# CHAPTER 1: Introduction and Literature Review

*Chapter-1*

# Introduction and Literature Review

## 1.1 Introduction

Cancer is a leading cause of death which deleteriously affects the life expectancy globally. Studies suggest that worldwide burden of cancer incidences is expected to be 28.4 million cases in 2040, a 47% rise from 2022 [1]. Researchers and scientists across the world are continually working to develop novel therapeutics to cope with this dreadful disease. Conventional methods like radiation, surgery and chemotherapy are now combined with novel approaches like targeted therapies and immunotherapies which have revolutionized the outcome of difficult-to-treat cancers [2]. Though these combination therapies have improved cancer survivability, new anti-cancer medications are responsible for substantial side effects on daily life that can endure for many years. Compared to people without a history of cancer, cancer survivors experience higher functional impairment, which includes decreased mobility [3]. One of the serious emerging side effects of chemotherapy is damage to peripheral nervous system leading to neuropathy which also lacks viable curative approaches [4]. Chemotherapy induced neuropathic pain (CINP) manifests as a painful condition which can progress, in severe cases, to loss of sensory perceptions. Impairments in sensory functions can lead to a lowered pain threshold in response to various stimuli giving rise to mechanical allodynia, tingling, burning, paresthesia and dysesthesia triggered by contact with warm or cool temperatures [5]. The patient's quality of life is negatively impacted by CINP, which might result in dosage adjustments or even treatment

discontinuation [6]. Some of the medications currently being used for the management of CINP include serotonin and norepinephrine reuptake inhibitors (SNRIs) such as duloxetine, anticonvulsants like gabapentin and pregabalin, tricyclic antidepressants (TCAs) and patches of lidocaine, capsaicin and both weak and strong opioids. Unfortunately, a substantial proportion, approximately 70%, of patients do not get effective pain relief from these medications [7]. Managing CINP is a significant challenge for healthcare providers, as it can impact the quality of life and treatment outcomes for cancer patients. Ongoing research aims to enhance our understanding of the underlying mechanisms behind CINP in order to develop more effective and safer strategies for both prevention and management of this distressing side effect.

In recent years, numerous reports have highlighted the involvement of various molecular mechanisms in CIPN [8]. These mechanisms are diverse and affect different components of the peripheral nervous system (PNS). The Dorsal Root Ganglion (DRG), which lacks an effective blood-brain barrier (BBB) [9], is particularly vulnerable to neurotoxic damage, contributing to the sensory symptoms associated with CIPN. Platinum (Pt) compounds induce DNA damage through the formation of Pt adducts, leading to changes in the nucleoli of DRG sensory neurons and disrupting the transcription machinery [10]. The accumulation of taxanes and vinca alkaloids in the DRG appears to cause nucleolar abnormalities [11] and alterations in neurofilaments [12]. These agents also impact microtubule conformation by inducing tubulin acetylation.

Bortezomib (BTZ) and vinca alkaloids further disrupt axonal transport by reducing the availability of trophic factors and energy production, or by enhancing

Wallerian degeneration, resulting in potentially irreversible neurological damage. Mitochondrial damage within axons, leading to energy depletion, is another contributor to the neurotoxicity associated with various chemotherapeutic agents [13–15]. BTZ also compromises the integrity of the endoplasmic reticulum, particularly in Schwann cells, leading to degeneration of the myelin sheath.

The modulation of axonal ion channels is another key factor in CIPN. Dysfunctions in sodium ($Na^+$) channels, primarily induced by oxaliplatin, but also by paclitaxel and vincristine, increase Na+ currents in the DRG, which can predispose patients to paresthesia [16–18]. Calcium ($Ca^{2+}$) and potassium ($K^+$) channels are also implicated in the toxicity of paclitaxel [19] and oxaliplatin [20], respectively. Furthermore, alterations in proteins involved in $Ca^{2+}$ signaling, such as calpains and caspases, can trigger apoptotic events in the DRG [21].

Changes in the expression levels of transient receptor potential (TRP) channels—TRPV, TRPA, and TRPM—as well as in molecules related to glutamate signaling, are induced by Pt compounds and treatments with paclitaxel and BTZ [22–26]. These changes lead to hyper-responsiveness of nociceptors, making patients more susceptible to neuropathic pain and the development of peripheral neuropathy. Chemotherapeutic agents also increase the expression of mitogen-activated protein kinases (MAPKs), contributing to neurotoxicity [27]. Vincristine, paclitaxel, and BTZ provoke inflammation by elevating pro-inflammatory cytokines in peripheral nerves and increasing the number of antigen-presenting cells in the skin. Additionally, chemotherapy induces the production of reactive oxygen species (ROS) and elevates $Ca^{2+}$ levels in the DRG, both of which contribute to neuronal cytotoxicity [28–30].

