Mechanisms inhibiting Transient Receptor Potential ion channel activation via lipid rafts

Doctoral (PhD) thesis

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1. Research Concept

Neuropathic pain remains one of the most difficult pain conditions to treat effectively. Conventional pharmacological therapies, including opioids, non-steroidal anti-inflammatory drugs (NSAIDs), and various adjuvant analgesics, often fail to provide sufficient pain relief across diverse patient populations. Therefore, neuropathic pain represents a considerable clinical, economic, and psychosocial burden. In recent decades, several members of the transient receptor potential (TRP) ion channel family—most notably TRPV1 and TRPA1—have emerged as key molecular mediators of nociceptive signaling and were therefore proposed as attractive therapeutic targets. Intensive efforts have been made to develop selective TRP channel antagonists. Despite promising results in preclinical pain models, clinical translation has been hindered by severe side effects, including disturbances in body temperature regulation and impaired heat sensation. These failures clearly indicate the limitations of direct receptor blockade and underscore the need for alternative analgesic strategies.

A novel approach involves targeting the lipid microenvironment of the plasma membrane. Lipid raft microdomains are cholesterol-, sphingolipid-, and ganglioside-rich ordered regions of the plasma membrane. They play a crucial role in organizing signaling complexes that are essential for nociceptive processing. These domains facilitate the spatial and functional coupling of multiple pain-related proteins, including TRPV1 and TRPA1. Disruption of lipid raft integrity can therefore interfere with nociceptive signaling at multiple levels within the peripheral nervous system.

One experimental tool to achieve such modulation is the use of cyclodextrins (CDs), which bind and extract cholesterol from the plasma membrane. Through lipid raft destabilization, CDs attenuate TRP channel activity and reduce neurogenic inflammation when applied topically. This strategy offers the possibility of modifying pain signaling without abolishing essential sensory functions.

Targeted manipulation of the membrane microenvironment represents a promising new perspective for the development of peripherally acting analgesics. By preserving physiological thermosensation and minimizing central nervous system adverse effects, this approach may overcome key limitations of current therapies. Moreover, as lipid rafts regulate not only TRP ion channels but a broad range of pain-related receptors, membrane-focused interventions hold the potential for broader and more effective modulation of nociceptive pathways.

2. Introduction

2.1. The physiology of pain, basic concepts

According to the definition of the International Association for the Study of Pain (IASP), pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (1). Modern pain research emphasizes that pain is not merely the result of peripheral stimuli, but a complex, multilevel neurobiological process that is also modulated by cognitive, emotional, and social factors (2, 3).

Nociceptive, inflammatory, and neuropathic mechanisms may all be involved in the development of pain. Nociceptive stimuli can be mechanical, thermal, or chemical in origin and are detected by nociceptors specialized for sensing tissue-damaging effects (4). Nociceptive afferents can be divided into thinly myelinated Aδ fibers and unmyelinated C fibers, which transmit signals with different conduction speeds and pain sensations (G. I. Lee & Neumeister, 2020).

The cell bodies of primary afferent neurons are located in the dorsal root ganglia and certain cranial nerve ganglia, and their central projections project into the dorsal horn of the spinal cord, where nociceptive information is relayed to ascending pain pathways. The spinothalamic system transmits nociceptive signals via the thalamus to cortical and subcortical structures, ensuring the conscious processing of pain (5, 6).

Pain processing is classically divided into four phases: transduction, transmission, perception, and modulation, the latter of which is regulated by spinal networks and descending inhibitory pathways (3, 7). Disruption of these processes can lead to various clinical phenomena, such as hyperalgesia, allodynia, or analgesia (8).

Chronic pain can become a distinct pathology in which pain persists even after the triggering stimulus has ceased. It is primarily driven by peripheral and central sensitization, as well as neuroimmune interactions, supplemented by abnormal ectopic neural activity in neuropathic conditions (5, 9). The types of chronic pain—inflammatory, neuropathic, and nociplastic—are based on different pathophysiological mechanisms, but their common feature is the persistent rewiring of the nociceptive system (10, 11). This complex background explains the self-perpetuating nature of chronic pain and justifies the need for complex, mechanism-based therapeutic approaches.

2.2. Potential targets and unresolved areas in pain management

Therapeutic solutions for pain relief have advanced significantly, nevertheless, the management of chronic pain remains a major challenge. Currently used medications—opioids, NSAIDs, antidepressants, and gabapentinoids—typically act on a single molecular target and exert their effects

primarily at the level of nociceptive transduction, central neurotransmission, or descending pain modulation. These approaches often have only limited efficacy in chronic, neuropathic, nociplastic or mixed-origin pain, as pain maintenance is based on complex, multilevel pathophysiological processes (12).

NSAIDs remain first-line agents for acute and inflammatory pain; however, their long-term use is limited by significant side effects (13). Targeted modulation of ion channels expressed on peripheral nociceptors represents a promising therapeutic approach but has not yet resulted in widely applicable clinical formulations. Opioids acting on the central nervous system provide strong analgesia; however, due to the development of tolerance, dependence, and severe side effects, they are not sustainable long-term solutions for chronic pain (14). Non-opioid centrally acting agents can be used for certain types of chronic pain, but their efficacy is often partial, and their side effects limit their long-term use (5, 11).

Overall, the limitations of current therapies indicate that the treatment of chronic pain cannot yet be considered resolved. Due to the complex neurobiological background, modulation of a single molecular target is rarely sufficient; thus, future strategies may focus on multimodal approaches targeting multiple mechanisms simultaneously and the identification of new, non-classical targets.

2.3. The history and significance of capsaicin (CAPS) research

CAPS (8-methyl-N-vanillyl-6-nonenamide), the active compound responsible for the pungency of *Capsicum* species, is one of the key molecules in pain physiology research. Although the compound has been known since the 19th century, its neuropharmacological significance only became clear in the second half of the 20th century, when it was discovered that it selectively activates a subset of nociceptive C-fibers, thereby providing an excellent tool for studying primary sensory neurons. The foundations of CAPS research were laid by Hungarian researchers. Endre Hőgyes was the first to describe the compound's effect on sensory nerves (15), and Gábor Jancsó demonstrated that CAPS is not merely an irritant but can selectively desensitize nociceptive afferents, resulting in a sustained analgesic effect (16). Further studies demonstrated that the activation of CAPS-sensitive nociceptors triggers neurogenic inflammatory responses—vasodilation and plasma extravasation—which can be inhibited by desensitization or denervation (17, 18). These findings led to the hypothesis of a specific CAPS receptor. Subsequent molecular and pharmacological studies identified the binding sites for CAPS and the ultrapotent analog resiniferatoxin (RTX) (19, 20), and the use of the first antagonist, capsaicin, enabled functional testing of the receptor (21). Following cloning of the receptor, the protein was classified into the vanilloid subfamily of the transient receptor potential (TRP) channel family, under the name TRPV1 (22).

