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Review

A systematic mapping review of neuropathic pain therapeutics from marine organisms

[Una revisión de mapeo sistemático de terapéuticas para el dolor neuropático derivadas de organismos marinos]

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Abstract

Context: Neuropathic pain remains difficult to treat because current therapies provide limited efficacy and are often associated with adverse effects. Marine organisms are a source of structurally diverse bioactives that may modulate pain-relevant molecular targets.

Aims: A systematic evidence map (SEM) was constructed to identify and characterize the antineuropathic potential of marine-derived bioactives, with emphasis on mechanisms of action, translational evidence, and limitations affecting therapeutic development.

Methods: A PRISMA-guided review was conducted in PubMed, Scopus, Web of Science, and ScienceDirect for studies published from January 2010 to April 2025. Preclinical *in vivo* studies, *in vitro* or *in silico* target-engagement studies, and clinical studies evaluating marine-derived compounds, extracts, fractions, or synthetic equivalents relevant to neuropathic pain were considered.

Results: Ninety-one studies were included. Most evidence was preclinical, and only three clinical reports were identified. The strongest translational evidence was associated with compounds derived from *Conus* species, particularly conotoxins, and with tetrodotoxin. Marine bioactives were reported to modulate voltage-gated sodium and calcium channels, nicotinic acetylcholine receptors, acid-sensing ion channels, transient receptor potential channels, G protein-coupled receptors, and neuroimmune pathways. Major translational barriers included limited selectivity across species, peptide instability, delivery constraints, incomplete safety profiling, and insufficient use of disease-relevant models.

Conclusions: Marine-derived bioactives constitute promising leads for mechanism-based neuropathic pain therapy. Future studies should prioritize human-target validation, disease-relevant models, blinded and adequately powered experimental designs, long-term safety assessment, and scalable synthesis or delivery strategies.

Keywords: analgesics; conotoxins; marine toxins; neuropathic pain; sodium channels; tetrodotoxin.

Resumen

Contexto: El dolor neuropático sigue siendo difícil de tratar, ya que las terapias actuales ofrecen una eficacia limitada y se asocian con efectos adversos. Los organismos marinos constituyen una fuente de compuestos bioactivos estructuralmente diversos capaces de modular dianas moleculares relacionadas con el dolor.

Objetivos: Se construyó un mapa de evidencia sistemática para identificar y caracterizar el potencial antineuropático de compuestos bioactivos de origen marino, con énfasis en los mecanismos de acción, la evidencia traslacional y las limitaciones que afectan el desarrollo terapéutico.

Métodos: Se realizó una revisión siguiendo las directrices de PRISMA en las bases de datos PubMed, Scopus, Web of Science y ScienceDirect (enero de 2010-abril de 2025). Se consideraron estudios preclínicos *in vivo, estudios de* interacción con dianas *in vitro* o *in silico*, y estudios clínicos que evaluaran compuestos, extractos, fracciones o equivalentes sintéticos de origen marino relevantes para el dolor neuropático.

Resultados: Se incluyeron noventa y un estudios, en su mayoría preclínicos y solo tres clínicos. La evidencia traslacional más sólida se asoció con compuestos derivados de especies de *Conus* —en particular conotoxinas— y con la tetrodotoxina. Se reportó que los compuestos bioactivos marinos modulan los canales de sodio y calcio dependientes de voltaje, los receptores nicotínicos de acetilcolina, los canales iónicos sensibles al ácido, los canales de potencial de receptor transitorio, los receptores acoplados a proteínas G y las vías neuroinmunes. Las principales barreras traslacionales incluyen la selectividad limitada entre especies, la inestabilidad de los péptidos, las limitaciones en la administración, un perfil de seguridad incompleto y el uso insuficiente de modelos relevantes para la enfermedad.

Conclusiones: Los bioactivos marinos constituyen candidatos terapéuticos prometedores para el dolor neuropático basados en mecanismos específicos. Los estudios futuros deberían priorizar la validación de dianas humanas, los modelos relevantes para la enfermedad, diseños experimentales cegados y con potencia estadística adecuada, la evaluación de la seguridad a largo plazo y estrategias de síntesis o de administración escalables.

Palabras Clave: analgésicos; canales de sodio; conotoxinas; dolor neuropático; tetrodotoxina; toxinas marinas.

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Abbreviations: 5-HT: 5-hydroxytryptamine; **5-HT7R:** 5-hydroxytryptamine 7 receptor; **ACC:** anterior cingulate cortex; **ASIC:** acid-sensing ion channels; **AST:** astaxanthin; **BBB:** blood-brain barrier; **BDZ:** benzodiazepine; **BM-21:** *Thalassia testudinum* seagrass crude hydroethanolic extract; **CB:** cannabinoid; **CB1/2R:** cannabinoid receptors 1 and 2; **CCI:** chronic constriction injury; **CD31:** cluster of differentiation 31; **CIPN:** chemotherapy-induced peripheral neuropathy; **CNS:** central nervous system; **conG:** conantokin-G; **CREB:** cAMP response element-binding protein; **CTX:** conotoxin; **DRG:** dorsal root ganglia; **EC_{50}:** the median effective concentration; **EMA:** European Medicines Agency; **ERK:** extracellular signal-regulated kinases; **FDA:** The Food and Drug Administration; **GABA:** gamma-aminobutyric acid; **$GABA_{A/B}R$:** gamma-aminobutyric acid A and B receptor; **GFAP:** glial fibrillary acidic protein; **GIRK:** G protein–gated inwardly rectifying potassium channels; **GPCRs:** G-protein-coupled receptors; **HIF-1α:** hypoxia-inducible factor 1 alpha; **IC_{50}:** the median inhibitory concentration; **IDO:** indoleamine 2,3-dioxygenase; **IL-1β:** interleukin 1 beta; **IL-4/6/10/17:** interleukin-4,6,10,17; **IM:** intramuscular route; **IT:** intrathecal route; **IV:** intravenous route; **JNK:** c-Jun N-terminal kinases; **KAR:** kainate receptor; **KCC2:** K^+-Cl^- co-transporter 2; **KO:** knockout; **K_V:** voltage-gated potassium channels; **LDHA:** lactate dehydrogenase A; **LTCTs:** low-threshold cold thermosensors; **MAPK:** mitogen-activated protein kinases; **MMP-9/2:** matrix metalloprotease-9 and 2; **nAChRs:** nicotinic acetylcholine receptors; **NAM:** negative allosteric modulator; **NFκ-B:** nuclear factor-κB; **NMDAR:** N-methyl-D-aspartate receptor; **NP:** neuropathic pain; **Nrf2:** nuclear factor erythroid 2-related factor 2; **NTS:** neurotensin; **NTSR1/2:** neurotensin receptors 1 and 2; **OXA:** oxaliplatin; **PAM:** positive allosteric modulator; **PCT:** proximal C-terminus; **PDK:** pyruvate dehydrogenase kinase; **PHN:** post-herpetic neuralgia; **PNI:** peripheral nerve injury; **PNS:** peripheral nervous system; **PSL:** partial sciatic nerve ligation; **PTX:** paclitaxel; **PWL:** paw withdrawal latency; **PWT:** paw withdrawal threshold; **SCI:** spinal cord injury; **SNI:** spared nerve injury; **SNL:** spinal nerve ligation; **SST:** somatostatin; **SSTR1/4:** somatostatin receptors 1 and 4; **STX:** saxitoxin; **STZ:** streptozotocin; **TB:** thalassiolin B; **TGF-β:** transforming growth factor β; **TNF-α:** tumor necrosis factor alpha; **TRP:** transient receptor potential channels; **TRPA1:** transient receptor potential ankyrin 1; **TRPM8:** transient receptor potential M8; **TRPV1:** transient receptor potential vanilloid 1; **TTX:** tetrodotoxin; **TTX-R:** TTX-resistant; **TTX-S:** TTX-sensitive; **UVB:** ultraviolet B; **VEGF:** vascular endothelial growth factor; **VFT:** Venus Fly Trap domain; **VGCC or Ca_V:** voltage-gated calcium channels; **VGSC or Na_V:** voltage-gated sodium channels.

INTRODUCTION

Neuropathic pain (NP) is a common global health issue affecting an estimated 6.9% to 10% of the general population, with a significant impact on patients' quality of life (van Hecke et al., 2014). The International Association for the Study of Pain (IASP) defines NP as pain caused by a lesion or disease of the somatosensory system (Treede et al., 2008). NP typically persists continuously or manifests as recurrent episodes beyond the acute phase of injury, evolving into a chronic and disabling condition (Scholz et al., 2019).

Neuroanatomical changes in response to neural injury generally involve a combination of peripheral and central adaptive and maladaptive mechanisms in both pronociceptive and antinociceptive systems, resulting in complex, heterogeneous pathophysiology (Campbell & Meyer, 2006; Latremoliere & Woolf, 2009). Despite current understanding of these underlying mechanisms, largely based on animal models, NP remains challenging for both basic and clinical researchers. There is consensus that the therapeutic approach to NP should be based on specific mechanisms rather than on etiological conditions (Jensen & Baron, 2003; Woolf, 2010). However, development of an individualized treatment algorithm remains challenging because it requires identification of a classification based on underlying physiology and stratified clinical trials to evaluate novel drugs that target specific mechanisms (Finnerup et al., 2021).

Most available drug therapies fail to address the complexity of peripheral or central mechanisms involved in NP (Bouhassira & Attal, 2018). Current pharmacotherapy for NP mainly includes drugs acting on α_2-δ subunits of calcium channels, sodium channels, and descending modulatory inhibitory pathways; unfortunately, their efficacy and safety are limited (Soliman et al., 2025). Therefore, innovative analgesics represent a critical need for patients with NP, and a considerable number of novel molecular entities acting on specific targets are under development; however, clinical translation of preclinical results has encountered several difficulties (Attal & Bouhassira, 2019; Bouhassira & Attal, 2018; Yezierski & Hansson, 2018).

For analgesic drug development to be successful, it must follow a rational cycle from target selection through animal studies and clinical trials, using relevant endpoints directly linked to NP pathophysiology and the drug candidate's mechanism of action (Woolf, 2010; Yezierski & Hansson, 2018). Several cell-cell interactions and molecular signaling pathways that orchestrate NP processes offer opportunities to develop novel therapeutic strategies and to revisit existing targets, including ion channels, enzymes, and G protein-coupled receptors (GPCRs) (Yekkirala et al., 2017). Over the past decades, researchers have introduced targeting therapies to glial-derived mediators, glia, or infiltrating immune cells as a novel approach to NP therapy (Austin & Moalem-Taylor, 2010; De Leo et al., 2006; Milligan & Watkins, 2009).

Compared with the therapeutic index of available drugs and in the context of a continuing public health opioid crisis, natural products have attracted notable interest in the research and development of novel analgesics with greater efficacy, reduced adverse effects, and lower predisposition to abuse (Altmann, 2017;

More et al., 2023). Marine organisms, which have evolved and adapted to unique ecological conditions, constitute a valuable source of natural bioactive molecules; consequently, they can synthesize molecules that have no counterparts in terrestrial organisms (Haque et al., 2022). In the marine environment, all kingdoms of living organisms are represented, from bacteria to multicellular plants and animals (Costello et al., 2013). The majority of phyla and more than 90% of all living classes of organisms are found in the oceans, which extend over more than 70% of the planet's surface (Calado et al., 2022). In addition to their vast biodiversity and extent, marine organisms are characterized by their exposure to extreme abiotic factors, high incidence of predation, and herbivory, which generate powerful adaptive defense mechanisms that can lead to novel chemical structures with relevant biological activities (Calado et al., 2022; Gono et al., 2022; Lopes et al., 2024; Romano et al., 2022). These compounds may act as modulators of several cell signaling pathways involved in NP, making them especially important for drug discovery and development of a novel class of analgesics (Cappello & Nieri, 2021; Gao et al., 2017).

In particular, the large family of predatory marine cone snails provides an extensive repository of conotoxins that have attracted increasing attention in NP drug discovery because of their highly specific actions on key components of excitable membranes and chemical synapses (Gao et al., 2017; Sadeghi et al., 2017). Ziconotide represents a notable success in the development of marine-based non-opioid analgesics—a synthetic equivalent of a peptide (ω-conotoxin MVIIA) isolated from the venom of *Conus magus* (McIntosh et al., 1982)—which has been approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the management of intractable chronic pain (Cappello & Nieri, 2021; Safavi-Hemami et al., 2019). However, off-target effects and the need for intrathecal administration limit its use (Rubiu et al., 2024). Additionally, researchers have identified and selected other natural products and related peptidic or non-peptidic compounds from marine organisms as promising drug leads for extended preclinical evaluation (Kayser et al., 2010; Li et al., 2021; Liao et al., 2019; Si & Zhu, 2022).

The antineuropathic potential of marine-derived compounds has been increasingly investigated, but the available evidence remains heterogeneous across compound classes, biological targets, experimental models, and translational stages. Previous reviews have centered primarily on conotoxins or marine-derived compounds in pain broadly; recently, an interesting narrative review focused on the pharmacology and therapeutic potential of these compounds in the context of NP (Mishra et al., 2025); however, their systematic evaluation specifically targeting validated NP mechanisms and their translational status has not been conducted. A critical synthesis is therefore needed to distinguish compounds supported by disease-relevant *in vivo* or clinical evidence from those supported only by target-engagement data. This review evaluates marine-derived bioactives with potential relevance to NP, assesses the reliability and limitations of the evidence, and identifies experimental priorities for translation into safer, more effective mechanism-based therapies.

MATERIAL AND METHODS

Review methodology

This review was structured as a systematic evidence map (SEM) combined with a critical review and adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) recommendations (Khalil et al., 2025; Page et al., 2021). The protocol for this systematic review has been registered with the International Prospective Register of Systematic Reviews (PROSPERO), registration number: CRD420261381895. Minor deviations from the initial protocol were made during the screening phase and are described in this Methods section.

Eligibility criteria

Studies were considered eligible when they reported primary evidence on marine-derived isolated compounds, extracts, fractions, or synthetic equivalents/analogs relevant to neuropathic pain. Eligible evidence included: (i) *In vivo* studies using validated neuropathic pain models. In addition to validated neuropathic pain models, studies using other experimental models will be included only when they demonstrate evidence of nerve injury and provide mechanistic evidence regarding molecular targets relevant to neuropathic pain. (ii) *In vitro* or *in silico* studies demonstrating interaction with or modulation of targets implicated in neuropathic pain mechanisms. *In vivo* experiments in other pain models shall not be an exclusion criterion, provided the mechanistic evidence comes from *in vitro* or *in silico* assays. (iii) Clinical studies in patients with neuropathic pain.

Reviews, letters, book chapters, protocols, non-marine sources, and studies limited to non-neuropathic endpoints were excluded unless they provided essential background not available from a primary source. Studies without a control were excluded, depending on the type of design. The exclusion of studies without control will not apply to all *in silico*, clinical, or mechanistic studies. Studies were limited to English-language publications from January 2010 to April 2025.

Information sources

A comprehensive search was conducted across four academic databases: PubMed, Scopus, Web of Science, and ScienceDirect. The search terms applied were consistent across all four search systems. Boolean operators, specifically OR and AND, were used to expand and refine search results, ensuring exhaustive coverage of relevant literature.

Search strategy

A preliminary Google search was conducted to define the search terms, focusing on "marine organisms" and "neuropathic pain." From the results, potential search terms were identified. A search strategy was formulated using these terms, and additional relevant terms were identified. All authors approved the search strategy. Boolean operators, exact phrases, and keywords in English (individually or combined) such as the following were used: general terms (marine or sea organisms, marine or sea bioactive, marine natural products), as well as for each organisms of different kingdoms and particular phylum or species (marine bacteria, cyanobacteria, marine protozoans, marine chromists, phytoplankton, seaweeds, algae, marine fungi, marine plants, seagrasses, marine angiosperms, halophytes, mangroves, marine animals, cnidarians, sea anemone, sponges, marine worms, molluscs, cones, snails, conotoxins, conopeptides, mussels, crustaceans, echinoderms, sea stars, corals, jellyfish, bryozoans, fish, tetrodotoxin), analgesic, antinociceptive, antihyperalgesic, antihypernociceptive, antiallodynic, neuropathic pain, and mechanisms or Na_V, Ca_V, K_V, NMDA, neuroimmune interactions, nAChR, GABA, GPCR ASIC; TRP, neuroinflammation, neuroprotection. For each of the six marine kingdoms, two analysts independently searched the articles by reading the titles and abstracts of the studies identified in the electronic searches. The results of each search, along with the corresponding search terms, were forwarded to four reviewers for validation. These reviewers also identified relevant studies. A full search strategy is available in Table S4.

Selection of articles

To indicate the different stages of the systematic review methodology, the PRISMA diagram (Page et al., 2021) was used (Fig. 1), generated in Software Advice – Opera Reviews & Ratings. All search results were exported in "ris" and "txt" formats and uploaded to the Rayyan program (Ouzzani et al., 2016), which compiled them. Subsequently, the program resolved duplicates, reducing the total number of results. Two analysts then used this program to analyze and select articles. Each analyst read the titles, keywords, and abstracts to determine whether the articles were useful for this review. Inter-reviewer agreement was 99% during title/abstract screening and 98% during full-text assessment. When eligibility remained uncertain after full-text assessment, disagreements were resolved through discussion with a third reviewer.

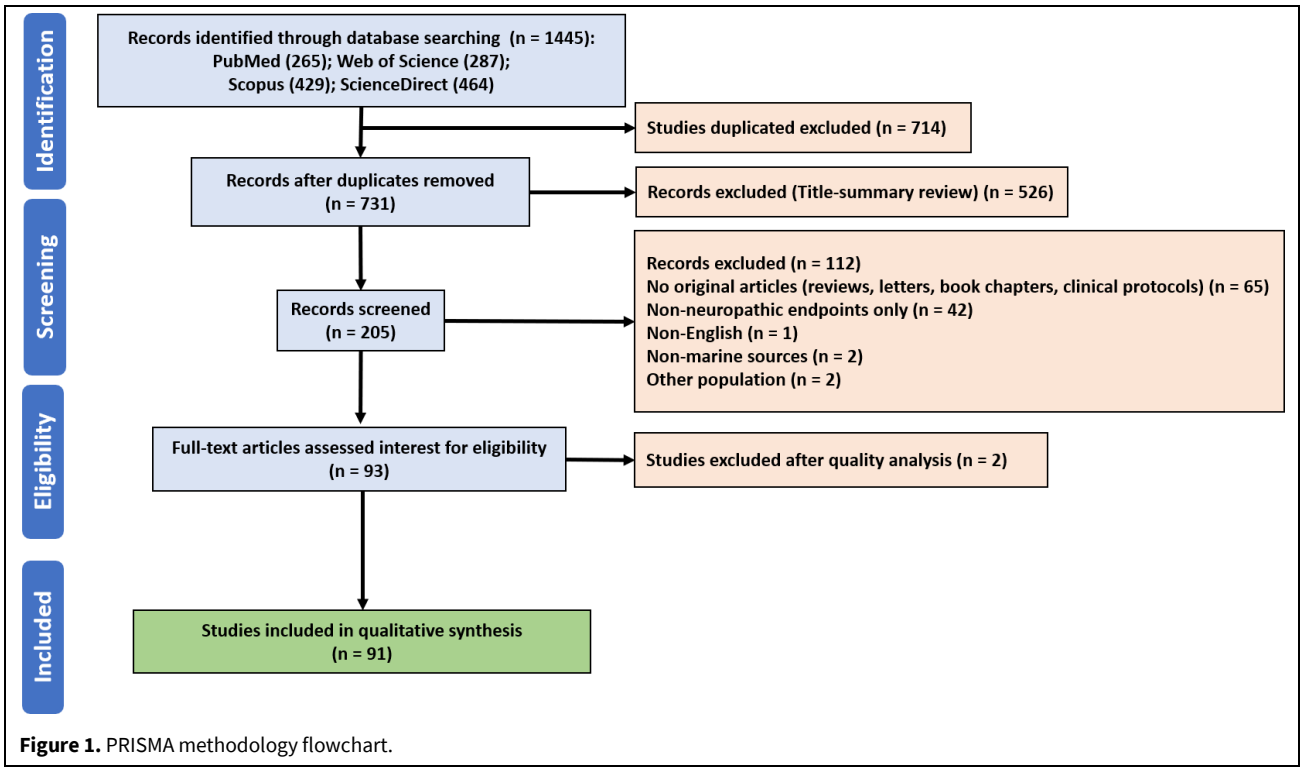

Figure 1. PRISMA methodology flowchart.

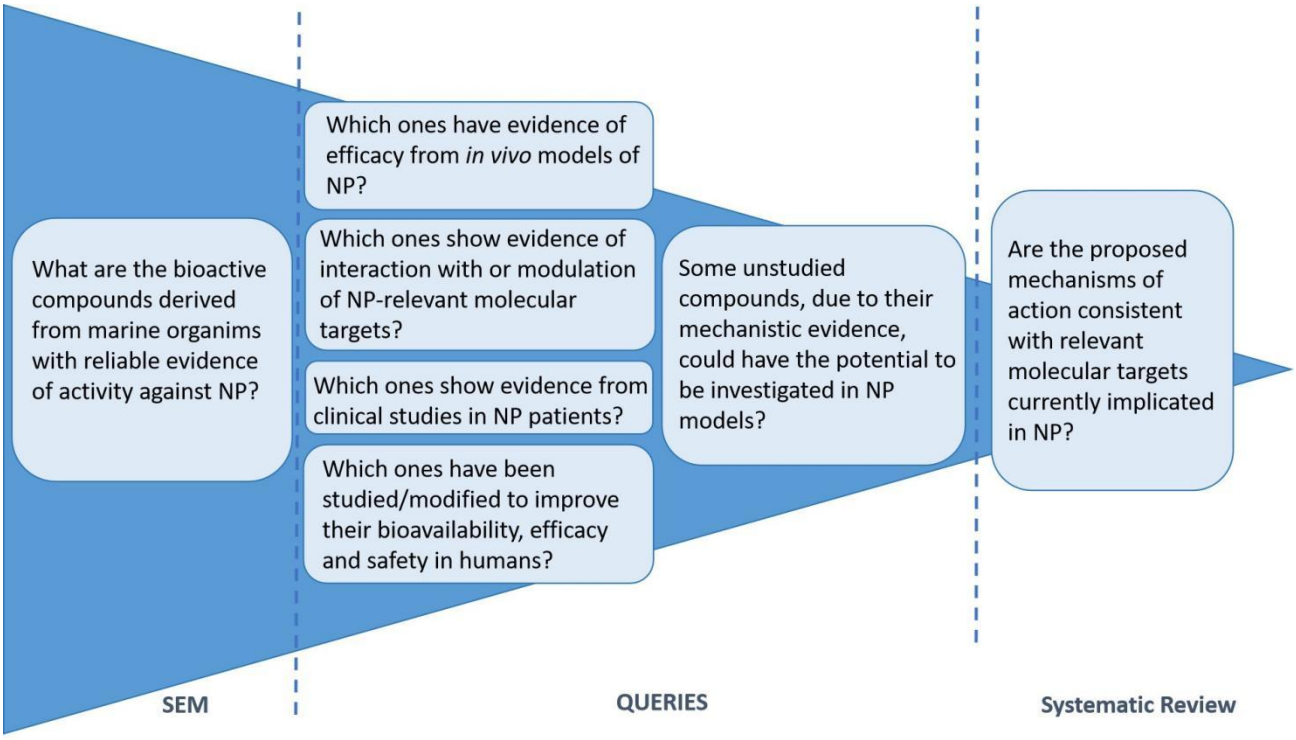

Figure 2. The process of identifying trends and exploring the evidence landscape through queries of the Systematic Evidence Map (SEM) database.

The figure illustrates the scope of the research questions—with an increasingly specific focus—addressed at each stage of data exploration.

Qualitative analysis and data mining

A table was created in Microsoft Excel® that listed the variables extracted from the articles uploaded to the Rayyan program: article title, authors, year, article type, journal, keywords, and abstract. This information was reviewed in depth and, if necessary, complemented by reading the full text of the article to exclude documents that did not meet all the inclusion criteria. Once the articles were chosen, another table was created in Excel, which, in addition to the previous fields, contained the following: study design preclinical (*in vivo* and/or *in vitro* and/or *in silico*) or clinical, aim, approach and significant main results of study, marine product used, type (isolated compound, extract, fraction or synthetic equivalent/analogue, marine species scientific name and family which were verified on World Register of Marine Species (WoRMS) website: (www.marinespecies.org), molecular target/signaling pathways/cellular process towards which the mechanistic hypothesis was directed, experimental NP model, damage, other models, animal species, strain of the animals, sex, pain outcomes measures, randomization, blinding, sample size calculation, laboratory environmental, reporting quality, replication, interventions [doses (dose-response relationship), routes], controls, exposure time, experimental methods, target engagement, type of neuropathic pain (peripheral or central), ethiopathological condition, adverse effects.

To identify trends and explore the evidence landscape—once the data have been extracted and the information from the literature coded—it was possible to explore the SEM through a series of queries with an increasingly specific focus (Fig. 2). These data were used for further analysis in order to explore the validity of the results. Additionally, a basic complementary statistical analysis of the variables was carried out, including qualitative analysis, using graphs generated in Microsoft Excel 2010. The results were expressed as frequencies and percentages.

Three domains—encompassing 22 aspects that affect the reliability of preclinical or clinical research—were considered to qualitatively assess the strength of the evidence, accounting for differences by study type. Domain 1 (A1-A5) addressed methodological aspects and reporting quality to detect potential experimental bias. These include randomization, blinding, sample size calculation, protocol availability or reporting that includes all expected outcomes, and laboratory environmental factors affecting pain sensitivity. Domain 2 (A6-A20) addressed aspects relevant to clinical translation, involving the refinement of NP models and outcome measures, mechanisms of action, and the efficacy and safety of the compounds. These include sex balance, dose-response relationship, treatment regimen (repeated doses), route relevance, positive control, safety outcome and/or health status, species

translation, target engagement, prediction-based mechanistic evidence (bioinformatic, computational to predict potential binding site, and mechanistic modeling), experimental mechanistic evidence, number of *in vivo* NP models, clinically relevant model/outcome measure, pharmacokinetic (PK) characterization, and replication. Domain 3 (A21-A22) addressed aspects related to strategies for improving effectiveness in humans such as potency, selectivity, stability, and oral bioavailability [chemical modifications and/or knowledge for developing improved synthetic analogs, analog pharmacological or functional evaluation (A21)], as well as progression to clinical studies (A22). These variables and the frequency of research on each marine product were incorporated into a heat map. After assessing the reliability of the evidence, a hierarchy was categorized as: preliminary, moderate preclinical, strong preclinical, and clinical (Fig. 3).

Preliminary: Results only from *in vitro* assays. Evidence provided exclusively from *in vivo* studies reporting activity in one validated NP models/outcomes without experimental mechanistic evidence and/or bioinformatic predictive studies (pharmacological families, functional annotation). Evidence could primarily come from studies evaluating extracts of marine organisms.

Moderate preclinical: Preclinical evidence provided by studies using validated NP models/outcomes that mainly simulate traumatic peripheral neuropathy, with *in vivo* efficacy and/or safety evidence, but generally using a single regimen of administration to determine the time-response course. They may or may not provide relevant routes or repeated regimens of administration and evidence of cross-species translation. Experimental mechanistic evidence (*in vitro* target engagement or functional modulation, *in vivo* mechanistic analysis, causal validation) could be associated with computational predictions combining several more robust lines of computational evidence. Therefore, in addition to bioinformatic annotation, docking and molecular dynamics simulations (MDS) studies are incorporated. Additionally, evidence of chemical modifications and/or information to develop improved synthetic analogs and their functional or pharmacological characterization could be provided.

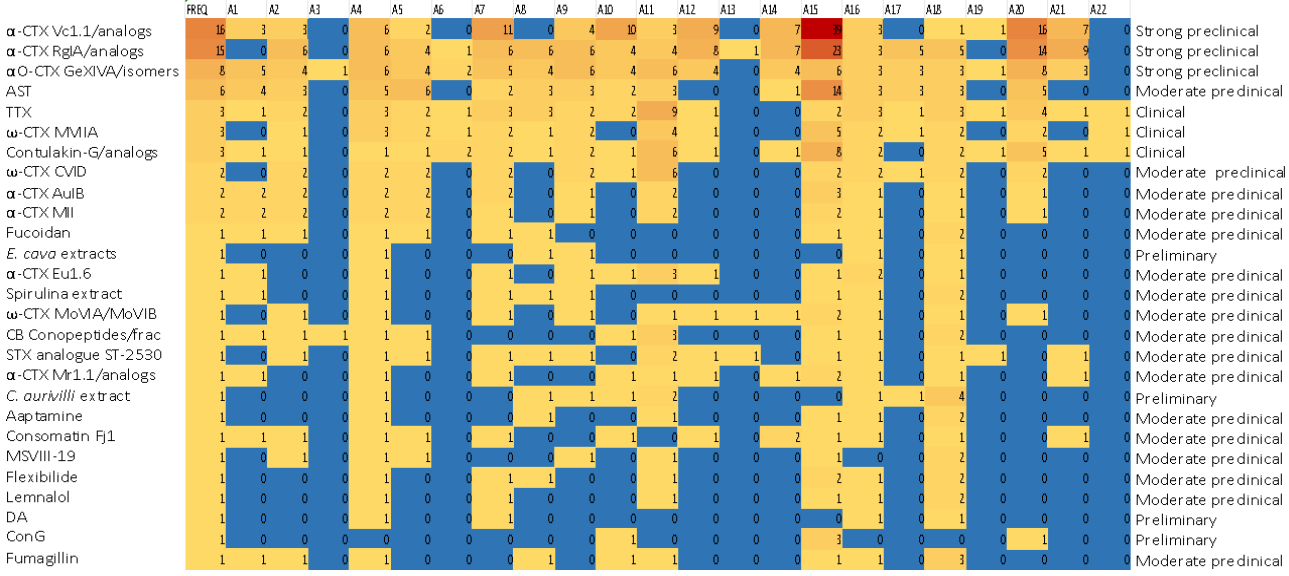

Figure 3. The heat map reflects frequency values for the variables considered in estimating the strength of evidence for each marine-derived product investigated for neuropathic pain treatment.

These variables include aspects impacting the reliability (A1-A5) and translational potential (A6-A22) of the evidence. It also includes the frequency with which each isolated compound, synthetic equivalent, extract or fractions was investigated. Aspects: randomization (A1), blinding (A2), sample size calculation (A3), reporting quality (A4), laboratory environmental factors affecting pain sensitivity (A5), sex balance (A6), dose-response relationship (A7), repeated regimen (A8), route relevance (A9), positive control (A10), safety outcome and/or health status (A11), species translation (A12), target engagement (A13), prediction-based mechanistic evidence (A14), experimental mechanistic evidence (A15), neuropathic pain *in vivo* models (A16), clinical relevant model (A17), clinical relevant outcome measure (A18), pharmacokinetic study (A19), replication (A20), strategies for improving effectiveness in humans/chemical engineering and/or knowledge for developing improved synthetic analogues (A21), candidate progression to clinical studies (A22). The hierarchy was categorized as: preliminary, moderate preclinical, strong preclinical and clinical.

Strong preclinical: Preclinical evidence from studies with better methodology and reporting quality, *in vivo* efficacy and safety evidence using one or various clinically relevant NP models/outcomes, positive controls, relevant route and regimen of administration, as well as species translation. Robust experimental mechanistic evidence (*in vitro* target engagement or functional modulation, *in vivo* mechanistic analysis, causal validation), which could be associated with bioinformatic and/or computational predictions of the binding site and mechanistic modeling to guide and strengthen the mechanistic research. PK characterization and sex balance are criteria that strengthen this category. Evidence of strategies to reduce limitations, whether modifications and/or information to develop improved analogs that are well characterized and have the potential to advance to clinical phases.

Clinical: Clinical evidence from exploratory interventional clinical studies or different phases of clinical development (Phase I to Phase IV).

The criteria used to define each aspect are specified in Annex 1. Interpretations were formulated regarding the translational perspective of these marine-derived bioactive compounds; however, the proposed priorities represent expert synthesis of the available evidence rather than a formal ranking process.

Article quality analysis

Risk of bias was assessed using tools appropriate to study design. Animal intervention studies were evaluated with SYRCLE's risk-of-bias tool (Hooijmans et al., 2014). Randomized clinical trials (RCTs) were evaluated with the revised Cochrane risk of bias tools: RoB 2, version 22 Aug 2019 (Sterne et al., 2019), and nonrandomized clinical studies with version 2 of the ROBINS-I tool (ROBINS-I V2) for follow-up (cohort) studies, 20 Nov 2025 (www.riskofbias.info). *In vitro* and *in silico* studies were appraised using predefined criteria addressing target validation, reference controls, concentration-response assessment, assay reproducibility, and species relevance. The certainty of evidence was summarized by compound and target, considering study design, replication, model relevance, dose-response, blinding, randomization, safety assessment, and translational consistency (Fig. 3).

Protocol deviations

During the screening process, it was identified that some studies provided analgesic activity and relevant mechanistic evidence using experimental models that did not correspond to classic NP models but exhibited characteristics of nerve injury. To improve consistency in selection, the inclusion criterion (i) was refined to allow the inclusion of these studies when they provided experimental evidence on molecular targets relevant to NP. Regarding criterion (ii), the wording already included a clarification of the pivotal role played by *in vitro* and *in silico* studies providing mechanistic evidence, whereas evidence from other non-neuropathic pain models—due to a mix of these studies—would serve only a complementary function. With the aim of reducing discrepancies between reviewers and improving the applicability of these criteria, it was decided to revise the wording to reduce ambiguity. Likewise, it was identified that the comparison criteria could not be realistically applied according to the eligible evidence, in particular to all *in silico* , clinical, or mechanistic studies. Therefore, these exclusion criteria were refined by taking into account the study design. These refinements did not alter the research questions or the objectives of the review. Instead, they enrich the synthesis of the mechanisms of action of marine bioactive compounds in the context of nerve injury.

RESULTS

Selection of sources of evidence and study designs

The database search identified 1445 records: 265 from PubMed, 287 from Web of Science, 429 from Scopus, and 464 from ScienceDirect. After removal of 714 duplicates, 731 records were screened by title and abstract. Of these, 526 were excluded, and 205 full-text articles were assessed for eligibility. A further 112 articles were excluded according to the predefined criteria. After quality assessment, 91 articles were included in the qualitative synthesis. Fig. 1 details the information processing and stages involved in the systematic literature review. Data obtained from the 91 articles chosen were used to construct the SEM database.

A broad subset of the evidence base addressing various specific research questions was compiled. Consequently, different topics were identified for which sufficient data exist to conduct a systematic review. Fig. 2 illustrates the scope of the research questions addressed at each stage of data exploration. Upon exploring the SEM database, four trends were identified regarding the evidence provided in the studies: (group 1) studies that provide *in vivo* preclinical evidence of analgesic activity in models of NP or other experimental models with evidence of nerve injury demonstrated (n = 43), (group 2) studies providing preclinical evidence of interaction with or modulation of a potential target involved in NP, mostly derived from *in vitro* and/or *in silico* research (n = 25), (group 3) studies that provide clinical evidence of the analgesic activity and safety in NP patients (n = 3), (group 4) studies that provide strategies to reduce limitations and improve efficacy and safety in humans (n = 20). As is typical in the complex, long, and rigorous process of drug discovery,

the majority (97%) of the evidence comes from preclinical research, while only 3% comes from clinical studies in which three bioactive compounds including contulakin-G, MVIIA (Ziconotide), and tetrodotoxin (TTX), were tested in patients with NP (Brinzeu et al., 2019; Goldlust et al., 2021; Sang et al., 2016) (Fig. 4). The most significant aspects of the design and results of these studies which translate some preclinical results into patients with NP are summarized in Table 1.

In preclinical studies, most authors (49%) employed mixed designs combining *in vivo*, *in vitro,* and *in silico* procedures. Meanwhile, 30% of the studies were designed exclusively *in vivo*, 18% *in vitro*, and 3% *in silico*. As shown in Fig. 4, *in vivo* studies—and their combinations with *in vitro* and/or *in silico* procedures—were observed most frequently in group 1, whereas *in vitro* studies predominated in group 2, and *in vitro*/*in silico* mixed studies in group 4. The main preclinical pharmacological findings and some interesting strategies for optimizing marine-derived bioactives are discussed in the following subsections and compiled in Tables S1, S2 and S3.

Some bioactives across this broad timeline have already advanced in their research and development phases, such as α-conotoxins Vc1.1 and RgIA (Callaghan & Adams, 2010; Cuny et al., 2012). In particular, some compounds or extracts reported from group 2 and group 4 show mechanistic evidence involving a potential NP-related target; however, their activity has not been reported in *in vivo* models of NP but rather—for the most part—in algesimetric tests or other models that do not mimic NP. Additionally, one study provided relevant mechanistic evidence using an experimental model that did not correspond to a classic NP model but exhibited characteristics of peripheral nerve injury (Chen et al., 2019). Nevertheless, based on the evidence, evaluation in animal models that mimic clinical manifestations, regardless of peripheral or central origin, is recommended. Throughout this review, these marine-derived bioactives are identified as potential candidates for future research in relevant NP models (Table 2).

Overall, most studies showed positive results, whereas only one preclinical study reported negative results when evaluating changes in mechanical sensitivity in a model mimicking comorbid NP and depressive disorders; however, that study reported significant results for depressive-like behavioral outcomes (Lax et al., 2018).

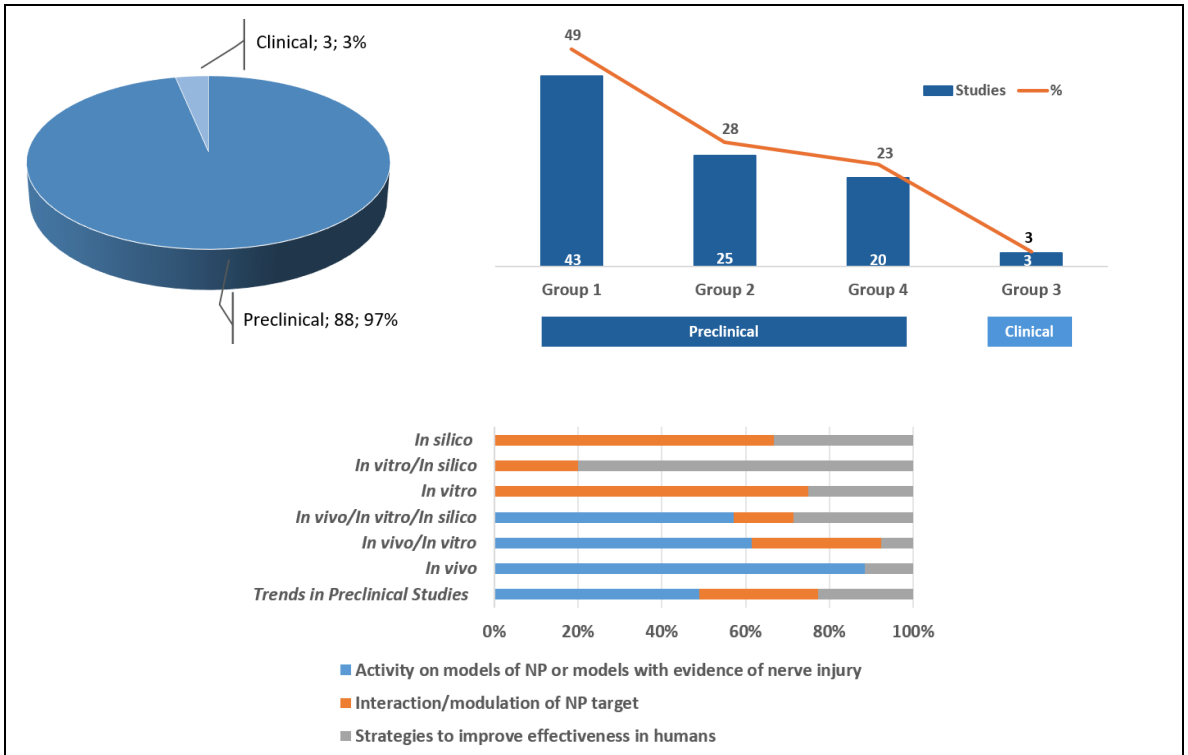

Figure 4. Trends identified while exploring the SEM database regarding the evidence provided in the studies.