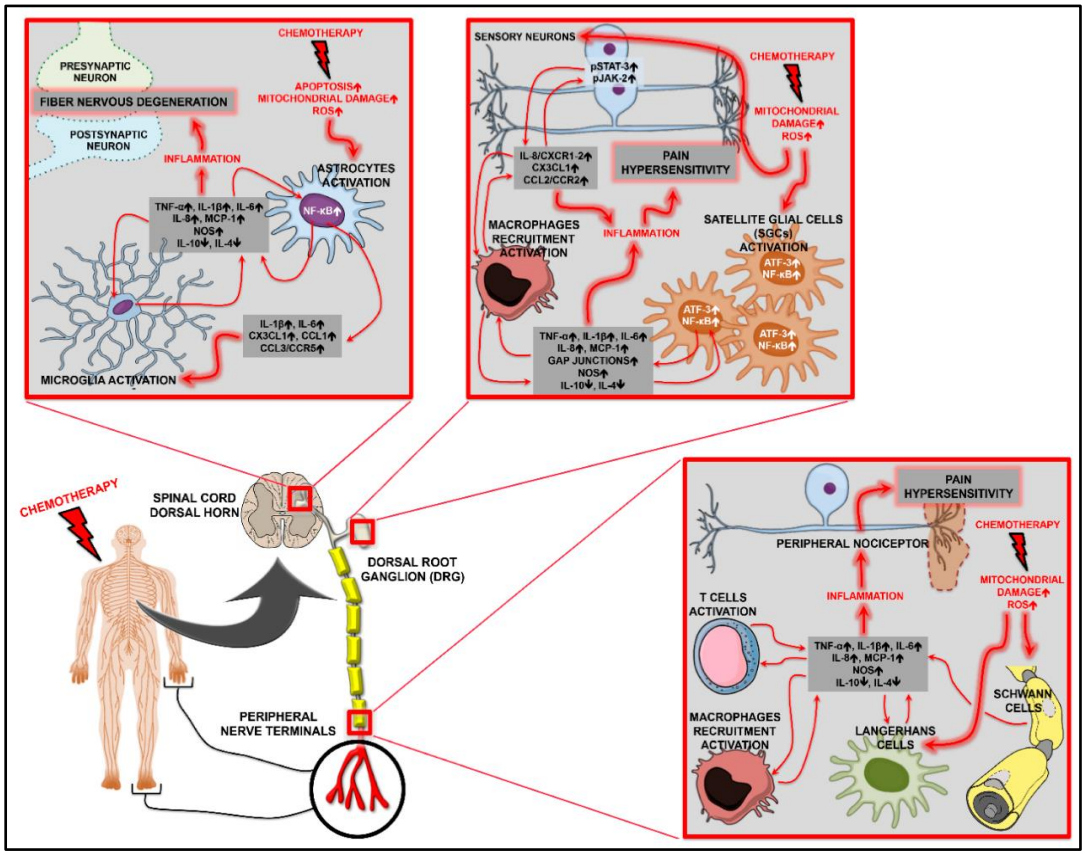


**Figure.1.1 Overview of the key factors contributing to CINP** [31]

## 1.2 Chemotherapy- Induced Neuropathic Pain

CINP is a complex condition characterized by damage and dysfunction of the peripheral nervous system resulting from exposure to chemotherapeutic agents. The culprits include platinum agents, taxanes, vinca alkaloids, thalidomide, bortezomib, and others [32]. This adverse effect is not only prevalent but also manifests differently based on the type of chemotherapy, total dose, frequency, and duration of treatment, all of which contribute to varying degrees of neuronal damage. CINP typically emerges in approximately 68% of individuals within the initial month post-chemotherapy, with a persistent prevalence of 30% even six months after completing the treatment [33].

The severity of CINP is dose-dependent, highlighting the importance of understanding the cumulative impact of chemotherapy on the peripheral nervous

system. Various risk factors contribute to the development of CINP, including pre-existing neuropathies such as those associated with diabetes, age-related factors, smoking, alcohol abuse [34].

### 1.2.1 CINP Manifestations: Unique Symptomatology

Patients grappling with CINP commonly report a distinctive pattern of sensory disturbances, with symptoms initiating at the extremities and progressing proximally. This progression often follows a characteristic "stocking and glove" distribution. The sensory symptoms encompass a spectrum of abnormal sensations, ranging from spontaneous or evoked paresthesia and dysesthesias to numbness, burning sensations, shooting pains, and electric shock-like discomfort [35]. Additionally, patients may experience heightened sensitivity, expressed as allodynia, where non-painful stimuli induce pain, or hyperalgesia, where there is an exaggerated response to normally painful stimuli. These multifaceted symptoms underscore the complexity of CINP and the need for targeted interventions that consider both its diverse manifestations and underlying mechanisms [36].

CINP is predominantly a sensory neuropathy that may be accompanied by motor and autonomic deficits, depending on the chemotherapy regimen. Sensory dysfunctions can generate positive symptoms as well as negative symptoms [37].

**Positive sensory symptoms can be stimulus-evoked or spontaneous and include**:

- Hyperalgesia (increased response to a normally painful stimulus)
- Allodynia (increased response to a normally non-painful stimulus), resulting from thermal and mechanical (dynamic, static, and vibration) stimuli

- Spontaneous (i.e., stimulus-independent, ongoing, or paroxysmal) shooting pain, electric shocks, or burning pain
- Dysesthesia (abnormal, unpleasant, and/or painful sensation felt in the skin)
- Paresthesia (abnormal sensation that is distracting but not generally painful)

**Negative sensory symptoms include:**

- Reduced responses to either normally non-painful or painful stimuli in the damaged nerve territory (i.e., hypoesthesia or hypoalgesia) causing a feeling of numbness
- Impaired fine motor skills (e.g., difficulties in closing buttons or holding a pen)
- Disturbance of vibratory and proprioceptive sensations

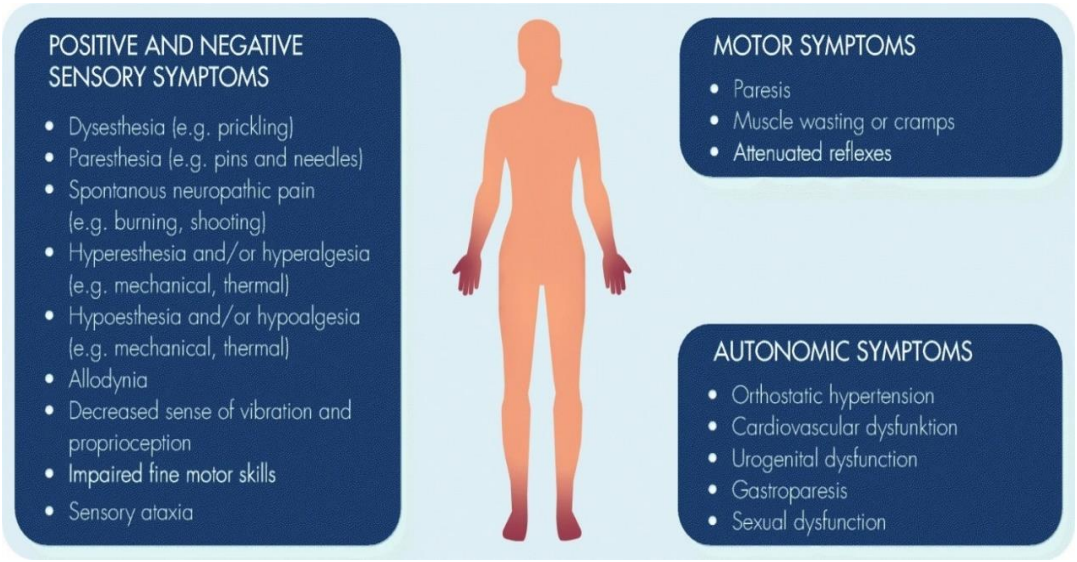


**Figure.1.2 Clinical features of CINP** [7]

## 1.2.2 Neural Basis of Chemotherapy-Induced Neuropathic Pain

Any damaging stimuli that persist for long-duration cause accumulation of cytokines and release of a variety of mediators from immune cells that participate in the neurobiology of chronic pain. In preclinical studies, the mechanism of chronic pain

can be distinguished as inflammatory and neuropathic, based on the model of induction such as complete Freund's adjuvant and nerve injury [38]. Involvement of ion channels including TRP family members, sodium channels, calcium channels etc. across the peripheral nervous system is one of the most prominent features of chronic pain neurobiology. Moreover, at central level it involves heightened transmission in ascending pain pathway and suppressed activity across the descending inhibitory pain pathway [39]. Some other common features of chronic pain pathophysiology are activation of C fibers and Aδ fibers, peripheral sensitization, and central sensitization [38,40,41]. The term sensitization defines as the lowering of threshold and enhanced response of nociceptors to supra-threshold stimuli and spontaneous activity [42]. The sensitization at peripheral or central levels is key pathophysiology of chronic pain and a brief about them is discussed below.