2.4. TRP ion channels

Transient receptor potential (TRP) ion channels are widely expressed in various tissues and, upon activation by physical (thermal and mechanical) as well as endogenous and exogenous chemical stimuli, mediate non-selective cation influx. Based on their sequence and structural similarities, they can be classified into six major subfamilies; a total of 28 TRP ion channels are known in mammals, and ten of these exhibit distinct temperature sensitivity (23, 24). A common structural feature of TRP ion channels is the presence of six transmembrane domains (S1–S6) and a tetrameric structure, which enables the integration of various stimuli and polymodal function (25). The heat-sensitive TRP ion channels, especially TRPV1, TRPA1, TRPM3, and TRPM8 are of particular importance for nociceptive signaling. Expressed on primary sensory neurons and peripheral nerve endings they play a key role in detecting noxious stimuli (26, 27). Among these channels, TRPV1 and TRPA1 are often co-expressed and participate in the perception of painful and inflammatory stimuli (28, 29).

The TRPV1 ion channel is activated by painful heat stimuli (>43°C), in acidic environments, and by numerous exogenous and endogenous vanilloid ligands (e.g., CAPS, RTX). Its activation and sensitivity are further enhanced by various inflammatory mediators, thus playing an important role in the development of peripheral sensitization and inflammatory pain (30–32). TRPV1 function is refined by mechanisms involving membrane lipids and intracellular regulatory proteins, enabling dynamic regulation of nociceptive signaling (33).

TRPA1 is primarily activated by noxious cold stimuli (<17 °C), mechanical stress, and a wide range of irritant and electrophilic chemical compounds. A distinctive structural feature of TRPA1 is its large number of N-terminal ankyrin repeats, which enables activation by covalently acting agonists. Consequently, TRPA1 is particularly well suited to sensing endogenous damaging mediators generated during oxidative stress and inflammation (34–36).

Activation of both channels induces Ca^{2+} and Na^{+} influx, leading to depolarization of nociceptive neurons, neuropeptide release, and pain signal transmission. The co-activation of TRPV1 and TRPA1 is associated with increased pain perception, particularly in an inflammatory environment, contributing to peripheral and central sensitization (37). Based on these properties, TRPV1, and TRPA1 are not only essential nociceptive sensors but also promising therapeutic targets for the development of new pain-relief strategies.

2.5. The Function of CAPS-Sensitive Sensory Nerve Endings

CAPS-sensitive nociceptive fibers expressing TRPV1 and TRPA1 play a role in pain perception and signal transmission via three functions. During the classical afferent function, the rise in Ca^{2+} levels in activated sensory nerve endings triggers primary neuronal signaling processes. These signals reach the appropriate nociceptive areas of the central nervous system, where nociception occurs. The local

efferent function is the activation triggered by Ca^{2+} influx, that results in neuropeptide (e.g., CGRP, substance P) release. This process induces vasodilation, plasma protein extravasation, and immune cell activation, thereby generating neurogenic inflammation (17, 38). The literature also describes this process as one of the central mechanisms of TRPV1- and/or TRPA1-mediated inflammation, which plays a key role in neurogenic and chronic inflammatory processes in the skin and other peripheral tissues (39, 40). To date, there is no satisfactory therapy for this neurogenic inflammation, making it an active area of research. However, upon activation, not only inflammatory neuropeptides but also anti-inflammatory neuropeptides, opioid peptides, pituitary adenylate cyclase-activating polypeptide (PACAP), and somatostatin (SOM) are released. Upon entering the systemic circulation, these exert analgesic and anti-inflammatory effects in other areas of the body (18). Together, these three processes constitute the triple function of CAPS-sensitive sensory nerve endings.

2.6. Lipid rafts

The structure and function of TRP ion channels are well characterized, whereas the lipid environment of the plasma membrane surrounding the receptors is significantly less explored. According to the fluid mosaic model, lipids are arranged homogeneously in the plasma membrane, while membrane proteins are distributed randomly within the phospholipid bilayer (41, 42). In contrast, the lipid raft model stated that the plasma membrane is laterally heterogeneous: it is segmented into liquid-ordered (L_o) and liquid-disordered (L_d) microdomains (43, 44).

Lipid rafts are dynamic microdomains with diameters ranging from 10 to 200 nm that are rich in cholesterol and sphingolipids and exhibit distinct physicochemical properties compared to the rest of the membrane. These structures serve as functional platforms for membrane proteins and fundamentally influence the organization and stability of signaling complexes (45). The cholesterol content of lipid rafts plays a key role in maintaining their integrity, which is critical for the function of the receptors and ion channels localized within them.

Lipid rafts are also as fundamental regulatory units of nociceptive and inflammatory signaling. The function of numerous ion channels and receptors that play a key role in pain perception, particularly TRP channels, is closely linked to the membrane's cholesterol and sphingolipid content (46, 47). A significant proportion of nociceptive TRP channels (e.g., TRPV1, TRPA1) function is facilitated by the ordered membrane environment (46, 48). Pharmacological disruption of lipid rafts—through cholesterol depletion, sphingomyelin hydrolysis, or inhibition of glycosphingolipid synthesis—reduces agonist-induced activation of these channels; however, the extent and nature of this effect may be agonist- and channel-specific (49–51). Animal models have clearly demonstrated that structural and compositional changes in lipid rafts contribute to the maintenance of neuropathic and inflammatory pain, as well as to the development of mechanical allodynia. Pharmacological disruption of lipid raft

integrity exerts a peripheral antinociceptive effect, affecting multiple nociceptive TRP channels (52–54). In addition, so-called inflammarafts, which form during central nervous system neuroinflammation, are key components of glial-mediated inflammatory signaling and contribute significantly to central sensitization (47).

2.7. The significance of CD derivatives in lipid raft research and their potential role in pain relief

CDs constitute one of the earliest introduced and most widely used family of compounds in the pharmacological study of lipid rafts. Their primary mechanism of action is based on modulating the cholesterol content of the membrane, which directly influences the structure and integrity of lipid rafts. The approach to lipid raft disruption using CDs became widespread in the late 1990's, when it was described that the targeted removal of cholesterol is a suitable method for the functional study of raft-dependent receptor and signal transduction processes (55, 56).

The most commonly used CD derivatives are β-cyclodextrin (BCD) and its modified forms, particularly methyl-β-cyclodextrin (MCD), hydroxypropyl-β-cyclodextrin (HPBCD), and sulfobutylether-β-cyclodextrin (SBECD). Due to its rapid and efficient cholesterol-removing capacity, MCD is the gold standard in lipid raft research; however, because of its strong membrane-modifying effects, it is primarily suitable for acute *in vitro* studies. HPBCD and SBECD are allowing finer modulation of membrane lipids, ensuring better tolerability in various model systems (56, 57).

