, Writing - Revising & Editing. All authors read and approved the final version of the manuscript.

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