#### 1.2.2.1 Peripheral sensitization

Peripheral sensitization is the increased responsiveness of nociceptive neurons in the periphery due to the continuous presence of a stimulus and several signaling pathways are associated with this phenomenon [43,44]. After tissue injury release of different mediators occurs at the site including bradykinin, nerve growth factors, adenosine tri-phosphate, histamine, interleukins, etc. These mediators stimulate the ion channels present on the nociceptive terminal by direct (phosphorylation) or indirect mechanisms (prostaglandin pathway) [38,43,45]. Stimulation of nociceptors initiates the peripheral sensitization often accompanied by cytokines storm, protein phosphorylation, and ion channel activation which in chronic terms modify the gene transcription [38,40,45,46]. Activation of G protein-coupled receptors (GPCR) or serine/threonine kinases such as protein kinase A and protein kinase C occur which further activate the downstream signaling and modulate cellular activity especially the

electric impulse across the PNS. The vicious cycle of inflammation and nociceptor activation gets developed due to continuous stimulation of ion channels (e.g., TRPV1, $Na_V1.9$) and the release of inflammatory mediators. Another mechanism of peripheral sensitization is altered intracellular signaling and change in substrates for activation which is independent of altered nociceptive threshold and relies upon the heightened sensitivity and cross-interactions of pathways inside the cell.

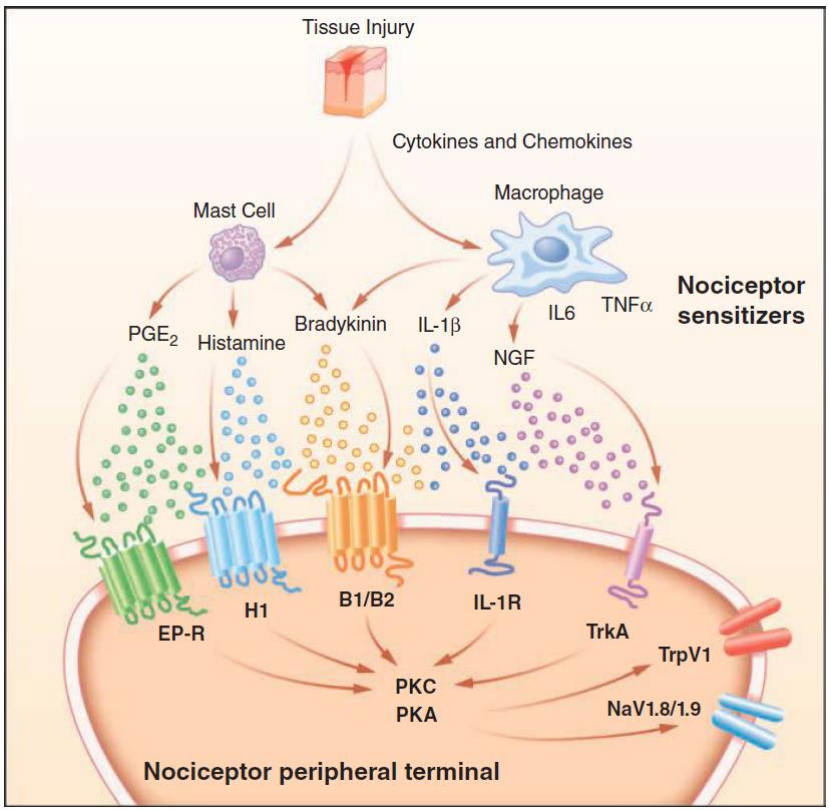


**Figure 1.3 Peripheral sensitization in CINP** [38].

### 1.2.2.2 Central sensitization

Central sensitization is a phenomenon that occurs in spinal cord and brain regions and increases pain responsiveness [47,48]. This feature of chronic pain is very unique as, unlike peripheral sensitization, it can persist without the presence of the stimulus and is adequate independently to develop hypersensitivities [38]. In presynaptic sites of the central nociceptor terminal activation of various cytokine and-

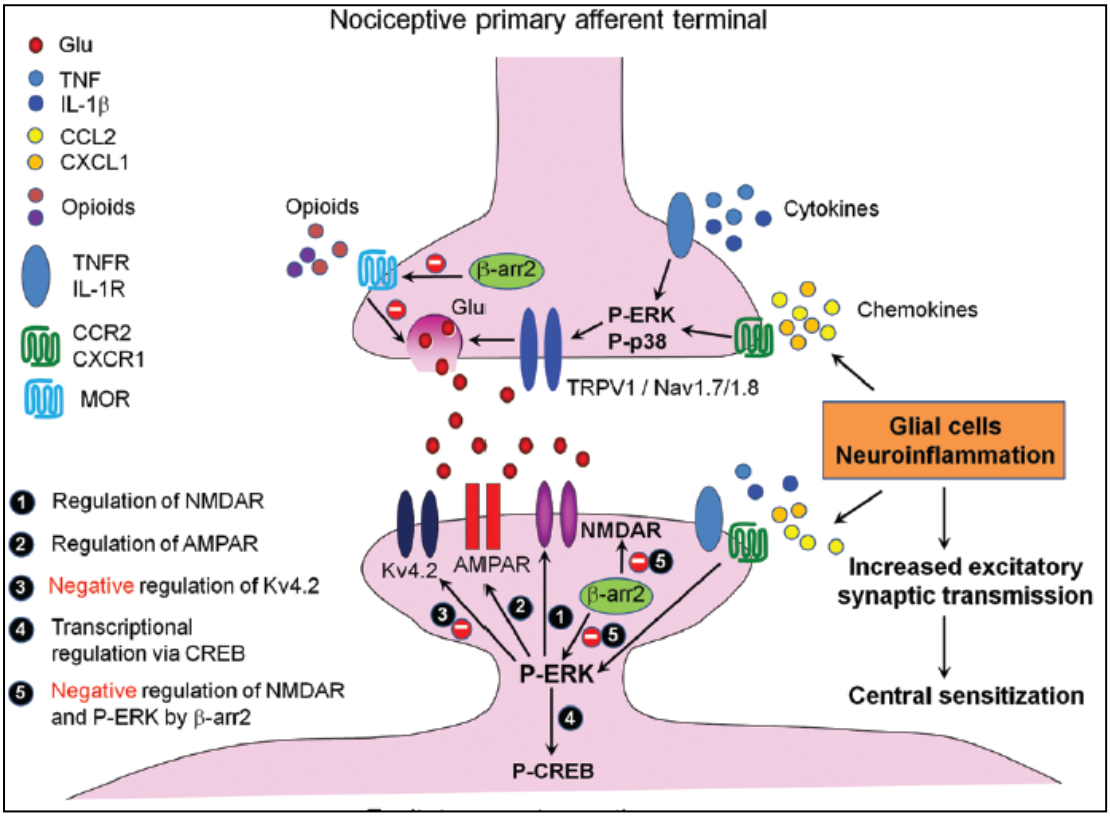


**Figure 1.4 Central sensitization in CINP.** Reprinted or adopted with permission by American Society of Anesthesiologists from source reference **[47]**.