CD-based approaches allow rapid, reversible, and precisely controlled manipulation of plasma membrane lipid composition, although their limited selectivity requires cautious interpretation. This methodology has been instrumental in establishing that the activity of many receptors and ion channels is dependent on lipid raft integrity. (57).

The significance of CDs extends beyond basic research: they have long been used in the pharmaceutical industry as excipients to enhance solubility and stability, as well as components of advanced drug delivery systems. Through chemical modifications, various pharmacokinetic and pharmacodynamic benefits can be achieved, and CDs are now present in numerous drug formulations (58, 59). Certain CD derivatives have also gained clinical relevance as independent therapeutic agents, such as the anesthesiologic use of sugammadex (SBECD) and the investigation and application of HPBCD in the treatment of Niemann–Pick disease type C(60, 61).

Since numerous nociceptive receptors and ion channels—particularly TRP channels—are localized in cholesterol-rich lipid raft regions, the potential analgesic and anti-inflammatory role of CD derivatives has also been suggested. In preclinical pain models, targeted disruption of raft integrity has been shown to reduce nociceptive responses and modulate nociceptive signaling (53, 54, 62).

CDs are thus important tools in lipid raft research and modern drug development. In pain research, they emerge as multifunctional modulators capable of influencing complex nociceptive processes through the membrane lipid environment, and thus may form the basis for new therapeutic approaches.

3. Objectives

The limitations of current pain therapies highlight that many clinically used analgesics provide insufficient efficacy or are constrained by significant adverse effects. Although TRP ion channels—particularly TRPV1 and TRPA1—have long been recognized as promising targets for pain relief, classical receptor antagonist–based strategies have failed to be translated into clinical practice.

Consequently, increasing attention has shifted toward membrane-level modulation of nociceptive signaling, with particular emphasis on the targeted manipulation of lipid raft microdomains, which play a critical role in the function of several pain-sensing receptors. Owing to their cholesterol-sequestering properties, CDs are capable of disrupting lipid raft structures, thereby offering a novel, peripherally acting analgesic approach to attenuate neurogenic inflammation and TRP ion channel–mediated nociception.

This strategy holds promise for the development of innovative pain therapies that are effective while avoiding central nervous system–related adverse effects and impairment of thermosensation. The aim of this research is to investigate to what extent CD-based targeted modulation of lipid rafts can contribute to the development of new, safer, peripherally acting analgesic interventions.

Main scope of the doctoral thesis:

1. Comparison of CD derivatives with different chemical properties (methylated/unmethylated, anionic/cationic/nonionic) and degrees of isomeric purity in cellular models with regard to safety, structural effects on the plasma membrane, and functional effects on TRPA1 and TRPV1 ion channels.

2. Investigation of CD derivatives selected based on the results of *in vitro* studies in mouse models of TRPA1- and TRPV1-mediated acute pain and inflammation.

3. *in silico* investigation of the strength of the interaction between the selected CD derivatives and cholesterol.

4. Experimental models, experimental methods

4.1. The CD derivatives studied

The CD derivatives used in the experiments were obtained from CycloLab Cyclodextrin Research and Development Ltd. The key properties of the CD derivatives studied are summarized in ***Table 1***. The CD solutions used were freshly prepared each time prior to CD treatment: an appropriate amount of CD derivative was dissolved in the culture medium used in the given experiment (complete Dulbecco's Modified Eagle Medium (DMEM), extracellular solution (ECS), physiological saline, or ethanol).

CD derivative	Abbreviation	Molecular formula	Characteristics
(2-Hydroxypropyl)-gamma-cyclodextrin	HPGCD	$C_{48}H_{80-n}O_{40}$ $(C_3H_7O)_n$	Non-methylated, neutral
(2-Hydroxypropyl)-beta-cyclodextrin	HPBCD	$C_{42}H_{70-n}O_{35}$ $(C_3H_7O)_n$	Non-methylated, neutral
Sulphobutylethter-beta-cyclodextrin sodium salt	SBECD	$C_{42}H_{70-n}O_{35}$ $(C_4H_{8SNa})_n$	Non-methylated, anionic
(2-Hydroxy-3-N,N,N-trimethylamino)propyl-beta-cyclodextrin	QABCD	$C_{42}H_{70-n}O_{35}$ $(C_6H_{15}ONCl)_n$	Non-methylated, cationic
Randomly methylated beta-cyclodextrin	RAMEB	$C_{42}H_{70-n}O_{35}$ $(CH_3)_n$	Methylated, neutral
Heptakis(2,6-di-O-methyl)-beta-cyclodextrin	DIMEB-50	$C_{42}H_{70-n}O_{35}$ $(CH_3)_n$	Methylated, neutral isomeric purity: ~ 50%
Heptakis(2,6-di-O-methyl)-beta-cyclodextrin	DIMEB-50	$C_{42}H_{70-n}O_{35}$ $(CH_3)_n$	Methylated, neutral isomeric purity: ~ 95%
Heptakis(2,3,6-tri-O-methyl)-beta-cyclodextrin	TRIMEB	$C_{63}H_{112}O_{35}$	Methylated, neutral

Table 1 Main properties of the tested CD derivatives (based on CycloLab Cyclodextrin Research and Development Ltd., https://cyclolab.hu/).

4.2. Cell line maintenance

Native CHO cells and CHO cells expressing TRPA1 and TRPV1 receptors were cultured in an incubator under standard cell culture conditions (37 °C, 5% CO_2). Adherent cells were grown in T-25 culture flasks and passaged upon reaching 70–80% confluence.

4.3. Cell viability assays

The CellTiter-Glo® Luminescent Cell Viability Assay is based on the measurement of intracellular ATP levels in live cells, thereby providing indirect information on the proportion of live cells following treatment. Native CHO cells were seeded into 96-well culture plates, and after overnight incubation (37 °C, 5% CO_2), the medium was replaced with CD solutions of increasing concentrations. After 24 hours of treatment, the luminescent signal was measured using a microplate reader.

4.4. Mitochondrial membrane potential analysis

The MitoTracker™ Red CMXRos fluorescent staining method is based on the measurement of mitochondrial membrane potential in cells. Native CHO cells were seeded onto 10-well plates, then treated with CD solutions for 24 hours, and finally stained. We acquired Z-stack (optical section) images at 1 µm intervals throughout the entire depth of the cells using a laser scanning confocal microscope.

4.5. Examination of cell membrane organization by fluorescence spectroscopy

Fluorescence spectroscopy performed following Laurdan staining is a suitable method for investigating the order of membrane lipid phases. Native CHO cells were cultured in a 12-well plate and then treated with CD solutions for 45 minutes. Fluorescence spectroscopy measurements were performed following Laurdan staining. The Laurdan emission spectrum was recorded between 400 and 600 nm under 360 nm excitation, while the excitation spectrum was measured in the 300–420 nm range at 432 nm emission. Membrane order was determined based on the generalized polarization (GP) value using emission intensities (435 and 500 nm). Membrane fluidity was characterized by fluorescence anisotropy calculations from polarized emission intensities.