Group 1: studies that provide *in vivo* preclinical evidence of analgesic activity in models of neuropathic pain (NP) or other experimental models with demonstrated nerve injury (n = 43). Group 2: studies providing preclinical evidence of interaction with or modulation of a potential target involved in NP, mostly derived from *in vitro* and/or *in silico* research (n = 25). Group 3: studies providing clinical evidence of analgesic activity and safety in NP patients (n = 3). Group 4: studies that provide strategies to reduce limitations and improve efficacy and safety in humans (n = 20). The majority of the evidence comes from preclinical studies, which predominantly used mixed designs combining *in vivo*, *in vitro*, and *in silico* procedures. The horizontal bar chart at the bottom shows the distribution of design types by group.

Table 1. Clinical evidence of the marine-derived bioactive analgesic activity and safety in neuropathic pain patients.

Bioactive	Species	Target/ signaling pathways/cellular process	Type of clinical study/sample	Outcomes/doses/routes/positive control	Significant findings	Author and year	Overall risk of bias
Contulakin-G (CGX-1160, linear O-glycosylated synthetic conopeptide)	*Conus geographus*	NTSR 2 in the spinal cord	Phase IA open-label, dose-escalation study in central NP patients after SCI (n = 4, first human clinical study of CGX-1160)	Safety: [adverse effects, neurological examination (MMSE), Cognitive function (LNS, SSS), vital signs, physical examination, clinical laboratory tests] Efficacy: Intensity of spontaneous and evoked pain (change in Gracely score) Pharmacokinetic: Serum and cerebrospinal fluid (CSF) concentrations Route: IT (Continuous Infusion System) Doses: 3 consecutive escalating doses starting at 25 µg/h over 6 hours	- 6-hour infusion of CGX-1160 (up to 1000 µg/h) is safe and may reduce pain intensity -The dose-limiting toxicity was diarrhea (MTD = 900 µg/h over 6 hours) -Analgesic Efficacy (Maximal reduction from the baseline pain intensity was 63%) -CGX-1160 shows a rapid redistribution phase followed by a slower terminal elimination phase in CSF. Peak analgesic effect occurred after the peak intrathecal concentration -The addition of the effect site compartment to the PK model improved its ability to represent the concentration and effect profiles, which implies that the compound needs to circulate from the site of IT administration within the CSF before drug effect is measurable	Sang et al., 2016	**Critical**
ω-Conotoxin MVIIA (Ziconotide)	*Conus magus*	N-type VGCCs	A prospective cohort study (Pilot study) in pharmaco-resistant NP related to SCI patients (n = 20, First study in a specific homogeneous population without other available treatments)	Efficacy: a decrease in VAS score of 40% or more; 11 patients were followed up for around 3.59 years Route: IT -Boluses (Lumbar puncture injection test) at 72-hour intervals, with progressively increasing dosages of 0.5, 1.0, 1.5 µg diluted in 2 ml of saline -Implanted intrathecal infusion pump (2 to 10 µg maximum per day with an increment of 1 µg every 3 days (Average effective dose of 7.2 µg/day) Safety: Adverse effects, laboratory tests	-Over half of the 20 patients (55%) responded to the test, and 40% (8 of 20) benefited from long-term treatment -15% severe adverse effects during the test phase (Too rapid titration) -This data justifies the development of a long-term RCT: * Protocol of Multicenter, randomized, comparative, placebo-controlled, double blind clinical trial (crossover of random alternating periods of 6 months) in severe refractory NP due to SCI (n = 44) (NCT03942848) Efficacy and Safety: Pain intensity vs. placebo. Continuous evaluation of pain intensity, % of patients with at least 30% pain reduction, patient satisfaction level, adverse effects, quality of life, spontaneous and provoked pain (Brinzeu et al., 2024).	Brinzeu et al., 2019	**Critical**

Table 1. Clinical evidence of the marine-derived bioactive analgesic activity and safety in neuropathic pain patients (continued...)

Bioactive	Species	Target/ signaling pathways/cellular process	Type of clinical study/sample	Outcomes/doses/routes/positive control	Significant findings	Author and year	Overall risk of bias
Tetrodotoxin (TTX) (NCT01655823)	*Tetraodontidae* Pufferfish (genus Fugu)	Na_V channels TTX-S/TTX-R	Phase II randomized, double-blind, dose-finding multicenter study in patients with taxane- or platinum-related CIPN (moderate to severe) (n = 125) (A population with limited therapeutic options)	NPRS score during the 4-day treatment period, as well as Days 7, 14, 21, and 28. QOL, SF-36 post-treatment. Route: s.c. Doses: (7.5 µg twice daily, 15 µg twice daily, 30 µg once daily, and 30 µg twice daily) for 4 consecutive days vs. Placebo Safety: Adverse effects	-Changes in mean NPRS score were not statistically different between cohorts, due to small trial size and influence of a few robust placebo responders. -Analgesic signal at TTX 30 µg twice daily (after cumulative responder analysis) -Most AE were mild or moderate (oral paresthesia and hypoesthesia)	Goldlust et al., 2021	**Some Concerns**

Table 2. Potential candidates for future research in neuropathic pain models.

No.	Bioactive	Target/ signaling pathways/cellular process	Experimental models	Concentration/ dose-response relationship	Route/regimen relevance	Safety	Positive control	Species relevance	Mechanistic evidence	Author and year
1	*Thalassia testudinum* crude extract (BM-21) and isolated thalassiolin B	ASICs	*In vitro*: rat DRG neurons EF *In vivo*: hot plate test/formalin test/writhing test	Yes No, increasing doses	Yes, p.o./single	Yes/Motor performance	Yes/Yes	No	Experimental Target modulation	Garateix et al., 2011
2	Sea anemone toxin APETx2	TTX-R (Na_V 1.8 channels)	*In vitro*: DRG neurons and *Xenopus laevis* oocytes EF	Yes	NA	No	Yes	Yes, hNa_V 1.8	Experimental Target modulation	Blanchard et al., 2012
3	µO-Conotoxin, MfVIA synthetic and its analog (E5K,E8K)MfVIA	Na_V1.8	*In vitro*: FLIPR Ca^{2+} assay in SH-SY5Y/Membrane potential assay in HEK293, ND7/23 and CHO cells *Xenopus leavis* oocytes, HEK293 and CHO, and DRG neurons EF Functional Na_V activity and lipid membrane binding affinity	Yes	NA	No	No	Yes, hNa_V1.8	Experimental Target modulation	Vetter et al., 2012 Deuis et al., 2016

Table 2. Potential candidates for future research in neuropathic pain models (continued...)

No.	Bioactive	Target/ signaling pathways/cellular process	Experimental models	Concentration/ dose-response relationship	Route/regimen relevance	Safety	Positive control	Species relevance	Mechanistic evidence	Author and year
4	Conantokins (ConPr1, ConPr2, and ConPr3)	GluN2B-containing NMDARs / Neuroprotection	*Ex vivo*: Rat hippocampal and mouse cortical neurons' EF	No	NA	No	Yes	No	Experimental Target modulation	Kunda et al., 2013
5	Ugr 9-1 peptide	ASIC-3	*In vitro*: *X. laevis* oocytes EF *In vivo*: Acetic acid writhing test/CFA model	Yes	Yes, IV/single	Yes, Crayfish toxicity/ Motor performance	No	Yes, hASIC3	Experimental Target modulation	Osmakov et al., 2013
6	*Sargassum thunbergii* ethanolic extract	Neuroinflammation	*In vitro*: LPS-stimulated BV-2 microglia	No/increasing concentrations	NA	No	No	No	Experimental Target modulation	Lee & Kang, 2015
7	Kunitz-Type Peptide HCRG21	TRPV1	*In vitro*: *X. laevis* oocytes EF *In silico*: HCRG21–TRPV1 homology modeling and docking	Yes	NA	Yes (hERG K+ channel)	Yes	No	Experimental Target modulation Prediction computational	Monastyrnaya et al., 2016
8	μ-Conotoxin TsIIIA	TTX-R Na_v ($Na_v1.5$, $Na_v1.8$, $Na_v1.9$)	*In vitro*: rat DRG neurons EF *In vivo*: Hot plate test	Yes	Yes, IV/single	No	Yes	No	Experimental Target modulation	Yang et al., 2017
9	Peptide Ms 9a-1	TRPA1	*In vitro*: Fluorescent Ca^{2+} influx in CHO-rTRPA1 cells and rat DRG neurons *Xenopus laevis* oocytes EF *In vivo*: Ms 9a-1 induces pain or thermal hyperalgesia/Hot plate test/allyl isothiocyanate (AITC)-induced pain and inflammation/CFA model/ capsaicin test/Paw edema *In silico*: Computation and structure modeling	Yes	Yes, IV/single	Yes, locomotor activity	No	No	Experimental Target modulation Prediction computational	Logashina et al., 2017; 2022

Table 2. Potential candidates for future research in neuropathic pain models (continued...)

No.	Bioactive	Target/ signaling pathways/cellular process	Experimental models	Concentration/ dose-response relationship	Route/regimen relevance	Safety	Positive control	Species relevance	Mechanistic evidence	Author and year
10	*Leptolyngbya* extract fractions (DUQ0002I)	5-HT7R	*In vitro:* Radioligand binding assay (5-HTRs and the 5-HT transporter (SERT) *In vivo:* SNI rats with comorbid depression/Murine depression and anxiety assays	No	No, ICV or CA1/single	Yes, locomotor activity	No	No	Experimental Target engagement	Lax et al., 2018
11	Butyrolactone-I (ZB5-1)	Neuroinflammation NFκB pathway	*In vitro:* BV-2 murine microglial cells *In silico:* Docking of ZB5-1 with the active site of NF-κB	No/increasing concentrations	NA	Yes, cytotoxicity (MTT assay)	No	No	Experimental Target modulation Prediction computational	Zhang et al., 2018
12	CoM22, RtM11 ArL1, ArD1, ArI110 LiA58, ArI21 ArO130, LiM43, LiT8 LiO32, ArCII1 ArCII16, ArCII12	NMDAR AMPAR nAChR (α) Na_V (ι) K_V (κ) α1-adrenoceptors (ρ)	*In silico*: Identification of pharmacological families of the CTXs of 11 species of cone snails collected in Bali/ConoServer (https://www.conoserver.org/). Protein modeling, prediction, and analysis of the representative mature toxin sequences/Phyre2 server (https://www.sbg.bio.ic.ac.uk)	NA	NA	NA	NA	NA	Bioinformatic prediction	Sudewi et al., 2019
13	Fucoxanthin	TRPV1 Neuroimmune activation Neuroinflammation	*In vivo*: Ultraviolet B radiation (UVB)-induced photokeratitis with inflammatory pain and a neuropathic component (mixed trigeminal pain)	No/increasing doses	Yes, p.o./ repeated	No	No	No	Experimental Target modulation	Chen et al., 2019
14	μ-Conotoxin TsIIIA	Na_V.1.8	*In vitro*: HEK293 or ND7/23 cells subtypes EF	Yes	NA	No	No	Yes, hNa_V1.1-hNa_V1.8	Experimental Target modulation	Yang & Zhou, 2020
15	Conotoxin-Ac1 and its variant conotoxin-Ac1-O6P	NMDA receptor subunit 2B (NR2B)	*In vitro*: Rat hippocampal neuron cells and HEK293 cells EF *In vivo*: Hot plate test and tail-flick test	Yes No/increasing doses	No	No	Yes	Yes, hNR2B	Experimental Target modulation	Liu et al., 2020a

Table 2. Potential candidates for future research in neuropathic pain models (continued...)

No.	Bioactive	Target/ signaling pathways/cellular process	Experimental models	Concentration/ dose-response relationship	Route/regimen relevance	Safety	Positive control	Species relevance	Mechanistic evidence	Author and year
16	δ-Conotoxin TxVIA	T-Type VGCC ($Ca_V3.2$)	*In vitro*: FLIPR Cell-based assays in SH-SY5Y, HEK293 and HEK293T cells EF *In vivo*: Zebrafish bioassays	Yes	Yes, IM	Yes, adverse effects (pain-related behaviors or paralysis)	No	Yes, hCa_V3	Experimental Target modulation	Wang et al., 2020
17	Native and synthetic conosteroids A–E	$GABA_AR$ (neurosteroids)	*In vitro*: Calcium imaging in DRG neurons HEK cells EF *In vivo*: Hot plate test/formalin test	Yes No, increasing doses	Yes, i.p. (peptide peripherally injected)	Yes, locomotor activity/anxiety tests (potential adverse effect)	Yes	Yes, $hGABA_A$ subtypes	Experimental Target modulation	Niu et al., 2021
18	Tunicate metabolite 2-(3,5-Diiodo-4-methoxyphenyl)ethan-1-amine (DIMTA)	$K_{Ca}1.1$, $K_V3.1$, and $K_V11.1$ *Cell-type selectivity vs. molecular target selectivity	*In vitro*: mouse DRG neurons, *Xenopus laevis* oocytes expressing channels, and HEK-293 cells EF *In vivo*: Naive CD1 mice. Cold Plate Assay	Yes	No	Yes, hERG K^+ channel	Yes	Yes, hKV7.2, hKV3.1, and hKCa1.1	Experimental Target modulation	Paguigan et al., 2021
19	Consomatin Ro1	SSTR4 and SSTR1	*In vitro*: Panel of GPCRs/ PRESTO-Tango β-arrestin recruitment assay *In vivo*: Tail flick test/ Acute post-surgical pain model	Yes	No	Yes, fish bioassays (swimming and breathing)	Yes	Yes, hSSTRs	Experimental Target modulation	Ramiro et al., 2022

Table 2. Potential candidates for future research in neuropathic pain models (continued...)

No.	Bioactive	Target/ signaling pathways/cellular process	Experimental models	Concentration/ dose-response relationship	Route/regimen relevance	Safety	Positive control	Species relevance	Mechanistic evidence	Author and year
20	α-Conotoxin PeIA and its analogue PeIA[S4Dap, S9Dap]	G protein-coupled $GABA_B$/$Ca_V2.2$ and GIRK channels α9α10 nAChR	*In silico*: Predictions using computational peptide docking and MD simulations *In vitro*: HEK293T cells coexpressing $hGABA_BR$ (WT and 15 $GABA_BR$ mutants) and $hCa_V2.2$ EF/mouse DRG neurons and HEK293T cells co-expressing either heteromeric hGIRK1/2 or homomeric GIRK2 subunits, with $hGABA_BR$ (GABA1 and GABA2 subunits)/ *X. laevis* oocytes expressing hAChRs EF	Yes	NA	No	Yes	Yes, human isoforms	Computational prediction Experimental Target modulation	Bony et al., 2022a, 2022b Li et al., 2024
21	Peptide toxins (from tentacles, column, and mesenterial filaments)	Na_V channels, K_V channels, ASIC, TRPV1, and TRPA1	- Transcriptome analysis (HTS technology), RNA extraction *In silico*: Homology modeling prediction and phylogenetic analysis	NA	NA	NA	NA	NA	Bioinformatic prediction	Guo et al., 2024

NA: not applicable; EF: electrophysiology.

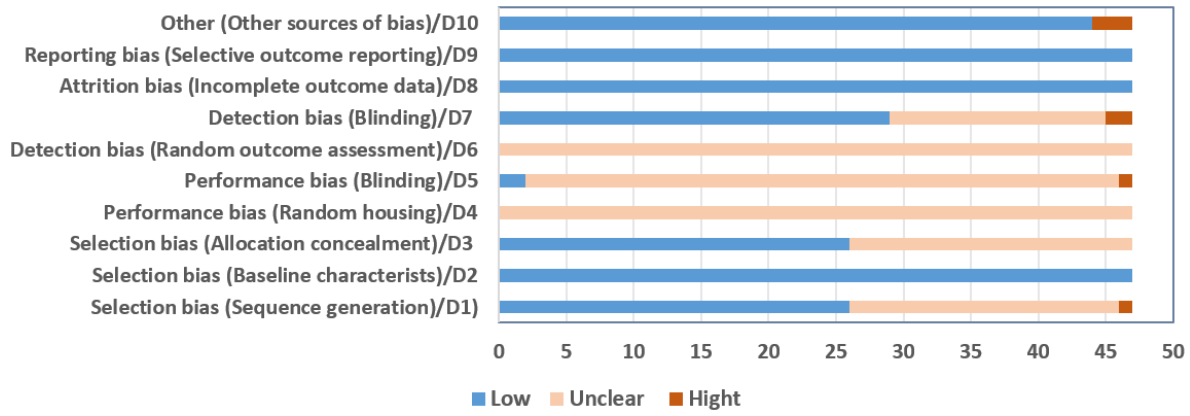

Figure 5. Risk of bias summary plot from the SYRCLE assessments of animal intervention studies (number of studies).

*Assessment of animal intervention studies relevant for mechanistic evidence (n = 47) including all studies from Group 1 (n = 43) and some from Group 4 (n = 4). This count does not include studies that used other non-neuropathic pain models due to their mixed designs, which serve only a complementary function regarding the evidence.

Risk of bias in studies

Forty-seven animal intervention studies were evaluated using the SYRCLE tool. Risk-of-bias judgments varied across domains, a low risk was consistently observed for baseline characteristics (D2, 100%), incomplete outcome data (D8, 100%), selective outcome reporting (D9, 100%), other potential sources of bias (D10, 94%), outcome assessor blinding (D7, 62%), and sequence generation and allocation concealment (D1 and D3, 55% each). High-risk judgments were uncommon, being identified in only 2% of studies for sequence generation, 2% for blinding of caregivers/investigators, 4% for blinding of outcome assessors, and 6% for other sources of bias. In contrast, substantial uncertainty was observed for random housing (D4, 100%), blinding of caregivers/investigators (D5, 94%), and random outcome assessment (D6, 100%); allocation concealment (D3, 45%) and outcome assessor blinding remained unclear in 34% of studies. Although several domains were frequently rated as low risk, incomplete reporting of random housing, caregiver blinding, and random outcome assessment limited confidence in the animal evidence (Fig. 5).

Although Poth et al. (2021) and Zhu et al. (2022) evaluate marine-derived peptides in animal models, they are primary PK studies rather than intervention studies. Hence, the SYRCLE domains measuring randomization and blinding could not be assessed and were read as "unclear". The quality of these studies was further evaluated by considering methodological aspects specific to PK studies that are not covered by this tool—specifically: animal selection, sample size, sampling strategy, dosage and route of administration, reported validated bioanalytical method and the PK analysis method, reported parameters (plasma AUC_{0-inf}, C_{max}, $t_{1/2}$, bioavailability), protein binding, and tissue distribution. In Zhu et al. (2022), the requirements for the preclinical population PK model (PopPK) and mechanistic PK-pharmacodynamic (PD) model were considered, in addition to experimental PK. All *in vitro* and *in silico* studies met the predefined quality criteria for their evaluation.

As shown in Table 1, the non-randomized clinical studies by Sang et al. (2016) and Brinzeu et al. (2019) presented a critical risk of bias for the analgesic efficacy outcomes, mainly due to the absence of a suitable comparator and thus inadequate control for confounding. Additionally, Brinzeu et al. (2019) had a significant risk of selection bias. Limitations related to pain measurement and potential selective reporting of outcomes added further uncertainty. Therefore, these studies were included in the review, as their results should be interpreted primarily as exploratory or hypothesis-generating evidence. Some details of the rationale for judgment are discussed. Meanwhile, in the study by Goldlust et al. (2021), the randomization sequence, blinding of participants and personnel, and availability of data for the primary outcome were considered at low risk of bias. The pre-specified primary outcome showed no statistically significant difference between the groups, but the authors clearly identified several post-hoc analyses as exploratory, including responder analysis and analysis conducted after excluding data from a site with a high placebo response. Although these analyses were clearly distinguished from the planned analyses, their use to explore a potential dose-response relationship and support future studies raised concerns about the selection of reported outcomes and analyses. Consequently, the study raised concerns about bias, particularly regarding the selection of reported outcomes.

Chemical diversity of marine-derived bioactives

Throughout the SEM database, 67% of the authors studied equivalents or analogs of marine-derived compounds obtained via chemical synthesis, while 4%

studied equivalents obtained through biological synthesis. Meanwhile, 10% studied natural compounds isolated from these organisms. Additionally, 7% evaluated the activity of natural extracts or their fractions. Other authors combined the study of isolated natural compounds and synthetic ones (1%) or extracts and isolated compounds (2%). In 8 studies (9%), the origin of the compounds investigated was not specified (Fig. 6A).

During the period under analysis, a diversity of 27 isolated natural molecules and/or their synthetic equivalents or analogs and natural extracts were directly investigated for NP treatment, mostly compounds of peptidic nature. Previously identified α-conotoxins (CTXs) stood out primarily due to the frequency with which they were studied. Vc1.1 and/or its analogs were investigated in 16 studies (21%), while RgIA and/or its analog RgIA4 were investigated in 15 studies (19%). Additionally, AuB and MII were evaluated in 2 studies each, and Mr.1.1 and its analogs in 1 study. αO-conotoxin GeXIVA and/or isomers were reported in 8 articles (10%), followed by the marine ketocarotenoid astraxanthin (AST) in 6 studies (8%), the guanidinium toxin tetrodotoxin (TTX) in 3 studies (4%), as well as ω-conotoxin MVIIA and contulakin-G or analogs, which were investigated in 3 studies each (4%, respectively). Other ω-conotoxins (CVID, MoVIA and MoVIB), a novel α-conopeptide Eu1.6, consomatin Fj1; ST-2530, a synthetic saxitoxin (STX) analog and non-peptide molecules [lemnalol, fucoidan, aaptamine, flexibilide, synthetic amino acid dysiherbaine (MSVIII-19), synthetic dihydroaustrasulfone alcohol (DA), and fumagillin] were also reported. Although conantokin-G (conG) was not evaluated *in vivo*, it was included in the analysis because mechanistic *in vitro* studies have continued during this period (Balsara et al., 2012), and its antiallodynic effect in a validated model of NP had been previously demonstrated (Malmberg et al., 2003). Furthermore, 4 studies evaluated extracts or their fractions from *Ecklonia cava*, *Conus* snails, *Spirulina platensis*, and *Cliothosa aurivilli* (Ali Safakhah et al., 2018; Karmakar et al., 2022; Jergova et al., 2021; Kim et al., 2014) (Fig. 6B).

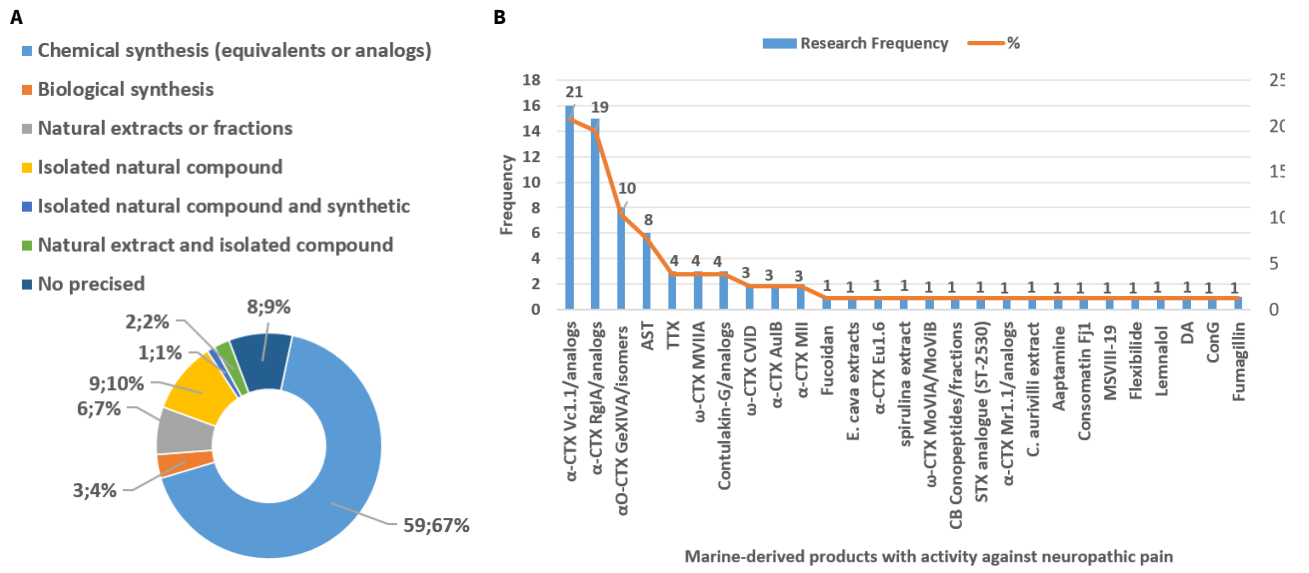

Figure 6. Chemical diversity of marine-derived products.

(A) Type of marine product (isolated natural molecules and/or their synthetic equivalents or analogs and natural extracts/fractions) investigated throughout the SEM database. Most authors studied equivalents or analogs obtained via chemical synthesis. The denominator comprises the articles reporting experimental or clinical investigations of marine-derived products (n = 88), rather than the total number of included studies (n = 91). Three studies were excluded from this analysis because they did not directly investigate the products experimentally or clinically: two relied exclusively on *in silico* approaches, whereas one combined transcriptomic and *in silico* analysis. Articles covering different types of marine-derived products were assigned to the corresponding combined categories, where applicable. **(B)** Distribution of marine-derived products according to their frequency of investigation for neuropathic pain treatment. The denominator represents the total number of marine products targeting NP reported (n = 77), rather than the number of included studies investigating them (n = 70), because some studies reported more than one product. This analysis excluded studies reporting marine products identified through this review as potential candidates for future research in neuropathic pain models (n = 21).

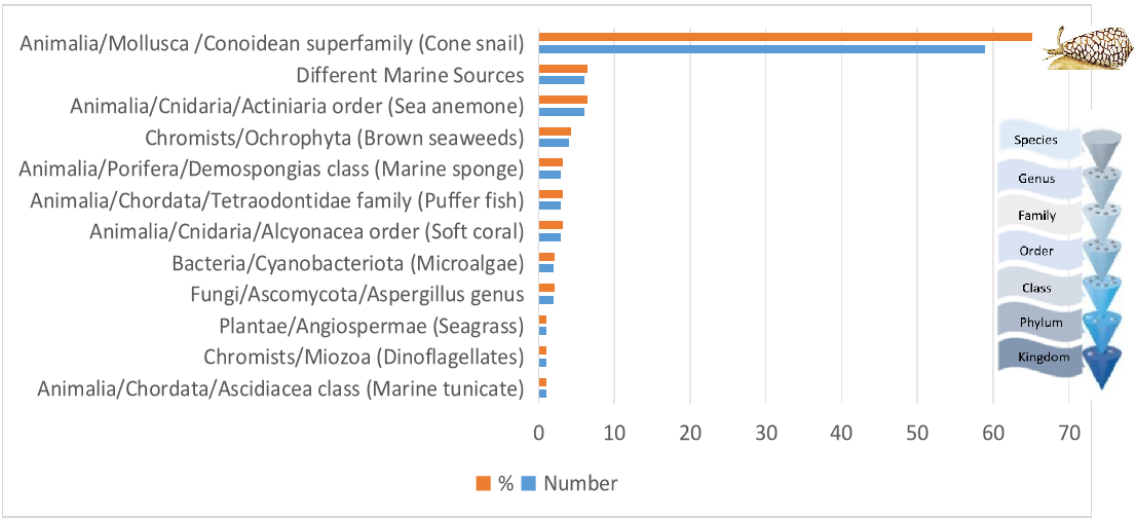

Figure 7. Marine organisms as a source of bioactive compounds investigated throughout the SEM database.
The species were grouped by kingdom, phylum, class, or order, based on Linnaean taxonomy.

Additional molecules and extracts with interesting mechanisms and potential for investigation include ConPr1-3, μO-conotoxin MfVIA, δ-conotoxin TxVIA, fucoxanthin, butyrolactone-I, μ-conotoxin TsIIIA, *Thalassia testudinum* crude extract and thalassiolin B, *Sargassum thunbergii* ethanolic extract, extract fractions from *Leptolyngbya*, conosteroids from *Conus geographus*, α-conotoxin PeIA, tunicate metabolite DIMTA, and sea anemone peptides (APETx2, Ms 9a-1, Ugr 9-1, HCRG21) among others (Table 2).

Marine organisms as a source of promising drug leads for neuropathic pain

The most significant findings regarding *in vivo* pharmacological efficacy in relevant animal models of NP, and in clinical reports, describe the pharmacological activity of compounds or extracts from the phylum Mollusca, all from the genus *Conus*. Nevertheless, bioactives from other invertebrates grouped in phylum Porifera, such as marine sponges (*Cliothosa aurivilli, Aaptos aaptos* and *Lendenfeldia chondrodes*), as well as soft corals (*Cladiella australis, Lemnalia cervicorni* and *Sinularia flexibilis*) from phylum Cnidaria were also studied. Given that TTX produced by microorganisms in marine ecosystems bioaccumulates in the liver and gonads of the pufferfish (family Tetraodontidae), these vertebrates served as the source for obtaining it. In addition, organisms from other kingdoms, especially chromists such as brown seaweeds from the phylum Gyrista/Ochrophytina and dinoflagellates from the phylum Miozoa, the bacterium *Spirulina platensis* and *Leptolyngbya* from the phylum Cyanobacteria, and Fungi (*Aspergillus fumigatus* and *Aspergillus terreus*) from the phylum Ascomycota, were also examined in preclinical studies (Fig. 7).

Preclinical models/outcome measures

Most authors (71%) modeled post-traumatic painful peripheral neuropathies, in particular the chronic constriction injury (CCI) model (Bennett & Xie, 1988), the spinal nerve ligation (SNL) model (Kim & Chung, 1992), the partial sciatic nerve ligation (PSL) model (Seltzer et al., 1990), and the spared nerve injury (SNI) model (Decosterd & Woolf, 2000). Nevertheless, more clinically relevant models of peripheral neuropathy induced by diseases—such as streptozotocin (STZ)-induced diabetic neuropathy (DN), post-herpetic neuralgia (PHN), chemotherapy-induced peripheral neuropathy (CIPN) models (22%) or central NP (6%) after spinal cord injury (SCI)—were implemented by several authors (Christensen et al., 2017; Fakhri et al., 2018; 2019; Hong et al., 2018; Huynh et al., 2020a; 2022; Karmakar et al., 2022; Kolosov et al., 2010; Ohashi et al., 2024; Pacini et al., 2016; Romero et al., 2017; Wang et al., 2019; 2024) (Fig. 8A-B).

As shown in Fig. 9A, stimulus-evoked pain such as heat hyperalgesia (4%), mechano-hyperalgesia (2%), mechano-allodynia (31%), and cold allodynia (7%), which retain utility in scenarios of sensory gain, were widely used throughout this review. Other authors reported their combinations (13%), especially the combination of mechano-allodynia and heat hyperalgesia (27%), which was frequently reported. However, a few authors associated the evaluation of these sensory abnormalities with spontaneous pain behavior (7%) (Chen et al., 2014; Di Cesare Mannelli et al., 2014; Wen et al., 2021), or comorbidity such as locomotor dysfunction/anxiety (4%) (He et al., 2025; Karmakar et al., 2022) or anxiety- and depression-like behaviors (4%) (Jiang et al., 2018; Lax et al., 2018).

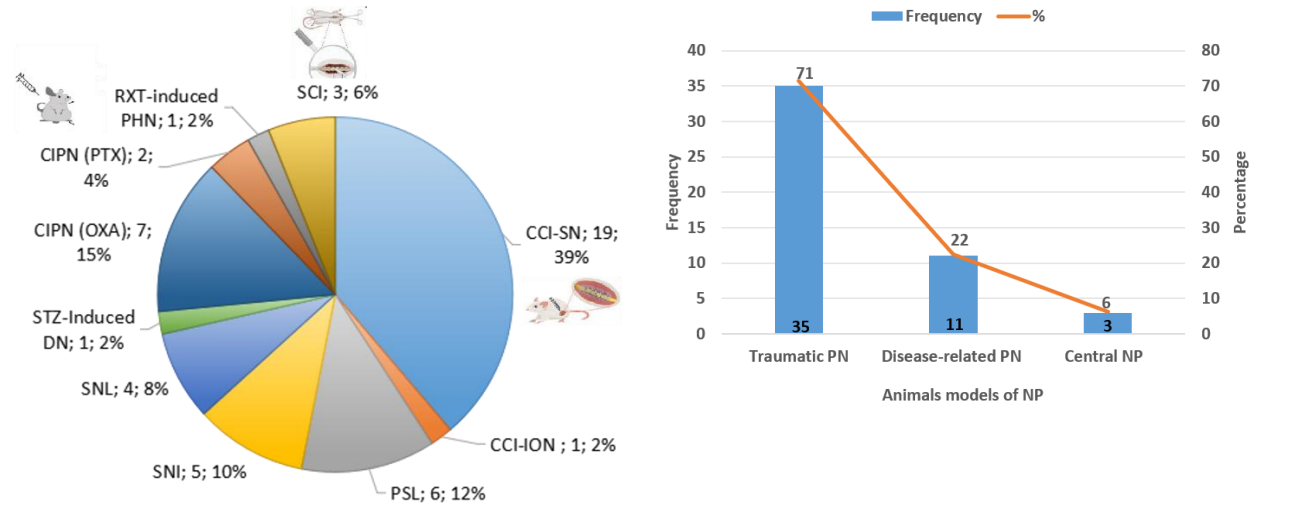

Figure 8. Neuropathic pain (NP) models throughout the SEM database.

(A) Distribution of preclinical studies according to NP model used in *in vivo* efficacy and/or mechanistic investigations. Sciatic nerve chronic constriction injury (CCI-SN) model, infraorbital nerve chronic constriction injury (CCI-ION) model, the spinal nerve ligation (SNL) model, the partial sciatic nerve ligation (PSL) model, the spared nerve injury (SNI) model, streptozotocin (STZ)-induced diabetic neuropathy (DN), resiniferatoxin (RTX)-induced post-herpetic neuralgia (PHN), paclitaxel (PTX) and oxaliplatin (OXA)-related chemotherapy-induced peripheral neuropathy (CIPN) models, or central NP after spinal cord injury (SCI). **(B)** Proportion of preclinical studies that modeled post-traumatic painful peripheral neuropathies (PN) compared to those that used more clinically relevant models of disease-related PN (STZ-induced DN, PHN, CIPN) or central NP. The denominator represents the total number of models reported (n = 49), rather than the number of included studies using NP models (n = 45), because some studies employed more than one model. One study involving neural injury that did not correspond to a classical NP model was excluded from this count.

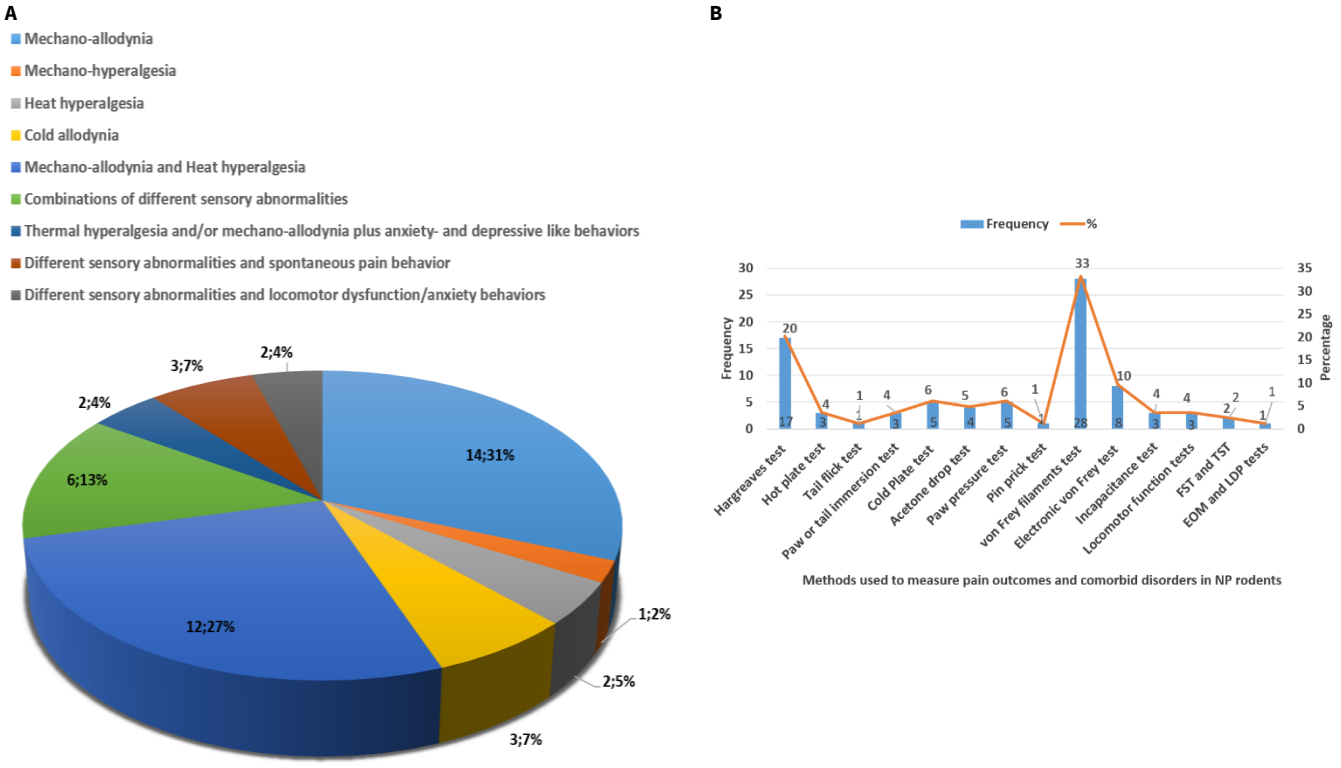

Figure 9. (**A**) Proportion of preclinical studies using different pain outcome measures. A predominance of pain evoked by mechanical, thermal, or chemical stimuli, and very often tested multiple stimulus modalities, was observed. A few authors associated the evaluation of these sensory abnormalities with spontaneous pain behavior or comorbidity, such as locomotor dysfunction/anxiety or anxiety- and depression-like behaviors. The denominator represents the number of included studies using NP models where these outcomes were evaluated (n = 45). One study involving neural injury that did not correspond to a classical NP model using the eye-wipe test was excluded from this count. (**B**) Behavioral tests used to explore pain outcomes in neuropathic pain (NP) models. Conventional behavioral tests of stimulus-evoked pain were extensively used (von Frey filaments test and its electronic version, as well as the Hargreaves test). The denominator represents the number of behavioral tests reported (n = 84), rather than the number of included studies using NP models (n = 45).