chemokine receptors, and ion channels occur that result in the initiation of ERK and p38 MAPK pathway which in turn facilitate the release of glutamate into the synaptic cleft [47]. These series of events activate NMDA and AMPA receptors with simultaneous inhibition of potassium channels (e.g., Kv4.2 and Kv1.2), which further mediates several downstream pathways thereby inducing the nociceptive gene expression [38,49,50]. Temporal windup or summation are the terms used to define the central sensitization where repeated stimulation led to persistent pain even after the removal of stimuli. Glutamate and its receptors N-methyl-d-Aspartate (NMDA participate in the development and maintenance of central sensitization which is Ca++ impermeable under normal conditions but due to certain mediators (e.g., Nerve injury associated molecules) the Mg+ block is removed and calcium enters the cell causing

the neuronal excitability [50]. The process is maintained by activity of various enzymes such as MAPK, PKA, PKC extracellular signal-related kinase (ERK) and Src [47]. Ion channels such as Kv4.2 also promote the NMDA induced excitotoxicity as the ERK inhibits these channels which resist the neuronal homeostasis. Apart from this different cytokine, prostaglandin, BDNF, substance P also excite NMDA receptors and promotes thermal and mechanical hypersentivity. Microglia, astrocytes, axonal degeneration, immune cell infiltration, protein phosphorylation and enhanced intracellular trafficking are other key mediators of central sensitization [44,47,48,51].

## 1.3 Chemotherapeutic agents and their mechanisms

The detailed mechanisms of chemotherapy drugs, known for their strong ability to slow down the growth of tumor cells and trigger cell death, have been thoroughly studied and are widely understood. Nonetheless, the potential deleterious effects on non-proliferating sensory neurons, distinct pharmacological contributions, and the genesis of CINP remain less understood [52]. While peripheral neuropathy is a well-acknowledged side effect of chemotherapy drugs, it doesn't occur universally with all such medications, suggesting the involvement of additional contributing processes. Notably, oxaliplatin and cisplatin both trigger CINP, although with different sets of symptoms. In contrast, carboplatin mainly affects the blood-forming system and typically doesn't lead to CINP. Within the vinca alkaloid class, the likelihood of inducing CINP varies, with vincristine having a higher chance compared to vinblastine, vinflunine, and vinorelbine [53]. Similarly, taxanes contribute to the nuanced variability in CINP. Specific taxane drugs, such as paclitaxel and docetaxel, exhibit distinct patterns in causing neuropathic pain, further emphasizing the need for a more profound

understanding of the unique molecular and cellular factors that govern these outcomes [54]. Despite the common pathogenic mechanisms, neuropathy induced by paclitaxel or vincristine involves a strong inflammatory component that seems to be less severe during oxaliplatin-induced CINP. This inflammatory component results from the activation of microglia, astrocytes and satellite glial cells in the dorsal horn of the spinal cord, which leads to the production and release of proalgesic mediators, such as tumour necrosis factor (TNF) and IL- 1β [55–57]. Paclitaxel, oxaliplatin and vincristine have toxic effects on the DRG neurons [58]. These substances increase activity of both voltage-gated and ligand-gated ion channels—such as voltage-dependent sodium channels (Nav), voltage-dependent calcium channels (Cav), and transient receptor potential (TRP) channels—and the production of reactive oxygen species (ROS), which can disrupt the mitochondrial electron transport chain and may alter ATP production in sensory neurons [59–62].

While each drug may have its unique pathways influencing peripheral nerve damage, there are commonalities in the overarching processes leading to neuropathic pain. The following figure provides a schematic representation of the diverse underlying mechanisms involved in the development of CINP, offering a visual insight into the intricate processes at play.

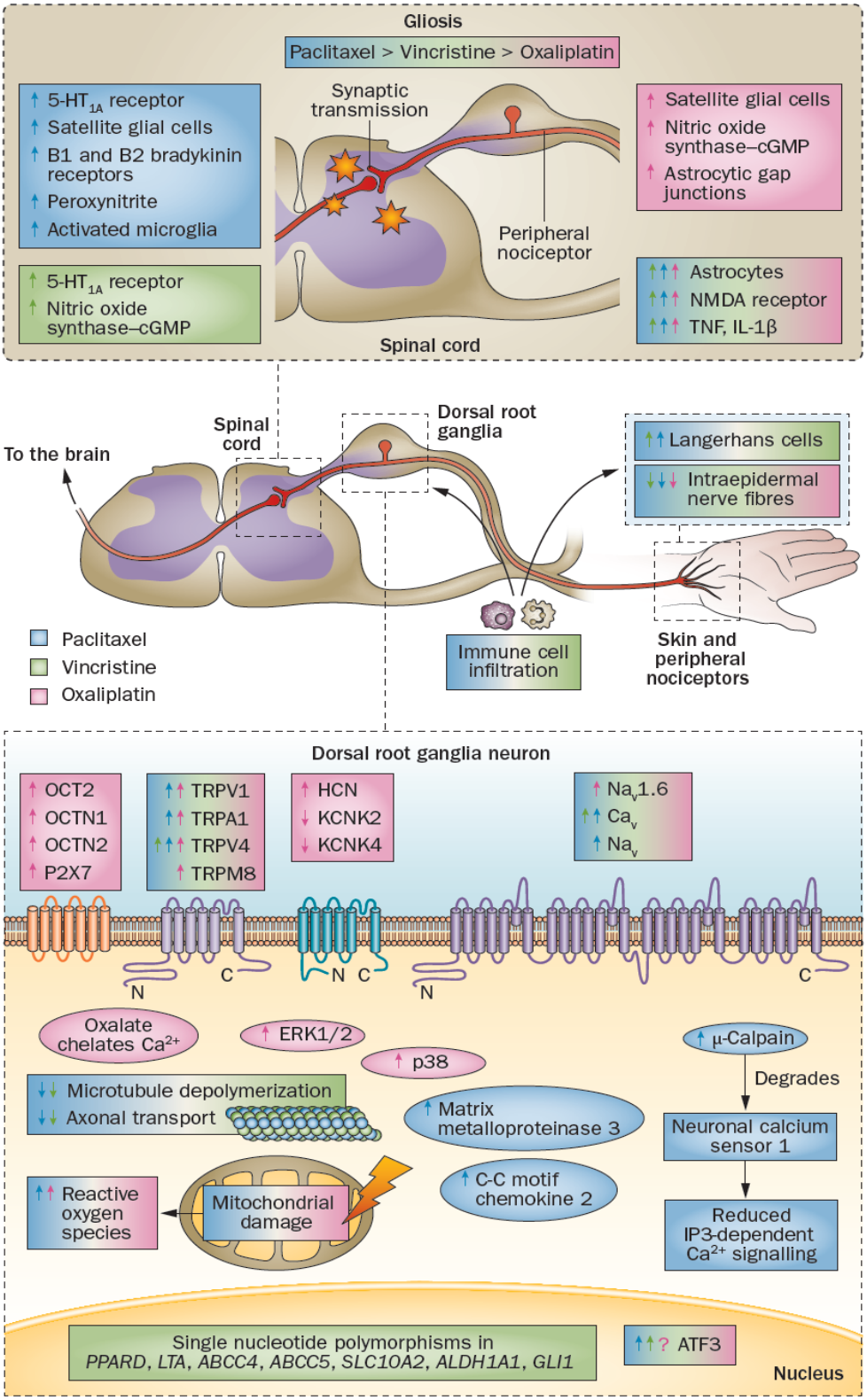


**Figure.1.5 Mechanisms of chemotherapy-induced neuropathic pain.** Pathophysiological alterations triggered by paclitaxel (blue), vincristine (green) and oxaliplatin (pink) in the spinal cord, dorsal root ganglia, and PNS and skin. Reprinted with permission from source reference [63].