4.6. Mapping cholesterol depletion using fluorescence microscopy

We determined the intracellular cholesterol content using filipin III fluorescent staining. Native CHO cells were cultured on 10-well cell culture plates and then treated with CD derivatives dissolved in ECS for 1 hour (37°C, 5% CO_2). Cholesterol staining was performed with a filipin III solution for 2 hours at room temperature. The samples were visualized using a confocal microscope with a DAPI filter set. Fluorescence intensity per cell was determined using ImageJ software after background correction. Intensity values for each treatment group were compared to untreated controls via cell-level quantitative analysis.

4.7. Measurement of radioactive calcium isotope influx in TRPV1- or TRPA1-expressing CHO cells

We examined the effect of CD derivatives on TRPA1 and TRPV1 receptor activation by measuring $^{45}Ca^{2+}$ isotope influx. CHO cells stably expressing these receptors were cultured in 72-well plates and then

treated with CD solutions at increasing concentrations for 30 minutes. The cells were incubated with a test solution containing a TRPA1 (allyl isothiocyanate, AITC, 200 μM) or TRPV1 (CAPS, 100 nM) agonist and $^{45}Ca^{2+}$ isotope for 2 minutes at room temperature. After stopping the reaction, the cells were washed several times, then dried and lysed to recover the accumulated $^{45}Ca^{2+}$ isotope. The radioactivity of the lysate was measured using a liquid scintillation counter in counts per minute (CPM). The results were compared to untreated controls.

4.8. Formalin-induced acute inflammatory pain model

We investigated the effect of CD derivatives on acute nociception in a formalin-induced somatic pain model. Thirty minutes prior to intraplantar (i.pl.) administration of the TRPA1 agonist formalin (2.5%, 20 μL), the animals were pretreated with CD (20 μL). We recorded the duration of the characteristic nociceptive behavior (shaking, licking, and lifting) during the early phase (0–5 minutes), associated with direct activation of acute sensory nerve endings, and during the late phase (20–45 minutes), dominated by neurogenic inflammation.

4.9. RTX-induced thermal hyperalgesia and mechanical allodynia model

We investigated the effect of CD derivatives on TRPV1-mediated nociceptive responses in an RTX-induced neurogenic pain model. The thermal pain threshold was measured using a gradually heating hot plate, while mechanical sensitivity was assessed using a dynamic plantar esthesiometer (DPA). The animals received a CD pretreatment (20 μL, i.pl.) 30 minutes prior to RTX administration (0.1 μg/mL, 20 μL, i.pl.). Thermonociceptive thresholds were assessed 10, 20, and 30 minutes after the RTX injection, while mechanical hyperalgesia was assessed 30 and 90 minutes after treatment. Animals pretreated with a vehicle and then treated with RTX served as positive controls.

4.10. Mustard oil (MO)-induced acute skin inflammation mouse model

We investigated the vascular effects of CD pretreatment in a model of acute neurogenic dermatitis induced by mustard oil (MO). We applied ethanol (control) to one ear of the animals and a CD solution dissolved in ethanol to the other (10–10 μL, bilaterally). Thirty minutes later, a 1% MO solution was applied to the left ear, while paraffin oil (vehicle) was applied to the right ear as a self-control in the same volume. Immediately after MO treatment, we began monitoring skin perfusion using laser speckle contrast analysis for 20 minutes. By selecting areas of the same size on the treated and reference ears, we compared the percentage difference in perfusion values between the experimental groups. The degree of ear edema was measured with an engineering micrometer before treatment and at 60, 120, 180, and 240 minutes following MO application.

4.11. Colorimetric measurement of free cholesterol levels in mouse ear and plantar skin tissue

Following CD pretreatment, the free cholesterol content in mouse ear and plantar skin was determined by a colorimetric method using the Cholesterol/Cholesteryl Ester Quantitation Assay kit. The animals were treated with CD solutions or an appropriate vehicle, then euthanized after 30 minutes, and tissue samples were collected. We performed organic solvent extraction on the tissues, then concentrated the lipid phase and dissolved it in assay buffer. The amount of free cholesterol was determined based on a colorimetric reaction performed according to the manufacturer's protocol, using optical density measured at 570 nm. The values were corrected for sample weight and dilution factor, then compared to a standard calibration curve.

4.12. Molecular modeling and *in silico* studies

The crystal structure of β-cyclodextrin (BCD) was obtained from a known CD-enzyme complex (PDB: 3cgt). The substituents of the CD derivatives (HPBCD, SBECD, RAMEB) were added using the PyMOL program based on the degree of substitution (DS) and literature data. The cholesterol molecule was built using the Maestro program. Energy optimization of all structures was performed using PM7 parameters and the MOPAC software package. Docking calculations were performed using AutoDock 4.2.6, applying flexible cholesterol and rigid host molecules. During the calculations, we used blind docking, a Lamarckian genetic algorithm, and local search. We evaluated the results based on the calculated free binding energy and structural similarity and selected the most favorable binding mode as the representative conformation.

4.13. Animals and ethical considerations

We used 12–16-week-old male NMRI mice for the experiments, kept under standard housing conditions following a habituation period of at least two weeks. All animal experiments were conducted in accordance with relevant Hungarian legislation and with the approval of the Animal Experimentation Ethics Committee of the University of Pécs (approval number: BA02/2000–22/2023).

4.14. Statistical Methods

In vitro experiments were performed in at least three independent replicates. Sample size was determined using *a priori* power analysis. Data are presented as mean ± SEM. For statistical analyses, following a normality test, we used parametric or nonparametric tests, as well as multiple comparison tests using GraphPad Prism and OriginPro software. The significance level was set at $p < 0.05$.

5. Results

5.1. Methylated,but not non-methylated CD derivatives reduce CHO cell viability

According to CellTiter-Glo® measurements, unmethylated CD derivatives (HPGCD, HPBCD, SBECD, QABCD) did not reduce viability up to 10 mM, and HPGCD remained safe even at 50–100 mM. Among the methylated derivatives, cytotoxicity appeared in the order DIMEB-95 (0.75 mM) > DIMEB-50 (1 mM) = TRIMEB (1 mM) > RAMEB (3 mM). For the hydroxypropylated derivatives (HPGCD, HPBCD), an elevated luminescent signal was detected, which can be associated with increased ATP production in the cells.

5.2. Certain non-methylated CD derivatives increase, while methylated derivatives decrease the mitochondrial membrane potential of CHO cells

The intensity of the MitoTracker™ Red CMXRos fluorescent dye—which indicates active mitochondria—increased significantly following 24-hour treatment with HPGCD, HPBCD, and QABCD (10 mM) treatments. In contrast, the methylated derivatives reduced fluorescence intensity even at concentrations that did not affect cell viability, except for DIMEB-50.