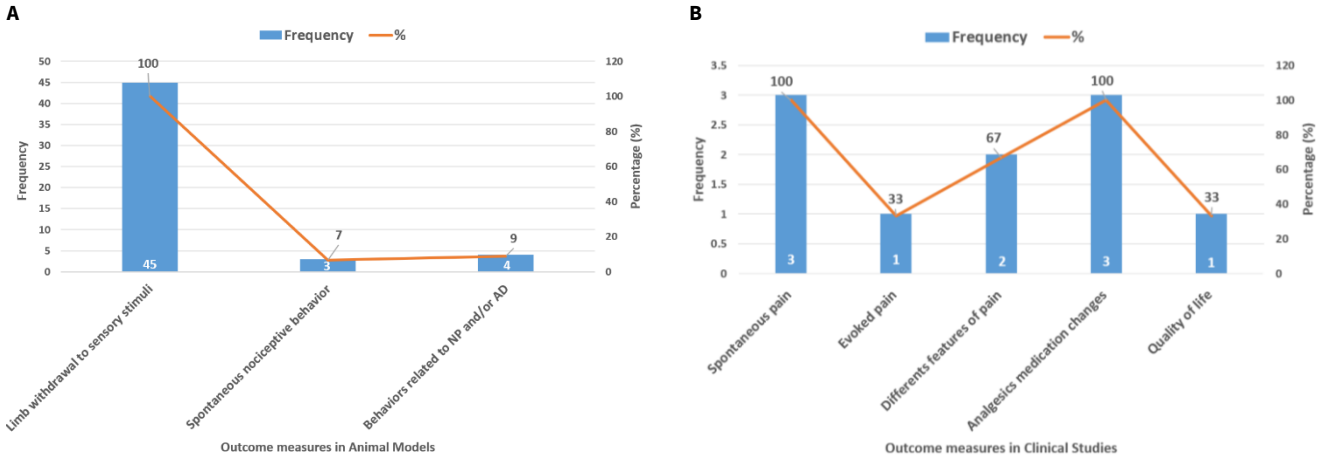

Figure 10. Discrepancies between outcome measures evaluated in preclinical studies and those in clinical studies involving patients with neuropathic pain (NP).

(**A**) All preclinical studies tested stimulus-evoked pain and habitually neglected social behavior. However, a few studies also tested spontaneous pain behavior (changes in hind limb weight-bearing) and affective disorder (AD)-like behaviors, which are associated with NP. (**B**) The three clinical studies assessed average daily pain with numeric or visual questionnaires [Numeric Pain Rating Scale (NPRS) Score, Gracely scale, visual analog score (VAS)], which asked about spontaneous pain intensity and sometimes evoked pain, as well as some psychosocial descriptors. Evoked pain was experimentally quantified in only one study.

Consequently, conventional behavioral tests of stimulus-evoked pain were extensively used in the reviewed studies, particularly the von Frey filaments test (34%) and its electronic version (10%), as well as the Hargreaves test (20%). The incapacitance test—used to determine changes in weight-bearing as an indicator of spontaneous pain behavior—was employed less frequently (4%). Similarly, spontaneous locomotor activity studies using open and closed fields—aimed at indirectly linking the findings to NP and/or associated affective disorders (AD)—were employed, as were other tests indicative of anxiety-like behaviors [elevated zero maze (EZM) and light-dark preference test (LDP)] or depression-like behaviors, such as the tail suspension test (TST) and the forced swim test (FST) (Fig. 9B).

Fig. 10 shows a discrepancy between outcome measures evaluated in preclinical studies (Fig. 10A) and those in clinical studies involving patients with NP (Fig. 10B). While spontaneous pain was assessed in all clinical studies and only Sang et al. (2016) reported on evoked pain, all preclinical studies focused on assessing withdrawal responses to various sensory stimuli; conversely, only three authors reported spontaneous nociceptive behaviors. Other outcomes assessed in clinical settings included changes in analgesic medication, pain characteristics, and quality-of-life (QoL) assessments using various validated tools. Only three preclinical study authors focused on the affective disorders associated with NP by evaluating anxiety- and depression-like behaviors.

Marine-derived compounds targeting neuropathic pain underlying molecular and cellular mechanisms

Significant peripheral nervous system (PNS), central nervous system (CNS), and disease-specific molecular targets involved in NP mechanisms have been the focus of investigations across this review (Campbell & Meyer, 2006). Fig. 11 summarizes 29 molecular targets and/or pathogenic mechanisms putatively implicated in the mechanisms of action of the marine-derived compounds, as reported. Compounds that, in addition to experimental mechanistic evidence, have demonstrated *in vivo* efficacy in NP models (orange bars) are distinguished, as are compounds or extracts showing prediction-based mechanistic evidence or functional modulation of relevant targets, indicating potential for evaluation in NP models (blue bars).

In vivo mechanistic evidence was provided for the antineuropathic activity of these bioactive compounds targeting nicotinic acetylcholine receptors (nAChRs), GPCRs [γ-aminobutyric acid type B receptor ($GABA_B$), neurotensin (NTS), somatostatin (SST), and cannabinoid (CB) receptors], neuroimmune activation/neuroinflammation, voltage-gated ion channels (sodium and calcium channels), and ionotropic glutamate receptors (iGluRs). In particular, the GluN2B subunit of the N-methyl-D-aspartate receptor (NMDARs), which is also linked to neuroprotective mechanisms, and the GluK1 subunit of kainate receptors (KARs) (Table S1). One study focused on the enzyme indoleamine 2,3-dioxygenase and its implications for NP in the depression-chronic pain complex (Jiang et al., 2018).

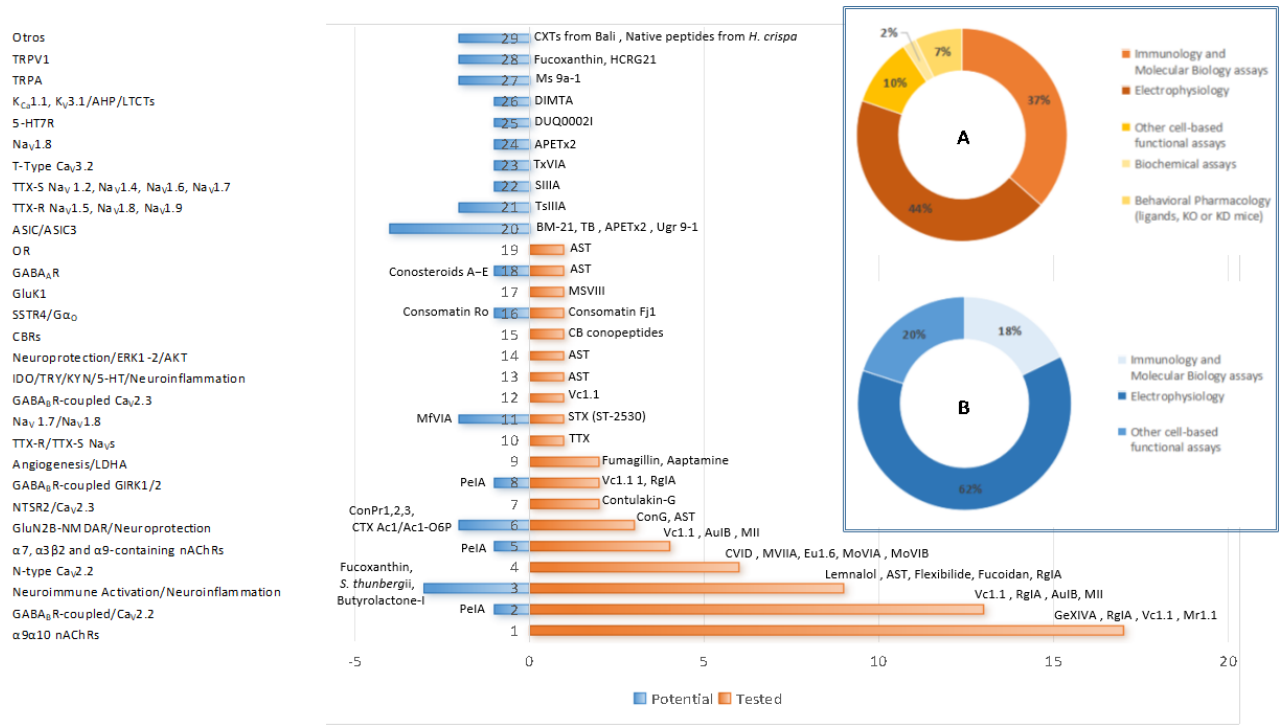

Figure 11. Marine-derived compounds targeting neuropathic pain (NP) underlying molecular and cellular mechanisms.

The figure summarizes 29 molecular targets and/or pathogenic mechanisms putatively implicated in the mechanisms of action of the marine-derived compounds, as reported. Orange bars represent compounds that, in addition to experimental mechanistic evidence, have demonstrated *in vivo* efficacy in NP models. Blue bars differentiate the compounds or extracts showing prediction-based mechanistic evidence or functional modulation of relevant targets, indicating potential for evaluation in NP models. Respective donut charts on the right represent the frequency with which different experimental methods were used to establish this mechanistic evidence. Electrophysiological techniques, as well as immunological and molecular biology assays, were widely used.

Other preclinical evidence was provided for marine bioactive interactions with potential targets involved in NP, such as ligand-gated ion channels [acid-sensing ion channels (ASIC), transient receptor potential (TRP) channels], voltage-gated potassium channels, ionotropic glutamate α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA receptors, and ionotropic γ-aminobutyric acid type A ($GABA_A$) receptors (Table 2).

Across this SEM database, only four marine-derived products were investigated in efficacy pharmacological studies in NP models without a mechanistic approach. Particularly, extracts from *Ecklonia cava, Spirulina platensis,* and *Cliothosa aurivilli*, attributing their bioactivity mostly to anti-inflammatory and antioxidant properties (Ali Safakhah et al., 2018; Karmakar et al., 2022; Kim et al., 2014), as well as a synthetic austrasulfone precursor from *Cladiella australis*, also evaluated for its anti-inflammatory and neuroprotective activity (Wen et al., 2010) (Table S1).

Fig. 11 (right) uses donut charts to show how often different experimental methods were used to establish this mechanistic evidence. Likewise, orange represents compounds with evidence of efficacy in NP models, while blue represents candidates slated for evaluation. In both cases, electrophysiological techniques, as well as immunological and molecular biology assays, were widely used. The latter primarily provided indirect mechanistic evidence, as they assess intervention-induced changes in downstream signaling pathways and/or in molecular or cellular processes associated with the proposed target or mechanism. However, two-electrode voltage-clamp (TEVC) recordings in *Xenopus laevis* oocytes and *in vitro* whole-cell patch-clamp recordings in dorsal root ganglia (DRG) neurons or immortalized cell lines such as HEK 293 played a pivotal role in providing direct mechanistic evidence of the functional modulation of ion channels and receptors. Some authors supported their results with electrophysiological recordings in spinal cord slices and *in vivo* recordings (Jayamanne et al., 2013; Napier et al., 2012), including in injured animals (Ohashi et al., 2024). Additionally, cell-based functional assays using Fluorescent Imaging Plate Reader (FLIPR) calcium imaging and FLIR membrane assay were used by several authors to determine downstream cellular signaling and functional cellular changes (Deuis et al., 2016; Lee et al., 2015; Martin et al., 2022; Niu et al., 2021; Paguigan et al., 2021; Sousa et al., 2018; Vetter et al., 2012; Wang et al., 2020). Evidence of GPCR functional modulation came from bioluminescence resonance energy transfer (BRET)-based assays of G-protein activation and β-arrestin recruitment, or from immunofluorescence analyses of receptor internalization (Bjørn-Yoshimoto et al., 2025; Jergova et al., 2021; Ramiro et al.,

2022). Few studies utilized direct target engagement techniques such as radioligand binding assays and Cis-bio binding competition bioassays to demonstrate binding, negative or positive, to the principal target or others (Christensen et al., 2017; Lax et al., 2018; Romero et al., 2017; Sousa et al., 2018).

Causal validation, whether pharmacological (demonstrating functional dependence of the target) or genetic/structural (demonstrating molecular specificity of the target), was considered robust mechanistic evidence. Mechanisms of action of Vc.1.1, RgIA, AuIB, AST, flexibilide and conG were characterized from a pharmacological approach using selective antagonists or pharmacological rescue (Balsara et al., 2012; Berecki et al., 2014; Bony et al., 2022a; 2022b; Klimis et al., 2011; Napier et al., 2012). Moreover, the specificity of targets for Vc.1.1, RgIA, contulakin-G, GeXIVA, CVID, MVIIA and Mr1.1 were supported using knockout (KO) or knockdown (KD) mice (Callaghan & Adams, 2010; Cuny et al., 2012; Huynh et al., 2022; Martin et al., 2022; Romero et al., 2017), and/or site-directed mutagenesis (Azam et al., 2015; Azam & McIntosh, 2012; Berecki et al., 2014; Bony et al., 2022a; Chu et al., 2019; Huynh et al., 2015, 2020b; Jayamanne et al., 2013; Liang et al., 2022; Luo et al., 2015; Zhangsun, 2017).

Evidence grading and translational potential

The heat map displays variables relevant to estimating te reliability of the evidence and its support for the potential translation of these marine-derived bioactives (Fig. 3). Based on qualitative analysis of these variables, the strength of the evidence was categorized, therefore marine bioactives such as TTX, ω-conotoxin MVIIA and contulakin-G were found to have clinical evidence (Brinzeu, 2019; Goldlust, 2021; Hong, 2018; Jayamanne, 2013; Kayser, 2010; Lee & Kang, 2015; Martin et al., 2022; Sang et al., 2016; Ohashi et al., 2024). The α-CTXs Vc1.1 and RgIA and their analogs, as well as the αO-conotoxin GeXIVA, show strong preclinical evidence (Azam & McIntosh, 2012; Azam et al., 2015; Belgi et al., 2021; Berecki et al., 2014; Bony et al., 2022a; 2022b; Callaghan & Adams, 2010; Christensen et al., 2017; Chu et al., 2019; Clark et al., 2010; Cuny et al., 2012; Di Cesare Mannelli et al., 2014; He et al., 2025; Huynh et al., 2015; 2020a; 2020b; 2022; Klimis et al., 2011; Li et al., 2016; 2019; Luo et al., 2015; Napier et al., 2012; Pacini et al., 2016; St Clair-Glover et al., 2025; Pan et al., 2021; Poth et al., 2021; Romero et al., 2017; Tae et al., 2025; Wang et al., 2019; 2024; Xu et al., 2020; Zhang et al., 2025; Zhangsun et al., 2017; Zhu et al., 2022).

It was identified that 63%, the majority of the bioactives studied (AST, ω-CTX CVID, α-CTXs AuIB and MII, fucoidan, α-CTX Eu1.6, spirulina extract, ω-CTX MoVIA/MoVIB, CB conopeptides/fractions, STX analogue (ST-2530), α-CTX Mr1.1/analogues, aaptamine, consomatin Fj1, MSVIII-19, flexibilide, lemnalol and fumagillin) have moderate preclinical evidence (Ali Safakhah et al., 2018; Beckley et al., 2021; Bjørn-Yoshimoto et al., 2025; Chen et al., 2014; Fakhri et al., 2018; 2019; Hashemi et al., 2024; Hu et al., 2014; Jayamanne et al., 2013; Jergova et al., 2021; Jiang et al., 2018; Klimis et al., 2011; Kolosov et al., 2010; Liang et al., 2022; Lin et al., 2011; Liu et al., 2018; Napier et al., 2012; Qiu et al., 2011; Sharma et al., 2018; Sousa et al., 2018; Sung et al., 2023; Wen et al., 2021; Zhao et al., 2021). Four of them (*E. cava* extracts, *C. aurivilli* extract, DA, and conG) show preliminary evidence (Balsara et al., 2012; Karmakar et al., 2022; Kim et al., 2014; Wen et al., 2010).

DISCUSSION

Currently, except for ziconotide, most marine molecules in clinical use or under trial are intended for anticancer therapy (Cappello & Nieri, 2021; Haque et al., 2022). Over the last 15 years, several studies have explored the analgesic effects of marine-derived bioactive compounds in relevant animal models of NP, with most providing consistent mechanistic evidence aligned with validated NP mechanisms (Finnerup et al., 2021; Rice et al., 2018). Despite acknowledged obstacles to analgesic drug development, which further complicate the already complex field of marine pharmacology, research on marine bioactives targeting NP has increased during this period. However, in most cases, these promising molecules have become preclinical academic leads that have not reached the development stage (Sanchez-Campos et al., 2022). Overall, the SEM landscape reflects different stages of translational maturity, from mechanistic discovery to clinical development. Distinguishing biological promise from translational readiness is therefore essential, with progression requiring increasingly convergent evidence of efficacy, mechanism, PK/PD, safety, and clinical feasibility. This review provides a critical synthesis to distinguish compounds supported by the most robust evidence, based on an analysis of their reliability and limitations. Consequently, experimental priorities are identified for their translation into safer and more effective mechanism-based therapies. Additionally, and taking into account preliminary mechanistic evidence, research into other bioactive compounds in NP context is recommended.

Marine-derived bioactives modulating sodium channels

Clinical translation: tetrodotoxin in chemotherapy-induced peripheral neuropathy and safety considerations

Sodium channels, which—due to their altered gene expression, protein trafficking, and altered kinetics in the NP context—are pivotal in the development of ectopic pacemaker capability and ectopic neuronal discharge (Bennett et al., 2019). Several lines of evidence from animal models and humans suggest that TTX-sensitive (TTX-S) $Na_V1.3$, 1.6, and 1.7 channels, and TTX-resistant (TTX-R) $Na_V1.8$ channels are particularly important in the generation and maintenance of NP (Alles & Smith, 2021). Genetic evidence in humans has demonstrated that gain-of-function mutations in the SCN10A and SCN9A genes—which encode $Na_V1.8$ and $Na_V1.7$, respectively—cause painful neuropathies, underscoring the robustness of both channels as analgesic targets (Alles & Smith, 2021; Gordon et al., 2025). Thus, pharmacologically mimicking loss-of-function mutations has gained attention as a strategy for analgesia; however, despite promising preclinical results, $Na_V1.7$-selective inhibitors have yet to progress beyond phase II clinical trials (Eagles et al., 2020; Yang et al., 2025). In contrast, the FDA-approved suzetrigine (VX-548)—a potent and highly selective small-molecule inhibitor of $Na_V1.8$—for the treatment of acute moderate to severe pain (Jones et al., 2023). Given $Na_V1.8$'s role in NP, VX-548 is currently under clinical evaluation for painful diabetic peripheral neuropathy (NCT05660538, 2023), however, its efficacy in chronic NP has not been confirmed to date (Gordon et al., 2025).

Natural guanidinium toxins, such as TTX and saxitoxin (STX), are potent Na_V inhibitors that bind to voltage-gated sodium channels at a site comprising the domain I–IV S5–S6 extracellular pore loops to block the pore (Cardoso & Lewis, 2018). As previously reported, TTX exerts antineuropathic effects in several NP models (González-Cano et al., 2021; Kayser et al., 2010; Nieto et al., 2008). A functional preference of TTX for inhibiting A-fiber-mediated mechanical hypersensitivity (Aβ-fibers, as well as Aδ-fibers) has been recognized in correlation with electrophysiological findings (Clarke et al., 2003; Kayser et al., 2010; Nieto et al., 2008). Interestingly, unlike somatic nerve injury (SNL model) in which $Na_V1.3$ expression is upregulated in the lumbar DRG, damage to the mandibular branch of the trigeminal nerve does not affect its expression (Davies et al., 2006). Kayser et al. (2010) observed differential effects of TTX on allodynia caused by sciatic nerve CCI versus infraorbital nerve CCI, suggesting that the antiallodynic action of TTX might involve, at least in part, its ability to block overexpressed $Na_V1.3$ channels. Furthermore, enhanced efficacy of TTX combined with naloxone was observed, suggesting complex interactions with opioidergic systems in this context (Kayser et al., 2010).

It has been demonstrated that a translational correlation exists in the $Na_V1.7$ protein, with changes in DRG expression and function between rodents and humans with paclitaxel (PTX)-related CIPN (Li et al., 2018). During this period, one of the few clinical trials of these products in NP patients with CIPN focused on sodium channels, aligning preclinical and clinical goals (Kayser et al., 2010; Li et al., 2018; Nieto et al., 2008). Goldlust et al. (2021) provided the first randomized, double-blind, placebo-controlled clinical evidence supporting the translational potential of TTX for CIPN; however, a statistically significant treatment benefit was not demonstrated in the prespecified primary analysis. Exploratory findings suggested a possible analgesic signal, particularly with 30 μg twice daily, but confirmatory evidence is required before efficacy can be established. Nevertheless, the study provides an important proof of concept regarding the analgesic effects observed in a preclinical model of PTX-induced NP (Nieto et al., 2008).

Consistent with observations regarding cancer-related pain, additional safety data from this RCT suggest a potential favorable risk/benefit profile in a population with limited therapeutic options (Goldlust et al., 2021; Huerta et al., 2023). The authors preferred subcutaneous TTX administration, based on prior experience in several clinical trials for cancer pain (Huerta et al., 2023). The most common adverse events were mild or moderate, particularly oral paresthesia (29.6%) and oral hypoesthesia (24.8%). Therefore, from a translational perspective, the study provides reasonably encouraging evidence of short-term tolerability, but does not allow for the establishment of long-term safety due to the small number of patients and the relatively short follow-up. However, acute TTX toxicity can be serious, including CNS depression and neuromuscular blockade (González-Cano et al., 2021). Hong et al. (2018) conducted a pharmacological study comparing the efficacy of TTX pellets with intramuscular TTX injection in a rat model of postherpetic neuralgia (PHN), aiming to align late-stage preclinical testing with anticipated clinical trials. The highest TTX pellet dose (20 μg/kg) was administered orally once daily for seven consecutive days without notable side effects. This dosage in rats is equivalent to 55.5–222.2 μg/person in humans. Oral TTX pellets improved the therapeutic index and greatly enhanced safety compared with TTX injection (Hong et al., 2018). Nevertheless, for peripheral NP, localized rather than systemic formulations such as plasters or patches to deliver marine-derived Na_V inhibitors could be a safer alternative.

A $Na_V1.7$-selective inhibitor derived from saxitoxin. Moderate preclinical evidence

Although some evidence in an adult-onset $Na_V1.7$ knockout (KO) mouse model suggests that $Na_V1.7$ is not essential for NP (Shields et al., 2018), other data indicate that $Na_V1.7$ is required for mechanical allodynia, particularly when this channel is ablated from both sensory and sympathetic nerves in NP models (Grubinska et al., 2019; Minett et al., 2012). A fully synthetic STX analog (ST-2530) that selectively inhibits $Na_V1.7$ reversed mechanical allodynia in the SNI model. ST-2530 demonstrated efficacy at doses (1-3 mg/kg) that did not affect olfactory or motor behavior in mice, using a repeated dosing schedule in animals with established neuropathy more akin to clinical settings than studies based exclusively on a single dose (Beckley et al., 2021). This study was strengthened by the integration of complementary experimental approaches, including electrophysiological characterization, PK assessment, and behavioral evaluation of ST-2530 using a relevant subcutaneous route. ST-2530 does not merely work in a single peripheral neuropathy model; it demonstrated higher affinity for the human $Na_V1.7$ isoform than for the murine one, high selectivity over other human Na_V channels associated with adverse effects, and pharmacologically relevant systemic exposure. These elements establish a favorable preclinical profile for future translation. Nevertheless, although the compound was specifically optimized to retain sufficient activity against mouse Nav1.7 for *in vivo* studies, this interspecies difference complicated direct extrapolation of the effective exposure in mice to the human therapeutic setting. ST-2560, also derived from STX, affects cardiovascular reflexes in non-human primates at exposures associated with analgesic activity, highlighting a potential cardiovascular liability of $Na_V1.7$ inhibition that may not be readily detected in conventional rodent models (Mulcahy et al., 2024). Unlike $Na_V1.8$, $Na_V1.7$ is expressed in sympathetic neurons (Bennett et al., 2019; Gordon et al., 2025); therefore, ST-2530 also requires an investigation of autonomic side effects in species whose $Na_V1.7$ pharmacology more closely resembles humans.

Marine-derived bioactives modulating voltage-gated calcium channels

Ziconotide: Clinical efficacy and limitations

Peripheral nerve injury induces a marked upregulation of the α_2-δ auxiliary subunit of high-threshold voltage-gated calcium channels (VGCCs, Ca_V) in rat DRG and spinal dorsal horn, supporting its relevance to NP mechanisms (Gong et al., 2018). N-type channels play a fundamental role in ascending nociceptive pathways and are highly localized to presynaptic terminals in laminae I and II of the dorsal horn (Antunes et al., 2023). Especially, N-type $Ca_V2.2$ is upregulated in uninjured L4 DRG neurons following SNL, increasing neuronal excitability (Yang et al., 2018). Moreover, in rat models of NP, such as STZ-induced painful diabetic neuropathy and CCI, T-type channel current density ($Ca_V3.2$ and $Ca_V3.3$ subtypes) is remarkably increased (Harding & Zamponi, 2022). In particular, the $Ca_V3.2$ isoform has been considered critical for the excitability of pain circuits and in touch-pain pathophysiology (Bourinet et al., 2016). Interestingly, the critical role of $Ca_V2.2$ in the pain pathway was validated by the clinical efficacy of its selective antagonist ziconotide (SNX-111)—a synthetic version of the ω-conotoxin MVIIA derived from *Conus magus*—which decreases neurotransmission from primary nociceptive afferents (Antunes et al., 2023; Staats et al., 2004).

Ziconotide is used clinically either as monotherapy or in polytherapy regimens to treat refractory cancer or non-cancer pain, including cases of NP (Safavi-Hemami et al., 2019). Ziconotide and related ω-conotoxins exert beneficial effects in NP by non-opioid mechanisms, especially when first-line therapies are inadequate, and notably lack the addictive risks and undesirable problems of opioids (Antunes et al., 2023). However, they induce severe adverse effects, have a narrow therapeutic window, and face delivery challenges (Rubiu et al., 2024). This drug is administered intrathecally (IT) to selectively target $Ca_V2.2$ at the spinal level while avoiding other sites, such as the peripheral sympathetic system. The most common ziconotide-related adverse events include dizziness, nausea, nystagmus, somnolence, urinary retention, amblyopia, hypotension, confusion, asthenia, and abnormal gait (Rubiu et al., 2024). Nevertheless, cognitive and psychiatric disorders that can worsen when already present, and elevation of creatine kinase serum values, which can indicate cardiac, brain, or skeletal muscle damage, have been significant causes for concern (Burdge et al., 2018; Safavi-Hemami et al., 2019). Recent evidence suggests that since the adverse consequences of IT ziconotide infusion monotherapy do not exceed the benefits in drug-resistant patients compared to other routes, and when compared with IT morphine, its benefit/risk assessment is favorable. However, due mainly to high rates of treatment discontinuation, specific long-term, prospective, randomized studies are still lacking. The evidence also suggests the need for studies involving more clinically homogeneous groups (Rubiu et al., 2024). Thus, given the lack of effective therapeutic options for pharmacoresistant NP in SCI patients, some authors have focused on this population (Brinzeu et al., 2019; 2024). A pilot study with a follow-up of 3.59 years (±1.94) showed that of the 20 patients evaluated, 14 initially achieved pain reduction exceeding 40% following IT ziconotide test. Eleven patients underwent pump implantation, and sustained benefit

was reported in eight patients with an average dose of 7.2 μg/day (Brinzeu et al., 2019). These uncontrolled observations should be interpreted cautiously because treatment selection and the absence of a comparator limit causal inference. Nevertheless, the study provides evidence of feasibility and a signal of efficacy in humans with actual, persistent NP. The need to confirm this signal through controlled trials is reflected in the subsequent development of the SPIDOL study—a multicenter, randomized, comparative, placebo-controlled, double-blind clinical trial specifically designed to evaluate the long-term efficacy of IT ziconotide for NP associated with SCI (Brinzeu et al., 2024).

Neuroprotective potential in spinal cord injury

Given that NP can be a manifestation of neurodegeneration, the treatment of more prevalent peripheral painful neuropathies and central NP in SCI, multiple sclerosis, or post-stroke pain is currently approached using neuroprotective strategies (Bordet & Pruss, 2009). Ziconotide and related ω-conotoxins have potential as therapeutic strategies for SCI by targeting the cascade of secondary neurodegenerative events triggered early and involving ionic disturbances, excessive intracellular calcium accumulation, and VGCC upregulation (Ohashi et al., 2024). A neuroprotective ability of MVIIA in the context of brain traumatic injury and ischemia has been demonstrated (Verweij et al., 2000). Moreover, administration of MVIIA 4 hours after SCI protects against neuronal death and mitochondrial dysfunction (Oliveira et al., 2018). Consequently, Ohashi et al. (2024) investigated the spinal analgesic actions and cellular mechanisms underlying the analgesic effects of MVIIA in an SCI contusion injury model. MVIIA administration in the acute phase (4 hours after SCI), coinciding with peak intracellular calcium concentration, improved mechanical allodynia and locomotor function at 14 days, suggesting a neuroprotective effect. Immunohistochemical and electrophysiological experiments suggest that MVIIA induces analgesia by inhibiting N-type voltage-dependent calcium channels on Aδ- and C-fiber terminals in the spinal dorsal horn, thereby reducing neuronal excitability that is enhanced by SCI-induced pain (Ohashi et al., 2024). Thus, the window of opportunity to prevent NP development after SCI could be important for future clinical trial design. Unlike previous studies, these authors provide robust functional evidence of MVIIA's action on synaptic transmission into neurons from lamina II of the spinal dorsal horn, as the whole-cell voltage clamp recordings used slices from SCI—rather than healthy—animals (Jayamanne et al., 2013). It also includes *in vivo* extracellular recordings (both spontaneous and stimulus-evoked firing) from the spinal dorsal horn neurons in SCI rats, reflecting changes in these channels following neural injury. These preclinical findings support the relevance of $Ca_V2.2$ channels as an analgesic target after SCI, strengthening the biological and mechanistic plausibility of introducing ziconotide in this patient population, both preventively in the early stages after injury and therapeutically once NP is established.

Alternative conotoxins with improved profiles. Moderate preclinical evidence

In addition to MVIIA, other structurally related ω-conotoxins, including GVIA and CVID, which act selectively as N-type voltage-gated calcium channel blockers, have demonstrated efficacy for NP in animal studies (Jayamanne et al., 2013; Kolosov et al., 2010; Lewis et al., 2012). CVID isolated from the venom of *Conus catus* has been reported to have a better therapeutic index than MVIIA because it is more selective for $Ca_V2.2$ than $Ca_V2.1$ and preferentially interacts with sensory rather than sympathetic neurons, which express the β3 subunit in $Ca_V2.2$ channels (Lewis et al., 2012). Because the β3 auxiliary subunit markedly decreases CVID potency relative to other conotoxins in blocking $Ca_V2.2$ channels, it is expected to have fewer consequences for hemodynamics and autonomic reflexes (Kolosov et al., 2010). Additionally, CVID inhibition of VGCC currents in isolated neurons is more reversible after washout than MVIIA, due to a different residue at position 10 of the peptide sequence (Mould et al., 2004). However, Jayamanne et al. (2013) reported that intrathecal CVID provides only a marginal improvement in therapeutic index over MVIIA in the SNL model, which appears unrelated to binding-mediated reversibility.

Systemic CVID and its synthetic form CNSB004/AM336 (leconotide) have been shown to penetrate the CNS, suggesting that non-spinal parenteral delivery may be feasible without severe cardiovascular effects (Kolosov et al., 2010). In the clinically relevant STZ-induced DN model, leconotide at the highest no-side-effect dose (2 mg/kg intravenously) achieved an antihyperalgesic effect of 51.7%, compared with just 0.4% for the highest no-side-effect dose of ziconotide (0.02 mg/kg) (Kolosov et al., 2010). This approach strengthened the pharmacological interpretation and suggests a potentially wider therapeutic window for CNSB004. However, the translational relevance is limited by the predominantly acute assessment of drug efficacy, which does not establish sustained analgesia, tolerance, or long-term safety. In addition, claims about synergistic combinations with flupirtine (5 mg/kg i.p.) should be supported by isobolographic analysis, not only by additive behavioral improvement. Notably, the use of a synthetic analog and IV administration represents a favorable step toward clinical development; however, this peptide

has successfully completed preclinical studies, reached phase II in 2004, and remains under investigation, although high cytotoxicity was noted in phase IIa trials (Gao et al., 2017; Kolosov et al., 2010; 2011). Meanwhile, ω-conotoxin GVIA from *Conus geographus* has the highest affinity for $Ca_V2.2$ channels, but its virtually irreversible binding renders it less attractive as a therapeutic agent (Tranberg et al., 2012). Nonetheless, the highly constrained nature of peptides from piscivorous Conidae and the availability of structure–activity relationship data make them excellent starting points for developing peptidomimetics (Tranberg et al., 2012).

More recently, researchers identified MoVIA and MoVIB from *Conus moncuri*, the first ω-conotoxins discovered in vermivorous cone snails. This suggests that ω-conotoxins may have originally evolved to defend against predation in ancestral worm-hunting species and were later repurposed for fish hunting (Sousa et al., 2018). These new conotoxins preferentially target fish $Ca_V2.2$ channels and possess an arginine at position 13 instead of the functional tyrosine previously found to be crucial for piscivorous ω-conotoxin activity (Kim et al., 1995). Nevertheless, both MoVIA and MoVIB fully inhibited N-type $Ca_V2.2$ channel currents in rat DRG cells, with no significant effects on P/Q-type $Ca_V2.1$ channels. Furthermore, in human SH-SY5Y cells, neither MoVIA nor MoVIB (30 μM) inhibited Ca^{2+} influx associated with $Ca_V1.3$ and $Ca_V3.1$, positioning them as selective $Ca_V2.2$ inhibitors with pharmacological profiles similar to those from piscivorous cone snails. MoVIB's analgesic activity was confirmed upon intrathecal administration in the PNL model, with side effects common to ω-conotoxins, including shaking, tail twitching, and serpentine tail movement. However, the safety margin for MoVIB appears improved compared with MVIIA (Sousa et al., 2018). Despite mechanistic evidence being robust for these peptides, as the authors demonstrated activity against $hCa_V2.2$ and rat $Ca_V2.2$, as well as the displacement of [^{125}I]-GVIA in binding assays, its main translational limitation is that the therapeutic window remains narrow.

Another notable development is the identification of Eu1.6 from *Conus eburneus*, the first α-conopeptide to directly and reversibly inhibit N-type $Ca_V2.2$ channels with high potency. In contrast, it demonstrates weak activity on neuronal nAChR subtypes (α3β4 and α7) and TTX-R sodium channels. Eu1.6 does not interact with $GABA_B$ receptors to modulate $Ca_V2.2$, TTX-S sodium channels, $K_V7.1$ (KCNQ1) channels, opioid receptors, transient receptor potential vanilloid 1 (TRPV1), or L- and T-type VGCCs. In NP models (PNL and CCI), Eu1.6 administered at microgram per kilogram (μg/kg) doses via clinically relevant intramuscular (IM) and intravenous (IV) routes was more potent than a combination of morphine and gabapentin. Remarkably, Eu1.6 exhibits a wide safety margin, showing no significant side effects on locomotor activity, cardiac or respiratory function, or drug dependence after repeated administration (Liu et al., 2018). However, both MoVIB and Eu1.6 require validation in disease models beyond traumatic nerve injury.

Marine-derived bioactives as modulators of glutamatergic spinal neurotransmission: central sensitization after nerve injury

Central sensitization represents an activity-dependent increase in synaptic strength within nociceptive circuits, resulting from synaptic facilitation or disinhibition at multiple levels of the neuraxis, phenomena first described and extensively characterized in the dorsal horn of the spinal cord (Latremoliere & Woolf, 2009; Woolf & Salter, 2000). Pharmacological strategies that attenuate central sensitization by acting on voltage-gated calcium channel subunits to decrease presynaptic transmitter release, and on NMDA receptors to reduce postsynaptic transmitter action, could represent effective alternatives for treating NP (Latremoliere & Woolf, 2009). While ω-conotoxins targeting presynaptic N-type voltage-gated calcium channels have been the primary focus in this context, multiple investigations have focused on identifying NMDA glutamate receptor modulators from marine organisms (Hama & Sagen, 2009; Malmberg et al., 2003; Ohashi et al., 2024; Safavi-Hemami et al., 2019). Available NMDA receptor antagonists reduce NP in both preclinical and clinical settings; however, their clinical utility is severely limited by unacceptable adverse effects resulting from the disruption of NMDAR's essential physiological functions in learning, memory consolidation, and cognitive processing (Puşcaşu et al., 2024). Therefore, the development of selective negative allosteric modulators targeting NMDA subunits differentially expressed along nociceptive pathways has been proposed as a substantially safer alternative strategy (Malmberg et al., 2003). In particular, the GluN2B subunit exhibits the most abundant expression among GluN2 subunits within the spinal cord, localized both presynaptically on small-diameter primary afferent terminals and postsynaptically on neurons within the superficial laminae of the spinal dorsal horn (Boyce et al., 1999), as well as at supraspinal sites implicated in pain processing (Chizh et al., 2001; Wu & Zhuo, 2009). Accumulating preclinical and clinical evidence suggests that selective targeting of GluN2B yields analgesic and neuroprotective effects with fewer adverse effects than non-selective antagonists (Khalid et al., 2026; Kim et al., 2012; Wu & Zhuo, 2009).

Conantokins and other marine peptides as GluN2B-selective antagonists. Neuroprotective potential beyond symptomatic pain relief

Aligned with this strategic targeting approach, Malmberg et al. (2003) demonstrated the antinociceptive effects of cone snail venom-derived subtype-selective NMDA receptor antagonists termed conantokins. These peptides contain multiple conserved γ-carboxyglutamate (Gla) residues, which are central to their selective inhibition of ion flow through co-agonized glutamate/glycine channels of NMDA receptors (McIntosh et al., 1984). Conantokin-G (conG) is a 17-amino acid peptide isolated from *Conus geographus* that selectively inhibits the GluN2B subunit of the NMDA receptor at nanomolar concentrations. In contrast, the structurally related conantokin-T from *Conus tulipa* inhibits both GluN2A and GluN2B subunits (Klein et al., 2001). Spinal administration of the subtype-selective GluN2B antagonist conG produced dose-dependent suppression of nerve injury-induced thermal and mechanical allodynia in preclinical models. Moreover, both conantokins reduce the second phase of the formalin test (Malmberg et al., 2003), in which activity-dependent central sensitization is triggered (Coderre et al., 1990). An investigation evaluating the antihypernociceptive interaction between conG and ω-conotoxin MVIIA following intrathecal co-administration revealed additive antinociception in the SNL model, but robust synergistic antinociception following SCI (Hama & Sagen, 2009). Previously, overexpression of spinal GluN2B following SCI, along with the antiallodynic efficacy of selective antagonists ifenprodil and Ro25-6981, had been documented (Kim et al., 2012), providing mechanistic support for the enhanced efficacy of conG in this model.

The synthetic variant of conG, designated CGX-1007, progressed to early clinical development, primarily for refractory epilepsy, but its development was subsequently discontinued. Although conG showed promising antinociceptive activity in preclinical models of NP, clinical efficacy as an analgesic was never established (Balsara et al., 2012; Malmberg et al., 2003). This clinical progression validates the translational potential of subtype-selective NMDA receptor modulators derived from marine organisms. However, further clinical development will require demonstrating adequate CNS penetration following systemic administration, acceptable PK profiles, and confirming therapeutic windows that separate analgesic efficacy from potential cognitive or psychotomimetic adverse effects. During the period covered by this review, ongoing academic research identified neuroprotective mechanisms of conG, providing preliminary evidence. These mechanisms could also inform strategies to prevent and modify the neurodegeneration underlying NP states (Balsara et al., 2012; Bordet & Pruss, 2009). Accumulating evidence suggests that excitotoxicity is primarily mediated by signaling pathways coupled to extrasynaptically localized GluN2B-containing NMDA receptors, whereas GluN2A-containing receptors, which are predominantly located synaptically, mediate antiapoptotic and neuroprotective effects. Given that conantokin-G blocks pro-death extrasynaptic activation while permitting beneficial neuroprotective synaptic activation of NMDA receptors in rat hippocampal neurons, this peptide has been recognized for its dual neuroprotective and analgesic potential (Balsara et al., 2012).