### 1.3.1 Platinum- induced neuropathic pain

Platinum-based chemotherapeutic agents, including oxaliplatin, cisplatin, and carboplatin, are widely used to treat various solid tumors but are known to cause significant neurotoxicity, particularly chemotherapy-induced peripheral neuropathy (CIPN) [64]. Oxaliplatin, in particular, is associated with unique acute cold-induced neuropathy, likely due to its rapid transformation into reactive platinum complexes and oxalate, which has been implicated in this neuropathic response. Studies in laboratory animals have shown that both oxaliplatin and its oxalate-free analogue induce peripheral sensory neuropathy, with oxalate contributing to a delayed decrease in mechanical threshold [65,66].

The neurotoxicity of platinum agents is primarily initiated by the accumulation of platinum adducts in dorsal root ganglion (DRG) and trigeminal ganglion (TG) neurons [67–69]. This accumulation is thought to be the key mechanism driving neurotoxicity. Research indicates that oxaliplatin transporters, such as Octn1 and Mate1, are involved in platinum accumulation in DRG neurons, which correlates with the severity of neuropathic behavior [70].

While the exact mechanisms of platinum-induced neurotoxicity in humans remain under investigation, preclinical studies have provided valuable insights into the processes likely involved in the pathogenesis of CIPN. These mechanisms include disruptions in neuronal and glial cell function, neuroinflammation, DNA damage, and axonal degeneration, all of which are linked to the antitumor activity of these chemotherapeutic agents [71–73].

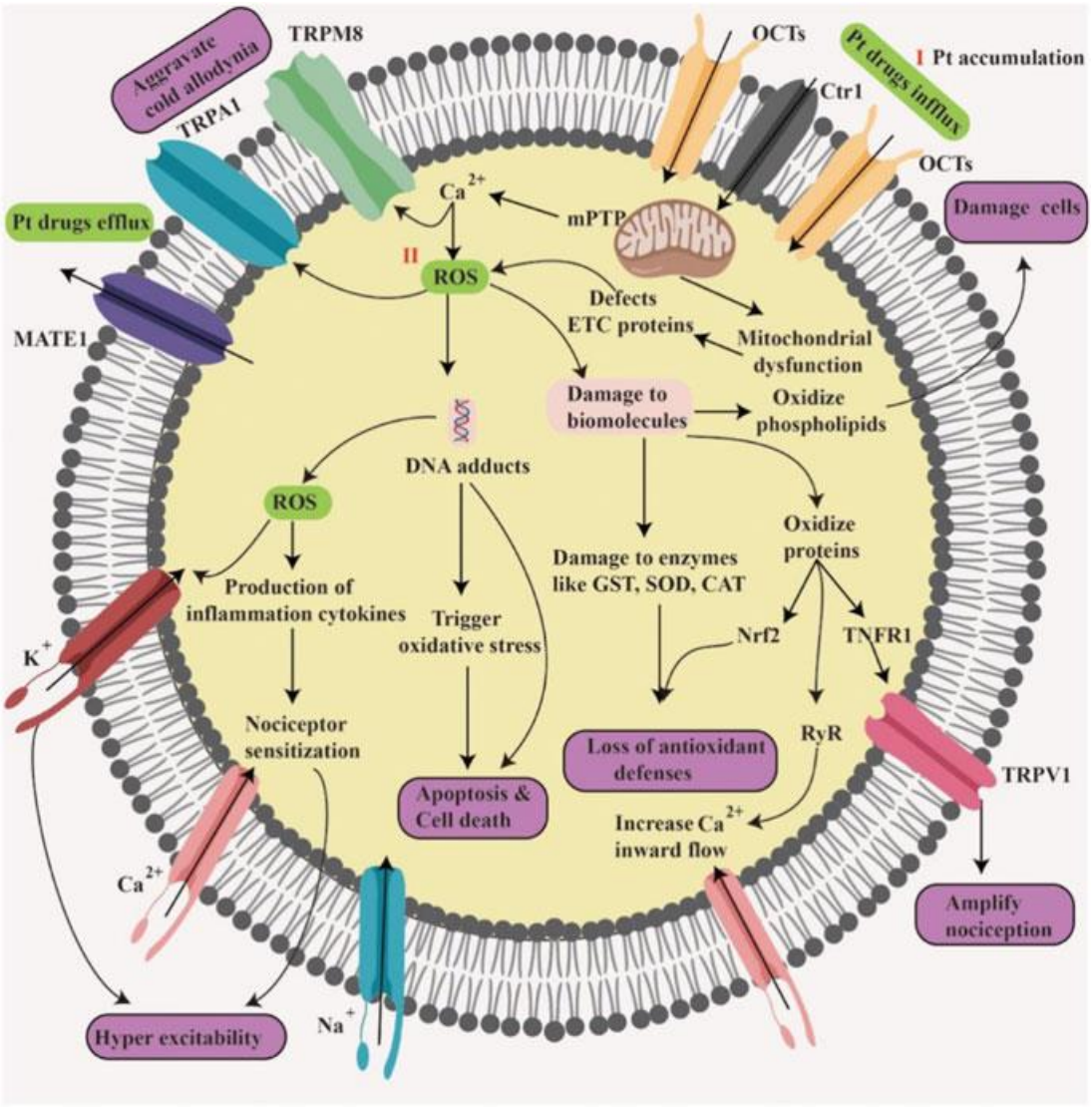


**Figure 1.6 The mechanisms of chemotherapy-induced peripheral neuropathy (CIPN) induced by platinum-based drugs** [74]

## 1.3.2 Epothilones induced neuropathic pain

Epothilones, including ixabepilone and sagopilone, are newer antineoplastic drugs that act similarly to taxanes by targeting tubulin to prevent cancer cell division. Ixabepilone is FDA-approved for breast cancer treatment in the US, while sagopilone is used for various cancers without FDA approval [75,76]. A common side effect of these drugs is chemotherapy-induced peripheral neuropathy (CIPN), with ixabepilone

showing a prevalence of around 67%, particularly in patients previously treated with other chemotherapies [77].