5.3. All CD derivatives reduce cholesterol levels in CHO cells

To investigate the cholesterol-lowering effect of CD derivatives, we compared the filipin III staining of untreated control CHO cells with that of cells treated with CD solutions at concentrations that did not affect cell viability. Treatment with CD derivatives significantly reduced the intensity of the filipin III signal, confirming cholesterol depletion.

5.4. Among the CD derivatives, DIMEB-50 disrupts membrane order the most

Based on spectroscopic analyses following Laurdan staining, DIMEB-50 and RAMEB caused the most significant spectral red shift, indicating a higher dielectric constant and a more disordered membrane microenvironment. For all CD derivatives examined, a slight shift toward the L_d state was observed compared to the control. A similar trend was observed in the fluorescence anisotropy spectra.

5.5. CD derivatives inhibit *in vitro* TRPV1 and TRPA1 receptor activation

CD treatments reduced TRPV1 activation in a concentration-dependent manner for all CD derivatives tested. A similar trend was observed for TRPA1: CD derivatives applied at the highest concentrations significantly inhibited AITC-induced $^{45}Ca^{2+}$ uptake; only DIMEB-50 did not show a significant inhibitory effect.

5.6. CD derivatives reduce acute nociceptive behavior induced by TRPA1 activation

For example, following a formalin injection, the animals spent an average of 157.8 ± 80.11 seconds and 264.0 ± 23.63 seconds, respectively, exhibiting nocifensive behavior during the first (0–5 min) and second (20–45 min) phases of the experiment. CD treatment did not significantly affect the duration of nocifensive behavior in the first phase of the experiment. In contrast, in the second phase associated with neurogenic inflammation, the duration of nocifensive behavior was significantly reduced.

5.7. CD derivatives attenuate acute mechanical allodynia induced by TRPV1 activation but have no effect on thermal hyperalgesia

Following i.p. treatment with the TRPV1 receptor RTX agonist, the animals' mechanonociceptive threshold decreased from the baseline. CD pretreatment attenuated acute mechanical allodynia but did not affect RTX-induced thermal hyperalgesia.

5.8. CD treatment reduces TRPA1-induced acute neurogenic vasodilation in the skin

In the TRPA1 receptor agonist MO-induced acute skin inflammation, based on laser speckle contrast analysis results, blood perfusion in the affected ear's skin increased compared to the reference ear. In contrast, this increase was significantly reduced in the CD-pretreated groups. Based on ear thickness measurements, edema developed in the affected ear following MO treatment. CD pretreatment mitigated this edema formation.

5.9. CD treatment reduces the total cholesterol content of the plantar skin and the ear

Based on the results of the colorimetric assay, the free cholesterol content of plantar skin and ear tissues of mice was significantly reduced compared to the control.

5.10. CD derivatives exhibit different cholesterol-binding properties

When modeling the interaction between CD derivatives and cholesterol *in silico*, cholesterol binding exhibited a similar pattern for HPBCD and SBECD, while the more closed conformation of RAMEB prevented deeper embedding of the ligand into the cavity. The structural observations were supported by energetics analysis: the slightly more positive binding free energy (ΔG_b) value of RAMEB suggests that a 1:1 binding ratio alone is insufficient for the formation of a stable complex.

6. Discussion, conclusions

In this study, we examined the effects of eight different CD derivatives *in vitro* and three selected derivatives *in vivo*, with particular focus on membrane cholesterol content, the function of TRPV1 and TRPA1 ion channels, and their effects on nociceptive and inflammatory processes.

Our *in vitro* experiments were the first to demonstrate that the cytotoxicity of CD derivatives, their effect on mitochondrial membrane potential, and their ability to deplete cholesterol are structure dependent. Methylated CD derivatives exhibited more pronounced cholesterol-removing and TRP ion channel-inhibiting effects at lower concentrations, but also demonstrated a less favorable safety profile. In contrast, the hydroxypropylated and ionic derivatives exhibited significant membrane effects even at lower concentrations, while maintaining favorable safety parameters.

Based on fluorescent membrane structure analyses and mitochondrial membrane potential measurements, CD treatments reduced membrane order, particularly in the case of methylated derivatives, suggesting disruption of lipid rafts. Concurrently, our functional assays showed that all tested CD derivatives concentration-dependently inhibited agonist-induced activation of TRPV1 and TRPA1 ion channels, as well as the associated Ca^{2+} influx. These results support the notion that a decrease in membrane cholesterol content and the reorganization of the lipid microenvironment play a key role in regulating the function of TRP ion channels.

Peripheral administration of the CD derivatives selected for the *in vivo* experiments (RAMEB, HPBCD, SBECD) reduced formalin-induced inflammatory nociceptive responses in the second phase of the experiment, which is associated with the release of inflammatory neuropeptides. CD pretreatment also attenuated RTX-induced mechanical allodynia, as well as MO-induced neurogenic inflammation and the associated vascular responses. An important observation was that the treatments did not significantly affect thermal nociception, which is a favorable property for the clinical applicability of interventions targeting TRP ion channels.

Measurement of tissue cholesterol levels confirmed that the observed *in vivo* effects are associated with cholesterol depletion. *In silico* docking studies revealed that methylated and unmethylated CD derivatives form complexes with cholesterol in different ways. In the case of RAMEB, the possibility of complex formation with a 2:1 stoichiometry may result in a more stable binding, which could explain its more pronounced cholesterol-depleting effect and increased cytotoxicity. In contrast, HPBCD and SBECD tend to form 1:1 complex, which are associated with a more moderate yet more favorable safety profile.

Overall, our results suggest that CD derivatives can influence the peripheral activation of TRPV1 and TRPA1 ion channels by modulating membrane lipid microdomains, thereby exerting antinociceptive

and anti-inflammatory effects. Given their favorable efficacy-safety profile, unmethylated CD derivatives may represent a promising starting point for the development of new, peripherally acting analgesic and anti-inflammatory therapeutic strategies, whereas the potential toxicity of methylated derivatives requires further optimization.

7. Summary of new findings

In our study, we investigated the inhibition of TRPV1 and TRPA1 ion channel activation through the disruption of lipid rafts by cholesterol depletion, combining *in silico*, *in vitro,* and *in vivo* approaches. First, we comprehensively evaluated how CD derivatives with different chemical properties and isomeric compositions influence the membrane-level regulation of nociceptive signaling.

1. We found that the chemical modifications and isomeric purity of CD derivatives determine their cholesterol-binding capacity, their cellular safety profile, and their functional effects on plasma membrane structure and ion channel activation. We identified CD derivatives that effectively disrupt lipid rafts while exhibiting favorable safety profiles.