Although less studied, kainate receptors (KARs)—also members of the iGluRs family but with metabotropic, non-canonical signaling—have been identified for their role in modulating nociceptive transmission and integration, acting through both pre- and postsynaptic mechanisms in primary sensory neurons and spinal circuits. Consequently, they are recognized as more prominent targets than NMDA and AMPA receptors in pathological pain (Degiorgi & Lerma, 2026). MSVIII-19 (8,9-dideoxy-neodysiherbaine), a synthetic analog of the toxin dysiherbaine isolated from the marine sponge *Lendenfeldia chondrodes*, was characterized as a functional antagonist of homomeric GluK1 receptors. Unlike other conventional antagonists, MSVIII-19 potently desensitizes the receptors without activating the channel (Frydenvang et al., 2009). GluK1 is an essential subunit for assembling functional KAR in DRG neurons (Degiorgi & Lerma, 2026). However, MSVIII-19 also shows lower potency on heteromeric GluK1/GluK5 or GluK2/GluK5 receptors, also expressed in these cells (Frydenvang et al., 2009; Qiu et al., 2011). Spinally but not peripherally administered MSVIII-19 reduces thermal and mechanical hypersensitivity in the CCI model, highlighting the therapeutic potential of spinal GluK1-containing KARs blocked in NP (Qiu et al., 2011). These authors suggest that the receptors involved in the antihyperalgesic effects of MSVIII-19 could be localized in central terminals of primary afferent neurons, in dorsal horn neurons, or both. Although MSVIII-19 has not undergone clinical development, it was proposed as a lead compound or template molecule for developing related molecules that retain GluK1 selectivity, as well as an important pharmacological tool for studying it within nociceptive pathways (Degiorgi & Lerma, 2026). Current literature continues to regard NMDA and GluK1-containing KARs as potential targets for analgesia, but the gap between that preclinical validation and an approved drug remains significant.

Marine-derived bioactives as modulators of neuroinflammation and neuroimmune activation

Strategic considerations for glial modulation

Recognition of neuronal-glial and glial-glial interactions, and the prospect of inhibiting glial activation as a treatment strategy for NP, has led to the repurposing of several drugs, including propentofylline, ibudilast, and antibiotics such as minocycline, doxycycline, ceftriaxone, and azithromycin (Sisignano et al., 2022). However, glial cells can be either protective or destructive depending upon the immune and chemical microenvironment, the type and burden of stimuli, and the timing of their activation (Ji et al., 2013; Manning, 2006; Milligan & Watkins, 2009; Tiwari et al., 2014). Astrocytes play a key role in maintaining synaptic transmission by producing L-lactate via the astrocyte-neuron lactate shuttle, thereby providing a crucial additional energy source for neurons, especially under stress and continuous neuronal stimulation (Kong et al., 2022). Glial cells also release anti-inflammatory factors such as interleukin-4 (IL-4), interleukin-10 (IL-10), and transforming growth factor-β (TGF-β) that protect against neurotoxicity and restore normal nociceptive processing. In fact, because of their lack of specificity and potential detrimental effects on neurons, glial inhibitors have not shown a significant impact on NP relief in clinical trials (Finnerup et al., 2021; Zimmerman et al., 2025). Therefore, enhancing the protective anti-inflammatory actions of glial cells may be a more powerful strategy than preventing global glial activation (Tiwari et al., 2014). Marine organisms, rich sources of anti-inflammatory compounds, have also been explored in the search for more selective glial-modulating agents that preserve supportive and protective roles of glia. Several isolated compounds and extracts from different marine organisms have demonstrated analgesic activity in validated NP models, with effects mechanistically linked to—or suggested to modulate—neuroimmune activation, neuroinflammation, and/or nitroxidative stress (Ali Safakhah et al., 2018; Chen et al., 2014; Fakhri et al., 2018; Hashemi et al., 2024; Karmakar et al., 2022; Kim et al., 2014; Lin et al., 2011; Sung et al., 2023; Wen et al., 2010; 2021; Zhao et al., 2021).

Bioactives from soft corals, brown seaweeds, and marine fungi

Lemnalol (8-isopropyl-5-methyl-4-methylene-decahydro-1,5-cyclo-naphthalen-3-ol), a ylangene-type sesquiterpenoid isolated from the soft coral *Lemnalia cervicorni* (Duh et al., 2004), attenuates CCI-induced mechanical allodynia and thermal hyperalgesia in rats (Lin et al., 2011). Microglia both release and respond to pro-inflammatory cytokines, including interleukin-1β (IL-1β), IL-6, tumor necrosis factor-α (TNF-α), and interferon-γ, which are involved in the induction of cell adhesion molecule expression, recruitment of immune cells into the lesion site, and astrocytic activation (Manning, 2006). Given the dominant role of TNF-α in inducing neuronal hyperexcitability in the spinal cord, the antihyperalgesic effects of lemnalol were linked to its ability to inhibit microglial and astrocytic activation and suppress the expression of this proinflammatory cytokine from these cells (DeLeo et al., 2006; Kawasaki et al., 2008). It has been recognized that TNF-α inhibits the expression and activity of astrocytic glutamate transporters in the spinal cord; consequently, by increasing extracellular glutamate concentrations, it promotes excitatory neurotransmission through both AMPA and NMDA receptors (Austin & Moalem-Taylor, 2010).

Other bioactives derived from different species of marine soft corals, such as capnellene isolated from *Capnella imbricata* and sinularin/flexibilide from *Sinularia flexibilis*, have demonstrated antihyperalgesic activity linked to modulation of spinal glial activation and regulation of proinflammatory proteins (cyclooxygenase-2 and/or inducible nitric oxide synthase) in inflammatory and neuropathic pain models, respectively (Chen et al., 2014; Huang et al., 2012; Jean et al., 2009). Interestingly, sinularin has been shown to prevent the development of thermal hyperalgesia and weight-bearing deficits in CCI rats, through the upregulation of spinal TGF-β production (Chen et al., 2014). TGF-β has been reported to attenuate spinal neuroinflammation and dysfunction of the excitatory amino acid system in neuropathic rats, making sinularin attractive for this strategy (Chen et al., 2013).

Likewise, isolated compounds from different species of brown seaweeds, such as fucoxanthin and sulfated polysaccharides such as fucoidans, have the potential to interfere with tissue derangement or maladaptive changes in the nervous system that lead to NP (Chen et al., 2019; Hu et al., 2014). These compounds are well known for their anti-inflammatory, antioxidant, and immunomodulatory activities (Hu et al., 2014; Jegadeshwari & Rajaram, 2024; Kim et al., 2014; Nagahawatta et al., 2023). Specifically, intrathecal administration of fucoidan attenuates SNL-induced mechanical allodynia and thermal hypersensitivity in rats, possibly by inhibiting spinal glial activation, extracellular signal-regulated kinases (ERK) phosphorylation, and subsequent production of proinflammatory cytokines (Hu et al., 2014). It has been reported that fucoidan inhibits Toll-like receptors and interferes with the activation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways (Nagahawatta et al., 2023). Some extracts from *Ecklonia cava* and *Sargassum thunbergii* have shown promising

results from this perspective (Kim et al., 2014; Lee & Kang, 2015).

Astaxanthin

Astaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione), a marine ketocarotenoid with strong neuroprotective properties, has shown promising results in pain-related behaviors in CCI, SNL, and SCI models of NP (Fakhri et al., 2018; 2019; Hashemi et al., 2024; Jiang et al., 2018; Sharma et al., 2018; Zhao et al., 2021). The mechanisms of action of astaxanthin (AST) have been studied by various research groups focusing on relevant targets from different neurotransmission pathways, including NR2A and NR2B subunits of NMDA receptors, the serotonergic system, the $GABA_A$/benzodiazepine (BDZ) receptor complex, and opioid receptors (Fakhri et al., 2018; Hashemi et al., 2024; Jiang et al., 2018). However, all investigators agree that AST can limit central neuroinflammatory responses by stabilizing microglial and astrocytic resting phenotypes. In this pathophysiological context, glial cells also release nitroxidative species that contribute to the dysfunction of the glutamatergic pathway (Chen et al., 2010; Salvemini et al., 2011). It has been reported that AST treatment decreased lipopolysaccharide-induced nitroxidative stress in C6 glial cells, as well as their reactive form, exhibiting more complex and hypertrophic morphology with elevated glial fibrillary acidic protein (GFAP) expression (Sharma et al., 2018).

Oral administration of AST has therapeutic effects on CCI-induced thermal and mechanical hyperalgesia and comorbid depressive behaviors in mice, thereby mitigating the expression of TNF-α, IL-6, and IL-1β in both the hippocampus and the spinal cord (Jiang et al., 2018). Furthermore, intraperitoneal injection of AST improved CCI-induced cold allodynia and thermal hyperalgesia in a naloxone- and flumazenil-sensitive manner, suggesting the involvement of opioid and $GABA_A$/BDZ receptors in its effects. Following neural injury, researchers have reported paradoxical antihyperalgesic effects of systemic BDZs and the activity of subtype-selective modulators targeting spinal α2/α3 $GABA_A$Rs (Price et al., 2009; Qian et al., 2023). AST decreases matrix metalloprotease-9 (MMP-9) and -2 (MMP-2) activity following peripheral nerve injury (PNI); upregulation of these gelatinases is necessary for the development of the early and late phases of NP, respectively (Hashemi et al., 2024; Tajerian & Clark, 2015). In this context, AST also increases catalase activity and glutathione levels while suppressing nitrite production (Hashemi et al., 2024). AST activation of nuclear factor erythroid 2-related factor 2 (Nrf2) has been linked to its antioxidant and antiallodynic activity in the CCI model (Ciapała et al., 2023).

Within spinal glial cells, activation of the MAPK family is critical for NP development; in particular, cytoplasmic ERK1/2, p38, and c-Jun N-terminal kinases (JNK) signaling pathways link a broad array of extracellular stimuli to receptor activation and to the expression and release of pro- and anti-inflammatory glial mediators (Manning, 2006). An interesting study by Zhao et al. (2021), which focused on the ability of AST to inhibit spinal inflammatory responses induced by activation of the MAPK family and NF-κB following SNL, demonstrated the antihypernociceptive effect of AST. On day 11 post-surgery, AST decreased SNL-induced upregulation of the activated neuronal marker c-fos, the microglial marker IBA-1, and the astrocyte marker GFAP in the L5 spinal dorsal horn. AST treatment for 7 days also abolished increases in p-ERK1/2 and p-p38 levels, blocking SNL-induced nuclear translocation of NF-κB p65. Notably, AST regulated the balance between spinal proinflammatory and anti-inflammatory cytokines in this context, reducing overexpression of TNF-α, IL-1β, IL-6, and IL-17, while increasing expression of IL-10 and IL-4, which SNL reduced (Zhao et al., 2021). Notably, AST targets MAPK signaling pathways to regulate the release of glial mediators and also acts on MMP-9/2, which are upstream activators of glia (Ji et al., 2013).

AST treatment attenuates SCI-induced cold allodynia and motor dysfunction, which is related to its ability to decrease spinal NMDAR/NR2B expression and the active phosphorylated form of p38 MAPK (p-p38), as well as TNF-α, all of which participate in central sensitization and excitotoxicity-induced neurodegeneration (Fakhri et al., 2018). AST exhibits antiapoptotic and neuroprotective effects, decreasing demyelination and motor neuron loss induced by compression and contusion in rodent models of SCI (Fakhri et al., 2018; Masoudi et al., 2017). Notably, AST treatment protects spinal cord tissue at the site of injury and in the rostral and caudal regions following clip compression injury (Fakhri et al., 2018; 2019). The beneficial effects of AST on sensory-motor outcomes are likely related to its ability to prevent the increase in activated ERK1/2 and the reduction of pro-survival activated Akt following SCI, which plays an important role in the recovery of spinal cord function after secondary injury (Fakhri et al., 2019; He et al., 2022). These findings are further supported by previous results demonstrating that the antiapoptotic effects of AST were linked to reductions in caspase-3 and the Bax/Bcl-2 ratio, downstream targets of phosphorylated Akt that regulate apoptosis after SCI (Masoudi et al., 2017; Si & Zhu, 2022). Thus, preclinical evidence suggests that AST behaves as a multi-target compound with potential synergistic mechanisms, suggesting its benefits in treating NP, even following SCI. However, further mechanistic, dose-response, PK, and oral bioavailability studies are

required to improve their translational consistency. Recently, Ciapala et al. (2024) strengthened the evidence for the reproducibility and potential therapeutic utility of AST in a diabetic neuropathy model, particularly under repeated twice-daily treatment conditions and including both male and female animals. Although AST was not administered via a clinically relevant route, repeated treatment produced a sustained anti-hypersensitivity effect and attenuated opioid-induced hyperalgesia (Ciapala et al., 2024).

Fumagillin and aaptamine target neuroinflammation/angiogenesis processes

The formation of new blood vessels is associated with increased metabolic demand, as occurs when the dorsal horn nociceptive processing system is sensitized following neural injury (Latremoliere & Woolf, 2009; Sharma et al., 2023). Thus, alongside spinal neuroinflammation, angiogenic processes contribute to the development and maintenance of NP; in particular, vascular endothelial growth factor (VEGF) signaling has been implicated in prolonged NP behaviors following PNI (Sharma et al., 2023; Wen et al., 2021). Additionally, spinal overexpression of cluster of differentiation 31 (CD31), a glycosylated transmembrane adhesion protein highly expressed on endothelial cells, has been observed in CCI and SCI rats (Wen et al., 2021). Sung et al. (2023) reported that CCI upregulates neuronal lactate dehydrogenase A (LDHA) expression in the ipsilateral spinal cord; LDHA is an essential glycolytic enzyme that catalyzes the conversion of pyruvate to lactate. By regulating neuroinflammation and angiogenesis, LDHA has been proposed as a potential therapeutic target for treating NP (Cheng et al., 2024; Sung et al., 2023). The pyruvate dehydrogenase kinase (PDK)-lactic acid axis modulates glial and neuronal phenotypes and activities, as well as the functional polarization of peripheral immune cells (Devanney et al., 2020; Kong et al., 2022).

Fumagillin, a mycotoxin produced by *Aspergillus fumigatus* with known antiangiogenic activity, reduced CCI-induced nociceptive behaviors. Although administered intrathecally, its effect was compared to that of the anti-VEGF antibody following a 14-day preventive treatment regimen. In addition to stimulus-evoked responses, the study assessed spontaneous pain through changes in hindpaw weight distribution. The effect was linked to its ability to inhibit spinal angiogenesis, astrocyte activation, and proinflammatory cytokine production (Wen et al., 2021). From this novel perspective, Sung et al. (2023) explored, for the first time, the antihypernociceptive effect of aaptamine, an alkaloid isolated from the sponge *Aaptos aaptos* (Nakamura et al., 1982), in the CCI model. These authors demonstrated that aaptamine alleviates mechanical allodynia and thermal hyperalgesia, possibly by downregulating CCI-induced spinal angiogenic markers—VEGF and CD31—as well as LDHA expression in the spinal cord (Sung et al., 2023). Interestingly, VEGF was expressed predominantly in astrocytes, suggesting that the antiangiogenic effect of aaptamine could be mediated by inhibiting astrocytic VEGF release. The results suggest that aaptamine exerts its antihyperalgesic effect not only by decreasing the expression of enzymatically active LDHA in spinal dorsal horn neurons but also by inhibiting its nuclear translocation. Previously, aaptamine has been recognized as a full agonist at the δ-opioid receptor and only a weak partial agonist at the μ-opioid receptor (Johnson et al., 2017). Since the δ-opioid receptor has been implicated in the regulation of hypoxia-inducible factor 1 alpha (HIF-1α) expression, the authors proposed that this bioactive compound downregulates LDHA expression and angiogenesis through primary activation of this receptor—a mechanism that could provide additional analgesic synergies; however, further research is needed to elucidate the complete analgesic pathway of aaptamine. This evidence, combined with its low toxicity, indicates potential for further drug development (Sung et al., 2023). Both compounds show a consistent analgesic signal in the CCI model and point toward neuroinflammatory and vascular-metabolic mechanisms, yet their mechanistic robustness and translational value are limited, with direct target validation not fully established and feasibility on the IT route and a single model of traumatic peripheral neuropathy. A subsequent study by Wen et al. (2024) linked fumagillin's effects to HIF-1α-regulated angiogenic and glycolytic proteins in astrocytes and neurons; thus, fumagillin showed relatively stronger mechanistic support owing to convergent findings across preclinical studies (Wen et al., 2021; 2024). However, the translational maturity of both compounds remains preliminary and requires validation in clinically relevant models, alongside PK, long-term safety assessment, and clinical feasibility, especially because the antiangiogenic mechanism raises a translational safety issue.

Marine-derived bioactives targeting nicotinic acetylcholine receptors: Relevance for NP treatment

α-Conotoxins: Unexpected analgesic activity

Nicotinic acetylcholine receptors (nAChRs) have long been investigated as targets for pain management; however, no novel analgesics targeting these receptors have yet achieved clinical approval. Although nAChR agonists produce analgesia in NP models and humans, their lack of selectivity also causes off-target activity at α3-containing subtypes, leading to autonomic nervous system side effects, especially in the cardiovascular and gastrointestinal systems (Hone & McIntosh, 2018;

2023; Rowbotham et al., 2009). Nicotine stimulation of α3β4 receptors may also have a hyperalgesic effect, and the α7 subtype is known for its dual role in nociceptive pathways (Hone & McIntosh, 2018). Thus, given the diversity and broad distribution of these receptors, the development of subtype-selective ligands is a pivotal factor in avoiding both adverse and counteracting effects (Hone & McIntosh, 2018; 2023). However, a new approach emerged from unexpected *in vivo* findings related to the antiallodynic and antihyperalgesic effects of some members of the α-conotoxin family from cone snails across several NP models. Vc1.1, RgIA, MII, and AuIB have demonstrated excellent analgesia in traumatic neuropathy and CIPN models (Huynh et al., 2020a; Napier et al., 2012; Satkunanathan et al., 2005; Vincler et al., 2006) despite being classified pharmacologically as competitive antagonists of neuronal, rather than muscle, nAChRs (Gao et al., 2017; Li et al., 2025). Specifically, Vc1.1 and RgIA selectively target α9/α10 nAChRs, which are widely distributed on immune cells (Richter & Grau, 2023). Precisely, their exquisite nAChR subtype selectivity allows them to discriminate between α9 and α7 subtypes, making them good candidates for NP treatment with neuroprotective and/or neurorestorative effects (Di Cesare Mannelli et al., 2014; Hone & McIntosh, 2018; Satkunanathan et al., 2005; Vincler et al., 2006). Similar potent nAChR antagonism, preferentially at the α7 subtype, has been reported by α-CTX Mr1.1 isolated from *Conus marmoreus* venom duct. However, synthetic Mr1.1 analogs have been engineered to display increased selectivity for human α9α10 receptors, and one variant (Mr1.1 S4Dap) decreased NP behavior in CCI rats (Liang et al., 2022). Hence, the possibility of influencing neuroimmune interactions and neuroinflammation mediated by nAChR modulation has emerged as another attractive alternative (Hone & McIntosh, 2023).

These observations generated intense debate regarding the relative contribution of α9α10 versus other nAChR subtypes—and even non-nAChR targets—to the analgesic mechanisms of α-conotoxins. Genetic and pharmacological studies using α9 KO mice yielded mixed results, with some experiments supporting α9 dependence of analgesia (Huynh et al., 2020a; 2022; Romero et al., 2017), while others suggested α9-independent mechanisms (Mohammadi & Christie, 2015). Parallel work demonstrated that a subset of α-conotoxins (RgIA, Vc1.1, PeIA, AuIB) inhibit high-voltage-activated N-type calcium channels in DRG neurons via $GABA_B$ receptor ($GABA_BR$) activation and G-protein coupling, offering an alternative or complementary mechanism for their antinociceptive effects (Adams & Berecki, 2013; Adams et al., 2012; Callaghan et al., 2008; Huynh et al., 2015; Klimis et al., 2011). However, this $GABA_BR$-dependent mechanism is not consistently observed across expression systems, and Vc1.1 analogs that retain α9α10 activity but lack $GABA_BR$ stimulation failed to produce analgesia in some studies, further fueling controversy (McIntosh et al., 2009; Napier et al., 2012; Nevin et al., 2007; Wright et al., 2015).

Despite these complexities, accumulating evidence over the past 15 years supports a dual-mechanism model in which α9α10 nAChR antagonism confers long-lasting, disease-modifying and neuroimmune-modulating effects, while $GABA_BR$-mediated inhibition of N-type Ca^{2+} channels underlies rapid symptomatic analgesia (Callaghan et al., 2008; Christensen et al., 2017; Di Cesare Mannelli et al., 2014; Huynh et al., 2020a; 2022; Klimis et al., 2011; Li et al., 2016; Liang et al., 2022; Luo et al., 2015; Romero et al., 2017; Wang et al., 2019, 2024). RgIA, for example, not only reverses CCI-induced mechanical allodynia and hyperalgesia but also attenuates Wallerian degeneration-related changes in the injured nerve, reduces infiltration of CD86-positive M1 macrophages, protects against axonal demyelination, and prevents spinal microglial and astrocytic activation, indicating a neuroprotective and anti-inflammatory profile (Di Cesare Mannelli et al., 2014). In oxaliplatin (OXA)-induced neuropathy, RgIA and its analogs also normalize nucleolar alterations in DRG neurons and modulate glial activation patterns, suggesting that nAChR targeting may promote glial protective responses rather than solely suppress glial function (Pacini et al., 2016).

Strengthening of a9a10 nAChR as a translational target. Significance of RgIA and aO-CTX GeXIVA for CIPN

Vc1.1 entered phase II human clinical trials as a drug for NP, conducted by Metabolic Pharmaceuticals Limited in 2005, but it was dropped due to its lower potency in humans (IC_{50} = 1 μM) compared with rat (IC_{50} = 20-100 nM) α9α10 nAChRs (Li et al., 2021; Yu et al., 2018b). This species difference is postulated to be determined by residue 59 (Thr in rat and Ile in human) at the α9 subunit (−) component (Yu et al., 2013). Similarly, RgIA is 300-fold less potent in humans than in rats at the α9α10 nAChR (Azam & McIntosh, 2012). Therefore, to improve RgIA affinity for the human target and clarify mechanistic controversies surrounding α9α10 nAChR as an analgesic target, the McIntosh group developed RgIA4, an analog of α-conotoxin RgIA containing two unnatural amino acids (citrulline at position 9 and 3-iodotyrosine at position 10). This analog retains highly selective targeting of α9α10 nAChRs but shows no activity on $GABA_B$ receptors in DRG or immortalized cell lines. RgIA4 is 1,000-fold more selective for α9α10 nAChRs compared with other molecular targets, including opioid and $GABA_B$ receptors, and shows high affinity for both rodent and

human α9α10 nAChRs (IC_{50} rat = 0.9 nM; IC_{50} human = 1.5 nM) (Christensen et al., 2017; Romero et al., 2017).

Earlier studies reported that α9-nAChR KO mice exhibit only chronic mechanical hyperalgesia, lacking thermal or mechanical allodynia, thus raising questions about the congruence between the sensory behavioral phenotype of these animals and the analgesic profile of α-conotoxins (Mohammadi & Christie, 2015). Pacini et al. (2016) and Romero et al. (2017) demonstrated the activity of RgIA and RgIA4, respectively, using robust *in vivo* experimental designs and clinically relevant behavioral assessments in models of OXA-induced peripheral neuropathy, where both innate and adaptive immune responses are implicated (Boyette-Davis et al., 2015; Makker et al., 2017). While the full clinical phenotypic heterogeneity of NP has yet to be replicated in experimental models, CIPN models offer a closer approximation to clinical painful polyneuropathies (Rice et al., 2018). OXA-induced neurotoxicity typically results in persistent cold sensitivity in wild-type mice, mirroring the sensory profile observed in patients after chemotherapy (Attal et al., 2009).

Repeated administration of both RgIA and RgIA4 clearly prevented OXA-induced chronic cold allodynia and mechanical hypersensitivity (Pacini et al., 2016; Romero et al., 2017). Notably, cold allodynia was absent in α9 KO mice or when RgIA4 was co-administered with OXA, demonstrating a critical role for α9α10 nAChR in the progression of chemotherapy-induced NP (Romero et al., 2017). Christensen et al. (2017) corroborated the efficacy of RgIA4 in alleviating OXA-induced cold allodynia, with a long-term effect persisting for 21 days after treatment discontinuation, despite the peptide's short plasma half-life (<20 minutes). They also confirmed the lack of activity of this analog at $GABA_B$Rs and demonstrated that Vc1.1 and AuIB do not activate $GABA_B$Rs as measured by G-protein-based BRET assays in HEK cells (Christensen et al., 2017).

In the same CIPN model, subcutaneous administration of RgIA4 (40 μg/kg) prevented acute cold allodynia in wild-type mice but not in mice lacking the α9 nAChR-encoding gene, chrna9 (Huynh et al., 2022). Similarly, RgIA-5524, a methylene thioacetal analog of RgIA, lost its analgesic activity following deletion of the α9 nAChR subunit (Zheng et al., 2021). RgIA4 also failed to reverse allodynia in mice depleted of CD3+ T cells, indicating that both α9 nAChR and T-cell mechanisms are required for its protective effects against OXA-induced acute cold allodynia (Huynh et al., 2022). These authors observed an increase in CD3+ T cells, predominantly helper T cells (Th), following oxaliplatin treatment. Most findings highlight a role for T cells in both the enhancement and resolution of NP (Austin & Moalem-Taylor, 2010; Manning, 2006). Specifically, Th1 cytokines (interferon gamma, TNF-α) and Th17-derived IL-17 promote pain sensitivity (Austin & Moalem-Taylor, 2010), whereas Th2-derived cytokines (IL-4, IL-10) may exert protective effects (Tiwari et al., 2014). Additionally, the analgesic effect of β-endorphin-containing T cells after nerve injury has been documented (Labuz et al., 2010).

Although the underlying mechanisms of CIPN remain incompletely understood, animal models that simulate this condition exhibit pathophysiological differences depending on the chemotherapeutic agent, as well as general maladaptive changes in the peripheral and central nervous systems (Boyette-Davis et al., 2015). Huynh et al. (2020a) propose that blockade of α9α10 nAChRs represents a convergent target for the prevention and/or treatment of NP across various CIPN models, regardless of the differing neuropathological mechanisms of each chemotherapeutic class. To investigate this, they employed a prophylactic approach in a PTX-induced peripheral neuropathy model in rats (Polomano et al., 2001), administering RgIA4 concurrently with PTX. Although RgIA4 did not prevent mechanical allodynia during the induction period (days 0–16), animals receiving 80 μg/kg subcutaneously exhibited significant recovery from mechanical allodynia by day 31. This effect persisted for 12 days after the last RgIA4 dose, suggesting a rescue mechanism (Huynh et al., 2020a).

Vc1.1, its analogs, and RgIA/RgIA4 are supported by extensive and mechanistically convergent preclinical evidence from *in vitro* receptor modulation and multiple *in vivo* relevant models of NP. However, the translational strength of this evidence is tempered by uncertainties regarding the precise analgesic mechanisms, species-dependent differences in α9α10 nAChR pharmacology, and limited reproducibility of preclinical efficacy in humans, as exemplified by the clinical failure of Vc1.1. RgIA4 (KCP-400) represents a more translationally advanced candidate owing to its improved selectivity and potency at the human α9α10 receptor, although clinical validation and comprehensive PK, safety, and long-term efficacy data remain lacking. Despite progressing to advanced preclinical development, however, it did not enter clinical trials.

On the other hand, the discovery and characterization of the previously unidentified O1-superfamily conotoxin GeXIVA from *Conus generalis*, as well as the identification of its most effective isomers for antagonizing α9α10 nAChRs, further support the therapeutic validity of this target (Li et al., 2016; Luo et al., 2015; Wang et al., 2019; Zhangsun et al., 2017). GeXIVA is structurally distinct from other O1 gene superfamily members, possessing four rather than six cysteine residues. Although ω-conotoxins also belong to this superfamily, they interact pharmacologically with

VGCCs and show weak activity against nAChRs; in contrast, GeXIVA does not block VGCCs and shows potent antagonist activity at α9α10 nAChRs (Luo et al., 2015). Unlike α-conotoxins, the bead isomer GeXIVA[1,2] is more potent at α9α10 nAChRs than the globular isomer GeXIVA[1,4] (IC_{50} = 4.6 nM vs. IC_{50} = 7 nM), and the binding site does not overlap with that of the competitive antagonist RgIA. All three disulfide bond isomers of GeXIVA show only ~5-fold differences in potency between human and rat α9α10 receptors (Zhangsun et al., 2017).

A single intramuscular dose of GeXIVA[1,2], likely acting peripherally, improved CCI-induced mechanical hypersensitivity as assessed by von Frey filament and electronic von Frey tests, with effects comparable to morphine and without causing motor incoordination (Luo et al., 2015). Additionally, both isomers demonstrated sustained antihypersensitive effects without tolerance after repeated dosing for 7 or 14 days in CCI rats, with GeXIVA[1,2] maintaining efficacy up to two weeks after treatment cessation—slightly surpassing GeXIVA[1,4]. Treatment regimens initiated 10–12 days post-surgery allowed differentiation of their therapeutic effects. Importantly, GeXIVA[1,2] and GeXIVA[1,4] did not affect acute thermal nociception in the tail flick model in non-injured animals, indicating selective activity under pathological conditions of neural injury (Li et al., 2016).

Wang et al. (2019) extended investigations into GeXIVA in an OXA-induced peripheral neuropathy model with established NP, employing single and 16-day repeated dosing to distinguish PK influences from homeostatic compensation following sequential administration (Yang et al., 2025). Compared with gabapentin, GeXIVA[1,2] alleviated mechanical and cold allodynia after a single IM injection. Unlike gabapentin, the cumulative long-term analgesic effect of GeXIVA[1,2] on mechanical allodynia persisted for 10 days after discontinuation (Wang et al., 2019). Furthermore, GeXIVA[1,2], administered preventively alongside OXA, also inhibited the development of mechanical allodynia induced by chronic OXA treatment (Wang et al., 2024). In chronic pain states, transcriptional changes driven by receptor-coupled cAMP response element-binding protein (CREB) and other transcription factors upregulate genes that result in long-lasting central sensitization (Latremoliere & Woolf, 2009). Given the critical role of spinal cord circuits in pain processing and the ability of other peripherally acting nAChR antagonists to affect CNS glial cells, Wang et al. (2024) focused on gene expression changes in L4–L6 spinal cord segments. They identified 209 differentially expressed genes in the CIPN model, some of which were reversed by concurrent GeXIVA[1,2] treatment, particularly those associated with immune-related pathways, such as cytokine–cytokine receptor interactions. OXA-induced mechanical hypersensitivity has been linked to hyperactivation of spinal astrocytes, increased proinflammatory cytokine levels, and decreased anti-inflammatory cytokine levels, but not lymphocytic mobilization (Janes et al., 2015). Thus, the authors speculate that inhibition of overactive neuron–glial signaling may underpin the antiallodynic effect of GeXIVA[1,2] in this context (Wang et al., 2024). Overall, due to their promising preclinical results in clinically relevant NP models, αO-conotoxin GeXIVA disulfide-bond isomers are emerging as promising translational candidates, benefiting from interdisciplinary research and sustained development progress, but further validation of the relationship between target modulation and the *in vivo* analgesic phenotype is required.

Interestingly, despite the poor stability of GeXIVA[1,2] in human, rat, and dog serum, *in vivo* PK studies report that its terminal half-life in rats and dogs is extremely short relative to its analgesic effect, which lasts up to 6 hours (Yu et al., 2018a; Zhu et al., 2021; 2022). PK modeling-based mechanistic assessment suggests that after GeXIVA[1,2] binds to α9α10 nAChRs, it modulates downstream signaling pathways, possibly producing its antiallodynic effect by inhibiting endogenous factors (Zhu et al., 2022). This inferential evidence provides a biologically plausible explication supporting the mechanistic hypothesis, which must be corroborated experimentally. However, these authors reported low systemic bioavailability and short systemic half-life of GeXIVA after IM administration, highlighting important PK limitations. In this context, He et al. (2025) reported an innovative technological strategy to reduce GeXIVA degradation and improve administration via transdermal delivery. This multidisciplinary team encapsulated GeXIVA[1,2] in an optimized 4:3 (w/w) polyvinyl alcohol–sucrose composite hydrogel, which acts as a physical barrier to enhance stability. *In vivo* efficacy experiments confirmed that a single microneedle patch application increased paw withdrawal threshold (PWT) for three days in CCI and SNI models and improved locomotor function in SNI mice. The hydrogel microneedle patch platform extends GeXIVA[1,2]'s analgesic duration from hours to days via sustained release, while addressing administration challenges, representing a promising approach for chronic NP treatment (He et al., 2025). Although this technological strategy enhances the translational potential of GeXIVA, further PK/PD evidence is needed to determine whether the new formulation effectively improves systemic exposure and therapeutic durability. The activity of GeXIVA in NP models has been confirmed outside the originating laboratory (Luo et al., 2015), an important aspect supporting its efficacy; however, regarding

safety, the evidence is insufficient to establish its tolerability during prolonged or long-term administration. This is particularly relevant for the sustained-release transdermal formulation.

Marine-derived bioactives interacting with non-opioid GPCRs

The chemical diversity of marine snail venoms provides a large, underexploited source of effective, selective peptides with the potential to modulate GPCRs (Adams & Berecki, 2013; Gao et al., 2017; Sadeghi et al., 2017). Nonetheless, most venom peptide-based analgesic candidates in preclinical and clinical development target ionotropic receptors to modulate membrane excitability, whereas relatively few venom peptides displaying analgesic properties act via GPCRs (Li et al., 2021; Liao et al., 2019; Mackieh et al., 2021; Safavi-Hemami et al., 2019). This discrepancy may be attributed to the predominance of channel-targeted peptides in cone snail venoms, consistent with their toxic mechanisms. However, it may also reflect a tendency in NP mechanism-based peptide discovery to focus on established ionotropic targets (Daniel & Clark, 2017). However, over the past 15 years, this balance has begun to shift, with a growing interest in GPCR-targeting conopeptides that have demonstrated *in vivo* efficacy in NP models or in Phase Ia clinical trials involving patients with central NP (Belgi et al., 2021; Bjørn-Yoshimoto et al., 2025; Jergova et al., 2021; Klimis et al., 2011; Martin et al., 2022; Sang et al., 2016). In particular, modulation of VGCCs by marine-derived bioactives acting via GPCRs, including $GABA_BRs$, is recognized as an important alternative in the management of NP (Adams & Berecki, 2013; Adams et al., 2012; Li et al., 2025; Sadeghi et al., 2017). Furthermore, in the clinical setting, intrathecal baclofen—a selective $GABA_BR$ agonist—has been reported to have antihyperalgesic effects in patients with SCI (Kumru et al., 2024).

α-Conotoxins as $GABA_BR$ positive allosteric modulators

Callaghan et al. (2008) demonstrated that α-conotoxins Vc1.1 and RgIA attenuated native N-type $Ca_V2.2$ channel currents in dissociated rat DRG neurons. The inhibition of these channels was not reversed by depolarizing prepulses. However, it was abolished by pertussis toxin, intracellular GDP-βS, or a selective inhibitor of pp60c-Src tyrosine kinase, suggesting an indirect mechanism mediated by a Gi/o-coupled GPCR pathway. Interestingly, this effect was blocked by $GABA_BR$ antagonists CGP 55845, CGP 54626, or phaclofen. This group proposed a novel mechanism by which α9α10 nAChR-selective conotoxins modulate $Ca_V2.2$ channels in primary afferent neurons by acting as positive allosteric modulators (PAMs) via G protein-coupled $GABA_BRs$ (Callaghan et al., 2008). Moreover, they demonstrated in α9 KO mice that the inhibition of N-type Ca^{2+} channels by Vc1.1 and RgIA is not mediated by α9α10 nAChR expression in DRG neurons (Callaghan & Adams, 2010). Additionally, Cuny et al. (2012) found that siRNA knockdown of both human $GABA_BR$ subunits in DRG neurons impaired the α-conotoxin (Vc1.1, RgIA, and AuIB)-mediated inhibition of $Ca_V2.2$. However, this blockade was restored in HEK293 cells expressing human $GABA_BR$ subunits and $Ca_V2.2$. The pharmacological significance of this mechanism is further supported by findings that administration of the selective $GABA_BR$ antagonists CGP 55845 or SCH 50911 completely reversed the analgesic effects of Vc1.1 in CCI, PNL, and chronic visceral hyperalgesia models (Castro et al., 2017; Clark et al., 2010; Klimis et al., 2011).

Functional selectivity and biased signaling

A potential advantage of α-CTXs has been suggested: functional selectivity at $GABA_BR$ and inhibition of VGCCs via a novel voltage-independent mechanism, distinct from the traditional Gβγ-mediated voltage-dependent inhibition exerted by conventional agonists (Daniel & Clark, 2017; Sadeghi et al., 2017). In addition to $Ca_V2.2$, Vc1.1 differentially inhibits $Ca_V2.1$ and $Ca_V2.3$ when heterologously expressed in HEK293 cells; unlike conventional agonists, which inhibit both subtypes comparably, Vc1.1 preferentially inhibits $Ca_V2.3$ (Berecki et al., 2014). These channels are considered less susceptible to direct Gβγ modulation than $Ca_V2.1$ and $Ca_V2.2$, due to structural differences (Shekter et al., 1997). While the physiological role of $Ca_V2.3$ in nociceptive pathways is less well understood, it is expressed in human DRG neurons and trigeminal ganglia, and is regarded as an important target for NP analgesics (Castro et al., 2017; Ferreira & Ferreira, 2024). Interestingly, $Ca_V2.3$ appears to be primarily involved in long-lasting synaptic plasticity rather than rapid synaptic transmission (Dietrich et al., 2003).

Huynh et al. (2015) used a heterologous $GABA_BR$ expression system, electrophysiology, and mutagenesis to propose a model of signaling bias in $Ca_V2.2$ inhibition, resulting from allosteric ligand binding to GPCRs at sites distinct from the endogenous ligand binding domain. They observed that the R576D mutation in the $GABA_B2$ subunit, which inhibits baclofen-induced G protein activation, does not affect Vc1.1 activity. In contrast, truncation of the proximal C-terminus (PCT) of the $GABA_B1$ subunit leads to loss of sensitivity to Vc1.1 but does not affect baclofen signaling. These events suggest that the PCT domain may couple $GABA_BR$ and $Ca_V2.2$ during Vc1.1 activation (Huynh et al., 2015). Moreover, S246A and S270A mutations in the $GABA_B1$ Venus Fly Trap (VFT) domain, which

render the receptor completely insensitive to baclofen and GABA, do not affect the ability of CTXs like Vc1.1 to inhibit VGCCs, and these peptides do not compete with orthosteric ligands. Collectively, these data indicate that α-conotoxins inhibit $Ca_V2.2$ and $Ca_V2.3$ channels via a novel mechanism involving the PCT domain and the dissociated Gαi/o subunit, without direct channel binding, and mediated by c-Src kinase-dependent phosphorylation sites in the channel's C-terminus (Berecki et al., 2014; Huynh et al., 2015). Recent studies by Bony et al. (2022a) identified an allosteric binding site at the interface of the VFT domains of the $GABA_BR$ subunits for analgesic α-conotoxins. Unlike conventional $GABA_BR$ agonists, which typically bind the $GABA_B1$ VFT domain, α-CTXs interact with VFT residues in both subunits. The critical $GABA_BR$ residues implicated in the interaction include K168 and R207 on the $GABA_B2$ subunit, and S130, S153, R162, E200, F227, and E253 on $GABA_B1$. However, further functional assays are required to definitively confirm the binding site of α-CTXs.