The underlying mechanisms of epothilone-induced CIPN involve microtubule disruption, leading to impaired axonal transport and Wallerian degeneration, which in turn causes altered ion channel activity and peripheral neuron hyperexcitability. Additionally, epothilones damage mitochondria, increasing the production of reactive oxygen species (ROS). This oxidative stress damages enzymes, proteins, and lipids within neurons, triggering apoptotic changes and further altering neuronal excitability [78]. ROS also attract and activate immune cells, such as T-lymphocytes and monocytes, leading to the release of pro-inflammatory cytokines and the development of neuroinflammation [35]. While epothilone-induced CIPN is generally less severe than that caused by taxanes, more research is needed to fully understand these mechanisms.

### 1.3.3 Thalidomide-induced peripheral neuropathy

Thalidomide, an immunomodulatory drug used to treat multiple myeloma, exerts anticancer effects primarily through its antiangiogenic properties and inhibition of tumor necrosis factor alpha (TNF-α) and NF-kB, which lead to neuronal cell death [79]. However, thalidomide-induced peripheral neuropathy (TIPN) affects 25–75% of patients, with severity increasing in a dose-dependent manner. TIPN typically presents with sensory symptoms, motor impairment, and autonomic dysfunction, and in severe cases, it may necessitate treatment discontinuation. Risk factors include advanced age and prior neuropathy [80].

The pathophysiology of TIPN is not fully understood but likely involves multiple mechanisms. Thalidomide's inhibition of TNF-α and NF-kB disrupts neurotrophin regulation, accelerating neuronal cell death [81]. Additionally, its antiangiogenic effects can lead to secondary ischemia and hypoxia in nerve fibers, causing irreversible damage to sensory neurons. There is also evidence that thalidomide's dihydroxy metabolite may trigger the release of reactive oxygen species (ROS) and DNA cleavage, contributing to neurotoxicity, though more research is needed to confirm this mechanism [82].

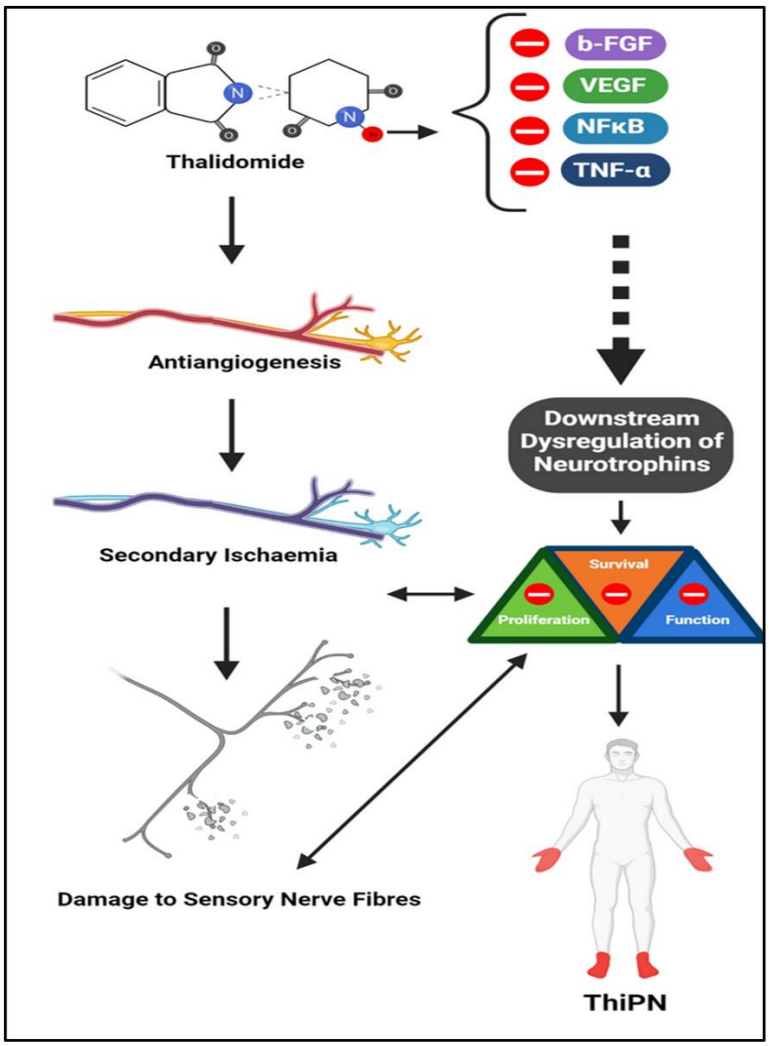


**Figure 1.7 The mechanisms of peripheral neuropathy induced by Thalidomide** [83]

### 1.3.4 Vinca alkaloids induced neuropathic pain

Vinca alkaloids, derived from the Madagascar periwinkle plant, include vincristine, vinblastine, vinorelbine, and vindesine. These drugs are widely used to treat various cancers, including Hodgkin and non-Hodgkin lymphoma, testicular cancer, and non-small cell lung cancer. They work by inhibiting microtubule assembly and promoting their disassembly, which disrupts axonal transport in peripheral nerves [84]. Vincristine is the most neurotoxic of this group, with neurotoxicity becoming evident at cumulative doses as low as 4 mg/m². Symptoms of vincristine-induced peripheral neuropathy (VIPN) typically begin with distal numbness, tingling, and pain in the hands and feet, progressing to muscle weakness and cramping [85]. The neuropathy affects both motor and sensory fibers and can also involve autonomic fibers, leading to a range of sensory and motor deficits.

The neurotoxic effects of vinca alkaloids are primarily due to their binding to tubulin, which blocks microtubule formation and disrupts axonal transport. This leads to distal axonopathy, cytoskeletal disorganization, and Wallerian degeneration [86]. Additionally, the drugs induce neuroinflammation by activating immune cells and increasing the release of pro-inflammatory cytokines. Genetic factors, such as Charcot-Marie-Tooth disease and polymorphisms in the CEP72 gene, can increase the severity of VIPN [87,88]. Preventive measures, such as the use of tropisetron and targeting genes like WldS and SARM1, have shown promise in reducing neurotoxicity in experimental models [89].