2. We demonstrated *in vitro* that the selected CDs significantly reduce TRPV1- and TRPA1-mediated Ca^{2+} responses—not through receptor antagonism, but by inhibiting raft-dependent activation. This offers a new mechanistic approach to the modulation of TRP channels.

3. *In vivo*, we demonstrated that CD derivatives effectively attenuate TRPA1- and TRPV1-mediated acute nociceptive and inflammatory responses in mouse models, while not affecting physiological thermosensation, thus possessing safer analgesic potential with peripheral action.

4. Our *in silico* studies revealed the structure- and affinity-dependent nature of the interaction between CDs and cholesterol, which correlated well with the experimental membrane-modulating effects and supports the design of specifically optimized CD-based therapies.

Overall, our results establish a novel, membrane-level approach to reducing TRP ion channel-mediated pathological nociceptive processes and contribute to the development of safer, peripherally acting analgesic strategies.

8. References

1. International Association for the Study of Pain. 2026. IASP Terminology.
2. Hoegh, M., and P. Hodges. 2025. Pain Science in Practice (Part 8): *Nociceptive, Neuropathic, and Nociplastic Pain*. *Journal of Orthopaedic & Sports Physical Therapy*. **55**: 555–560.
3. Maio, G. Di, I. Villano, C. R. Ilardi, A. Messina, V. Monda, A. C. Iodice, C. Porro, M. A. Panaro, S. Chieffi, G. Messina, M. Monda, and M. La Marra. 2023. Mechanisms of Transmission and Processing of Pain: A Narrative Review. *Int. J. Environ. Res. Public Health*. **20**.
4. Tracey, W. D. 2017. Current Biology Nociception.
5. Dehghan, B., N. Abolhasanzadeh, B. Shademan, and A. Nourazarian. 2024. Deciphering pain: molecular mechanisms and neurochemical pathways–challenges and future opportunities. *Front. Mol. Biosci.* **11**.
6. Galdino, G., F. P. Veras, and T. dos Anjos-Garcia. 2024. The Role of the Thalamus in Nociception: Important but Forgotten. *Brain Sci.* **14**.
7. Marsh, J. D. 2013. *In* *Pain* (Copstead, L. S., and Banasik, J. L., eds.). 6th ed., pp. 960–972.
8. Lee, G. I., and M. W. Neumeister. 2020. Pain: Pathways and Physiology. *Clin. Plast. Surg.* **47**: 173–180.
9. Seidel, M. F., T. Hügle, B. Morlion, M. Koltzenburg, V. Chapman, A. MaassenVanDenBrink, N. E. Lane, S. Perrot, and W. Zieglgänsberger. 2022. Neurogenic inflammation as a novel treatment target for chronic pain syndromes. *Exp. Neurol.* **356**.
10. Catalisano, G., G. M. Campione, G. Spurio, A. N. Galvano, C. P. di Villalba, A. Giarratano, A. Alongi, M. Ippolito, and A. Cortegiani. 2024. Neuropathic pain, antidepressant drugs, and inflammation: a narrative review. *Journal of Anesthesia, Analgesia and Critical Care*. **4**.
11. Thapa, R., and D. Ang. 2025. Nociceptive pain: A practical guide to chronic pain management in the primary care setting. *Cleveland Clinical Journal of Medicine* **92**: 236–247.
12. Soliman, N., N. Soliman, X. Moisset, M. C. Ferraro, D. Ciampi De Andrade, R. Baron, J. Belton, D. L. H. Bennett, M. Calvo, P. Dougherty, I. Gilron, A. J. Hietaharju, K. Hosomi, P. R. Kamerman, H. Kemp, E. K. Enax-Krumova, E. Mcnicol, T. J. Price, S. N. Raja, A. S. C. Rice, B. H. Smith, F. Talkington, A. Truini, J. Vollert, N. Attal, and N. B. Finnerup. 2025. Pharmacotherapy and non-invasive neuromodulation for neuropathic pain: a systematic review and meta-analysis. [online] www.covidence.org.
13. Labianca, R., P. Sarzi-Puttini, S. M. Zuccaro, P. Cherubino, R. Vellucci, and D. Fornasari. 2012. Adverse Effects Associated with Non-opioid and Opioid Treatment in Patients with Chronic Pain.
14. Fairbanks, C. A., and C. D. Peterson. 2023. The opioid receptor: emergence through millennia of pharmaceutical sciences. *Frontiers in Pain Research*. **4**.
15. Kataria, S., U. Patel, K. Yabut, J. Patel, R. Patel, S. Patel, J. H. Wijaya, P. Maniyar, Y. Karki, M. P. Makrani, O. Viswanath, and A. D. Kaye. 2024. Recent Advances in Management of Neuropathic, Nociceptive, and Chronic Pain: A Narrative Review with Focus on Nanomedicine, Gene Therapy, Stem Cell Therapy, and Newer Therapeutic Options. *Curr. Pain Headache Rep.* **28**: 321–333.
16. Ovsepian, S. V., and S. G. Waxman. 2023. Gene therapy for chronic pain: emerging opportunities in target-rich peripheral nociceptors. *Nat. Rev. Neurosci.* **24**: 252–265.
17. Mattioli, R., A. Mariano, A. Torpey, E. Bellow, V. Samojedny, S. Ahluwalia, A. Desai, W. Caldwell, and S. Bergese. 2024. Nanotechnology in Pain Management. [online] https://doi.org/10.3390/pharmaceutics16111479.
18. Hőgyes, E. 1878. Contributions to the Physiological Effects of the Components of Capsaicum aniim. *Arch. Exp. Pathol Pharmakol.* 117–130.

19. Jancsó, M. 1955. Storage. Accumulation of substances in the reticuloendothelial system and in the kidney.

20. Szolcsányi, J. 2004. Forty years in capsaicin research for sensory pharmacology and physiology. *Neuropeptides*. **38**: 377–384.

21. Szolcsányi, J. 1996. Chapter 20. Capsaicin-sensitive sensory nerve terminals with local and systemic efferent functions: facts and scopes of an unorthodox neuroculatory mechanism. *Prog. Brain Res.* **113**: 343–359.

22. Szállási, Á., and P. M. Blumberg. 1990. Specific binding of resiniferatoxin, an ultrapotent capsaicin analog, by dorsal root ganglion membranes. *Brain Res.* **524**: 106–111.

23. Wood, J. N., J. Winter, F. James, H. P. Rang, J. Yeats, and S. Bevan. 1988. Capsaicin-Induced Ion Fluxes in Dorsal Root Ganglion Cells in Culture.

24. Bevan, S., S. Hothi, G. Hughes, I. F. James, H. P. Rang, K. Shah, C. S. J. Walpole, and J. C. Yeats. 1992. Capsazepine: a competitive antagonist of the sensory neuron excitant capsaicin. *Br. J. Pharmacol.* **107**: 544–552.