GIRK channel potentiation

Previous studies have shown that Vc1.1 and Rg1A do not activate G protein–gated inwardly rectifying potassium (GIRK) channels heterologously co-expressed with $GABA_BRs$ in *Xenopus laevis* oocytes (McIntosh et al., 2009). However, Bony et al. (2022b) reported a novel analgesic mechanism for α-conotoxins Vc1.1, RgIA, and PeIA, based on their ability to potentiate GIRK-mediated K^+ currents in HEK293T cells recombinantly expressing human GIRK1/2 channels and $GABA_BRs$. Moreover, $GABA_B$ receptor-dependent GIRK channel potentiation by Vc1.1 was demonstrated in mouse DRG neurons, where it hyperpolarized the cell membrane potential, reducing excitability (Bony et al., 2022b). In the context of NP, reduced GIRK1/2 expression and function have been associated with sensory neuron hyperexcitability, and ectopic firing has been demonstrated (Smith, 2020). Thus, the synergistic action to inhibit $Ca_V2.2/2.3$ and potentiate GIRK channels via allosteric α-conotoxin-$GABA_BR$ agonism, reducing neuronal excitability, offers another element in favor of these bioactives as an alternative non-opioid therapeutic for NP. Nevertheless, most studies have assessed these peptides in heterologous $GABA_BR$ expression systems and isolated murine DRG neurons; strategies using human pluripotent stem cell-derived sensory neurons offer a promising avenue for elucidating these mechanisms in human tissue. Although both Vc1.1 and baclofen reduced membrane excitability via $GABA_BR$-mediated modulation of high-threshold VGCCs in these cells, GIRK channels were not required for this effect, despite their expression and functionality. Therefore, the evidence from this model suggests a limited interaction between $GABA_BRs$ and GIRK channels in humans (St Clair-Glover et al., 2025).

Overcoming species differences: Rational design of a-conotoxin analogs and its contribution for the development of next-generation a9a10 nAChR-targeting therapies

Species-specific differences in target sensitivity, low stability with short metabolic half-lives, and poor oral bioavailability pose significant challenges to the clinical development of marine-derived bioactives (Daniel & Clark, 2017; Gao et al., 2017). Various authors have implemented chemical re-engineering or novel technological strategies to enhance the biopharmaceutical properties of conopeptides for potential clinical translation (Clark et al., 2010; He et al., 2025; Lee et al., 2015; Li et al., 2024; Xu et al., 2020; Zhang et al., 2025). Others, using mutagenesis and *in silico* peptide–receptor interaction studies, have sought to elucidate the determinants of interaction with specific molecular targets in rodents and humans, thereby achieving rational design of analogues with improved potency and selectivity (Azam & McIntosh, 2012; Azam et al., 2015; Bony et al., 2022a; Chu et al., 2019; Deuis et al., 2016; Huynh et al., 2020b; Leipold et al., 2011; Li et al., 2019; Logashina et al., 2022; Pan et al., 2021; Tae et al., 2025; Zhangsun et al., 2017).

Overall, in the α-CTX family, the preclinical evidence can be considered robust for demonstrating pharmacological activity and antihypernociception, but moderate for supporting the prediction of clinical efficacy-precisely due to the experience from the clinical development of the linear Vc1.1 (ACV1) and interspecies differences (Li et al., 2021; Yu et al., 2018b). Subsequently, Clark et al. (2010) developed an orally active peptide by cyclizing the α-conotoxin Vc1.1, joining its N- and C-termini via a six-residue linker. Backbone-cyclized Vc1.1 (cVc1.1) exhibits higher selectivity and potency for $GABA_BR$-mediated N-type Ca^{2+} channel current inhibition than the parent linear peptide. Thus, cVc1.1 altered the activity profile, providing a significant *in vivo* analgesic effect 4 hours after oral administration. The potency of cVc1.1 to decrease mechanical hypersensitivity in CCI rats was more than two orders of magnitude superior to gabapentin. Backbone cyclization has been applied to other analgesic conotoxins such as RgIA and GeXIVA to improve their serum stability and folding yield (Halai et al., 2011; Wu et al., 2017). Despite being a peptide engineering success, cVc1.1 did not become a clinical candidate; *in vivo* PK characterization showed that the improved stability conferred by backbone cyclization *in vitro* did not translate into higher oral bioavailability or a longer systemic half-life than the linear peptide. Although negligible brain penetration and predominant renal distribution were favorable from a safety perspective,

the persistently low oral bioavailability (0.2–0.3%) represents an important limitation for further development (Poth et al., 2021). Notably, no subsequent clinical development of cVc1.1 has been established, suggesting that the PK profile remained a major translational barrier. Vc1.1/cVc1.1 continue to be investigated as a scientific platform (Castro et al., 2017; Chu et al., 2019; Tae et al., 2025; Zhang et al., 2025).

In particular, Chu et al. (2019) investigated the Vc1.1 structure-activity relationship at the human α9α10 nAChR, using minimal side-chain replacement. MD simulations identified the involvement of the hydroxyl group of Vc1.1, Y10, which forms a hydrogen bond with the carbonyl group of α9 N107. Furthermore, they determined the activity of systematically generated unnatural amino acid analogs of Vc1.1 at heterologously expressed human α9α10 nAChRs in *Xenopus laevis* oocytes using two-electrode voltage clamp electrophysiology. Notably, the second-generation mutants of Vc1.1 [S4 Dab, N9A] and [S4 Dab, N9W] increased potency at the α9α10 nAChR by 20-fold compared with that of Vc1.1. Some authors focused on the rational design of Vc1.1-derived α-CTXs with enhanced nAChR subtype selectivity. Tae et al. (2025) explored the molecular determinants of potency and selectivity across human nAChR subtypes, finding preferential inhibition of the α3β2 (which can be associated with hypotension) and α9-containing subtypes involved in neuroinflammatory mechanisms of NP. MD simulations identified β2 E86 and α9 N179 and N213 as key molecular determinants of Vc1.1 potency. Recently, the group of Clark successfully synthesized polyethylene glycol (PEG)-cVc1.1 using solid-phase peptide synthesis and enzymatic cyclization with structural, stability, and activity profiles similar to cVc1.1. They used PEG linkers with varying lengths as substitutes for peptide backbone cyclization linkers, highlighting the essential role of helicity in maintaining peptide function and stability (Zhang et al., 2025).

Regarding the limitations of RgIA, through the systematic substitution of residues found in the human α9 subunit into the homologous position in the rat α9 subunit, Azam and McIntosh (2012) revealed that a single point mutation—from Thr56 (rat) to Ile56 (human)— is the primary determinant of species-specific differences in potency. Lastly, this group reports an additional binding site for ACh in the rat α9α10 nAChR between the α10(+)/α9(−) interface, which provides a high-affinity binding site for α-CTX RgIA. They also propose a similar interaction for Vc1.1 with this interface (Azam et al., 2015). Other authors continued to predict the interaction mechanism between RgIA/Vc1.1 and the rat α9α10 nAChR. They identified novel residues, including Tyr95 and Trp151 at the α9(+)/α10(−) interface and Tyr196 and Arg59 at the α10(+)/α9(−) interface in these models, which are determinant and particularly highlight the role of electrostatic interactions. The interaction of RgIA is mediated by negatively charged residues, whereas positively charged residues mediate that of Vc1.1 (Li et al., 2019). Pan et al. (2021) reported for the first time the co-crystal structure of RgIA and RgIA4 in complex with *Aplysia californica* acetylcholine binding protein (Ac-AChBP) at a resolution of 2.6 Å. Based on the structure of the complexes, together with MD simulation, they predicted the key residues of α9α10 nAChR in determining its high affinity for RgIA and its analog RgIA4. In addition, using a mutational approach, Huynh et al. (2020b) determined that the primary contributors to activity are the side-chain properties of RgIA residues rather than backbone conformation. They observed that replacement of the C-terminal Arg13 with Tyr significantly increased RgIA potency for human α9α10 nAChR by 243-fold.

Although Vc1.1, RgIA, and their analogs did not themselves achieve clinical translation, the extensive academic and preclinical evidence generated around these peptides contributed to the development of KCP-506, which has subsequently progressed to first-in-human Phase 1 clinical development for chronic NP (NL-OMOM28854). Thus, their translational contribution should be considered not only in terms of individual drug candidates but also as a cumulative platform for the development of next-generation α9α10 nAChR-targeting therapies.

Marine-derived bioactives modulating GPCRs from endogenous pain modulation pathways

Activating endogenous pain-inhibitory mechanisms by enhancing descending influences from the brainstem or higher centers has long been a conventional strategy for managing NP. Antidepressant medications, recommended as first-line treatments for NP, exert their effects by augmenting inhibitory control through modulation of the serotonergic and noradrenergic systems (Soliman et al., 2025). Within this framework, considerable research has focused on targeting the endocannabinoid system, either by increasing endogenous cannabinoid levels or by administering exogenous ligands for CB receptors (Finn et al., 2021; Moore et al., 2021). Furthermore, the peptides neurotensin and somatostatin have attracted significant attention, not only for their well-established neuroendocrine roles but also for their recognized actions as neurotransmitters and neuromodulators within the CNS (Dobner, 2006; Viollet et al., 2008). Importantly, crucial components of these endogenous systems are expressed throughout the nociceptive pathway—including supraspinal regions, primary sensory neurons, and the dorsal horn of the spinal cord—where they can be

modulated pharmacologically (Dobner, 2006; Finn et al., 2021; Kántás et al., 2019).

Marine-derived bioactives modulating neurotensin receptors

Contulakin-G

Contulakin-G is a 16-amino-acid glycopeptide isolated from the venom of *Conus geographus* with homology to the mammalian peptide NTS at the C-terminus, and it binds three subtypes of neurotensin receptors with submicromolar potency (Craig et al., 1999). While neurotensin receptor 2 (NTSR2) is primarily known for its antinociceptive action, neurotensin receptor 1 (NTSR1) exhibits a broader range of effects, including hypothermia, hypotension, cell proliferation, and modulation of the dopaminergic system (Allen et al., 2007; Eiselt et al., 2019). Thus, the development of neurotensin analogs that selectively target NTSR2 is a safety necessity and represents a promising opportunity for treating NP (Eiselt et al., 2019). Contulakin-G exhibits potent analgesic activity in rats following intrathecal delivery with a minor increase in body temperature and no significant cardiovascular effects, motor incoordination, or tolerance (Allen et al., 2007; Martin et al., 2022; 2025). Following SNL and PNL, intrathecal contulakin-G reverses mechanical and thermal hypersensitivity in two different species in a sex-independent manner (Martin et al., 2022). Furthermore, in a cancer-induced bone pain (CIBP) model, which includes a neuropathic component (Mantyh & Thompson, 2014), contulakin-G attenuated both spontaneous pain behaviors and evoked mechanical hypersensitivity (Martin et al., 2025).

Mechanism of action: NTSR2/Ca_V2.3 signaling

Martin et al. (2022) evaluated the effects of editing the ntsr2 and cacna1e genes on contulakin-G analgesia in PNL mice. Contrary to morphine, contulakin-G did not induce antinociception when spinal NTSR2 or Ca_V2.3 were edited. Subsequently, the dependence of the analgesic effect exerted by contulakin-G on the expression of NTSR2 or Ca_V2.3 was also demonstrated in the CIBP model (Martin et al., 2025). Based on pharmacological and genetic evidence, they proposed that the antinociceptive effect of contulakin-G is mediated by activation of the NTSR2/Ca_V2.3 signaling pathway, confirming, through anatomical studies, the co-expression of NTSR2 and Ca_V2.3 in sensory neurons and presynaptic terminals. These authors observed that neither the expression levels nor the pre- versus post-synaptic distribution of NTSR2 and Ca_V2.3 were changed in the spinal cord after PNI (Martin et al., 2022). NTSR2 was observed in both pre- and post-synaptic compartments of the spinal cord, while Ca_V2.3 was detected only in the presynaptic fraction. Thus, by integrating electrophysiological data with this expression pattern, they proposed that the spinal inhibitory action of contulakin-G is predominantly presynaptic via inhibition of Ca_V2.3 (Martin et al., 2022). Interestingly, RNAScope and immunoblotting analyses revealed that NTSR2 is expressed in both the dorsal and ventral horns, whereas Ca_V2.3 is minimally expressed in the ventral horn, which could explain the sensory selectivity of contulakin-G (Martin et al., 2025).

Proteolytic stability and receptor desensitization

Because contulakin-G lacks disulfide bridges, it becomes a linear peptide susceptible to rapid degradation; however, it has been observed to exhibit high resistance to proteolysis in serum and brain homogenates (Kern et al., 2007; Lee et al., 2015). The presence of a glycoamino acid in its peptide sequence protects contulakin-G from degradation and modulates interactions with target receptors. Thus, contulakin-G shows weaker agonist properties than NTS (0.96 μM vs. 1.4 nM), inducing less desensitization of target NTSRs, thereby improving its analgesic properties (Craig et al., 1999; Lee et al., 2015). These facts support the conclusion that, in *in vivo* experiments, it behaves as a potent and long-lasting agonist, a considerable advantage since receptor desensitization is a recognized mechanism of analgesic tolerance to GPCR agonists (Che, 2021; Kern et al., 2007). Moreover, unlike morphine, a potent μ-opioid agonist, chronic intrathecal infusion of contulakin-G demonstrated maintained analgesic effects with reduced tolerance in the CIBP model (Martin et al., 2025). Post-translational glycosylation of contulakin-G confers an evolutionary advantage for *Conus geographus* by protecting this secreted peptide from metabolic degradation in prey and is critical for its pharmacological properties (Lee et al., 2015).

Blood-brain barrier considerations and primary sensory neuron selective targeting

However, because of its polar character, glycosylation may also limit contulakin-G penetration across the blood-brain barrier (BBB), thereby interfering with its interactions with CNS target receptors (Allen et al., 2007; Lee et al., 2015). Nevertheless, NTSR2 are predominantly colocalized with Ca_V2.3 in the spinal cord, around the central terminals of primary sensory neurons, and are the primary site of action for this bioactive after PNI (Martin et al., 2022). Given that systemic administration of BBB-permeable NT analogs to target the CNS has significant safety alerts, contulakin-G's natural selective targeting of NTSR2 on primary sensory neurons could itself represent a strategy for specifically targeting peripheral GPCRs in NP (Uniyal et al., 2023).

BBB-penetrant analogs

For marine bioactives targeting central NP mechanisms, BBB impermeability presents a significant obstacle. While the peripheral selectivity of many CTXs can be advantageous for avoiding CNS side effects (Daniel & Clark, 2017), certain NP conditions—particularly central post-stroke pain or SCI-related pain—may benefit from central penetration. From this perspective, Lee et al. (2015) developed an analog of contulakin-G in which they replaced glycosylated Thr10 with a non-natural, more hydrophobic residue (a Glu10-coupled memantine moiety) to improve BBB penetration. This analog shows comparable agonist potency and less desensitization than the native peptide, and produces analgesic activity in the tail-flick test after intraperitoneal administration in rodents (Lee et al., 2015). This result opens the door to a potentially less invasive route of administration. However, given the proliferative risk of NTS analogs and other undesirable systemic effects, NTSR2 selectivity is a fundamental prerequisite.

Clinical evidence and translational value

Contulakin-G (coded as CGX-1160) received United States FDA orphan-drug designation in 2005 and advanced into clinical development for chronic intractable pain following IT administration in patients with SCI. A first-dose-escalation human clinical study of CGX-1160 in 4 patients demonstrated preliminary tolerability and PK data, supporting advancement to later development phases. Although CGX-1160 (up to 1000 µg/h) after 6 hours of infusion reduced central NP intensity by 63% from baseline, the assessment of analgesic effect was exploratory (Sang et al., 2016). Regarding analgesic efficacy, the main methodological concern was the absence of a control group, which makes it impossible to distinguish observed changes in pain from the natural course of the disease, regression to the mean, spontaneous pain fluctuations, or effects related to treatment expectations. Unfortunately, despite its favorable signal, the clinical development of contulakin-G was discontinued when the sponsoring company developing this peptide shut down (Safavi-Hemami et al., 2019). The story of contulakin-G illustrates both the promise and challenges of marine peptide drug development for NP. The peptide demonstrates a validated mechanism of action, favorable preclinical efficacy across multiple NP models, sex balance, reduced tolerance compared to opioids, and positive phase I clinical signals in a challenging patient population with SCI-related central NP (Martin et al., 2022; 2025; Sang et al., 2016). However, non-scientific factors ultimately limited its development. This highlights the critical importance of sustained funding and corporate commitment to marine natural product development, particularly for conditions like NP, where clinical trials are lengthy and expensive. The NTSR2/$Ca_V2.3$ pathway validated by contulakin-G studies remains an attractive target for future peptide and small-molecule drug development efforts.

Marine-derived bioactives modulating somatostatin receptors

Discovery of consomatins: From evolutionarily optimized SST analogs to their pharmacological optimization

SST-like peptides in venoms of a deep-water lineage of fish-hunting cone snails were recently discovered by combining molecular phylogenetics with behavioral observations of predation strategy used by the *Asprella* clade, and the changes elicited by individual venom components in mice (Ramiro et al., 2022). These authors observed that prey capture by these cone snail species is extremely slow, with a delay of 1 to 3 hours before venom action. They hypothesized that the venom of *Asprella* snails may contain toxins that target GPCRs expressed in the somatosensory or neuroendocrine systems of prey. Consomatin Ro1 from *Conus rolani* was identified and characterized by mass spectrometry and transcriptome sequencing from a subfraction of the venom that induced a slow-onset phenotypic response in mice similar to that of fish after toxin exposure (Ramiro et al., 2022). Comparative sequence mining of consomatin Ro1 against an in-house database of known GPCR ligands revealed its similarity to SST, sharing a disulfide bond and three of the four core residues (Trp7-Lys8-Thr9) known to be important for SSTR1–5 activation (Moller et al., 2003). Given that the SSTRs in fish are highly similar to their human orthologs, these peptides represent promising new drug leads that, unlike other analogs, evolved from an endogenous SST-like signaling peptide under the principles of natural selection. This peptide is considered an evolutionarily optimized, stable analog of SST due to the presence of a D-amino acid in the receptor-binding motif, which confers resistance to proteolytic degradation and subtype selectivity (Weckbecker et al., 2003). The cyclic structure of consomatins, formed through a disulfide bond, further constrains the peptide backbone to adopt a receptor-binding conformation, improving both potency and metabolic stability (Ramiro et al., 2022).

Consomatin Ro1 preferentially activates SSTR1/SSTR4, with little or no activity at SSTR2, SSTR3, or SSTR5, and does not activate opioid receptors, yet exhibits *in vivo* analgesic effects. Interestingly, unlike morphine, consomatin Ro1 did not increase tail-flick latency in mice after intrathecal administration. However, when administered intraperitoneally, this peptide exhibits a dose-dependent analgesic effect

similar to morphine, suggesting a peripheral mode of action. Furthermore, in a mouse model of acute post-surgical pain involving inflammatory mechanisms, consomatin Ro1 reversed mechanical hypersensitivity lasting up to 3 hours (Ramiro et al., 2022). Several lines of evidence show that the broad anti-inflammatory, antinociceptive, and antihyperalgesic effects of SST are mediated by SSTR4 without affecting endocrine functions (Kántás et al., 2019). Although the molecular mechanisms of SSTR4-mediated antinociception are not fully understood, SSTR4 activation in nociceptive DRG neurons may lead to downstream Gβγ-mediated activation of GIRKs and inhibition of voltage-gated calcium channels, thereby hyperpolarizing cells involved in pain sensing and/or transmission (Gorham et al., 2014a). Additionally, SSTR4 activation can reduce capsaicin-induced TRPV1 currents in DRG neurons via Gαi signaling, thereby further hyperpolarizing primary nociceptive neurons (Gorham et al., 2014b). Thus, SSTR4-selective ligands provide analgesia by inhibiting peripheral pain signals and do not require invasive CNS administration or BBB penetration (Bjørn-Yoshimoto et al., 2025).

Nevertheless, consomatin Ro1 exhibits only micromolar potency at SSTR4 and is equipotent at SSTR1. To improve upon consomatin Ro1, researchers performed computational selection, synthesis, and receptor profiling of eight candidate toxins from the venom gene datasets of 247 species. They identified consomatin Fj1 from *Conus fijisulcatus*, a small cyclic peptide with potent and selective SSTR4 agonism, which activated SSTR4 with an EC_{50} value of 22 nM, showing 173-fold selectivity over SSTR1 (EC_{50} = 3.8 μM) and no measurable activity at the other three receptor subtypes when tested at concentrations up to 100 μM (Bjørn-Yoshimoto et al., 2025). Importantly, acute systemic administration of consomatin Fj1 alleviates mechanical hypersensitivity after SNI in mice. This represents a significant advance, as it demonstrates efficacy in a validated traumatic nerve injury model of NP, not just acute inflammatory pain. Additionally, structure-activity studies using molecular dynamics simulations, combined with analog design, revealed the peptide's minimized binding mode and opportunities to improve Fj1's potency and selectivity for future drug design and development (Bjørn-Yoshimoto et al., 2025). Fj1 is an example of lead evolution/optimization based on prior evidence, progressing from a peptide that was active but had sub-optimal pharmacological properties (Ro1) to one with potency and selectivity far more compatible with therapeutic development.

Translational advantages of SSTR4-selective agonists

The localization of SSTR4 on primary sensory neurons, especially in peripheral terminals and cell bodies in DRG or trigeminal ganglia, offers several translational advantages for NP treatment (Kántás et al., 2019). First, systemic administration of SSTR4-selective agonists like consomatin Fj1 can produce analgesia without requiring invasive intrathecal delivery, in contrast to ziconotide and contulakin-G. Second, the lack of activity at SSTR2, SSTR3, and SSTR5—which mediate endocrine effects including inhibition of growth hormone, insulin, and glucagon secretion—minimizes the risk of metabolic and hormonal side effects (Viollet et al., 2008). Third, the peripheral mechanism of action suggests a lower likelihood of CNS-mediated adverse effects such as sedation, dizziness, and cognitive impairment that complicate the clinical use of centrally acting analgesics. Fourth, the absence of activity at opioid receptors suggests that SSTR4 agonists lack the adverse side effects associated with opioid analgesics. Nevertheless, the receptor-selectivity profile provides a rationale for investigating whether these compounds differ from opioid analgesics in their adverse-effect profiles. Effects on respiration, gastrointestinal function, endocrine regulation, and abuse liability require direct assessment.

The discovery of consomatins validates SSTR4 as a druggable target for peripherally acting, systemically deliverable NP therapeutics (Uniyal et al., 2023). Consomatin Ro1 provided an initial proof of concept for venom-derived SSTR agonists as peripheral analgesics, combining *in vitro* target identification with *in vivo* antinociception and the potential to be investigated in an NP context. However, its moderate micromolar potency and lack of SSTR4 selectivity limited its translational perspective. Building on this platform, Fj1 showed substantial nanomolar potency and high selectivity for human SSTR4, along with analgesic efficacy after peripheral administration in a mouse model of NP. Nevertheless, the current evidence remains limited by the small sizes, acute dosing paradigms, restricted number and non-clinically relevant NP model/outcome, and lack of comprehensive PK/PD and long-term safety data. Thus, Fj1 represents a more compelling translational candidate than Ro1, but the therapeutic potential and safety remain to be validated beyond the early preclinical stage.

Marine-derived bioactives modulating cannabinoid receptors

Altered endocannabinoid levels and cannabinoid receptors 1 and 2 (CB1R and CB2R) expression/function have been observed following PNI, underscoring the endocannabinoid system as a therapeutic target for NP (Maldonado et al., 2016). In rodent NP models, including PNI, CIPN, and STZ-induced DN, CB1R and CB2R agonists, fatty acid amide hydrolase (FAAH) enzyme inhibitors, and cannabidiol consistently

demonstrated antihypernociceptive efficacy (Finn et al., 2021). Whereas CB1R agonists produce severe psychotomimetic effects and have large abuse potential, selective CB2R agonists produce analgesia in preclinical models without any major CNS side effects (Yekkirala et al., 2017). The differential distribution and functional roles of CB1R and CB2R provide opportunities for subtype-selective therapeutic strategies. In particular, CB2R-selective approaches promise peripherally mediated analgesia with favorable side effect profiles, particularly through modulation of neuroinflammatory processes (Finn et al., 2021; Guindon & Hohmann, 2008; Yekkirala et al., 2017). Although there has been limited clinical translation of these results, some reports indicate positive effects in patients with NP (Moore et al., 2021). Recently, evidence of its lack of efficacy for NP treatment has been replicated, and recommendations against its use have been issued (Soliman et al., 2025).

Cannabinoid receptor agonists from Conus venoms

Jergova et al. (2021) hypothesized that *Conus* species could produce CB receptor ligands among the cocktail of conopeptides in their venoms, based on the presence of CB receptors in fish, some crustaceans, and nematodes, which are known *Conus* prey (McPartland et al., 2006). They used crude venom extracts from six *Conus* species (*C. textile*, *C. miles*, *C. quercinus*, *C. magus*, *C. geographus*, and *C. radiatus*) and assayed them for CB1R internalization in HEK293 cells expressing hemagglutinin-tagged CB1R. Extracts from *C. textile* and *C. miles* showed the most potent CB1R internalization and were serially subfractionated by high-performance liquid chromatography (HPLC) to identify their active fractions. These subfractions showed antihypernociceptive effects after intrathecal injection in the tonic phase of the formalin test and the CCI model. One subfraction from the second generation of *C. textile* attenuated neuropathic behaviors and diminished late-tonic-phase formalin responses, reducing c-Fos expression in the spinal cord. Pretreatment with AM251, a CB1R antagonist, reversed both effects, suggesting potent CB1R agonism in this subfraction, likely peptidergic, since CB1R internalization was protease-sensitive. From this selected subfraction, two particular fraction pools with similarly strong CB1R activity were identified (Jergova et al., 2021).

Safety behavioral analyses showed less severe side-effect profiles, particularly for the fraction with greater analgesic potency, including reduced hypothermia and motor incoordination compared to synthetic CB receptor agonists, and less or no development of catalepsy, even after systemic administration. When exposed to CB1- and CB2-selective antagonists, the two subfractions showed different profiles of receptor selectivity, suggesting the activation of CB2Rs by one of them—a promising finding given that selective CB2R agonists lack psychoactive and additive effects (Finn et al., 2021; Yekkirala et al., 2017). The identification of these two CB-acting peptidergic fractions from *C. textile* opens a new avenue for bioactives derived from marine invertebrates in the search for novel analgesics. However, structural identification and total synthesis of the active CB-acting peptides from *C. textile* are needed to enable comprehensive pharmacological characterization and medicinal chemistry efforts to optimize them for potency, selectivity, and PK properties while maintaining the favorable safety profile of the native peptides. Likewise, the evaluation of these peptides in disease-relevant NP models beyond traumatic nerve injury, and assessment of long-term efficacy, tolerance development, and withdrawal phenomena after chronic administration, are required.

Marine-derived bioactives modulating noradrenergic and serotonergic pathways

Conopeptides targeting a_2-adrenergic receptors

The noradrenergic descending inhibitory pathway represents a validated target for NP treatment, as evidenced by the clinical efficacy of serotonin-norepinephrine reuptake inhibitors (SNRIs) such as duloxetine (Soliman et al., 2025). Xen2174, an analog of the χ-conopeptide Mr1A isolated from *Conus marmoreus*, produces antiallodynic effects in NP models through highly selective, non-competitive inhibition of the norepinephrine transporter (NET) (Nielsen et al., 2005; Patel et al., 2018). By blocking NET in the spinal cord, Xen2174 augments descending noradrenergic inhibition by increasing norepinephrine availability at pre- and postsynaptic α_2-adrenergic receptors in a yohimbine-sensitive manner (Nielsen et al., 2005). Given the impairment of the descending noradrenergic system in chronic pain, including NP (Patel et al., 2018), Xen2174 appeared to be a promising candidate and advanced to clinical trials for cancer pain and post-surgical pain. However, clinical development was halted in 2012 because it required an invasive intrathecal route of administration (Hamad et al., 2018). This experience highlights the critical importance of delivery route feasibility in marine peptide drug development.

Astaxanthin in co-morbid depressive like behaviors in neuropathic pain model

Following PNI, alterations in spinal 5-hydroxytryptamine (5-HT) receptor expression and function contribute to the development and maintenance of NP. Convergent data showed that among all 5-HT receptor subtypes—5-HT1A, 5-HT1B/D, 5-HT2A, 5-HT2C, 5-HT3, 5-HT4, 5-HT6, and 5-HT7 receptors—are considered as potential targets for pain therapy, with special

interest with regard to NP (Liu et al., 2020b). Both direct actions of excitatory or inhibitory receptors and indirect mechanisms via inhibitory interneurons underlie the modulatory effects of 5-HT on nociceptive pathways (Viguier, 2013). Consequently, the pronociceptive effects of 5-HT have been proposed to explain the lower efficacy of selective serotonin reuptake inhibitors (SSRIs) compared with SNRIs or tricyclic antidepressants for NP treatment (Ansari, 2000). While marine-derived direct modulators of 5-HT receptors have not been extensively characterized for NP applications, several marine bioactives exhibit indirect serotonergic effects that may contribute to their analgesic properties (Jiang et al., 2018; Lax et al., 2018).

As in the spinal cord, plastic changes induced by NP have been described in the anterior cingulate cortex (ACC), a key brain area for pain processing. The ACC encodes the emotional/aversive component of pain, being linked to the comorbid symptoms of chronic pain like depression and anxiety (Barthas et al., 2015; Zhuo, 2016). In addition to sensory behaviors, it is recommended to assess depression-and anxiety-related behaviors, as well as cognitive impairments in NP models as a strategy to enhance their clinical resemblance (Palazzo et al., 2021). Jiang et al. (2018), using this experimental paradigm, demonstrated that AST exhibits concomitant analgesic and antidepressant effects on CCI-induced hyperalgesia and comorbid depressive behaviors. Indoleamine 2,3-dioxygenase (IDO) activity is associated with reduced 5-HT levels and alterations in pro-inflammatory cytokines via kynurenine pathway metabolites, mechanisms implicated in depression and NP (Sublette & Postolache, 2012). Thus, Jiang et al. (2018) proposed that AST's antihyperalgesic effects may be partially mediated by the descending serotonergic system through downregulation of IDO activity, linking the dependence of the effects to 5-HT through the use of an 5-HT inhibitor. Although the concomitant improvement of sensory and affective dimensions of NP increases the clinical relevance of this approach, further studies are required to establish its PK/PD profile, mechanism of action, sex balance, efficacy across NP models, and translational feasibility.

Cyanobacterial metabolites improve affective dimension behaviors of neuropathic pain

In contrast, Lax et al. (2018) identified a fraction from *Leptolyngbya* genus extract with significant affinity for 5-HT7R that, following intracerebroventricular (ICV) injection, decreased depression- and anxiety-like behaviors in males but had no effects in 5-HT7 receptor (5-HT7R) KO or female mice. Notably, blockade of the 5-HT7R in the CA1 region of the hippocampus decreases depression-like behaviors in rodents (Wesolowska et al., 2006). In this initial screening, the administration of this fraction (DUQ00021) bilaterally to the CA1 region of the hippocampus was sufficient to induce antidepressant-like effects in male animals.

Based on the behavioral phenotype of DUQ00021, which mimics the effects of known 5-HT7R antagonists such as SB258719, the authors suggest that the active compound in DUQ00021 is most likely a 5-HT7R antagonist. Interestingly, this fraction shows antidepressant effects in SNI animals, a model of NP with comorbid depression, suggesting a role for the 5HT7R in the development of comorbid pain and depression. However, DUQ00021, when targeted to the CA1, had no behavioral effect on SNI-induced mechanical hypersensitivity (Lax et al., 2018). Despite contradictory findings, most studies suggest that activation of the 5-HT7R mediates antinociceptive effects at both spinal and supraspinal sites (Liu et al., 2020b; Santello et al., 2017). Upregulation of 5-HT7R in the dorsal horn of the spinal cord has been demonstrated in NP, and 5-HT7R agonists exert analgesic effects via activation of inhibitory GABAergic interneurons in the spinal cord (Liu et al., 2020b; Viguier, 2013). In contrast, other studies have found that systemic or spinal administration of the 5-HT7R antagonist SB-269970 can reduce tactile allodynia in a dose-dependent manner in L5/L6 SNL models (Amaya-Castellanos et al., 2011). Lax et al. (2018) provided moderate preclinical evidence for a potential role of marine cyanobacterial metabolites in the affective dimension of NP; although the DUQ00021 fraction did not significantly improve the sensory component of NP in the SNI model, it reduced depression-like behavior in animals with NP, with pharmacological and genetic findings supporting involvement of 5-HT7R. However, the translational relevance is limited by the absence of analgesic efficacy, ICV administration, and the fact that the active material was a complex fraction rather than a chemically defined compound. The loss of activity following further purification, suggesting possible synergistic effects among multiple constituents, further complicates the dose-response relationship, PK/PD, safety, and drug-development considerations. Thus, the study provides valuable proof-of-concept evidence for targeting the affective component of NP, but limited evidence for the development of a defined analgesic candidate. However, the authors proposed a potential floor effect as a limitation of the sensory assay and suggested further evaluation in alternative pain models; therefore, this study provides preliminary evidence with rationale for further investigation, particularly using alternative administration strategies and NP models.

Stages of translational maturity of marine-derived candidates for neuropathic pain treatment through the systematic evidence map

The 27 marine-derived candidates displayed different levels of translational maturity, ranging from early mechanistic discovery/preliminary *in vivo* evidence to extensive preclinical validation and, for a limited number of candidates in clinical development. Notably, the accumulation of candidates at the preclinical stage contrasted with the small number progressing toward clinical investigation, highlighting a substantial translational gap between demonstration of biological activity and therapeutic development (Yezierski & Hansson, 2018). Thus, intensive research on marine bioactives in this field has yielded many promising leads to date, but few candidates have successfully navigated the PK/PD—formulation—safety—clinical development bottleneck.

(Level 1) Discovery/preliminary *in vivo* evidence/Identification of a target (target modulation *in silico/in vitro)* or initial pharmacological activity with still insufficient *in vivo* evidence to consider the candidate validated: *C. aurivilli* extract, spiruline extract, *E. cava* extracts, aaptamine, fumagilin, flexibilide, lemnalol, DA, Eu1.6, AuB, MII, conantokin-G, cyanobacterial extract fractions/DUQ0002I, CB conopeptides fractions.

(Level 2) Preclinical validation/Target interaction, functional modulation and *in vivo* efficacy: [GeXIA (advanced), MoVIA/MoVIB, Mr.1 and analogs, MSVIII-19, STX synthetic analog (ST-2530), AST, fucoidan, consomatin Ro1/Fj1, contulakin-G, CVID, Vc1.1 and analogs (as preclinical platform), RgIA and analogs (RgIA4/KCP400)].

(Level 3) Translational development/Optimized candidate, PK/PD, formulation/delivery, safety and clinical research: TTX, ω-conotoxin MVIIA/ziconotide, α-CTXs Vc1.1/-derived clinical platforms (KCP506, 2020).

Potential candidates for future research in neuropathic pain models

Marine-derived bioactive compounds modulating voltage-gated ionic channels

In general, VGSCs are among the most important potential targets for marine toxins (Mackieh et al., 2021). Genetic and pharmacological validation suggest $Na_V1.8$ as an attractive analgesic target in NP (Alles & Smith, 2021). The TTX-R isoform $Na_V1.8$ is most selectively expressed in peripheral nociceptive neurons and generates the majority of inward Na^+ current during the upstroke of action potentials in nociceptors, inducing neuronal repetitive firing in response to stimulation and remaining functional even at depolarized thresholds (Bennett et al., 2019). After traumatic nerve injury, $Na_V1.8$ protein has been shown to accumulate within nerve endings or painful neuromas. Furthermore, a redistribution of its expression to the axons of uninjured unmyelinated afferent fibers has been described in both models and patients (Bennett et al., 2019; Finnerup et al., 2021). Previous studies have shown that the μO-conotoxins MrVIA and MrVIB from the venom of *Conus marmoreus* inhibit $Na_V1.8$ in mammalian sensory neurons with >10-fold selectivity over TTX-S Na_V isoforms (Cardoso & Lewis, 2018; Ekberg et al., 2006). In particular, MrVIB decreases mechanical and thermal hypersensitivity in the PNL model without causing motor deficits when compared with a non-selective local anesthetic; therefore, μO-MrVIB (CGX-1002) is in preclinical phases for treating NP (Ekberg et al., 2006; Gao et al., 2017). Therefore, μO-conotoxins could be promising leads for the development of novel $Na_V1.8$-selective molecules. Accordingly, Vetter et al. (2012) report the isolation, pharmacological characterization, and a regioselective approach for the synthesis of a novel μO-conotoxin (MfVIA) isolated from the venom of *Conus magnificus*. MfVIA was selective for $Na_V1.8$ and $Na_V1.4$ over other mammalian Na_V subtypes and displayed weak activity at the centrally expressed $Na_V1.2$ (Vetter et al., 2012). More recently, Yang et al. (2017) identified a novel first μ-conotoxin, TsIIIA, from a worm-hunting *Conus tessulatus*, which shows specificity to inhibit TTX-resistant ($Na_V1.5$, $Na_V1.8$, $Na_V1.9$) sodium currents but has no effect on TTX-sensitive ($Na_V1.1$, $Na_V1.2$, $Na_V1.3$, $Na_V1.4$, $Na_V1.6$, $Na_V1.7$) sodium currents in rat DRG neurons. *In vivo,* TsIIIA increases the pain threshold, with a stronger effect than ziconotide in a phasic acute nociceptive test. Later, the same authors identified these relevant molecular targets and tested TsIIIA across human Na_V subtypes ($hNa_V1.1$-$hNa_V1.8$). TsIIIA selectivity inhibited $hNa_V1.8$ currents with an IC50 value of 2.11 μM, highlighting its therapeutic potential (Yang & Zhou, 2020).

On the other hand, neurotoxins from sea anemones that modulate Na_V channels have served as classical biochemical tools for studying the structural organization and function of Na_V subtypes, and they hold potential for evaluation in NP models (Monastyrnaya et al., 2023). In particular, β-defensin-like peptides in sea anemone venom—including CgNa, Rc I, Am II, BDS I, APETx1, and APETx2—are potential toxins that may modulate voltage- and ligand-gated ion channels such as Na_V types 1/2/4, K_V type 3, and ASICs (Madio et al., 2019). APETx2 from *Anthropleura elegantissima* is the most potent rat ASIC3 inhibitor (IC_{50} of 63 nM), though it is less potent against human ASIC3 (IC_{50} of 175 nM); its lack of selectivity and susceptibility to rapid degradation *in vivo* are notable disadvantages

(Monastyrnaya et al., 2023). Interestingly, Blanchard et al. (2012) first reported that APETx2 also inhibits $Na_V1.8$ channels at *in vivo-relevant* concentrations, suggesting a dual mechanism of action. This is significant, as NP encompasses multiple heterogeneous pathophysiological mechanisms, providing potential therapeutic targets for multitarget molecules and rational combination drug therapies (Soliman et al., 2025).