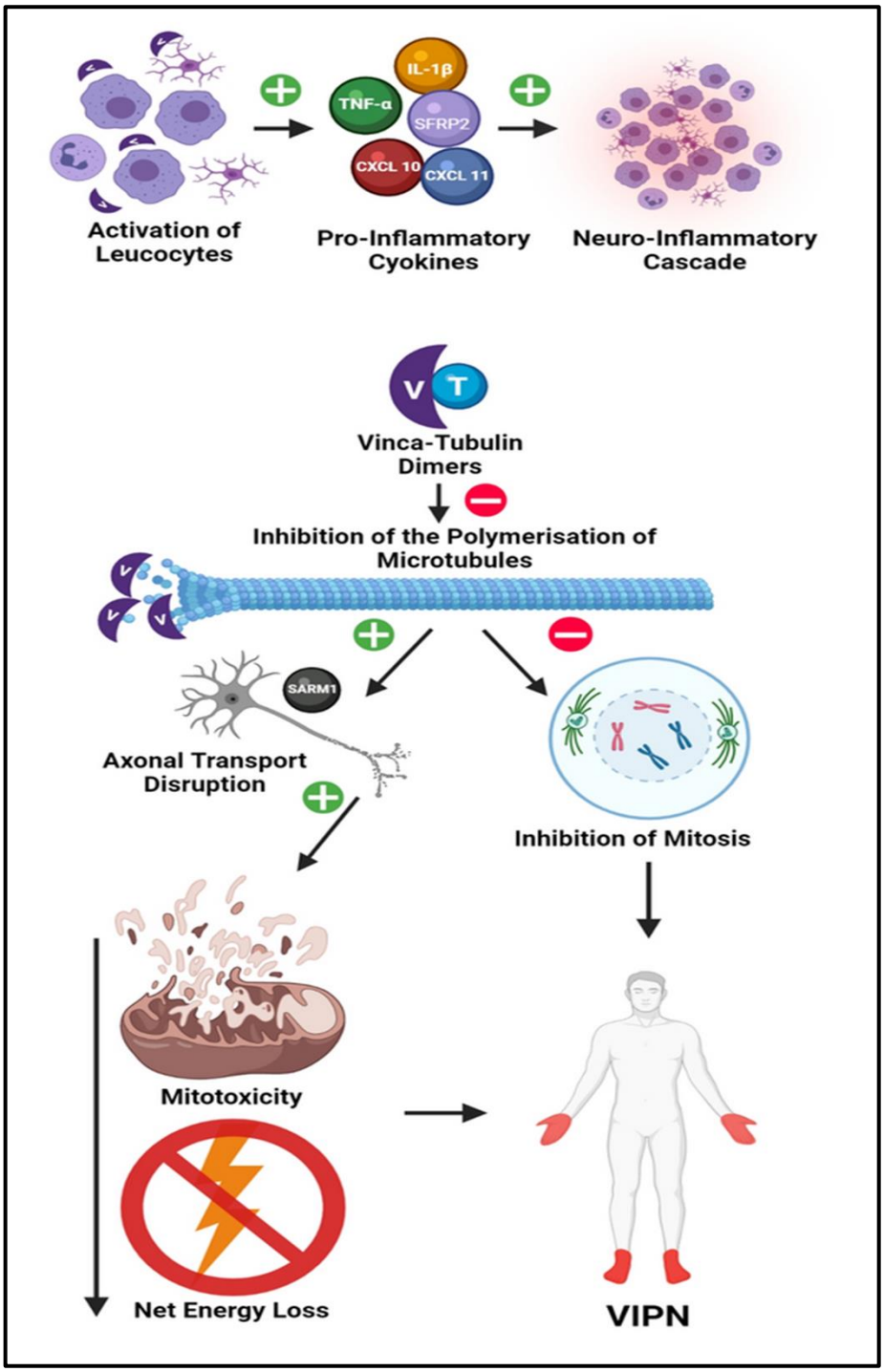


**Figure 1.8 The mechanisms of peripheral neuropathy induced by Vinca alkaloids** [83]

### 1.3.5 Protease Inhibitors induced neuropathic pain

Bortezomib and carfilzomib are proteasome inhibitors used to treat multiple myeloma and some lymphomas, but they are associated with significant neurotoxicity [90]. Approximately 34% of patients experience neuropathy, which is often painful and can be accompanied by muscle weakness and demyelinating neuropathy [91]. The neuropathy is typically dose-dependent and may persist for weeks, months, or even years after treatment cessation [35]. Subcutaneous bortezomib and oral ixazomib may have lower neuropathy incidence.

Bortezomib-induced neuropathy is linked to increased sphingolipid metabolism in astrocytes, leading to elevated levels of ceramide, sphingosine-1 phosphate (S1P), and dihydrosphingosine-1-phosphate (DH-S1P) [92–94]. These metabolites contribute to neuropathic pain by enhancing presynaptic glutamate release in the spinal cord. Additionally, bortezomib causes mitochondrial damage, increasing reactive oxygen species (ROS) production, which further impairs neuronal function and induces neuroinflammation [95].

Genetic factors, including SNPs in genes like PKNOX1 and CBS, and low vitamin D levels may influence the severity of neuropathy [96]. Targeting sphingolipid metabolism and ROS-related pathways could be potential strategies for mitigating bortezomib-induced neuropathic pain.