25. Caterina, M. J., and D. Julius. 2001. The Vanilloid Receptor: A Molecular Gateway to the Pain Pathway. *Annu. Rev. Neurosci.* **24**: 487–517.

26. Nilius, B., and G. Owsianik. 2011. The transient receptor potential family of ion channels. [online] http://genomebiology.com/2011/12/3/218.

27. Wang, H., and J. Siemens. 2015. TRP ion channels in thermosensation, thermoregulation, and metabolism. *Temperature*. **2**: 178–187.

28. Damann, N., T. Voets, and B. Nilius. 2008. TRPs in Our Senses. *Current Biology*. **18**.

29. Fernandes, E. S., M. A. Fernandes, and J. E. Keeble. 2012. The functions of TRPA1 and TRPV1: Moving away from sensory nerves. *Br. J. Pharmacol.* **166**: 510–521.

30. Ross, R. A. 2003. Anandamide and vanilloid TRPV1 receptors. *Br. J. Pharmacol.* **140**: 790–801.

31. Stratiievska, A., S. Nelson, E. N. Senning, J. D. Lautz, S. E. Smith, and S. E. Gordon. 2018. Reciprocal regulation among TRPV1 channels and phosphoinositide 3-kinase in response to nerve growth factor. *Elife*. **7**: 1–13. [online] https://elifesciences.org/articles/38869#abstract.

32. Gees, M., Y. A. Alpizar, B. Boonen, A. Sanchez, W. Everaerts, A. Segal, F. Xue, A. Janssens, G. Owsianik, B. Nilius, T. Voets, and K. Talavera. 2013. Mechanisms of Transient Receptor Potential Vanilloid 1 Activation and Sensitization by Allyl Isothiocyanate. *Mol. Pharmacol.* **84**: 325–334. [online] https://molpharm.aspetjournals.org/content/84/3/325 (Accessed January 9, 2023).

33. Prescott, E. D., and D. Julius. 2003. A Modular PIP2 Binding Site as a Determinant of Capsaicin Receptor Sensitivity. *Science (1979).* **300**: 1284–1288.

34. Guimaraes, M. Z. P., and S.-E. Jordt. 2007. TRPA1: A Sensory Channel of Many Talents. *TRP Ion Channel Function in Sensory Transduction and Cellular Signaling Cascades*. [online] https://www.ncbi.nlm.nih.gov/books/NBK5237/ (Accessed September 13, 2022).

35. Paulsen, C. E., J. P. Armache, Y. Gao, Y. Cheng, and D. Julius. 2015. Structure of the TRPA1 ion channel suggests regulatory mechanisms. *Nature*. **520**: 511–517.

36. Startek, J. B., B. Boonen, A. Ló Pez-Requena, A. Talavera, Y. A. Alpizar, D. Ghosh, N. Van Ranst, B. Nilius, T. Voets, and K. Talavera. 2019. Mouse TRPA1 function and membrane localization are modulated by direct interactions with cholesterol. [online] https://doi.org/10.7554/eLife.46084.001.

37. Julius, D. 2013. TRP Channels and Pain. *Annu. Rev. Cell Dev. Biol.* **29**: 355–384.

38. Mickle, A. D., A. J. Shepherd, and D. P. Mohapatra. 2015. Sensory TRP channels: the key transducers of nociception and pain. *Prog. Mol. Biol. Transl. Sci.* **131**: 73–118. [online] https://pubmed.ncbi.nlm.nih.gov/25744671/ (Accessed August 16, 2022).

39. Maggi, C. A. 1995. Tachykinins and calcitonin gene-related peptide (CGRP) as co-transmitters released from peripheral endings of sensory nerves. *Prog. Neurobiol.* **45**: 1–98.

40. Viana, F. 2016. TRPA1 channels: molecular sentinels of cellular stress and tissue damage. *J. Physiol.* **594**: 4151–4169.

41. Bautista, D. M., M. Pellegrino, and M. Tsunozaki. 2013. TRPA1: A gatekeeper for inflammation. *Annu. Rev. Physiol.* **75**: 181–200.

42. Singer, S. J., and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science*. **175**: 720–31.

43. Lehninger, A. L. 2013. Principles of Biochemistry. 6th ed.

44. Simons, K., and E. Ikonen. 1997. Functional rafts in cell membranes. *Nature*. **387**: 569–572.

45. Rietveld, A., and K. Simons. 1998. The differential miscibility of lipids as the basis for the formation of functional membrane rafts.

46. Simons, K., and R. Ehehalt. 2002. Cholesterol, lipid rafts, and disease. *Journal of Clinical Investigation*. **110**: 597–603.

47. Miller, Y. I., J. M. Navia-Pelaez, M. Corr, and T. L. Yaksh. 2020. Lipid rafts in glial cells: Role in neuroinflammation and pain processing. *J. Lipid Res.* **61**: 655–666.

48. Sviridov, D., and Y. I. Miller. 2020. Biology of lipid rafts: Introduction to the thematic review series. *J. Lipid Res.* **61**: 598–600.

49. Simons, K., and D. Toomre. 2000. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **1**: 31–39.

50. Liu, M., W. Huang, D. Wu, and J. V. Priestley. 2006. TRPV1, but not P2X3, requires cholesterol for its function and membrane expression in rat nociceptors. *European Journal of Neuroscience*. **24**: 1–6.

51. Szőke, É., R. Börzsei, D. M. Tóth, O. Lengl, Z. Helyes, Z. Sándor, and J. Szolcsányi. 2010. Effect of lipid raft disruption on TRPV1 receptor activation in trigeminal sensory neurons and a transfected cell line. *Eur. J. Pharmacol.* **628**: 67–74.

52. Startek, J. B., and K. Talavera. 2020. Lipid Raft Destabilization Impairs Mouse TRPA1 Responses to Cold and Bacterial Lipopolysaccharides. *Int. J. Mol. Sci.* **21**. [online] https://pubmed.ncbi.nlm.nih.gov/32481567/ (Accessed August 30, 2022).

53. Navia-Pelaez, J. M., J. Borges Paes Lemes, L. Gonzalez, L. Delay, L. Dos Santos Aggum Capettini, J. W. Lu, G. Goncalves Dos Santos, A. M. Gregus, P. M. Dougherty, T. L. Yaksh, and Y. I. Miller. 2023. AIBP regulates TRPV1 activation in chemotherapy-induced peripheral neuropathy by controlling lipid raft dynamics and proximity to TLR4 in dorsal root ganglion neurons. *Pain*. **164**: E274–E285.

54. Horváth, Á., T. Biró-Sütő, B. Kántás, M. Payrits, R. Skoda-Földes, E. Szánti-Pintér, Z. Helyes, and É. Szőke. 2020. Antinociceptive Effects of Lipid Raft Disruptors, a Novel Carboxamido-Steroid and Methyl β-Cyclodextrin, in Mice by Inhibiting Transient Receptor Potential Vanilloid 1 and Ankyrin 1 Channel Activation. *Front. Physiol.* **11**.