An interesting approach was proposed by Paguigan et al. (2021) based on the functional specialization of DRG neurons to relay specific sensory information to the CNS. A strategy that, by targeting functionally specific cell types rather than widely distributed individual channels, could be effective and safer. These authors demonstrated the neuromodulatory and ion channel blocker properties of the defensive amino-acid-derived metabolite 2-(3,5-diiodo-4-methoxyphenyl) ethan-1-amine (DIMTA) from a marine tunicate *Didemnum* sp. DIMTA at lower doses selectively targets low-threshold cold thermosensors (LTCTs) in DRG neurons by inhibiting $K_{Ca}1.1$, $K_V3.1$, but not $K_V7.2$ channels, which tend to keep impulse generation under control (Alles & Smith, 2021). Therefore, DIMTA reduces the magnitude of afterhyperpolarization, leading to faster inactivation of Na_V channels, thereby causing desensitization and also increasing the baseline intracellular calcium concentration in these cells (Paguigan et al., 2021). Although LTCTs are characterized by their response to menthol, reflecting the role of the transient receptor potential M8 (TRPM8) in the transduction of cold sensation, DIMTA unexpectedly does not modulate this channel but increases the cold-sensation threshold by >3°C (Marwaha et al., 2016). However, high concentrations (25 µM) of DIMTA can non-selectively target other ion channels, including voltage-gated calcium channels and $K_V11.1$ (also known as hERG), thereby increasing the risk of ventricular arrhythmia (Zhou et al., 1998). This metabolite provides a novel lead scaffold for the design of cell-type selective potassium channel blockers that could inhibit cold allodynia in NP conditions and minimize adverse effects (Paguigan et al., 2021).

Regarding VGCCs, while most research has focused on $Ca_V2.2$ as a well-established molecular target, $Ca_V3.2$ is also considered a promising therapeutic target for NP (Bourinet et al., 2016). Recently, the native δ-conotoxin TxVIA from *Conus textile* was found to be inactive on mammalian $Na_V1.2$ and $Na_V1.7$ channels, but preferentially inhibited $Ca_V3.2$ currents in HEK293 cells. TxVIA did not induce pain-related behaviors or paralysis in zebrafish bioassays, consistent with a lack of excitatory effect on nociceptive pathways (Wang et al., 2020). Thus, TxVIA warrants further evaluation in NP models.

Peptides from sea anemones modulating acid-sensing ligand-gated ion and transient-receptor potential channels

The importance of ASIC3-sustained current inhibition in controlling acidosis-related pain has been recognized, thereby justifying the evaluation of the analgesic activity of ASIC3 inhibitors from sea anemones (Andreev et al., 2018). Osmakov et al. (2013) isolated the peptide Ugr 9-1 (π-actitoxin-Ugr1a) from the sea anemone *Urticina grebelnyi*, which has an unusual β-hairpin-like structure. While APETx2 inhibited only transient currents, Ugr9-1 decreased both transient and sustained components of pH-induced ASIC3 currents (Andreev et al., 2018; Osmakov et al., 2013). In addition, the Hcr 1b-1 peptide from *Heteractis crispa* has been recognized as a selective ASIC3 inhibitor (Kozlov et al., 2012). Other APETx-like peptides from this species (Hcr 1b-2, Hcr 1b-3, and Hcr 1b-4) are capable of inhibiting ASIC1a, and Hcr 1b-2 has demonstrated an antihyperalgesic effect in the acid-induced muscle pain model. However, Hcr 1b-4 has been identified as the first potentiator of ASIC3 (Kalina et al., 2020). After SNL, which imposes acidic conditions, increased expression of ASIC1b in L4 DRG and ASIC3 in L5 DRG versus a decrease in ASIC1a in L4 DRG, specifically in unmyelinated peptidergic neurons, has been reported. The upregulation of ASIC3 channels, which show a transient component followed by a sustained slow component, may play an important role in neuropathic hypersensitivity (Papalampropoulou-Tsiridou et al., 2020). ASIC3 is also overexpressed in the spinal cord dorsal horn in hyperalgesic SNL rats. Furthermore, intrathecal treatment with non-selective ASIC channel antagonists (amiloride and benzamil) reduced mechanical allodynia in these animals (Jeong et al., 2013).

On the other hand, several lines of evidence support the role of TRPV1 in ectopic transduction of endogenous or exogenous thermal and chemical stimuli that may initiate spontaneous pain from injured sensory neurons (Basso & Altier, 2017; Marwaha et al., 2016). Clinically, low doses of capsaicin, a TRPV1 agonist, alleviate pain by inducing short-term receptor desensitization, while high doses, often used in PHN patients, elicit dysfunction that lasts for months, which may be related to the structural ablation of TRPV1-expressing nerve endings (Blair, 2018; Gao et al., 2024). Formulations based on both strategies are recommended as second-line treatment for peripheral NP (Soliman et al., 2025). TRPV1's interaction with TRPA1 channels, which are colocalized to a subset of TRPV1-positive sensory neurons, further influences NP onset and progression (Gao et al., 2024). A new sea anemone peptide, Ms 9a-1 (τ-metridiotoxin-Ms9a), isolated from *Metridium senile* venom, acts as a positive modulator of TRPA1 *in vitro* but does not have pronociceptive effects when injected into the hind paw of mice. Nevertheless,

Ms 9a-1, administered via the IV route, decreases the nociceptive and inflammatory responses to allyl isothiocyanate, a prototypic TRPA1 agonist, and reverses CFA-induced inflammatory hyperalgesia, probably due to desensitization of TRPA1-positive neurons (Logashina et al., 2017; 2022).

Electrophysiological data show that the Kunitz-type bifunctional peptide HCRG21, belonging to the *H. crispa* multigene HCRG peptide subfamily, fully inhibits its capsaicin-induced TRPV1 currents (maximum inhibition of 95%) (Monastyrnaya et al., 2016). Furthermore, HCRG21–TRPV1 molecular *in silico* simulations allow the authors to hypothesize potential pore-blocking interactions with the TRPV1 outer pore domain or intracellular binding to the regulatory channel domain, triggering conformational changes in the pore helix region. These authors highlight the importance of the combinatorial library of *H. crispa*, which yields promising peptides with variations in single charged residues in these sequences that could determine their subtype receptor and the types of stimulus specificity. Despite TRPV1 antagonists showing promise in preclinical studies, given TRPV1's significant role in thermosensation, some of these antagonists cause hyperthermia, thereby limiting their clinical introduction (Gao et al., 2024). Interestingly, APHC1, APHC2, and APHC3 Kunitz-type peptides isolated from sea anemone *Heteractis crispa*, acting as partial antagonists of TRPV1 channels, have shown *in vivo* analgesic activity and moderate hypothermia (Andreev et al., 2013).

Marine natural products and ASIC/TRPV1 modulation as neuroprotective strategy

Given that both TRPV1 and ASIC3 channels regulate neuron–immune cell interactions, thereby exacerbating neuroinflammatory responses, modulation of these channels presents an attractive neuroprotective strategy for addressing NP (Basso & Altier, 2017; Kong et al., 2017; Kung et al., 2020). Marine algae, a diverse group of multicellular photoautotrophs, produce complex metabolites that target multiple molecular signaling pathways (Jegadeshwari & Rajaram, 2024; Juárez-Portilla et al., 2019). Of particular interest are carotenoids—tetraterpenoid compounds that protect photosynthetic organisms from photoxidative stress and exhibit potent antioxidant and anti-inflammatory effects (Juárez-Portilla et al., 2019). Fucoxanthin, an orange carotenoid isolated from brown seaweeds, reduces TRPV1 expression in neurons and suppresses satellite glial cell activation in trigeminal ganglia after ultraviolet B (UVB)-induced photokeratitis in rats (Chen et al., 2019). Fucoxanthin decreases eye wipe behavior, an indicator of inflammatory pain, and increases the expression of antioxidant Nrf2 in corneal tissues (Chen et al., 2019). Although UVB-induced keratitis triggers an inflammatory response, the cornea is a densely innervated tissue that, upon UVB exposure, undergoes degeneration of nerves in the epithelial laminae (Bishop et al., 2007). Thus, in this context, corneal pain shows mixed mechanisms, including a neuropathic component. Actually, in the clinical setting, radiation keratopathy is one of the potential causes of neuropathic corneal pain (McNally & Figueiredo, 2024). The authors report a strong dose-dependent neuroprotective effect of orally administered fucoxanthin against UVB-induced corneal nerve endings degeneration, with beneficial effects that preserve the integrity of the corneal surface, elements that suggest its attractive to be evaluated in NP models (Chen et al., 2019).

Additionally, marine flavones and flavonols—mainly isolated from seagrasses and halophytes—are of increasing pharmacological interest (Ameen et al., 2024; Gono et al., 2022). Sulfated glycoside flavone thalassiolin B (chrysoeriol 7-β-D-glucopyranosyl-2″-sulfate), isolated from *Thalassia testudinum* seagrass crude hydroethanolic extract (BM-21), has been reported to have analgesic activity in phasic and tonic pain tests. In the formalin test, thalassiolin B (TB) decreases nociceptive behavior during both phases, while BM-21 exclusively shows activity on the late inflammatory phase. Interestingly, both BM-21 and TB selectively inhibited fast desensitizing ASIC currents in DRG neurons, suggesting that ASICs are involved in the analgesic effect (Garateix et al., 2011). These findings showed, for the first time, the presence of an ASIC inhibitor in a marine plant extract linked to a phenolic compound, an activity that in marine organisms is commonly reported for cnidarian peptide neurotoxins (Madio et al., 2019). BM-21 also shows neuroprotective effects in the acrylamide-induced neurotoxicity model, where nitroxidative imbalance and neuroinflammation are pivotal in the axonal degenerative process (Menéndez et al., 2014). These authors focused their investigation on BM-21's beneficial effects in acrylamide-induced motor dysfunction, documenting improvements in amplitude and conduction velocity of the compound muscle action potential, restoration of total lipoperoxide levels in the sciatic nerve, and normalization of antioxidant enzymatic activity. Although this study did not include sensory behavioral testing, acrylamide-induced neuropathy is a well-established animal model of progressive axonal degeneration that results in diffuse sensorimotor polyneuropathy with dysautonomia (Ling et al., 2005). Given these precedents, the extract's preventive and therapeutic potential regarding sensory variables in NP models warrants systematic investigation. Furthermore, the non-peptidic nature of TB confers potential advantages over ASIC-targeting peptides (APETx2, Ugr 9-1, Hcr 1b-2) in pharmaceutical development, owing to superior

oral bioavailability and proteolytic stability profiles (Garateix et al., 2011).

Conopeptides and conosteroids modulating NMDAR/GABA$_A$R activity

Other small peptides from *Conus parius* (Pr1, Pr2, and Pr3) with NMDAR antagonistic properties and greater selectivity for the GluN2B subunit can sustain CREB signaling, which is critical for neuronal survival (Kunda et al., 2013). Additionally, a novel conotoxin Ac1 and its variant conotoxin-Ac1-O6P, isolated from the venom duct of *Conus achatinus*, have demonstrated inhibitory activity on NR2B-containing NMDARs and analgesic effects in response to acute thermal stimuli (Liu et al., 2020a). Another potential NMDAR blocker, framework VI/VII O3, has been identified from conotoxins of various venomous marine cone snails in Bali, Indonesia (Sudewi et al., 2019).

Interestingly, a synthetic analog from a series of conosteroids isolated from the hypobranchial gland of *Conus geographus*, which acts as a GABA$_A$R negative allosteric modulator (NAM), exhibits subtype selectivity, most strongly antagonizing α1β1γ2 and α4β3γ2 receptors (Niu et al., 2021). Nevertheless, these authors designed an experimental set to evaluate only specific combinations of (α1-5)-containing γ2 receptors in the presence of β1 or β3 subunits, although it has been reported that GABAergic neuroactive steroids exhibit higher potency at α4δ-containing GABA$_A$Rs than at α4γ2-containing GABA$_A$Rs (Brown et al., 2002). Thus, considering the relevance of targeting δ-containing GABA$_A$Rs for analgesic drug discovery in NP, the potential of these bioactives to modulate extrasynaptic α4/6βδ subunits should be examined (Luo et al., 2021). The possibility of modulating endogenous neurosteroidogenesis and/or administering natural or synthetic neurosteroid analogs has been recognized as a strategy for treating NP (González & Ferreyra, 2022; Meyer et al., 2019). In particular, extrasynaptic receptors containing the δ-subunit are highly sensitive to neurosteroids and BDZ-insensitive, which appear to exhibit high affinity, display slow desensitization, and underlie tonic inhibitory currents in the brain and spinal cord (Price et al., 2009; Qian et al., 2023).

Niu et al. (2021) hypothesized that these compounds have analgesic potential based on their activity in different neuronal subsets of mouse DRG. The analog cholesta-3,5-dien-7-one, which increases Ca^{2+} influx into peptidergic and non-peptidergic nociceptors, C-low-threshold mechanoreceptors, and cold thermosensors, also demonstrates modest analgesic activity in the hot-plate test and data trending toward significance in the tonic phase of the formalin test (Niu et al., 2021). Although the effects of neurosteroids on GABA$_A$Rs are complex and depend on multiple factors, previous work has shown that both GABA$_A$R-positive allosteric modulators (PAMs) and NAMs differentially reverse signs of neuropathic hypersensitivity in CCI rats (Munro et al., 2011). Further studies are needed to determine the compound's complete analgesic profile, but it would be interesting to evaluate it in NP models, as its potential activity could become evident following diminished GABA$_A$R-mediated inhibition in the presence of nerve injury (Coull et al., 2005; Price et al., 2009). In addition, natural conosteroids and their analogs deviate from the structure-activity relationship pattern for steroid NAMs, as they lack a hydroxyl group or charge at the 3-position and instead possess a diene-ketone moiety and a longer side chain. These structural features, combined with the apparent lack of toxic, psychotropic, or anxiogenic effects, suggest their utility as scaffolds for new design principles in discovering GABA$_A$R modulators with greater selectivity and a superior pharmacological index (Niu et al., 2021).

Butyrolactone-I (ZB5-1) from Aspergillus terreus and neuroinflammation

Marine fungi are another important source of secondary metabolites, recognized for their widespread presence in several habitats and their symbiotic interactions with higher organisms (Pang et al., 2016). Butyrolactone-I (ZB5-1) from *Aspergillus terreus*, an endophytic fungus isolated from Puko Shore coral (*Porites pukoensis*), attenuates the neuroinflammatory response in LPS-stimulated BV-2 microglial cells. This effect was related to its ability to inhibit phosphorylation and nuclear translocation of NF-κB in these cells (Zhang et al., 2018). ZB5-1 is a small phenolic molecule that has potential for study in the neuroinflammatory context of NP.

α-CTX PeIA and nAChRS

Li et al. (2024b) report chemically synthesized Dab/Dap-substituted analogs of α-CTX PeIA; however, this conotoxin has not been evaluated *in vivo* NP models (Mohammadi & Christie, 2015). The most potent analog, PeIA[S4Dap, S9Dap], improved activity by 24-fold compared with the native peptide via extra interactions with the receptor. The potency of PeIA[S4Dap, S9Dap] is approximately two-fold higher than that of dimeric PeIA, and its synthesis is more facile. However, compared with the absence of inhibition by PeIA, the analog exhibits enhanced potency against human α7 nAChRs (IC50 of 161.7 nM), a finding worth following, as the α7 subtype has been linked to bidirectional effects in the context of NP (Hone & McIntosh, 2018).

Marine cyanobacteria metabolites and GPCRs

Marine cyanobacteria have been proposed as sources of secondary metabolites structurally similar to endogenous mammalian GPCR ligands, particularly those with significant activity at 5-HT receptors and/or 5-HT transporters, likely linked to the predator-prey relationship between *Aplysia* sea hares and marine cyanobacteria (Lax et al., 2016; 2018). Some extracts from the genus *Moorea* acting on CB receptors have also been reported (Gutierrez et al., 2011; Montaser et al., 2012). The fraction DUQ00021 reported by Lax et al. (2018) due to negative results, rather than being excluded as a potential lead for NP, warrants further investigation in this context.

Implications and future directions

The evidence presented here reflects over 15 years of exploration into bioactive compounds and extracts derived from marine organisms in both preclinical and clinical settings. The most significant findings in relevant animal models of NP and clinical studies report pharmacological activity of compounds or extracts from the phylum Mollusca, all from the genus *Conus*. Because the evidence stems primarily from preclinical studies, it is necessary to examine gaps and concerns about quality and experimental design that affect translational potential.

First, the preclinical evidence showed considerable uncertainty arose from incomplete reporting of key methodological safeguards, a pattern consistent with recognized limitations in reporting methodological procedures in preclinical animal research (Hooijmans et al., 2014; Rice et al., 2009). Although several authors report adherence to Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines, they do not explicitly describe these procedures. These findings highlight the need for more transparent reporting of preclinical methodology, particularly regarding randomization procedures, allocation concealment, housing allocation, investigator and caregiver blinding, and outcome assessment. Explicit reporting of these procedures would reduce uncertainty in risk-of-bias assessments, improve reproducibility, facilitate more robust evidence synthesis, and strengthen the translational value of preclinical research (Yezierski & Hansson, 2018). Another frequently missing factor is laboratory environmental stressors that affect pain sensitivity and are known to impact replication and translation of results (Mogil, 2017). In addition, in this context, potential confounding effects of sedation, muscle weakness, or motor impairment limit the strength of the efficacy interpretation; thus, complementary assessment, such as rotarod performance, open-field locomotor activity, grip strength, or a functional observation battery, can help determine the nocifensive behavioral specificity (Rice et al., 2009). Most authors (73%) who studied the analgesic activity of these compounds in NP models reported the assessment of these confounding factors.

Second, through these studies, female animals are widely underrepresented (Ciapala et al., 2024; Martin et al., 2022; Romero et al., 2017; Wang et al., 2024), despite the higher prevalence of chronic NP in women and the recognized sex differences in pain neurobiology impacting the sensory profiles and responses to interventions (Keogh, 2022; van Hecke et al., 2014). This suggests a significant sex bias, consistent with limitations reported in preclinical pain research that affect its translational relevance (Mapplebeck et al., 2016; Mogil & Chanda, 2005). Specifically, one factor that could explain the clinical failure of glial inhibitors is the role of spinal microglia/proinflammatory responses in nociception in males compared with females (Mapplebeck et al., 2016; Zimmerman et al., 2025). Therefore, marine bioactives that modulate neuroinflammation and neuroimmune activation should be evaluated with consideration of their potential sexual-dimorphic effects.

Third, the *in vivo* models of NP and outcome measures used in these investigations have been validated for their symptomatic resemblance, construct rationality, predictive (pharmacological) validity, and systematically assessed reliability (Bennett, 2001). However, experts have proposed the need for a refinement of preclinical methods to align with the clinical phenotypic heterogeneity of NP and to improve their predictive validity for testing the efficacy of novel compounds in RCTs (Rice et al., 2009; 2018). This review shows a similar pattern to that seen for years in the preclinical literature on NP, where traumatic nerve injury models dominate in more than 60% of cases—a condition examined in only ~8% of clinical trials that mainly recruit patients with CIPN, diabetic polyneuropathy, and PHN (Rice et al., 2018; Soliman et al., 2025; Yang et al., 2025). Overall, a significant discrepancy between outcome measures in preclinical studies—which have relied on limb withdrawal hypersensitivity to sensory stimuli—and the endpoints measured in human RCTs (Yezierski & Hansson, 2018). Therefore, these bioactive compounds need to be evaluated using assessment tools that reflect these clinical endpoints. Nevertheless, some authors conducted their studies in clinically relevant models using endpoints directly linked to mechanisms of generation, aiming to address forms of NP that are most resistant to available therapies—representing a substantial unmet medical need—such as CIPN and central pain after SCI. In CIPN, which restricts chemotherapy regimens for cancer patients and for which there are no effective preventive agents and only limited therapeutic options (Gewandter et al., 2017), CTXs targeting multiple mechanisms offer an attractive alternative, providing not only symptomatic

relief but also neuroprotective and neurorestorative effects. Some of these peptides appear safe at antiallodynic doses and bioavailable via clinically relevant non-invasive routes (IM, transdermal), but long-term safety evidence is still needed (Tosti et al., 2022).

Fourth, although the evolutionary principle of similarity supports the resemblance of nociceptive processing in rodents and humans, under both normal and pathological conditions, significant species differences have also been recognized (Bennett, 2001). In particular, these differences affect ion channel tissue expression, function, and sensitivity to pharmacological agents, which implies caution when extrapolating results (Alles & Smith, 2021; Smith, 2020; Tanner & Beeton, 2018). Therefore, differences in the phenotype and function of Na_V, especially $Na_V1.7$ and 1.8 (McDevitt et al., 2025; Yang et al., 2025), ASIC, TRP channels, and nAChRs, among other key targets proposed for these bioactive compounds across nociceptive pathways, have been reported (Azam & McIntosh, 2012; Gründer & Pusch, 2015; Tanner & Beeton, 2018). Cross-species potency shifts also limit *in vivo* efficacy studies and candidate selection. Among the compounds identified in this review, including Vc1.1/analogs, RgIA/analogs, GeXIVA, CVID, MVIIA, and STX-derived $Na_V1.7$ inhibitors, were characterized against their corresponding human targets *in vitro*. Whereas Vc1.1 and RgIA were also investigated using homology models based on human receptor sequences (Chu et al., 2019; Pan et al., 2021), and GeXIVA was studied in a model initially derived from rodent receptors but subsequently adapted to incorporate human sequences (Zhangsun et al., 2017). Hence, structural modeling predictions provided additional, although inferential, evidence of ligand-target interactions in the human orthologue. Subsequently, evidence obtained directly from human targets, followed by evidence derived from these models, can be considered progressively more informative for reducing interspecies uncertainty than evidence based exclusively on rodent receptors. However, the authors did not corroborate *in vivo* pharmacological activity in humanized animals.

Other aspects of animal experimentation inconsistent with the clinical setting—such as the use of young rodents with limited genetic variability, and the administration of a single dose to study the time course of the response, unlike the repeated-dose regimen used clinically (Yang et al., 2025)—represent a trend in this review, though not the rule. Several authors employed repeated-dose schedule to investigate their long-term therapeutic or preventive effects (Ali Safakhah et al., 2018; Hashemi et al., 2024; Hong et al., 2018; Hu et al., 2014; Jiang et al., 2018; Karmakar et al., 2022; Kim et al., 2014; Wen et al., 2021; Zhao et al., 2021) as well as both approaches (Beckley et al., 2021; Chen et al., 2014; Kayser et al., 2010; Li et al., 2016; Sung et al., 2023; Wang et al., 2019). Chronic administration was used to evaluate CTXs in the context of research into their disease-modifying effects (Christensen et al., 2017; Di Cesare Mannelli et al., 2014; Huynh et al., 2020a, 2022; Pacini et al., 2016; Romero et al., 2017; Wang et al., 2024). In particular, the demonstration of therapeutically relevant exposure at the site of action linked to efficacy and adverse effects, with appropriate repeated-dose assessment, is recognized as a key criterion for clinical progression (Yezierski & Hansson, 2018). Only four studies evaluating the PK of the compounds were identified: cVc1.1 vs. Vc1.1 (Poth et al., 2021), GeXIVA (Zhu et al., 2022), STX-2530 (Beckley et al., 2021), and TTX pellets (Hong et al., 2018).

Given the ethical and environmental conservation concerns associated with the risk of overexploiting marine resources, it is important that most molecules studied during this period were synthetic equivalents or analogs of the native ones. Currently, modern computational and "omics" methodologies help prevent indiscriminate exploitation of bioresources, safeguarding ecological balance (Cabral et al., 2024; Li et al., 2025). These techniques were also widely used by the authors, as exemplified by the high-throughput transcriptomic analysis conducted by Guo et al. (2024), which identified 1,049 transcripts from the venom glands of *Heteractis crispa*, accelerating peptide discovery while minimizing the need to collect the organisms.

Subsequently, the following priorities are suggested:

1. The advancement should preferentially focus on candidates supported by convergent evidence of target engagement, functional activity, *in vivo* efficacy, PK/PD relationships, and safety, rather than behavioral efficacy alone.

2. Disease-relevant validation beyond traumatic nerve injury. Promising compounds such as DA, lemnalol, MSVIII-19, flexibilide, Eu1.6, MoVIA and MoVIB, ST-2530, fumagillin, Mr1.1[S4Dap], aaptamine, and fucoidan should be tested in disease-relevant models, not only CCI/SNL/SNI models.

3. Both sexes should be included. Many pain mechanisms and immune mechanisms are sex-dependent. Future preclinical studies should include male and female animals and should be powered to detect sex-by-treatment interactions.

4. Randomization, blinding, and sample-size justification should be required to reduce risk of bias

5. Improved methodological transparency, reproducibility, and adherence to preclinical

reporting standards are required to reduce uncertainty in the existing evidence base and strengthen confidence in progression toward clinical development

6. Overall, more non-evoked pain outcomes should be added. Most studies rely on paw withdrawal threshold or latency. Operant pain assays, conditioned place preference/avoidance, grimace scales, burrowing, gait analysis, sleep disruption, and affective-pain endpoints would better reflect clinical neuropathic pain.

7. Human-target validation. Once the molecular targets underlying the antineuropathic effects of individual compounds have been identified, functional assays using human molecular targets should be conducted, particularly when existing evidence is limited to other species or based solely on computational predictions. This applies to isolated compounds such as AST, fucoidan, aaptamine, flexibilide, lemnalol, DA, and fumagillin. For chemically complex extracts and peptide fractions—including *E. cava*, spirulina, and *C. aurivilli* extracts, as well as CB conopeptide fractions—the bioactive constituent(s) responsible for the antineuropathic effects must first be identified and isolated to enable subsequent molecular target elucidation and functional validation using their human counterparts.

8. Comprehensive PK/PD characterization and delivery studies are needed. Many marine peptides have poor oral bioavailability and stability. Plasma half-life, CSF penetration, tissue distribution, peptide degradation, immunogenicity, and route-dependent efficacy should be reported. The demonstration of therapeutically relevant exposure at the site of action should become a key criterion for progression

9. Safety pharmacology should be expanded. hERG, cardiac conduction, motor coordination, respiratory depression, neuropsychiatric effects, immunotoxicity, and repeated-dose toxicity should be assessed before translational claims are strengthened.

10. Combination studies should be conducted rigorously. Claims about synergistic combinations should be supported by isobolographic analysis.

11. Sustainability and synthesis must continue to be a priority. Because natural extraction from marine organisms is not scalable, future studies should emphasize total synthesis, recombinant expression, or peptide-engineering routes

12. Mechanism-based stratification and translational biomarkers should be incorporated to better match candidate mechanisms with clinically relevant patient phenotypes.

Limitations of evidence

While the absence of a quantitative meta-analysis limits the ability to provide pooled estimates of treatment effects, this was considered appropriate given the marked heterogeneity of the evidence. The systematic evidence mapping approach instead allowed integration of complementary evidence domains, from molecular and mechanistic studies to *in vivo* efficacy, chemical optimization, drug-delivery strategies and clinical investigation, thereby providing a broader assessment of the maturity and translational potential of marine-derived compounds for NP. Although most preclinical studies were judged to have a low risk of selective outcome reporting according to the SYRCLE tool, publication and dissemination biases cannot be excluded in the available literature. Positive or statistically significant findings may be more likely to be published or disseminated than null or negative results, potentially leading to an overrepresentation of favorable evidence despite the absence of substantial concerns regarding selective reporting within individual studies. Furthermore, negative or inconclusive findings may remain underrepresented if they were not published or were less readily retrievable. Importantly, the inclusion of studies reporting null or partially negative findings was intended to mitigate this concern; however, it cannot compensate for potentially unpublished negative studies that were not accessible through the search strategy. Therefore, the apparent predominance of positive findings should be interpreted cautiously, as it may reflect not only the biological activity of the compounds investigated but also selective dissemination and reporting of underlying evidence. These limitations should be considered when interpreting the apparent predominance of positive preclinical findings and the translational potential of the compounds identified. Finally, although a comprehensive search strategy was applied across multiple databases, the possibility of false-negative retrievals cannot be excluded. The heterogeneous nomenclature of marine-derived compounds, variability in how their marine origin, molecular targets, or analgesic effects are described, and studies in which the marine origin or analgesic properties were not reflected in the title or abstract may have resulted in relevant studies being missing. This possibility was illustrated by the subsequent identification of studies investigating AST and fumagillin that met the conceptual scope of the review but were not retrieved by the original search (Ciapala et al., 2024; Wen et al., 2024).

CONCLUSION

Marine-derived compounds provide diverse molecular scaffolds for neuropathic pain research, particularly through modulation of sodium channels, calcium channels, nicotinic acetylcholine receptors, GPCRs, and neuroimmune pathways. However, the predominantly preclinical evidence, heterogeneous experimental designs, incomplete reporting of methodological safeguards, and limited controlled clinical evidence restrict conclusions about therapeutic effectiveness. Priorities should be guided by reproducible efficacy, human-target activity, adequate exposure, and direct safety assessment.

CONFLICT OF INTEREST

The research was conducted entirely free from any involvement in commercial, financial, or personal matters that might be perceived as a possible conflict of interest or that could have influenced the outcomes or interpretation of the study.

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The authors used Grammarly (Superhuman Platform) and Copilot (Microsoft Corp.) to improve the language and readability of the manuscript. The authors reviewed and edited the content and take full responsibility for its accuracy. No images were generated or manipulated using AI tools.

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Concepts or ideas	x						
Design	x	x					
Definition of intellectual content	x	x					
Literature search	x	x	x	x		x	x
Data acquisition	x	x	x	x			
Data analysis	x	x	x	x	x	x	x
Statistical analysis	x	x	x				
Manuscript preparation	x	x					
Manuscript editing	x	x					
Manuscript review	x	x	x	x	x	x	x

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Supplementary data

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models.

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
1	Synthetic precursor of austrasulfone dihydroaustrasulfone alcohol (DA)	Soft coral *Cladiella australis*	Only effect related to anti-inflammatory activity	*In vitro*: RAW264.7 cells activated by LPS *In vivo*: CCI Wistar rats.	Thermal hyperalgesia: PWL/Hargreaves method Route: IT Doses: 0.1, 0.5, 1.0, and 5.0 µg	- Antihyperalgesic effect (ED_{50} = 0.753 ± 0.05 µg) - Inhibition of the expression of iNOS and COX-2 proteins	Wen et al., 2010
2	Tetrodotoxin (TTX)	*Tetraodontidae* (puffer fish)	TTX-S and TTX-R Na_Vs	*In vivo*: CCI-sciatic nerve and CCI-infraorbital nerve (ION)	Mechanical hyperalgesia: PWT/vocalizations/Paw pressure test Randall-Selitto Mechanical allodynia: PWT/von Frey filaments test Thermal hyperalgesia: PWL/Paw immersion test Route: s.c. Doses: 0.3, 1, 3, or 6 µg/kg (acute) 3 µg/kg (subchronic, twice a day for 4 days) Co-administration of 5-HT1B, α2-adrenergic, opioid or CCK2 receptor ligands	- TTX suppressed allodynia and hyperalgesia for up to 3 h in sciatic nerve-CCI rats, but it was only moderately effective in rats with CCI-ION. - TTX prevented CCI-induced c-Fos IR in the lumbar SDH and hindbrain regions involved in nociception - The effect was promoted by naloxone and attenuated by morphine or a CCK2-receptor antagonist	Kayser et al., 2010
3	ω-Conotoxin CVID synthesized (CNSB004/AM336/ Leconotide)	*Conus catus*	N-type (Ca_V2.2) VGCC	*In vivo*: STZ-induced DN in Wistar rats	- Thermal hyperalgesia: PWL/Hargreaves method Route: IV infusion - Determination of maximum no-side-effect doses. Doses: Leconotide (2 mg/kg, IV) vs. Ziconotide (0.2 mg/kg, IV) Flupirtine (5 mg/kg, i.p.) -Pharmacological safety: Open field Noninvasive blood pressure and heart rate	- Both flupirtine and ziconotide had no anti-hyperalgesic effect when given alone or in combination. - Leconotide did cause an anti-hyperalgesic effect. - The efficacy of the subeffective dose of leconotide (0.02 mg/kg) was increased in combination with flupirtine - IV route, a potential advantage over ziconotide for clinical translation	Kolosov et al., 2010

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
4	Lemnalol	Formosan soft coral *Lemnalia cervicorni*	Spinal neuroimmune activation	*In vivo*: CCI in Wistar rats	Mechanical allodynia: PWT/von Frey filaments test Thermal hyperalgesia: PWL/Hargreaves method Route: IT Doses: 0.05 -10 µg	Inhibits spinal astrocytic and microglial activation and TNF-α expression in these cells	Lin et al., 2011
5	α-Conotoxins, Vc1.1, AuIB, and MII Synthesized	*Conus genus*	G protein-coupled $GABA_B$-modulated N-type ($Ca_V2.2$) channels	*In vitro:* Electrophysiology in *Xenopus laevis* oocytes and DRG neurons. *In vivo*: PSL in SD rats	Mechanical allodynia: PWT/von Frey filaments test Route: IM Doses: 0.36, 3.6, and 36 µg for each α-CTX	- α-CTXs selectively for $GABA_B$ receptor-dependent inhibition of N-type Ca^{2+} channels relieves allodynia - α3β2 nAChR antagonism also contributes to inhibiting allodynia - Inhibition of α9α10nAChRs is neither necessary nor sufficient for relief of allodynia	Klimis et al., 2011
6	8,9-dideoxy-neo-dysiherbaine (MSVIII) a synthetic derivative of dysi-herbaine	*Lendenfeldia chondrodes*	GluK1	*In vitro:* DRG neurons electrophysiology in *In vivo:* CCI in Swiss Webster mice Other models: Tail flick test, Formalin test, acetic acid-induced writhing test (Visceral), Acetic acid, CFA-induced Paw Edema	Mechanical allodynia: PWT/von Frey filaments test Thermal hyperalgesia: PWL/Hargreaves method Route: IT (central) /ipl. (peripheral) Doses: 0.5 nmol per mice	-Pre-application of MSVIII-19 efficiently reduced the amplitudes of currents evoked by a saturating concentration of glutamate (10 mM), consistent with activity as a functional antagonist. -MSVIII-19 effects were reversible, consistent with its activity on recombinant GluK1-containing receptors -Intrathecal MSVIII-19 significantly reduced both thermal hyperalgesia and mechanical hypersensitivity - Peripheral administration of MSVIII-19 had no analgesic efficacy - Intrathecal MSVIII-19 did not alter responses in Tail-flick tests or performance on rotarod.	Qiu et al., 2011
7	α-Conotoxins, Vc1.1, AuIB, and MII synthesized	*Conus genus*	nAChRs containing combinations of α3, α7 or other subunits	*In vitro:* Electrophysiology in lamina I/II spinal cord neurons. *In vivo*: PSL in SD rats	Mechanical allodynia: PWT/von Frey filaments test Route: IT Doses: 0.02, 0.2, and 2 nmol for each α-CTX	- Reduction of mechanical allodynia for up to 6 h without significant side effects. - Mode of action of these CTXs was not via a $GABA_B$-dependent mechanism, and is more likely related to their action at nAChRs containing combinations of α3, α7 or other subunits	Napier et al., 2012

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
8	ω-Conotoxins, CVID, MVIIA and hybrid peptides [K10R]CVID (mutation of residue 10 to arginine) and [R10K]MVIIA (mutation of residue 10 to lysine)	*Conus catus* *Conus magus*	N-type ($Ca_V2.2$) VGCC	*Ex vivo:* Electrophysiology (slices of the lumbar spinal cord) using naive rats *In vivo:* PSL in SD rats	Mechanical allodynia: PWT/von Frey filaments test Thermal hyperalgesia: PWL/Hargreaves method Motor performance (rotarod test) Maximal side-effect score Route: IT Doses: 0.01, 0,03, 0.1, and 0.3 nmol	- All ω-conotoxins produced dose- dependent reduction in mechanical allodynia and side effects (motor dysfunction) - CVID displayed a marginally better therapeutic index than MVIIA. - The hybrids had a lesser effect in the rotarod test than either of their parent peptides - CTXs presynaptically inhibited excitatory synaptic neurotransmission into the dorsal horn - Lack of correlation between the position 10 residue and recovery from inhibition	Jayamanne et al., 2013
9	Fucoidan (sulfated polysaccharide)	Brown seaweeds	Spinal neuroimmune activation MAPK pathways	*In vivo:* SNL in SD rats	Mechanical allodynia: PWT/von Frey filaments test Thermal hyperalgesia: TWL/Hargreaves method Route: IT Doses: 15, 50, 100 mg/kg	- Attenuation of mechanical allodynia and thermal hyperalgesia. - Inhibits spinal astrocytic and microglial activation, proinflammatory cytokine production, and MAPK activation	Hu et al., 2014
10	α-Conotoxin RgIA	*Conus regius*	α9α10 nAChR Neuroinflammation Neuroprotective strategy (disease-modifying effects)	*In vivo*: CCI model in SD rats	Mechanical sensitivity to noxious and non-noxious stimuli. PWT/ Paw-pressure test and von Frey test) Weight-bearing changes (Δ Weight)/Incapacitance test Route: IM ipsilateral Doses: 2 nmol/100 µL or 10 nmol/100 µL per rat for 14 days	- Reduces the number of macrophages (CD68) in the sciatic nerve - Prevents CCI-induced sciatic nerve Wallerian degeneration changes, DRG immune cell infiltrates and edema, as well as activation of spinal microglia and astrocyte cells	Di Cesare Mannelli et al., 2014
11	Brown seaweed extracts (phenols, carotenoids, and polysaccharides)	*Ecklonia cava*	Only Effect	*In vivo*: SNI in SD rats	Mechanical hypersensitivity: PWT/ von Frey filaments assay. Route: p.o. Doses: 300 mg/kg	- Decreases mechanical hypersensitivity	Kim et al., 2014

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
12	5,15-dioxatricyclo[12.3.1.0(4,6)]octadec-9-en-16-one (flexibilide/sinularin)	Sinularia flexibilis	Neuroinflammation/Nitroxidative stress	*In vivo:* CCI in Wistar rats	Weight-bearing deficits/Incapacitance test Thermal Hyperalgesia TWL/Hargreaves method Route: IT Doses: 1, 5, 10, 20, and 50 µg	-Single intrathecal administration of flexibilide attenuated CCI-induced thermal hyperalgesia at 14 days after surgery. Flexibilide 10 µg twice daily was able to prevent the development of thermal hyperalgesia and weight-bearing deficits in CCI rats -Flexibilide inhibited CCI-induced activation of microglia and astrocytes, as well as the upregulated proinflammatory iNOS in the ipsilateral spinal dorsal horn -Flexibilide attenuated CCI-induced downregulation of spinal TGF-β1 in a TGF-β type 1 receptor antagonist (SB431542) sensitive manner	Chen et al., 2014
13	αO-Conotoxin GeXIVA GeXIVA [1,2]	*Conus generalis*	α9α10 nAChR	*In vitro:* Electrophysiology in *Xenopus laevis* oocytes expressing *cloned* rat nAChR subtypes and DRG rat neurons *In vivo:* CCI model in SD rats *In silico:* Molecular modeling interactions	Mechanical hypersensitivity: PWT/ von Frey filaments and electronic von Frey assays Route: IM Doses: 0.3, 0.6, 1.2 nmol per rat vs. morphine	- Identification, cloning, synthesis, and characterization of GeXIVA, which decreases CCI-induced mechanical hypersensitivity - GeXIVA displays a lack of effect on Ca_V channels but potently antagonizes nAChRs, mostly the α9α10 subtype. - A voltage- dependent block suggests binding to an allosteric site or a channel pore - The binding site does not overlap with the binding site of the competitive antagonist RgIA - The most active disulfide isomer is the bead isomer	Luo et al., 2015
14	αO-Conotoxin GeXIVA[1,2] (bead isomer) and GeXIVA[1,4] (ribbon isomer)	*Conus generalis*	α9α10 nAChR	*In vivo:* CCI SD rats Tail-flick test/ radiant heat (no effect)	Mechanical hypersensitivity: PWT/ electronic von Frey test Route: IM Doses: 0.5 - 2 nmol per rat vs. morphine	Both isomers reduced mechanical allodynia in CCI rats without tolerance GeXIVA [1,2] long-term effect (2 weeks after discontinuation)	Li et al., 2016

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
15	Synthetic α-Conotoxin RgIA	*Conus regius*	α9α10 nAChR Neuroprotective	*In vivo*: OXA-induced neuropathic pain in SD rats.	Mechanical hyperalgesia: WRT or vocalizations/Paw pressure test Mechanical allodynia: PWL/electronic von Frey test Cold allodynia: cold latency/Cold Plate Test) Route: IM Doses: 2 and 10 nmol	- Concomitant treatment with RgIA reduces mechanical hypersensitivity to noxious and non-noxious stimuli and cold allodynia - RgIA prevents nucleolar changes of the DRG neurons - RgIA per se increases density and activation of microglia and astrocytes	Pacini et al., 2016
16	α-Conotoxin RgIA analog (RgIA4)	*Conus regius*	α9α10 nAChR	*In vitro:* Electrophysiology in *Xenopus laevis* oocytes expressing rat or human nAChR subtypes and rat DRG neurons Binding assays (5HT, GABA, opioids, other receptors, and transporters) *In vivo*: OXA-induced cold allodynia in SD rats and mice (wild-type CBA/CaJ and Germline α9 KO mice)	Mechanical hyperalgesia: withdrawal reflex thresholds (WRT) or vocalizations/Paw pressure test Cold allodynia: Cold latency/ Cold Plate Test Route: s.c. Doses: 0.128–80 µg/kg	- Development of (RgIA1-5) analogs - Selected RgIA (closing the affinity gap across the rodent and human α9α10 nAChRs vs. native) - Comparison of wild-type and α9 nAChR KO Mice. - The extended duration (24–72 h) of efficacy of RgIA4 in the rodent model is remarkable (plasma half-life in rodents is less than 20 min)	Romero et al., 2017
17	α-Conotoxin RgIA analog (RgIA4)	*Conus regius*	α9α10 nAChR	*In vitro:* Electrophysiology in *Xenopus laevis* oocytes expressing α9α10 nAChR. BRET assay (HEK293cells / heterotrimeric G protein dissociation) *In vivo:* OXA-induced cold allodynia in CBA/CaJ mice	Cold allodynia: cold latency/ Cold Plate Test Route: s.c. Doses: 40 µg/kg	- Long-lasting acting analgesic effects (for 21 days after its discontinuation) - Lacks activity at $GABA_BR$ expressed in HEK cells	Christensen et al., 2017

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
18	α-Conopeptide Eu1.6	*Conus eburneus*	N-type ($Ca_V2.2$) calcium channels	*In vitro:* Electrophysiology in isolated mouse DRG neurons and HEK293 cells *In vivo:* PSL and CCI in SD rats	Mechanical hyperalgesia: PWT/ Paw pressure test Route: IM, EV Doses: 2.5–5.0 µg/kg vs. α-conotoxin Vc1.1 and combination of morphine/gabapentin	- $Ca_V2.2$ inhibition/ GABAB receptor independent - Systemic administration exhibited potent analgesia	Liu et al., 2018
19	Spirulina (aqueous extract) C-phycocyanin	*Spirulina platensis* microalgae Cyanobacteria	Antioxidant mechanisms	*In vivo*: CCI Wistar rats	Thermal hyperalgesia: PWL/Hargreaves' test Mechanical allodynia: PWT/von Frey's test Route: p.o. Doses: 200, 400, and 800 mg/kg	- Antihyperalgesic effect (400 mg/kg) (preventive schedule) - Increased FRAP in serum	Ali Safakhah et al., 2018
20	ω-Conotoxins, MoVIA and MoVIB	*Conus Moncuri* *The first CTX identified in (vermivorous ω-CTXs)	human $Ca_V2.2$	*In vitro*: Fluorimetric calcium imaging assays in human SH-SY5Y cells Electrophysiology in rat DRG neurons *In vivo*: PSL in SD rats	Mechanical allodynia: PWT/Frey's test Route: IT Doses: 0.01–1 nmol per rat Side Effect Score	- An arginine at position 13 in MoVIA and MoVIB replaces the functionally critical tyrosine found in piscivorous ω-CTXs - Antiallodynic effect of MoVIB with apparent improvement in safety window vs. MVIIA	Sousa et al., 2018
21	Tetrodotoxin (TTX)	*Tetraodontidae* (puffer fish)	TTX-S and TTX-R Na_V	*In vivo*: Resiniferatoxin (RTX)-induced PHN model in SD rats Pharmacokinetic profile of TTX pellets (UPLC/MS/MS) and median lethal dose (LD_{50})	Mechanical allodynia: PWT/Frey's test Thermal allodynia: PWL/ Hargreaves' test Route: p.o. vs. IM Doses: TTX pellets 5, 10, and 20 µg/kg (for 4 days) vs. 1.3 and 6 µg/kg) vs. pregabalin	- Like pregabalin, oral TTX pellets prevent, in a dose-dependent manner, RTX-induced mechanical and thermal allodynia - Plasma Cmax of TTX pellets was around 2 h, with an elimination half-life time ($t_{1/2}$) of 3.23 ± 1.74 h - The median lethal dose (LD_{50}) of TTX pellets was 517.43 µg/kg via oral vs. the median effective dose (ED_{50}) was 5.85 µg/kg, Therapeutic index: 88.45 - Oral TTX pellets greatly enhance safety when compared with TTX injection	Hong et al., 2018

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
22	Trans-astaxanthin (AST)	Marine plants, algae, and seafood	Serotonergic system Neuroinflammation	*In vivo:* CCI in ICR mice	Mechanical allodynia: PWT/Frey's test Thermal hyperalgesia: PWL/Hargreaves' test Route: p.o. Doses: 10, 40, 80 mg/kg Force swimming and tail suspension tests Locomotor activity	- Repeated doses of trans-AST ameliorated CCI-induced NP behavior and co-morbid depressive-like behavior involving serotonergic system - AST reverses indoleamine 2,3-dioxygenase (IDO) expression in hippocampus and spinal cord, improving the 5-HT content, and reducing the levels of IL-1β, IL-6 and TNF-α	Jiang et al., 2018
23	Astaxanthin (AST)	Marine plants, algae, and seafood	Neuroimmune activation Nitroxidative stress NR2B/NMDAR	*In vitro*: LPS-induced astrocytic activation using C6 cells *In vivo*: CCI in SD rats *In silico*: Ligand-receptor molecular docking	Mechanical allodynia: PWT/electronic Frey's Thermal hyperalgesia: PWL/Hargreaves' test Route: i.p. Doses: 5 and 10 mg/kg vs. gabapentin	- AST attenuated NP behavior, showing affinity to inhibitory binding pocket of NR2B/NMDAR - AST reduces the nitroxidative stress and GFAP expression induced by LPS in C6 glial cells	Sharma et al., 2018
24	Astaxanthin (AST)	Marine plants, algae, and seafood	Neuroprotection NMDAR/NR2B p-p38MAPK TNF-α	*In vivo:* SCI in Wistar rats	Cold allodynia: Score/acetone drop test Route: IT Doses: 0.2 mM/10 µL Motor performance (inclined plane test)	- AST attenuates cold allodynia and motor dysfunction, decreasing myelinated white matter and motor neuron damage after SCI - AST decreased the spinal NMDAR/NR2B and p-p38MAPK along with TNF-α proteins	Fakhri et al., 2018
25	Extract fractions (small linear and cyclic peptides structurally like endogenous ligands to GPCR) (DUQ0002I)	Marine cyanobacteria *Leptolyngbya*	5-HT7R	*In vitro*: Target identification of 5-HTRs and the 5-HT transporter (SERT) in a radioligand binding assay *In vivo*: SNI rats with comorbid depression -Murine depression and anxiety assays (forced swim, tail suspension, elevated zero maze, and light-dark preference tests)	Mechanical sensitivity; PWT/von Frey filaments Route: ICV or CA1 hippocampus Doses: 40 µg per cannula	- Antidepressant and anxiolytic effects in male WT mice, but not have effects in 5-HT7R KO or female mice - DUQ0002I targeted to the CA1 had no behavioral effect on SNI-induced mechanical hypersensitivity (data not shown)	Lax et al., 2018

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
26	Fucoxanthin	Brown seaweeds (*Hizikia fusiformis*, *Laminaria japonica* and *Sargassum fulvellum*)	TRPV1 Glial stabilization, Antioxidant mechanisms/Nrf2 pathway, neuroprotective	*In vivo:* Trigeminal pain in ultraviolet B (UVB)-induced photokeratitis models (Inflammatory pain with neuropathic component).	Eye wipe test using hypertonic saline. Route: p.o Doses: 0.1, 1, 10 mg/kg	- Inhibition of UVB-induced corneal typical denervation (PGP 9.5) and inflammatory trigeminal pain (Prevention of NP) - Inhibition of UVB-induced corneal disorders including epithelial exfoliation - Decrease the number of phosphorylated p38 MAP kinase in neurons and neighboring satellite glial cells (SGCs), GFAP-positive SGCs, and TRPV1-positive neurons in trigeminal ganglia - Enhance the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) in the cornea	Chen et al., 2019
27	Astaxanthin (AST)	Marine plants, algae, and seafood	Neuroprotection Prevention of activation of ERK1/2 Promotion of AKT activation	*In vivo:* SCI in Wistar rats	Mechanical allodynia: PWT, Frey's test Thermal hyperalgesia: Paw licking latency/Hot plate Route: IT Doses: 0.2 mM/10 µL Open field test, Basso-Beattie-Bresnahan scoring scale, body weight- auricle temperature, blood glucose.	- AST attenuates SCI-induced NP behaviors, motor dysfunction, weight loss and ameliorates body temperature and blood glucose changes - AST decreases demyelination and motor neuron loss decreasing ERK1/2 activated and increased AKT activated	Fakhri et al., 2019
28	αO-Conotoxin GeXIVA[1,2]	*Conus generalis*	α9α10 nAChR	*In vivo*: OXA-induced neuropathic pain in SD rats. Other assays: Tail-Flick Latency, Grip Strength in naive rats	Mechanical hypersensitivity: PWT/ von Frey filaments test Cold allodynia: Score, Acetone drop test) Route: IM Doses: 32, 64, 128 nmol/kg vs. gabapentin	- GeXIVA 1,2 relieve and reverse OXA-induced mechanical and cold allodynia after single and repeated injections - α9α10nAChR antagonism (potentially via P2X7R activation induced IL-1β release inhibition) - GeXIVA[1,2] did not affect acute mechanical and thermal pain behaviors in normal rats	Wang et al., 2019

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
29	α-Conotoxin RgIA and a potent analog, RgIA4	*Conus regius*	α9α10nAChRs Neuroinflammation	*In vivo:* PTX-induced neuropathic pain in SD rats	Mechanical allodynia: PWT/von Frey filaments assay Thermal allodynia: PWL/Hargreaves' test Cold allodynia/cold PWL/Cold Plate Test Route: s.c. Doses: 16 and 80 µg/kg	- RgIA4 (80 µg/kg) accelerates recovery from mechanical allodynia - The therapeutic effects reached significance 12 days after the last administration of RgIA4, which is suggestive of a rescue mechanism	Huynh et al., 2020a
30	Astaxanthin (marine carotenoid)	Marine plants, algae, and seafood	Spinal neuroimmune activation MAPK and NF-κB pathways	*In vitro:* Primary cultures of astrocytes, BV2 microglial cell line and PC12 cells exposed to LPS *In vivo:* SNL in C57BL/6 mice	Mechanical allodynia: Paw withdrawal Response frequency (PWF) to von Frey filaments Thermal hyperalgesia: PWL/Hargreaves' test Route: i.p. Doses: 5 or 10 mg/kg	- AST attenuates SNL-induced NP behaviors - Inhibition of ERK1/2, p38, and NF-κB signals pathways in neuronal and glial cells - Decrease expression of pro-inflammatory (IL-1, IL-17, IL-6, and TNF-α) and increase anti-inflammatory IL-10 cytokines	Zhao et al., 2021
31	Conopeptides from venom extract fractions	*C. textile* *C. miles* *C. quericinus* *C. magus* *C. geographus* *C. radiatus*	CB1R and potentially CB2R	*In vitro*: HEK293 cells *In vivo*: formalin test CCI model in SD rats	Mechanical allodynia: PWT/von Frey filaments assay Heat hypersensitivity: PWL/Hargreaves' method Route: IT Doses: 5 µL of HPLC fractions (1 mg/mL)	- CB receptor agonism - *C. textile* and *C. miles* subfractions attenuate NP - Peptidergic nature of CBI active component	Jergova et al., 2021
32	Alkyne-Bridged α-Conotoxin Vc1.1	*Conus victoriae*	G protein-coupled $GABA_BR$-$Ca_V2.2$	*In vitro*: Electrophysiology in *Xenopus* oocytes expressing hnAChRs, HEK293T cells expressing hCaV subunits α1B, β3, and α2δ1, and co-transfected with $hGABA_BR$ subunits *In vivo*: SNL model in SD rats	Mechanical allodynia: PWT/von Frey filaments assay Route: IM Doses: 0.03, 0.06, and 0.1 mg/kg vs. gabapentin	- Bridge mutation of α-CTxVc1.1 produced a $GABA_BR$ selective agonist (alkyne, Vc1.1) with higher potency at hCav2.2 channels than that of native Vc1.1 - Vc1.1 analog is inactive at α9α10 nAChR but exhibits dose-dependent reversal of mechanical allodynia	Belgi et al., 2021

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
33	Saxitoxin (STX) synthetic analogue (ST-2530)	microalgae dinoflagellates, diatoms, and cyanobacteria	$Na_v1.7$	*In vitro:* Electrophysiology in HEK293 and CHO cells expressing hNa_v1 isoforms *In vivo:* SNI in C57Bl/6 mice Other assays: Hargreaves and tail flick test, formalin test, plantar incision model	Mechanical allodynia: PWT/von Frey filaments assay Route: s.c. Doses: 3 mg/kg	- ST-2530 reverses mechanical allodynia induced by the SNI at single and repeated doses. - At dose that does not affect motor and olfactory function	Beckley et al., 2021
34	Fumagillin	Aspergillus fumigatus derived from different niches (sponges, marine sediments, corals, algae, other invertebrates, seawater) (Ubiquitous environmental fungus)/terrestrial and marine (endophyte or saprophyte)	Neuronal metabolism, spinal angiogenesis, and neuroinflammation	*In vivo*: CCI model in Wistar rats	Mechanical allodynia: PWT/von Frey filaments assay Heat hypersensitivity: PWL/Hargreaves' method/ TFL/Hot-Water Immersion test / Changes in the hindpaw weight distribution/Weight Bearing test Route: IT Doses: 0.1 µg/day vs. anti-VEGF antibody	-Fumagillin and anti-VEGF-A reduced CCI-induced thermal hyperalgesia from day 5 to 14 and mechanical allodynia from day 3 to 14. -VEGF, cluster of differentiation 31 (CD31), von Willebrand factor (vWF) expressions and pro-inflammatory cytokine concentrations increased after CCI in the ipsilateral lumbar spinal cord. - Fumagillin reduced CCI-upregulated expressions of angiogenic factors and astrocyte activation. -Fumagillin differentially modulates CCI-induced spinal cytokine production, decreasing IL-16 and TNFα and increasing IL-10 levels	Wen et al., 2021
35	α-Conotoxin RgIA and its analog RgIA4	*Conus regius*	α9-containig nAChR within specific CD3+ T-cell subset Neuroinflammation Disease-modifying effects	*In vivo:* Acute OXA-Induced Cold Allodynia in wild-type CBA/CaJ mice and chrna9 KO mice	Cold allodynia: cold PWL/Cold Plate Test Route: s.c. Doses: 40 µg/kg	- Necessity of the α9 nAChR subunit - RgIA4 prevented cold allodynia in wild-type mice but not in mice lacking the α9 nAChR-encoding gene, chrna9 - RgIA4 also failed to reverse allodynia in mice depleted of CD3+ T-cells	Huynh et al., 2022

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
36	Synthetic α-conotoxin Mr1.1 and its analog Mr1.1[S4Dap]	*Conus marmoreus*	hα9α10 nAChR	*In vitro: X. laevis* oocytes expressing hnAChRs and rα9α10 electrophysiology *In silico:* MD-simulations *In vivo:* CCI model	Mechanical allodynia (PWT/electronic von Frey test Route: no precision Doses: 0.5, 2.5, and 25 µg/kg vs. Mr1.1 and gabapentin	- Most potent antagonist effect at h9α10 nAChR (IC_{50} = 92.0 nM) - Mr.1 bings at α10(＋)/α9(－) and α10(－)/α9(＋) sites via hydrogen bonds and salt bridges, stabilizing the closed conformation of channel - Several analogues were generated, the most potent antagonist Mr1.1[S4Dap] (IC_{50} = 4.0 nM) also exhibit analgesic effects in CCI model	Liang et al., 2022
37	Contulakin-G (CGX) (synthetic)	*Conus geographus*	Neurotensin receptor 2 (NTSR2)/R-type calcium channel ($Ca_V2.3$) pathway	*In vitro*: DRG primary cell cultures (calcium imaging and electrophysiology), spinal cord slices, electrophysiology *In vivo*: SNL, PSL in SD rats and C57Bl6/J mice (female and male) -PNL in mice knockdown/NTSR2 and $Ca_V2.3$ deficient (CRISPR/Cas9 editing) Other assays: Paw incision model of post-surgical pain	Thermal hyperalgesia: PWL/Hargreaves' test Naïve mouse tail-flick assay (latency) Mechanical allodynia: PWT/von Frey's test Route: IT Doses: 0.02, 0.1, 0.5 nmol per rat Doses: 0.15 nmol vs. morphine	- The efficacy of CGX was not sex dependent - Opioid-independent spinal mechanism of analgesia involving NTSR2-$Ca_V2.3$ pathway	Martin et al., 2022
38	Aqueous extract flavonoids, tannins, saponins, alkaloids, glycosides, sterols, and polyphenolic compounds	*Cliothosa aurivilli* *Marine sponge*	Only effect	*In vivo*: PTX-induced peripheral neuropathy in Swiss albino mice	Mechano-hyperalgesia: Score number of paw withdrawals/pin prick Thermal hyperalgesia: TWL/tail flick radiant heat test, tail immersion test, hot plate test Cold allodynia: Score number of paw withdrawal/ acetone drop test Route: p.o. Doses:100 and 200 mg/kg vs. methylcobalamin	- Improve mechano-hyperalgesia, cold allodynia and thermal hyperalgesia and motor function - Acute toxicology (no mortality)	Karmakar et al., 2022

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
39	Aaptamine (alkaloid composed of amines and halogenated cyclic nitrogen)	Sponge *Aaptos aaptos*	Neuronal metabolism, spinal angiogenesis, and neuroinflammation *δ-opioid agonism and μ-opioid agonism (Johnson et al., 2017).	*In vivo*: CCI Wistar rats	Mechanical allodynia: PWT/von Frey filaments Thermal hyperalgesia: PWL/Hargraves method Route: IT Doses: 5, 30, 100 μg per rat (single) and 30 μg repeated by 13 days	- Aaptamine shows acute and preventive analgesic effects - Inhibition of CCI-induced upregulation of angiogenic markers (VEGF and CD31) in spinal astrocytes - Inhibition of CCI-induced LDHA expression in spinal neurons - Inhibition of LDHA nuclear translocation	Sung et al., 2023
40	αO-Conotoxin GeXIVA[1,2]	*C. generalis*	α9α10 nAChR	*In vivo*: OXA-induced neuropathic pain in SD rats. (RNA sequencing of L4-L6 spinal cord and bioinformatic analysis)	Mechanical hypersensitivity: PWT/ von Frey filaments test Route: s.c. Doses: 0.0865 - 1.73 mg/kg Open field and elevated plus maze tests	- GeXIVA[1,2] prevents the chronic OXA-induced mechanical allodynia - Repeated injections for 3 weeks had no effect on the mice's normal pain threshold or locomotor activity and anxiety-like behavior - OXA-induced DEGs were reverted to control group level by GeXIVA[1,2] cotreatment - Reverted genes are associated with immune-related pathways, (cytokine–cytokine receptor interaction pathway)	Wang et al. 2024
41	Astaxanthin (AST)	Marine plants, algae, and seafood	Neuroinflammation Nitroxidative stress $GABA_A$/BDZ receptor Opioid receptors	*In vivo*: CCI in Wistar rats	Cold allodynia: Score/Acetone drop test Thermal hyperalgesia: PWL/hot plate test Route: i.p. Doses: 5 and 10 mg/kg vs. gabapentin Motor performance (open field test)	- AST improved allodynia, hyperalgesia, and locomotor activity after CCI, increasing catalase and glutathione activity while suppressing nitrite, MMP-2, and MMP9 levels in a naloxone/flumazenil sensitive manner - AST (10 mg/kg) improved myelin sheath integrity and Schwann cells in sciatic nerve after CCI, probably involving BDZ/opioid receptors	Hashemi et al., 2024

Table S1. Articles that provide *in vivo* preclinical evidence of the marine-derived bioactive analgesic activity in models of neuropathic pain or other experimental models with evidence of nerve injury demonstrated (Group 1). Rows shaded in blue represent potential candidates to be evaluated in neuropathic pain models (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model	Outcome measures/ doses/routes/positive control	Significant findings	Author and year
42	ω-Conotoxin MVIIA	*Conus magus*	N-type voltage-dependent Ca_V channels *Clinical study in SCI patients using ziconotide with positive results (Brinzeu et al., 2019)	*In vitro*: Electrophysiological experiments (whole-cell patch-clamp recording of SG neurons (using rats with SCI) and *in vivo* extracellular recording in SCI rats. *In vivo* SCI model in Wistar rats: Immunohistochemical analyses (pERK)	Mechanical allodynia: PWT/von Frey filaments test Route: IT Doses: 200 pmol/10 µL Basso-Beattie-Bresnahan scoring scale (locomotor functions)	- MVIIA inhibits presynaptic N-type Ca_V channels expressed on primary afferent Aδ- and C-fiber terminals and suppresses the presynaptic glutamate release from gelatinous substantia in the SDH - MVIIA decreases neuronal excitability enhanced by SCI-induced NP	Ohashi et al., 2024
43	Consomatin Fj1 Selective SSTR4 peptide	*Conus fijisulcatus*	Somatostatin receptor 4 (SSTR4) Gαo	*In vitro*: HEK293 cells (G protein dissociation, BRET-based assay) *In vivo:* SNI mice Other assays: Paw incision model *In silico*: Molecular modeling interactions (X-ray crystal structure and MD-simulations). Computational selection (data base), synthesis, and receptor profiling of eight candidate toxins	Mechanical allodynia: PWT/von Frey's filaments test Route: i.p.. Doses: 30 mg/kg vs. gabapentin	- Fj1 induces G protein dissociation of Gβγ subunits from the Gαo subunit at the SSTR4 - Synthetic Fj1 systemically administered alleviates SNI-induced mechanical hypersensitivity - Fj1 binds SSTR4 through the side chain interactions of D-Trp4 and Lys5 with receptor residues - Residues outside of Trp-Lys motif could be modified to improve in potency and selectivity while retaining SSTR4 binding (Fj1 analogs)	Bjørn-Yoshimoto et al., 2025

Exception: Bjørn-Yoshimoto et al., 2025 was identified in PubMed as a preprint during the search period and was provisionally retained because of its direct relevance. The record was subsequently updated to peer-reviewed publication.

Table S2. Articles that provided preclinical evidence of marine-derived bioactive interactions with or modulation of a potential target involved in neuropathic pain (Group 2).

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model/*in vivo* outcomes/doses/routes/positive control	Significant findings	Author and year
1	α-conotoxins Vc1.1 and Rg1A	*Conus victoriae* and *Conus regius*	G protein-coupled $GABA_B$-modulated N-type $Ca_V2.2$ channels	*In vitro*: Inhibition of N-type $Ca2^+$channels currents in DRG neurons of wild-type and α9 KO mice, Electrophysiology	- N-type VGCCs inhibition via a $GABA_B$ GPCR mechanism - α9α10 nAChRs cannot be involved in mediating this effect	Callaghan & Adams, 2010
2	*Thalassia testudinum* crude extract (BM-21) and isolated thalassiolin B	Sea grass *Thalassia testudinum*	ASICs	*In vitro*: rat DRG neurons electrophysiology *In vivo*: hot plate test/TWL, formalin test/number of lickings, writhing test/number of contortions in OF-1 mice. BM-21: 4, 20, 40, 400, 1000 mg/kg, p.o. Thalassiolin B: 100 mg/kg, i.p. vs. indomethacin	- BM-21 and thalassiolin B inhibit the fast-desensitizing ASIC currents - The nonpeptidic nature of thalassiolin B may have an advantage for future therapeutic applications	Garateix et al., 2011
3	Sea anemone toxin APETx2	*Anthropleura elegantissima*	TTX-R (Na_V 1.8 channels)	*In vitro*: DRG neurons and *Xenopus laevis* oocytes, Electrophysiology	Na_V 1.8 channel inhibition in addition to ASIC-3	Blanchard et al., 2012
4	α-Conotoxins Vc1.1 and Rg1A	*Conus victoriae* and *Conus regius*	G protein-coupled $GABA_B$-modulated N-type $Ca_V2.2$ channels	*In vitro*: $GABA_B$R knockdown by siRNA in rat DRG neurons and HEK 293 cell electrophysiology vs. baclofen	- The activity of these α-CTX on $Ca_V2.2$ needed functional expression of both human $GABA_B$R subunits (GBR1 and GBR2), like baclofen - The Vc1.1-mediated voltage-independent pathway is more sensitive to $GABA_B$R knockdown than the baclofen-mediated voltage-dependent pathway - The inhibition of VGCC currents exerted by Vc1.1 via $GABA_B$R could be due to $Ca_V2.2$ inactivation rather than channel internalization	Cuny et al., 2012
5	Conantokin-G synthetic	*Conus geographus*	GluN2B-containing NMDARs Neuroprotection *ConG decreases CCI-induced NP (Malmberg et al., 2003)	*In vitro*: Primary rat hippocampal neuron cultures, electrophysiology, real-time Ca^{2+} influx, Mitochondrial viability	NR2B-selective antagonist of NMDAR resulting in the opposing action of conG on differentially activated pools of NMDARs (synaptic and extrasynaptic locations)	Balsara et al., 2012

Table S2. Articles that provided preclinical evidence of marine-derived bioactive interactions with or modulation of a potential target involved in neuropathic pain (Group 2) (continued...)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model/*in vivo* outcomes/doses/routes/positive control	Significant findings	Author and year
6	µO-Conotoxin, MfVIA synthetic	*Conus magnificus*	$Na_V1.8$	*In vitro*: Membrane potential-sensitive dyes (FLIPR) High-throughput Ca^{2+} assay in SH-SY5Y human neuroblastoma cells HEK293, ND7/23 and CHO cells expressing $Na_V1.1$–$Na_V1.8$ isoforms HEK293 and CHO cells ($Na_V1.4$, $Na_V1.5$, $Na_V1.6$ and $Na_V1.7$), *Xenopus leavis* oocytes expressing r$Na_V1.2$ and h$Na_V1.8$ and DRG neurons electrophysiology	-MfVIA preferentially inhibits $Na_V1.8$ (IC_{50} 95.9±74.3 nM and $Na_V1.4$ (IC_{50} 81±16 nM), with significantly lower affinity for other Na_V subtypes - Improved approach for regioselective synthesis of µO-conotoxins	Vetter et al., 2012
7	Conantokins (ConPr1, ConPr2, and ConPr3)	*Conus parius*	GluN2B-containing NMDARs	*Ex vivo*: Rat hippocampal and mouse cortical neurons' electrophysiology	- Inhibition of NMDA/Gly-mediated Ca^{2+} influx through NMDAR channels - Antagonist function of these peptides on the NMDAR diminishes Ca^{2+} influx, but they do not completely eliminate the CREB signaling pathway essential for neuronal survival	Kunda et al., 2013
8	Ugr 9-1 peptide	Sea anemone *Urticina grebelnyi*	ASIC-3	*In vitro*: *X. laevis* oocytes expressing human ASIC3 electrophysiology *In vivo*: Acetic acid writhing test/Number of writhes CFA model/PWL in CD-1 mice 0.5, 0.1, and 0.01 mg/kg, IV	- Ugr 9-1 has an uncommon spatial structure, stabilized by two S-S bridges, with three classical turns and twisted -hairpin without interstrand disulfide bonds - This peptide inhibits both the transient and the sustained current of human ASIC3 channels - Ugr 9-1 decreases inflammatory hypernociception	Osmakov et al., 2013
9	α-Conotoxin Vc1.1 and c-Vc1.1	*Conus victoriae*	G protein-coupled $GABA_B$-modulated R-type $Ca_V2.3$ channels	*In vitro*: HEK-293 and HEK-293T cells coexpressing h$GABA_B$Rs and $Ca_V2.1$ or $Ca_V2.3$ channels electrophysiology (wild-type or mutant c-Src) Publicly available catalog of phosphorylation motifs (https://www.hprd.org/PhosphoMotif_finder)	- Vc1.1 only inhibits $Ca_V2.3$ channels, despite baclofen efficiently inhibiting both $Ca_V2.1$ and $Ca_V2.3$ channels. - c-Src phosphorylation of specific tyrosine residues (Y1761 and Y1765) in the human α-subunit C-terminus is sufficient to mediate Vc1.1 inhibition of $Ca_V2.3$ channels	Berecki et al., 2014
10	α-Conotoxin Vc1.1	*Conus victoriae*	G protein-coupled GABAB-modulated N-type CaV2.2 channels	*In vitro*: HEK-293 and HEK-293T cells coexpressing h$GABA_B$R and $Ca_V2.2$ channels electrophysiology (wild-type and mutated $GABA_B$1a subunits)	- Vc1.1 activates a distinct voltage-independent $GABA_B$R-$Ca_V2.2$ signaling pathway that requires the $GABA_B$1a proximal carboxyl terminus (PCT) domain to couple $GABA_B$R to $Ca_V2.2$, ultimately inhibiting $Ca_V2.2$ channels. This $GABA_B$R-biased signaling mechanism likely contributes to its superior selectivity and analgesia	Huynh et al., 2015

Table S2. Articles that provided preclinical evidence of marine-derived bioactive interactions with or modulation of a potential target involved in neuropathic pain (Group 2) (continued...)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model/*in vivo* outcomes/doses/routes/positive control	Significant findings	Author and year
11	*Sargassum thunbergii* ethanolic extract	Brown seaweed *Sargassum thunbergii*	Neuroinflammation	*In vitro*: LPS-stimulated BV-2 microglia	Neuroprotective potential Decreases iNOS expression, NO, and TNF-α release in activated microglial cells	Lee & Kang, 2015
12	Kunitz-Type Peptide HCRG21	Sea Anemone *Heteractis crispa*	TRPV1	*In vitro*: *X. laevis* oocytes expressing K_V1.1, 1.3, 1.4, 1.5, 1.6, Shaker IR, hERG, and TRPV1 electrophysiology *In silico*: HCRG21–TRPV1 homology modeling and docking	- Kunitz-type peptide rHCRG21, belonging to the *H. crispa* multigene HCRG peptide subfamily, did not exert any activity on the K_V channels, but fully inhibited capsaicin-induced currents through the TRPV1 channel (95%). - Potential peptide–channel interactions by pore-blocking or an intracellular binding with the regulatory domain triggering conformational changes of the pore helix region structure	Monastyrnaya et al., 2016
13	μ-Conotoxin TsIIIA	*Conus tessulatus*	TTX-R Na_V (Na_V1.5, Na_V1.8, Na_V1.9)	*In vitro*: Isolated rat DRG neurons electrophysiology *In vivo*: Hot plate test/TWL 20 pmol, IV vs. ziconotide	- A novel μ-conotoxin TsIIIA was identified from a worm-hunting and synthesized - TsIIIA selectively inhibits TTX-R but does not affect TTX-S sodium currents, showing a better analgesic effect than ziconotide	Yang et al., 2017
14	Peptide Ms 9a-1	Sea anemone *Metridium senile*	TRPA1	*In vitro*: Fluorescent Ca^{2+} influx in CHO-rTRPA1 cells and rat DRG neurons *Xenopus laevis* oocytes expressing rTRPA1 electrophysiology *In vivo*: Ms 9a-1 induces pain or thermal hyperalgesia (2.5 μg, Ipl.) Hot plate test/TWL, allyl isothiocyanate (AITC)-induced pain and inflammation/ time spent licking-guarding, CFA model/thermal hyperalgesia and inflammation/PWL, capsaicin test/ time spent licking. Edema/paw diameter. CD-1 mice 0.03 and 0.3 mg/kg, IV	- Ms 9a-1 neither induces pain nor thermal hyperalgesia, but decreases hypernociception and inflammation - Ms 9a-1 acts as a positive modulator, potentiating TRPA1 response to different agonists *in vitro* - Ms 9a-1 produces analgesia *in vivo* probably via desensitization of TRPA1-expressing sensory neurons	Logashina et al., 2017

Table S2. Articles that provided preclinical evidence of marine-derived bioactive interactions with or modulation of a potential target involved in neuropathic pain (Group 2) (continued...)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model/*in vivo* outcomes/doses/routes/positive control	Significant findings	Author and year
15	Butyrolactone-I (ZB5-1)	Coral-derived fungus *Aspergillus terreus*	Neuroinflammation/ NFκB pathway	*In vitro:* BV-2 murine microglial cells *In silico*: Molecular docking analysis	- ZB5-1 at tested concentrations had no cytotoxicity on LPS-induced BV-2 cell activation and significantly reduced the production of NO and IL-1β -ZB5-1 also down-regulated the expression of iNOS and COX-2 in a dose-dependent manner - ZB5-1 could inhibit the phosphorylation of p65 and IκB. -Molecular docking study suggested that ZB5-1 bound at the active sites of NF-κB to prevent its translocation to the nucleus	Zhang et al., 2018
16	CoM22, RtM11 ArL1, ArD1, ArI110 LiA58, ArI21 ArO130, LiM43, LiT8 LiO32 ArCII1 ArCII16 ArCII12	*C. arenatus, C.coronatus* *C. ebraeus* *C. imperialis* *C. lividus* *C. marmoreus* *C. quercinus* *C. rattus* *C. sponsalis* *C. varius* *C. virgo*	NMDAR AMPAR nAChR (α) Na_V (ι) K_V (κ) α1-adrenoceptors (ρ)	*In silico*: Pharmacological families of the CTXs of 11 species of cone snails collected in Bali (using ConoServer (https://www.conoserver.org/). Protein modeling, prediction, and analysis of the representative mature toxin sequences were conducted using the Phyre2 server (https://www.sbg.bio.ic.ac.uk).	Definitive pharmacological families of *conus* possibly targeting neuronal tissues (α, ι, κ, and ρ), as well as NMDA and AMPA receptor blockers, were identified	Sudewi et al., 2019
17	μ-Conotoxin TsIIIA	*Conus tessulatus*	Na_V.1.8	*In vitro*: HEK293 or ND7/23 cells expressing $hNa_V1.1$-$hNa_V1.8$ subtypes electrophysiology	Selective inhibition of hNav1.8	Yang & Zhou, 2020
18	Conotoxin-Ac1 and its variant conotoxin-Ac1-O6P	*Conus achatinus*	NMDA receptor subunit 2B (NR2B)	*In vitro*: Rat hippocampal neuron cells and HEK293 cells electrophysiology *In vivo*: Hot plate test and tail-flick test/TWL Route and doses: 10, 20, and 40 μg/kg, intracranial vs. morphine	- NMDA-containing NR2B inhibition - CTX-Ac1 and CTX-Ac1-O6P shows analgesic activity - Analysis of Ac-1 mutants show a key role of N1 and S12 for NR2B inhibition and analgesia	Liu et al., 2020a
19	δ-Conotoxin TxVIA	*Conus textile*	T-Type VGCC ($Ca_V3.2$)	*In vitro*: HEK293 cells expressing $Ca_V3.2$ and SH-SY5Y cells or transiently transfected $Na_V1.7$ HEK293T cells electrophysiology *In vivo*: Zebrafish bioassays (Bursts of erratic swimming) 250 ng/100 mg fish, IM	- TxVIA was inactive on mammalian $Na_V1.2$ and $Na_V1.7$ - TxVIA preferentially inhibits $Ca_V3.2$ current (IC_{50} = 0.24 μM) and enhances $Ca_V3.1$ current at higher concentrations - TxVIA does not induce signs of pain-related behaviors or paralysis	Wang et al., 2020

Table S2. Articles that provided preclinical evidence of marine-derived bioactive interactions with or modulation of a potential target involved in neuropathic pain (Group 2) (continued...)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model/*in vivo* outcomes/doses/routes/positive control	Significant findings	Author and year
20	α-Conotoxin Vc1.1	*Conus victoriae*	GIRK channels (inwardly rectifying K^+ currents) via G protein-coupled $GABA_B$ receptors	*In vitro*: Isolated mouse DRG neurons and HEK293 cells coexpressing either heteromeric hGIRK1/2 or homomeric GIRK2 subunits, with $GABA_BR$ electrophysiology	- Activation of GIRK channels by allosteric GABAB receptor agonism) hyperpolarizing the cell and decreasing neuronal excitability	Bony et al., 2022b
21	Conosteroids A–E	*Conus geographus*	$GABA_AR$ (neurosteroids)	*In vitro*: Cytosolic Ca^{2+} concentration in DRG cells HEK cells electrophysiology *In vivo*: Hot plate test/TWL and formalin test in mice 2, 10, and 50 mg/kg, i.p.	- Synthetic analog is a $GABA_AR$ negative allosteric modulator antagonizing α1β1γ2 and α4β3γ2 receptors - Modest analgesic activity in hot-plate test and tendency toward significant in the phase II of formalin test	Niu et al., 2021
22	Tunicate metabolite 2-(3,5-Diiodo-4-methoxyphenyl)ethan-1-amine (DIMTA)	*Didemnum sp.* tunicates	$K_{Ca}1.1$, $K_V3.1$, and $K_V11.1$ *Cell-type selectivity vs. molecular target selectivity	*In vitro*: mouse DRG neurons, *Xenopus laevis* oocytes expressing channels, and HEK-293 cells electrophysiology *In vivo*: Naive CD1 mice. Cold Plate Assay/Cold latency 1 and 10 mg/kg, i.p.	- DIMTA selectively affected low-threshold cold thermosensors by blocking K-channels, reducing the magnitude of the afterhyperpolarization (AHP), which results in faster inactivation of Na_V channels, thereby causing desensitization and also increasing the baseline intracellular calcium concentration of these cells - DIMTA increased the threshold of cold sensation by >3°C - DIMTA may serve as a lead in the further design of compounds that inhibit disturbances of the cold-sensory system, such as cold allodynia and other NP conditions - High concentration (25 µM) can nonselectively target other ion channels, including $K_V11.1$, which can cause ventricular arrhythmias, and VGCCs. - DIMTA does not block $K_V7.2$ and neither modulates TRPM8 channels	Paguigan et al., 2021

Table S2. Articles that provided preclinical evidence of marine-derived bioactive interactions with or modulation of a potential target involved in neuropathic pain (Group 2) (continued...)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Experimental model/*in vivo* outcomes/doses/routes/positive control	Significant findings	Author and year
23	Consomatin Ro1	*Conus rolani*	SSTR4 and SSTR1	*In vitro*: Panel of GPCRs using the PRESTO-Tango β-arrestin recruitment assay *In vivo*: Tail flick test and acute post-surgical pain model/mechanical hyperalgesia/IT (0.025-0.75 mg/kg) vs. systemic (i.p.) route (0.25-7.5 mg/kg) - Phylogenetic analysis, identification, extraction, and fractionation of venom, Mass determination and sequencing of consomatin Ro1, transcriptome sequencing, peptide synthesis, folding, and purification, plasma stability assay	- The first report of an SST-like peptide in venoms of a deep-water lineage of fish-hunting cone snails - Ro1 displays preferential activation of SST1/SST4 with little or no activity at SST2, SST3, and SST5; it also does not activate opioid receptors, exhibiting *in vivo* analgesic effects after a single dose - Ro1 is an evolutionarily optimized, metabolically stable analog of SST - Ro1 shows antinociceptive effect only by the systemic route (peripheral mode of action), and the effect lasts up to 3 hours	Ramiro et al., 2022
24	Peptide toxins (from tentacles, column, and mesenterial filaments)	Sea anemone *Heteractis crispa*	Na_V channels, K_V channels, ASIC, TRPV1, and TRPA1	- Transcriptome analysis (HTS technology), RNA extraction *In silico*: Homology modeling prediction and phylogenetic analysis (MEGA 7.0.14 software/SWISS-MODEL/UniProt and BLAST databases (https://www.uniprot.org/, https://blast.ncbi.nlm.nih.gov/)	1049 transcripts were identified and categorized into 60 families, of which 91.0% were proteins and 9.0% were peptides. The majority of peptides were ShKT domain, β-defensin Kunitz type, and EGF-like	Guo et al., 2024
25	α-Conotoxin Vc1.1	*Conus victoriae*	G protein-coupled $GABA_B$-modulated N-type $Ca_V2.2$ channels	*In vitro*: Human pluripotent stem cell (hPSC)-derived sensory neuron induction, molecular characterization, and transcriptomic analysis Functional characterization of $GABA_BR$ by electrophysiology	- Baclofen and Vc1.1 inhibited HVA Ca^{2+} channel currents, which were attenuated by the selective $GABA_BR$ antagonist CGP 55845 - Modulation of GIRK channels by $GABA_BRs$ was not observed in the presence of baclofen or Vc1.1, suggesting that functional GIRK1/2 channels were not coupled to $GABA_BRs$ in hPSC-derived sensory neurons	St Clair-Glover et al., 2025

Table S3. Articles that provide strategies to reduce limitations and improve the effectiveness of the marine-derived bioactives with potential to treat neuropathic pain (Group 4).