55. Horváth, Á., M. Payrits, A. Steib, B. Kántás, T. Biró-Süt, J. Erostyák, G. Makkai, É. Sághy, Z. Helyes, and É. Szőke. 2021. Analgesic Effects of Lipid Raft Disruption by Sphingomyelinase and Myriocin via Transient Receptor Potential Vanilloid 1 and Transient Receptor Potential Ankyrin 1 Ion Channel Modulation. *Front. Pharmacol.* **11**.

56. Aimee, C. E., M. P. Haynes, M. C. Phillips, and G. H. Rothblat. 1997. Use of cyclodextrins to manipulate cellular cholesterol content. *J. Lipid Res.* **38**: 2264–2272.

57. Zidovetzki, R., and I. Levitan. 2007. Use of cyclodextrins to manipulate plasma membrane cholesterol content: Evidence, misconceptions, and control strategies. *Biochim. Biophys. Acta Biomembr.* **1768**: 1311–1324.

58. Christian, A. E., M. P. Haynes, M. C. Phillips, and G. H. Rothblat. 1997. Use of cyclodextrins for manipulating cellular cholesterol content. *J. Lipid Res.* **38**: 2264–2272.

59. Loftsson, T., and M. E. Brewster. 2011. Pharmaceutical applications of cyclodextrins: Effects on drug permeation through biological membranes. *Journal of Pharmacy and Pharmacology*. **63**: 1119–1135.

60. Petitjean, M., I. X. García-Zubiri, and J. R. Isasi. 2021. History of cyclodextrin-based polymers in food and pharmacy: a review. *Environ. Chem. Lett.* **19**: 3465–3476.

61. Castagne, D., M. Fillet, L. Delattre, B. Evrard, B. Nusgens, and G. Piel. 2009. Study of the cholesterol extraction capacity of β-cyclodextrin and its derivatives, relationships with their effects on endothelial cell viability and on membrane models. *J. Incl. Phenom. Macrocycl. Chem.* **63**: 225–231.

62. Maarup, T. J., A. H. Chen, F. D. Porter, N. Y. Farhat, D. S. Ory, R. Sidhu, X. Jiang, and P. I. Dickson. 2015. Intrathecal 2-hydroxypropyl-beta-cyclodextrin in a single patient with Niemann-Pick C1. *Mol. Genet. Metab.* **116**: 75–79.

63. Talavera, K., J. B. Startek, J. Alvarez-Collazo, B. Boonen, Y. A. Alpizar, A. Sanchez, R. Naert, and B. Nilius. 2020. Mammalian transient receptor potential TRPA1 channels: From structure to disease. *Physiol. Rev.* **100**: 803.

9. List of Publications

9.1. Publications forming the basis of the dissertation

Andrea Kinga Nehr-Majoros, Ágnes Király, Zsuzsanna Helyes, Éva Szőke

Lipid raft disruption as an opportunity for peripheral analgesia

CURRENT OPINION IN PHARMACOLOGY 75 Paper: 102432, 11 p. (2024)

IF value: 4.2

Andrea Kinga Nehr-Majoros, János Erostyák, Éva Fenyvesi, Edina Szabó-Meleg, Levente Szőcs, György Sétáló, Zsuzsanna Helyes, Éva Szőke

Cyclodextrin derivatives decrease Transient Receptor Potential vanilloid 1 and Ankyrin 1 ion channel activation via altering the surrounding membrane microenvironment by cholesterol depletion

FRONTIERS IN CELL AND DEVELOPMENTAL BIOLOGY 12 Paper: 1334130, 13 p. (2024)

IF value: 4.1

Andrea Kinga Nehr-Majoros, Lajos Karakai, Maja Payrits, Noémi Bencze, Ágnes Kemény, György Sétáló, Rita Börzsei, Csaba Hetényi, Zsuzsanna Helyes, Éva Szőke

Cyclodextrins inhibit TRPV1- and TRPA1-mediated nociception via cholesterol depletion

JOURNAL OF LIPID RESEARCH 66 : 7 Paper: 100844 , 13 p. (2025)

IF value: 4.3

9.2. Other original publications

Mónika Ágnes Tóth, **Andrea Kinga Majoros**, Andrea Teréz Vig, Ede Migh, Miklós Nyitrai, József Mihály, Beáta Bugyi

Biochemical Activities of the Wiskott-Aldrich Syndrome Homology Region 2 Domains of Sarcomere Length Short.: WH2 domains in sarcomeric actin regulation

JOURNAL OF BIOLOGICAL CHEMISTRY 291 : 2 pp. 667-680. , 14 p. (2016)

IF value: 4.125

Bernadett Zsoldos, Nóra Nagy, Viktória Donkó-Tóth, Péter Keglevich, Márton Weber, Miklós Dékány, **Andrea Nehr-Majoros**, Éva Szőke, Zsuzsanna Helyes, László Hazai

Novel Piperazine Derivatives of Vindoline as Anticancer Agents

INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 25 : 14 Paper: 7929 , 13 p. (2024)

IF value: 4.9

Maja Payrits, Balázs Zoltán Zsidó, **Andrea Kinga Nehr-Majoros**, Rita Börzsei, Zsuzsanna Helyes, Csaba Hetényi, Éva Szőke

Lipid raft disruption inhibits the activation of Transient Receptor Potential Vanilloid 1, but not TRP Melastatin 3 and the voltage-gated L-type calcium channels in sensory neurons

FRONTIERS IN CELL AND DEVELOPMENTAL BIOLOGY 12 Paper: 1452306, 14 p. (2024)

IF value: 4.3

Ádám Horváth, Anita Steib, **Andrea Nehr-Majoros**, Boglárka Kántás, Ágnes Király, Márk Racskó, Balázs István Tóth, Eszter Szánti-Pintér, Eva Kudová, Rita Skoda-Földes, Zsuzsanna Helyes, Éva Szőke

Anti-Nociceptive Effects of Sphingomyelinase and Methyl-Beta-Cyclodextrin in the Icilin-Induced Mouse Pain Model

INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 25 : 9 Paper: 4637 , 13 p. (2024)

IF value: 4.9

Mónika Halmai, Dominika Mária Herr, Szabolcs Mayer, Péter Keglevich, Ejlal A. Abdallah, Noémi Bózsity-Faragó, István Zupkó, **Andrea Nehr-Majoros**, Éva Szőke, Zsuzsanna Helyes, László Hazai

Synthesis and In Vitro Anticancer Evaluation of Novel Phosphonium Derivatives of Chrysin

INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES 26 : 22 Paper: 11063 , 15 p. (2025)

IF value: 4.9

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