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Aim and experimental model	Significant findings	Author and year
1	Cyclic Vc1.1 analog (cVc1.1) *Efficacy in NP model (Satkunanathan, 2005)	*Conus victoriae*	$GABA_B$-modulated N-type ($Ca_V2.2$) channels	*To develop chemical re-engineering of Vc1.1 via cyclization, improving its rapid clearance and complex route of administration. *In vitro*: *Xenopus laevis* oocytes and DRG neuron electrophysiology Stability study [simulates gastric (SGF) and intestinal (SIF) fluids, Human serum] *In vivo*: CCI rats Mechanical allodynia/PWT (0.1, 0.3 and 3 mg/kg, p.o.) vs. gabapentin	- Orally active (improved stability and oral bioavailability) -Compared with linear Vc1.1, cVc1.1 is a more potent and selective inhibitor of the $GABA_B$-modulated N-type ($Ca_V2.2$) channel - cVc1.1 is more than two orders of magnitude more potent than gabapentin - Inhibition of HVA Ca^{2+} channel currents over α9α10nAChRs, which could mediate adverse effects on the auditory system	Clark et al., 2010
2	μ-Conotoxin SIIIA	*Conus striatus*	$Na_V1.2$, $Na_V1.4$, $Na_V1.6$ and $Na_V1.7$	*To identify the molecular epitopes of the channel protein determining toxin sensitivity. *In vitro*: Electrophysiology in HEK 293 cells transfected with wild-type Na_V channels or domain chimeras between $rNa_V1.4$ and $hNa_V1.5$ and single-site mutants. Neuro-2A expressing Na_V8 *Analgesia in Inflammatory pain model (Green et al., 2007) (potential for CIPN)	- Insensitivity of $hNa_V1.5$ and $r/hNa_V1.8$ for mSIIIA - Importance of the domain-2 pore loop as the major determinant for mSIIIA's subtype specificity - A residue, Ala728, in $rNa_V1.4$ is crucial for its high sensitivity toward mSIIIA - Asn889 at the homologous position in $hNa_V1.7$ is responsible for the channel's reduced mSIIIA sensitivity - Provides knowledge for the rational design of more specific antagonists	Leipold et al., 2011
3	α-Conotoxin RgIA	*Conus regius*	α9α10 nAChR	*To reveal molecular information to improve the potency of RgIA analogs by the construction of point mutations. *In vitro*: *Xenopus laevis* oocyte electrophysiology	- Differences between species in the potency of α-CTX on α9α10 nAChR (RgIA is 300-fold less potent on human vs. rα9α10 nAChR) - A single point mutation, Thr56 (rat) to Ile56 (human), primarily accounts for the difference -This species difference was conferred by only one residue in the (-) rather than (+) binding region of the α9 subunit. This result suggests that interaction might occur at an α10/α9 rather than an α9/α10 subunit interface	Azam & McIntosh, 2012

Table S3. Articles that provide strategies to reduce limitations and improve the effectiveness of the marine-derived bioactives with potential to treat neuropathic pain (Group 4) (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Aim and experimental model	Significant findings	Author and year
4	α-Conotoxin RgIA	*Conus regius*	α9α10 nAChR	*To elucidate the molecular interaction of RgIA with rα9α10 nAChR by the construction of point mutations. *In vitro*: *Xenopus laevis* oocyte electrophysiology *In silico*: Molecular modeling of the rα9α10 extracellular domain (ECD) based on the X-ray crystal structure of the hα9 ECD and its complexes with RgIA and ACch by MD simulation	-To test the possibility that the (−) face in the α9 and (+) face residues in the α10 subunit interact with RgIA [opposite of that suggested by Perez et al. (2009)], they mutated the α10(+) face and the α9(−) face residues at the respective homologous positions of the α9(+) face and α10(−) face. -These results strongly support the existence of an additional binding site for ACh in the α9α10 nAChR between the α10(+)/α9(−) interface. This interface is the high-affinity binding site for α-CTX RgIA - A similar interaction for α-CTX Vc1.1 with this interface of the rα9a10 nAChR is suggested	Azam et al., 2015
5	Contulakin-G-memantine	*Conus geographus*	NTSR	*To improve potency and systemic bioavailability/non-desensitizing properties for agonist-based drug leads. *In vitro*: cell line HT-29 expressing NTSR 1 fluorescence emission due to intracellular Ca^{2+} mobilization *In silico*: Structure-activity relationship studies and MD simulation *In vivo*: TFT and hot plate test in mice/latency (s) 4 mg/kg, i.p.	- Contulakin-G is a potent *in vivo* analgesic by being a metabolically stable and long-lasting agonist, which induces less desensitization of the target NTSR - The analog in which the glycosylated amino acid residue (Gal-GalNAcThr10) was replaced by the memantine-Glu10 residue preserves similar properties but also improved penetration across the BBB - Safety challenges (role of NT promoting cancer progression)	Lee et al., 2015
6	μO-Conotoxin MfVIA	*Conus magnificus*	Na_V.1.8	*To develop a μO-CTX analog with improved lipid membrane interactions and potency for channel Na_V1.8. Determination of the MfVIA three-dimensional structure and its membrane-binding properties. Alanine scanning mutagenesis and identification of important residues for MfVIA activity at hNa_V1.8. *In vitro*: Electrophysiology in CHO cells expressing hNa_V1.8 *In vivo*: Formalin test in C57BL/6J mice/parallel rod floor test (motor performance)	- The inhibition of Na_V1.8 by MfVIA is driven not only by peptide-ion channel interactions but also by peptide-lipid interactions, suggesting that the putative binding site is located in the membrane-embedded voltage-sensing domain (VSD). MfVIA is a gating modifier toxin that interacts with Lipid Membranes. - Building on these interactions leads to the more potent Na_V1.8 double mutant (E5K,E8K)MfVIA with analgesic activity in the formalin test (the peak effect at 20-30 min during phase II)	Deuis et al., 2016

Table S3. Articles that provide strategies to reduce limitations and improve the effectiveness of the marine-derived bioactives with potential to treat neuropathic pain (Group 4) (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Aim and experimental model	Significant findings	Author and year
7	αO-Conotoxin GeXIVA Disulfide Bond Isomers (GeXIVA[1,2], GeXIVA [1,3] and GeXIVA[1,4])	*Conus generalis*	α9α10 nAChR	*To elucidate the potential binding site of three disulfide bond isomers of GeXIVA with hα9α10 nAChR and subtype selectivity in human and rat *In vitro*: *Xenopus laevis* oocytes expressing nAChR subtypes and mutants, electrophysiology *In silico*: Molecular modeling	- GeXIVA isomers exhibit differential sensitivity for various nAChR subtypes but retain potency and selectivity for the hα9α10 subtype - The 'bead' isomer has the greatest affinity for hα9α10 nAChRs - The three isomers displayed only a ~5-fold difference in potency on the human vs. rα9α10 receptors and had similar affinities at wild-type rα9α10 nAChRs and rα9α10 receptor mutants. - GeXIVA binds different regions of α9α10 than those identified in α-CTXs (RgIA, Vc1.1) and αD-CTXs	Zhangsun et al., 2017
8	α-Conotoxin RgIA and Vc1.1 synthetic	*Conus regius* *Conus victoriae*	α9α10 nAChR	*To explore the interaction mechanism between RgIA/Vc1.1 and rα9α10 nAChR. *In silico*: Docking and molecular dynamics simulations	- Novel residues, including Tyr95, Trp151 in α9(+)/α10 (-) interface as well as Tyr196, Arg59 in α10 (+)/α9 (-) interface, were found in these models - Importance of electrostatic interaction. RgIA: the interaction was mediated by negatively charged residues (Glu197 in α10, Asp168 in α9, and Asp205 in α10). Vc1.1: the interaction was mediated by positively charged residues (Arg59, Arg81 in α9)	Li et al., 2019
9	α-Conotoxin Vc1.1	*Conus victoriae*	α9α10 nAChR	*To explore the structure-activity relationship of Vc1.1 at the hα9α10 nAChR by minimal side chain replacement generating unnatural amino acid analogs of Vc1.1 *In vitro*: *Xenopus laevis* oocytes expressing human α9α10 nAChRs, electrophysiology	- Vc1.1 S4 forms hydrogen bonds with α9D166 and D169; introducing positively charged residues at this position can improve potency. The P6 is near D119, and the introduced Hyp6 approaches D119 and forms a hydrogen bond. The hydroxyl group at the Y10 side chain forms several hydrogen bonds with residues at the (+) component of the α9 subunit. The side chain length and the number of negative charges are essential for the residue at the 11th position of Vc1.1 - Second-generation mutants of Vc1.1 [S4 Dab, N9A] and [S4 Dab, N9W] increased potency at the α9α10 nAChR by 20-fold vs. Vc1.1	Chu et al., 2019
10	α-Conotoxin RgIA synthetic and its analogs	*Conus regius*	α9α10 nAChR	*To characterize the chemical properties of RgIA that contribute to its activity against α9α10 nAChRs using a mutational approach. *In vitro*: *Xenopus laevis* oocytes expressing human and rα9α10 nAChRs electrophysiology	- Arg7 side chain length, charge, or functional group of the side chain in the first disulfide loop are critical for potency at both the rat and human receptor - Changes in the second disulfide loop can be fine-tuned to preferentially increase potency for the hα9α10 nAChR subtype - The C-terminal Arg13 is an engineerable hotspot - Structural analysis of analogs by NMR indicates that the primary contributors to activity are the side chain properties of RgIA residues, rather than backbone conformation	Huynh et al., 2020b

Table S3. Articles that provide strategies to reduce limitations and improve the effectiveness of the marine-derived bioactives with potential to treat neuropathic pain (Group 4) (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Aim and experimental model	Significant findings	Author and year
11	Disulfide-deficient analogs of αO-conotoxin GeXIVA	*Conus generalis*	α9α10 nAChR	*To implement a synthetic structure simplification through the disulfide bond deletion strategy improves synthetic yield *In vitro*: *Xenopus laevis* oocytes expressing human and rat nAChR subtypes electrophysiology. *In silico*: MD simulations	- The disulfide-deficient analogs of GeXIVA remained potent inhibitors of α9α10 nAChR - The disulfide bonds and termini of GeXIVA do not establish stable interactions with the receptor. Only a small segment might be responsible for the activity, suggesting that the peptide could be dramatically simplified	Xu et al. 2020
12	α-Conotoxin RgIA and its analog RgIA4	*Conus regius*	α9α10 nAChR	*To explore detailed interactions of RgIA and RgIA4 with Ac-AChBP to obtain the most selective inhibitor mechanisms. *In vitro*: Expression, purification, and crystallization of α-CTX/*Aplysia californica* acetylcholine-binding protein (Ac-AChBP) complex *In silico*: MD simulations	- Key residues of α9α10 nAChR (Ser79 and Arg113 of the α9 subunit) were suggested to determine its high affinity and selectivity for RgIA/RgIA4 -Through sequence alignment of GIC,LvIA, RgIA, and RgIA4, it is clearly shown that all conotoxins exhibited high homology in their N-termini, but the sequences in the C-termini were significantly different (variations in C-termini may contribute to the unique selectivity of different nAChRs or determine their preferential receptor antagonism - The first time that the co-crystal structure of pain-related CTXs in complex with Ac-AChBP was reported - The complementary side in the RgIA/RgIA4-α9α10 nAChR interface through the homologous protein AChBP was shown	Pan et al., 2021
13	Linear Vc1.1 and backbone-cyclized cVc1.1	*Conus victoriae*	G protein-coupled $GABA_B$-modulated N-type $Ca_V2.2$ channels	*To investigate the pharmacological influence of the peptidic bridge differentiating linear and cyclic Vc1.1 in various preclinical PK/PD rodent models, and to determine whether cyclization conferred a holistic advantage to this peptide drug as judged by the Drug Efficiency Index parameters. *In vivo*: PK, biodistribution, and brain penetrance study in SD rats, which contributes to assessing the safety profile	-While previous *in vitro* studies had indicated cyclization conferred increased stability for cVc1.1, *in vivo* both peptides exhibited similar half-lives (the order of 50 min) and oral bioavailabilities (0.31 ± 0.09 vs. 1.2 ± 1.1) -The ratios of free drug exposure metrics ($C_{max} \times f_{u,p}$ and $AUC_{0\text{-}inf} \times f_{u,p}$) following oral dosing vs. their respective *in vitro* IC_{50s} at the $GABA_B$ receptor were comparable for Vc1.1 and cVc1.1, indicating similar drug efficiency indices - The intact cyclopeptide and several metabolites persist in the GI tract for at least 4 h, long after the plasma levels of the intact peptide had fallen below the target IC_{50}. -Biodistribution analyses of IV-administered ^{125}I-labelled cVc1.1 revealed accumulation primarily in the kidneys consistent with renal elimination, and combined with insignificant uptake in brain, suggested a low likelihood of CNS-related side effects	Poth et al., 2021

Table S3. Articles that provide strategies to reduce limitations and improve the effectiveness of the marine-derived bioactives with potential to treat neuropathic pain (Group 4) (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Aim and experimental model	Significant findings	Author and year
14	Peptide MS 9a-1	Sea anemone *Metridium senile*	TRPA1 channel	*To reveal determinants for TRPA1 modulation. *In vitro*: *Xenopus laevis* oocytes expressing rTRPA1 channels electrophysiology *In silico*: Computation and structure modeling *In vivo*: AITC-induced nocifensive behavior/time spent/CFA-induced inflammation and thermal hyperalgesia/PWL/hot plate in CD-1 mice. Efficacy of Ms 9a-1 vs. peptide fragments (C-Ms, N-Ms) and homologous peptides (Ms 9a-2 and Ms 9a-3). (0.03, 0.1, and 0.3 mg/kg, IV). The peptide dosage was chosen to provide blood molarities similar to those of Ms 9a-1	- A mutual arrangement of the Ms 9a-1 C-tail and the non-homologous region between the second and third Cys residues is responsible for the proper binding to the TRPA1 surface, mediating a significant potentiating action. Substitutions (as in the case of Ms 9a-2 and Ms 9a-3) in this sequence lead to changes in the inward current - Both the N-terminal and C-terminal domains are essential for Ms 9a-1 activity - Other targets could be involved in the analgesic effects of Ms 9a-2 and Ms 9a-3 (homologous), since they are less effective in potentiating TRPA1 but can reduce *in vivo* nocifensive behavior with equal potency as Ms 9a-1	Logashina et al., 2022
15	α-Conotoxins Vc1.1, RgIA, and PeIA	*C. victoriae* *C. regius* *C.pergrandis*	G protein-coupled $GABA_B$-modulated N-type $Ca_V2.2$ channels	*To elucidate the potential binding site of α-CTXs with human $GABA_BR$ after the mutation of amino acid residues in the VFT domains of $GABA_BR$ subunits *In silico*: Predictions using computational peptide docking and MD simulations *In vitro*: HEK293T cells coexpressing $hGABA_BR$ (WT and 15 $GABA_BR$ mutants) and $Ca_V2.2$ electrophysiology	- The α-CTXs form close contacts with VFT residues in both B1 and B2 subunits (novel $GABA_BR$ allosteric binding site), unlike small-molecule agonists and antagonists whose interactions are confined to B1 VFT. - Mutations at the interface of VFT domains of both $GABA_BR$ subunits attenuated baclofen-sensitive IBa inhibition by α-CTXs - The key $GABA_BR$ residues involved in interaction with α-CTXs are K168 and R207 on the B2 subunit and S130, S153, R162, E200, F227, and E253 on the B1. The double mutant S130A+S153A abolished the inhibition of both baclofen and α-CTXs	Bony et al., 2022a

Table S3. Articles that provide strategies to reduce limitations and improve the effectiveness of the marine-derived bioactives with potential to treat neuropathic pain (Group 4) (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Aim and experimental model	Significant findings	Author and year
16	αO-Conotoxin GeXIVA[1,2]	*Conus generalis*	α9α10 nAChR	*To explore pharmacokinetics (PK) in experimental animals and validate a PK-PD (pharmacodynamic) model reflecting the mechanism of action of GeXIVA[1,2] *In vivo*: PK studies: Beagle dogs (IM doses: 0.035, 0.07, 0.14, or 0.28 mg/kg and IV dose 0.07 mg/kg) Rats (IM doses: 0.22, 0.44, and 0.88 mg/kg or an IV dose of 0.44 mg/kg) - PD study in OXA-induced NP in rats (IM doses: 0.11, 0.22, and 0.44 mg/kg)/mechanical allodynia/PWT -Mechanism-based PK modeling (Population PK (PopPK) modeling and human PK prediction; PK-PD modeling)	- The IM bioavailability in rats and dogs was 11.47% and 13.37%, respectively - The plasma exposure of GeXIVA[1,2] increased proportionally with the dose - The plasma protein binding of GeXIVA[1,2] differed between the tested animal species - The one-compartment model with the first-order absorption PopPK model predicted doses for healthy humans weighing 70 kg - The PK-PD relationships were characterized using an inhibitory loss-of-indirect-response model with an effect compartment. Model simulations provided potential mechanistic insights by inhibiting certain endogenous substances	Zhu et al., 2022
17	α-Conotoxin PeIA analogs with non-natural amino acids, PeIA[S4Dap, S9Dap]	*Conus pergrandis*	Inhibit α9α10 nAChR but also α3β2 Activate α7 (only analogs)	*To chemically synthesize Dab/Dap-substituted analogs of α-CTX PeIA and evaluate their activity. *In vitro*: *Xenopus laevis* oocytes expressing hAChRs electrophysiology *In silico*: MD simulations	- Non-natural amino acid analogs of α-CTXs ~24-fold improve potency vs. native peptide via extra interactions with the receptor - Side chain amino group of Dap4 formed additional hydrogen bonds with S168, and D169 of the receptor and Dap9 formed an extra hydrogen bond interaction with Q34, which is distinctive to PeIA -The formation of extra hydrogen bonds as well as the electrostatic interactions between Dap and residues at the α9(+)-α9(−) are postulated to contribute to the significantly enhanced activity of PeIA[S4Dap, S9Dap] compared to the native PeIA - The potency of PeIA[S4Dap, S9Dap] is approximately two-fold higher than that of dimeric PeIA, and the synthesis procedure for the former is more facile	Li et al., 2024
18	α-conotoxin Vc1.1	*Conus victoriae*	nAChRs Preferentially α3β2 and α9 subtypes.	*To evaluate the selectivity and potency of α-CTX Vc1.1 across various hnAChR subtypes. In addition, investigate the role of specific amino acid residue mutations in nAChR function. *In vitro*: *Xenopus laevis* oocytes expressing hnAChRs. electrophysiology *In silico*: MD simulations	- Vc1.1 is a selective antagonist for the α3β2 and α9 nAChR subtypes - Altered interactions between the mutant hα9 N179A, N213K, and N213R side chains and Vc1.1-D5 may partly explain the changes in potency	Tae et al., 2025

Table S3. Articles that provide strategies to reduce limitations and improve the effectiveness of the marine-derived bioactives with potential to treat neuropathic pain (Group 4) (continued…)

No.	Bioactive	Species	Target/ signaling pathways/cellular process	Aim and experimental model	Significant findings	Author and year
19	αO-Conotoxin GeXIVA[1,2]	*Conus generalis*	α9α10 nAChR	*To develop novel technological strategies to overcome the disadvantages of GeXIVA[1,2] due to rapid clearance and complex route of administration. A promising strategy for chronic NP management *In vivo*: SNI model and CCI model in SD rats. Mechanical allodynia/PWT. Open field test	- The innovative hydrogel microneedle patch platform extends GeXIVA[1,2]'s analgesic duration from hours to days through sustained release, while resolving administration challenges through transdermal delivery - A single microneedle patch application increases PWT by 3 days in both models and improves locomotor function in SNI mice -The quantitative relationship between dosage and effectiveness remains unclear, necessitating further investigation	He et al., 2025
20	PEG-linked cyclic Vc1.1 analogues (PEG-cVc1.1)	*Conus victoriae*	α9α10 nAChR	*To synthesize PEG-cyclized Vc1.1 using SPPS and enzymatic cyclization with structural, stability, and activity profiles similar to cVc1.1. They used polyethylene glycol (PEG) linkers with varying lengths as substitutes for peptide backbone cyclization linkers. *In vitro*: *X-laevis* oocytes expressing human α9α10nAChR electrophysiology/*In Vitro* Stability assay (human serum, SIF)	- The results highlight the essential role of helicity in maintaining peptide function and stability. -The SIF stability of PEG-cVc1.1 analogs increases with longer PEG linkers. P6-cVc1.1 shows slightly higher stability than cVc1.1, which also maintains activity similar to cVc1.1 on α9α10 nAChR	Zhang et al., 2025

Table S4. Searching strategy.

Academic search systems/ search dates	Search term	Filters
PubMed/ June 2024- April 2025	*General terms*: (Marine OR Sea natural product OR Marine OR Sea bioactive OR Marine OR Sea compound) AND (neuropathic pain OR hyperalgesia OR nociception OR hypernociception OR allodynia OR analgesic OR antihyperalgesic OR antinociceptive OR antihypernociceptive OR antiallodynic OR antinociception) *Particular terms*: (Marine bacteria OR cyanobacteria OR marine protozoans OR marine chromists OR phytoplankton OR seaweeds OR algae OR marine fungi OR marine plants OR seagrasses OR marine angiosperms OR halophytes OR mangroves OR marine animals OR cnidarians OR sea anemone OR sponges OR marine worms OR molluscs OR cones OR snails OR conotoxins OR conopeptides OR mussels OR crustaceans OR echinoderms OR sea stars OR corals OR jellyfish OR bryozoans OR fish OR tetrodotoxin) AND (analgesic OR antinociceptive OR antihyperalgesic OR antihypernociceptive OR antiallodynic OR neuropathic pain) AND (mechanisms OR Na_V OR Ca_V OR Kv OR NMDA OR neuroimmune interactions OR nAChR OR GABA OR GPCR OR ASIC OR TRP OR neuroinflammation OR neuroprotection)	Humans, Other animals, Exclude preprints, 15 years (Year 2010-2025), English language
Web of Science/ June 2024-April 2025	*General terms*: (Marine OR Sea natural product OR Marine OR Sea bioactive OR Marine OR Sea compound) AND (neuropathic pain OR hyperalgesia OR nociception OR hypernociception OR allodynia OR analgesic OR antihyperalgesic OR antinociceptive OR antihypernociceptive OR antiallodynic OR antinociception) *Particular terms*: (Marine bacteria OR cyanobacteria OR marine protozoans OR marine chromists OR phytoplankton OR seaweeds OR algae OR marine fungi OR marine plants OR seagrasses OR marine angiosperms OR halophytes OR mangroves OR marine animals OR cnidarians OR sea anemone OR sponges OR marine worms OR molluscs OR cones OR snails OR conotoxins OR conopeptides OR mussels OR crustaceans OR echinoderms OR sea stars OR corals OR jellyfish OR bryozoans OR fish OR tetrodotoxin) AND (analgesic OR antinociceptive OR antihyperalgesic OR antihypernociceptive OR antiallodynic OR neuropathic pain) AND (mechanisms OR Na_V OR Ca_V OR Kv OR NMDA OR neuroimmune interactions OR nAChR OR GABA OR GPCR OR ASIC OR TRP OR neuroinflammation OR neuroprotection)	Year 2010-2025, Clinical Medicine, English language; "Life Sciences & Biomedicine"
Scopus/ June 2024-April 2025	*General terms*: (Marine OR Sea natural product OR Marine OR Sea bioactive OR Marine OR Sea compound) AND (neuropathic pain OR hyperalgesia OR nociception OR hypernociception OR allodynia OR analgesic OR antihyperalgesic OR antinociceptive OR antihypernociceptive OR antiallodynic OR antinociception) *Particular terms*: (Marine bacteria OR cyanobacteria OR marine protozoans OR marine chromists OR phytoplankton OR seaweeds OR algae OR marine fungi OR marine plants OR seagrasses OR marine angiosperms OR halophytes OR mangroves OR marine animals OR cnidarians OR sea anemone OR sponges OR marine worms OR molluscs OR cones OR snails OR conotoxins OR conopeptides OR mussels OR crustaceans OR echinoderms OR sea stars OR corals OR jellyfish OR bryozoans OR fish OR tetrodotoxin) AND (analgesic OR antinociceptive OR antihyperalgesic OR antihypernociceptive OR antiallodynic OR neuropathic pain) AND (mechanisms OR Na_V OR Ca_V OR Kv OR NMDA OR neuroimmune interactions OR nAChR OR GABA OR GPCR OR ASIC OR TRP OR neuroinflammation OR neuroprotection)	Year 2010-2025, English language
ScienceDirect/ June 2024-April 2025	*General terms*: (Marine OR Sea natural product OR Marine OR Sea bioactive OR Marine OR Sea compound) AND (neuropathic pain OR hyperalgesia OR nociception OR hypernociception OR allodynia OR analgesic OR antihyperalgesic OR antinociceptive OR antihypernociceptive OR antiallodynic OR antinociception) *Particular terms*: (Marine bacteria OR cyanobacteria OR marine protozoans OR marine chromists OR phytoplankton OR seaweeds OR algae OR marine fungi OR marine plants OR seagrasses OR marine angiosperms OR halophytes OR mangroves OR marine animals OR cnidarians OR sea anemone OR sponges OR marine worms OR molluscs OR cones OR snails OR conotoxins OR conopeptides OR mussels OR crustaceans OR echinoderms OR sea stars OR corals OR jellyfish OR bryozoans OR fish OR tetrodotoxin) AND (analgesic OR antinociceptive OR antihyperalgesic OR antihypernociceptive OR antiallodynic OR neuropathic pain) AND (mechanisms OR Na_V OR Ca_V OR Kv OR NMDA OR neuroimmune interactions OR nAChR OR GABA OR GPCR OR ASIC OR TRP OR neuroinflammation OR neuroprotection)	Year 2010-2025, English language

Table S5. Excluded studies.
Table S6. SYRCLE.
Table S7. Variable Robins.
Tables S5-S7 can be seen at this **URL**

Annex 1. The criteria considered for defining each aspect to qualitatively assess the strength of the evidence
Frequency: Number of studies investigating the bioactive compound

Domain 1 (A1-A5) addressed methodological aspects and reporting quality to detect potential experimental bias. These include randomization, blinding, sample size calculation, protocol availability or reporting that includes all expected outcomes, and laboratory environmental factors affecting pain sensitivity.

A1: Randomization

A2: Blinding

A3: Sample size calculation

A4: Reporting quality: Protocol available or reporting that includes all expected outcomes

A5: Laboratory environmental factors affecting pain sensitivity.

*These aspects were evaluated based on whether or not (unclear) they were explicitly reported

*Reporting quality: Pre-specified outcomes in protocol were reported/incomplete outcome data adequately addressed

*Laboratory environmental factors/pain sensitivity to avoid stress-induced analgesia (SIA) or stress-induced hyperalgesia (SIH): Report on the management of stress-reducing factors (time of acclimatization, silent room, temp, non-restriction) for behavioral *in vivo* experiments

Domain 2 (A6-A20) addressed aspects relevant to clinical translation, involving the refinement of neuropathic pain models and outcome measures, mechanisms of action, and the efficacy and safety of the compounds. These include sex balance, dose-response relationship, treatment regimen (repeated doses), route relevance, positive control, safety outcome and/or health status, species translation, target engagement, mechanistic evidence, potential binding site prediction [structure activity relationship (SAR)], number of *in vivo* neuropathic pain models, clinical relevant model/outcome measure, pharmacokinetic (PK) characterization, and replication.

A6: Sex balance

A7: Dose-Response Relationship

A8: Repeated Regimen

A9: Route relevance

A10: Positive control

A11: Safety outcome and/or health status

A12: Species translation

A13: Target engagement

A14: Prediction-based mechanistic evidence

A15: Experimental mechanistic evidence

A16: Different neuropathic pain *in vivo* models

A17: Clinical relevant model

A18: Clinical relevant outcome measure

A19: PK study

A20: Replication

*Sex balance: Evidence of efficacy in species of both sexes

*Dose-Response Relationship: ED50 and/or IC50 reported (pharmacologic potency measure), if a mixed study (*in vivo*/*in vitro*) determined both outcomes for the compound counted as 1. Additionally, in efficacy studies, the reporting of other metrics (AUC, MPE) to quantify the magnitude and/or duration of analgesia—which strengthen the evidence of efficacy—was also taken into account as other qualitative factors because these metrics reflect analgesic efficacy with greater certainty compared with the mean values of the evaluated variable. At no point were these aspects homogenized to establish a single criterion.

*Route relevance for chronic pain in humans: Administration through a route established or realistically applicable for clinical drug delivery in humans, rather than exclusively experimental routes used for proof-of-concept studies. Very high and high relevance: oral, IV, S.C., IM, sublingual, and transdermal. Specific clinical relevance: IT route, especially to target SNC tissue. Experimental routes: ICV, intracerebral, intrahippocampal, intraplantar. In the case of a peptide demonstrating *in vivo* efficacy when administered systemically via i.p. route, this is considered relevant evidence that could impact its potential clinical translation.

*Positive control: A conventional positive control reported; if a mixed study (*in vivo*/*in vitro*) in both studies, counted as 1

*Replication: Replication of the result by both laboratories, number of additional reports with the same positive result or in different *in vivo* models, *in vitro* replicated experiments explicitly reported

*Safety outcome and/or health status: Number of tests to rule out adverse effects of the compound, toxicology, Therapeutic Index (TI), cytotoxicity assays, hERG K^+ channel. In some studies, assessing the animals' health status, which could interfere with the behavioral assessment (confounding effects) was also considered

*Species translation: Evidence of efficacy in different species (rats, mice) translates to humans. Given the broad scope of the review, some considerations were included to strengthen the potential translation value of the results, depending on the study type. In particular, the use of rodent and human receptor or channel subtypes and crystal structures of human receptor subunits (as templates for homology modeling) for *in vitro* or *in silico* studies, respectively. At no point were these aspects homogenized to establish a single criterion.

*Target engagement: Report direct target binding (SPR, ITC, CETSA, NanoBRET, radioligands)

*Prediction-based mechanistic evidence: Predicción bioinformatica, predicción computational (docking, MDS, QSAR) to predict potential binding site and mechanistic modeling (PK/PD) (inferential evidence)

*Experimental mechanistic evidence: Experimental evidence of interaction (target engagement) or functional modulation (target modulation) whether specific or compatible depending on the biological expression system. Target functional modulation using *in vitro* electrophyisiology (Patch Clamp), *ex vivo* and *in vivo* electrophyisiology, cell-based functional assays (FILPR, calcium imaging BRET, GPCR functional assays intracelular signaling). Mechanistic analysis *in vivo* using biochemical, immunology and molecular biology assays (Indirect/Mechanistic evidence molecular/downstream). Causal validation whether pharmacological demonstrating functional dependence of target (selective antagonists, pharmacological rescue) or genetic/structural demonstrating molecular specificity of target (KO, KD, CRISPR, site-directed mutagenesis)

*More clinical relevant model(disease/lesion-relevant animal models): Chemotherapy-induced peripheral neuropathy (CIPN), streptozotocin (STZ)-induced diabetic neuropathy, post-herpetic neuralgia (PHN), or central NP after spinal cord injury (SCI) models (corresponding to the disease or condition being studied in RCTs)

*Clinically relevant outcome measure: In addition to stimulus-evoked hypersensitivity (thermal hyperalgesia, mechanical hyperalgesia, mechanical allodynia, cold allodynia), which retain utility only in clinical scenarios of sensory gain, the inclusion of spontaneous pain, NP-related depressive comorbidity, motor and/or cognitive disorder outcome measures were considered.

Domain 3 (A21-A22) addressed aspects related to strategies for improving effectiveness in humans, whether chemical modifications and/or knowledge for developing improved synthetic analogues, and the evidence of candidate progression to clinical trials.

A20: Strategies/Chemical Engineering

A21: Clinical study

*Strategies/Chemical Engineering: Evidence of the strategies to reduce limitations and improve effectiveness and safety (potency, selectivity, stability and oral bioavailability, modifications and/or information to develop improved analogues, analogue pharmacological or functional evaluation.

*The reporting frequency of these variables (A1-A22) was tallied for each bioactive compound and the values were visualized in a heat map shown in Fig.5

Criteria for categories

Preliminary: Results only from *in vitro* assays. Evidence provided exclusively from the *in vivo* studies reporting activity in one validated neuropathic pain models/outcomes without experimental mechanistic evidence and/or bioinformatic predictive study (pharmacological families, functional annotation). Evidence could primarily come from studies evaluating extracts of marine organisms.

Moderate Preclinical: Preclinical evidence providing from studies using validated neuropathic pain models/outcomes which mainly simulate traumatic peripheral neuropathy, *in vivo* efficacy and/or safety evidences but generally using a single regimen of administration to determine time-response course. They may or may not provide relevant routes or repeated regimen of administration and evidence of cross-species translation. Experimental mechanistic evidence (*in vitro* target engagement or functional modulation, *in vivo* mechanistic analysis, causal validation) which could be associated with computational predictions combining several more robust lines of computational evidence. Therefore, in addition to bioinformatic annotation, docking and molecular dynamic simulations (MDS) studies are incorporated. Additionally, evidence of chemical modifications and/or information to develop improved synthetic analogs and its functional or pharmacological characterization could be provided.

Strong Preclinical: Preclinical evidence providing from studies with better methodology and reporting quality, *in vivo* efficacy and safety evidences using one or various clinically relevant NP models/outcomes, positive controls, relevant route and regimen of administration as well as species translation. Robust experimental mechanistic evidence (*in vitro* target engagement or functional modulation, *in vivo* mechanistic analysis, causal validation) which could be associated with bioinformatic and/or computational predictions of the binding site and mechanistic modeling to guide and strengthen the mechanistic research. PK characterization and sex balance are criteria that strengthen this category. Evidence of the strategies to reduce limitations whether modifications and/or information to develop improved analogues well characterized and with potential to advanced to clinical phases.

Clinical: Clinical evidence from exploratory interventional clinical studies or different phases of clinical development (Phase I to Phase IV).

Annex 2. PRISMA_2020_checklist.

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Title, p. 1
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Abstract, p. 2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Introduction, p. 2-3
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Introduction, final paragraph, p. 3
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Material and Methods - Eligibility criteria, p. 3
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Material and Methods - Information sources; Search strategy, p. 4; Supplementary Table S4, p. 87
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Material and Methods - Search strategy, p. 4; Supplementary Table S4, p. 87
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Material and Methods - Selection of articles, p. 4
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Material and Methods - Qualitative analysis and data mining, p. 5
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Material and Methods - Qualitative analysis and data mining, p. 5-7; Supplementary Table S7 (sheet variables)
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Material and Methods - Qualitative analysis and data mining, p. 5-7; Supplementary Table S7 (sheet variables)
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Material and Methods - Article quality analysis, p. 7; Supplementary Tables S6 and S7 (sheet Robins ROB2 and *in vitro, in silico)* can be seen at URL
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Not applicable
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Material and Methods - Qualitative analysis and data mining, p. 5; Fig. 2, Tables 1-2, Supplementary Tables S1-S3, S7, p. 61-86
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Material and Methods - Qualitative analysis and data mining, p. 5
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Material and Methods - Qualitative analysis and data mining, p. 5-7; Fig. 3, Supplementary Annex 1, p. 88-90.
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Material and Methods - Qualitative analysis and data mining; Fig. 2, p. 5 (no meta-analysis)
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	Not applicable; no formal subgroup analysis/ meta-regression performed
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	Not applicable; no sensitivity analyses performed
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Material and Methods - Article quality analysis, p. 7 and Supplementary Tables S6 and S7 (sheet Robins ROB2, *in vitro, in silico*)
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Material and Methods - Qualitative analysis and data mining, p. 5-7 (GRADE not applied; rationaly given); Fig. 3

RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Results - Selection of sources of evidence and study designs, p. 7 first paragraph; Fig. 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Results - Supplementary Tables S5 and S6 can be seen at URL
Study characteristics	17	Cite each included study and present its characteristics.	Tables 1-2, and Supplementary Tables S1-S3 and S7 (sheet variables)
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Supplementary Tables S6-S7 (sheet Robins ROB2 and sheet *in vitro, in silico*)
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Table 1; Supplementary Tables S1-S3, and S7 (sheet variables)
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Results - Selection of sources of evidence and study designs, p. 7-8 and Tables 1-2, Fig. 4; Risk of bias in studies, p. 15 and Fig.5
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Not applicable
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	Not applicable
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Not applicable
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Results - Risk of bias in studies, p. 15; Fig. 5, Table 1 - Implications and future directions, p. 45
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Results - Evidence grading and translational potential (expert synthesis of the available evidence), p. 21; Fig. 3 - Discussion - Implications and future directions - Limitations of evidence, p. 47
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Discussion, p. 21, first paragraph - Implications and future directions, p. 45-47 - Limitations of Evidence, p. 47 - Conclusion, p. 48
	23b	Discuss any limitations of the evidence included in the review.	Limitations of evidence, p. 47 - Discussion
	23c	Discuss any limitations of the review processes used.	Limitations of evidence, p. 47, first and final paragraphs
	23d	Discuss implications of the results for practice, policy, and future research.	Discussion - Implications and future directions, p. 45-47
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Material and Methods - Review methodology, p. 3
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	PROSPERO: CRD420261381895
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Material and Methods - Protocol deviations, p. 7
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Acknowledgments, p. 48
Competing interests	26	Declare any competing interests of review authors.	Conflict of Interest, p. 48
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Supplementary Tables S1-S7 and Annex 1

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. This work is licensed under CC BY 4.0. To view a copy of this license, visit https://creativecommons.org/licenses/by/4.0/

Annex 3. PRISMA 2020 abstract checklist.

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesise results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction of the effect (i.e. which group is favoured).	Yes
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	Yes
Registration	12	Provide the register name and registration number.	Yes

From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71. This work is licensed under CC BY 4.0. To view a copy of this license, visit https://creativecommons.org/licenses/by/4.0/