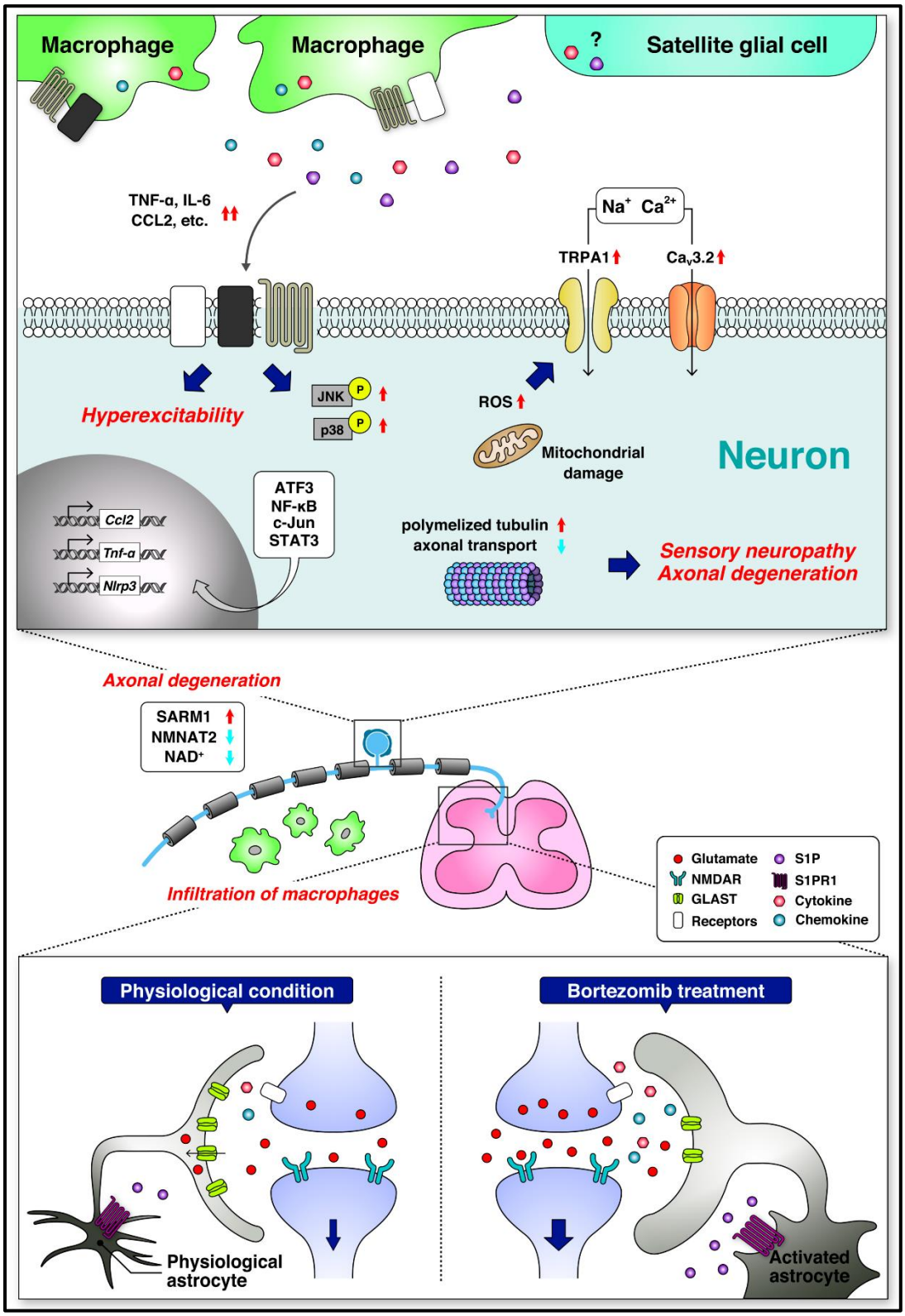


**Figure. 1.9 The mechanisms of peripheral neuropathy induced by bortezomib** [97]

### 1.3.6 Paclitaxel Induced Neuropathic Pain

Originally derived from the Pacific yew tree (*Taxus brevifolia*), paclitaxel has become a widely utilized and potent antineoplastic drug [98]. Paclitaxel is known to induce a distal sensory neuropathy and, in some individuals, a syndrome of subacute aches and pains referred to as paclitaxel-associated acute pain syndrome (P-APS). Notably, in P-APS, the intensity of pain reaches its highest point approximately 3- 4 days following the administration of the drug [99]. Paclitaxel exerts its impact on neurons by influencing both their excitability and survival through a multitude of mechanisms.

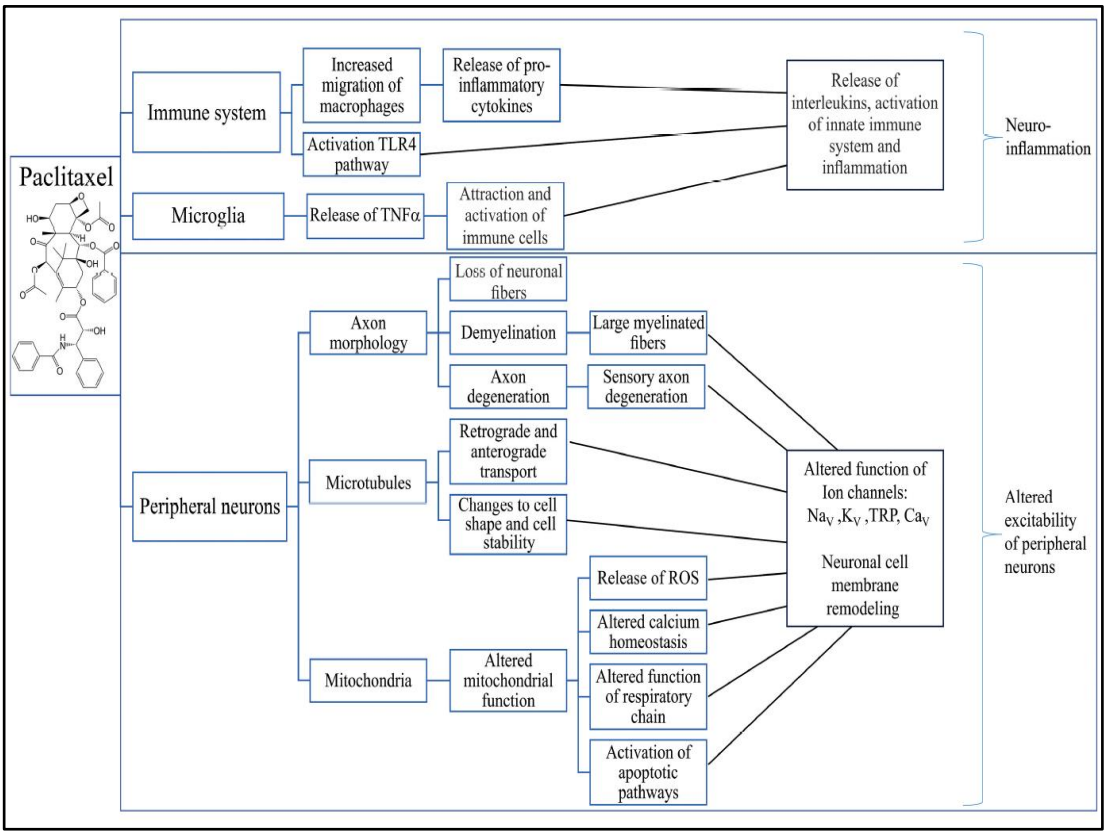


**Figure. 1.10 Putative mechanisms involved in the development of paclitaxel-induced peripheral neuropathy** [100]

### 1.3.6.1 Axonal transport disruption

In sensory neurons, axonal transport is a critical process that relies heavily on the dynamic and flexible adjustment of the cytoskeleton, particularly the microtubule network [101]. Microtubules, composed of tubulin subunits, are essential for the transport of organelles, proteins, and other cellular components along the axon [102]. This transport is vital for maintaining the structural integrity and functional connectivity of neurons, especially in the long axons of sensory neurons [103].

Paclitaxel, a widely used chemotherapeutic agent, is known for its role as a tubulin-stabilizing agent. It binds to the β-tubulin subunits within microtubules and stabilizes them by preventing their depolymerization, which is a necessary step for the dynamic remodeling of the cytoskeleton during normal cellular processes [104,105]. While this stabilization is beneficial in halting the rapid division of cancer cells, it has detrimental effects on neurons, particularly in the peripheral nervous system [19]. The stabilization of microtubules by paclitaxel impairs the normal dynamics required for efficient axonal transport [106]. One of the most severe consequences of impaired axonal transport is axonal degeneration [107,108]. The interruption of material flow along the axon, particularly the transport of mitochondria, which are essential for energy production and calcium buffering, leads to localized energy deficits and increased oxidative stress. This can trigger axonopathy, a pathological condition characterized by the degeneration of axons. [109,110]. Axonopathy manifests as the progressive loss of axonal integrity, which eventually results in the fragmentation of axons and the retraction of nerve endings. In sensory neurons, this process leads to a reduction in the density of epidermal nerve fibers, known as loss of epidermal

innervation [111]. This loss is directly correlated with the sensory symptoms experienced by patients, such as numbness, tingling, and pain, particularly in the extremities resulting in CINP.

### 1.3.6.2 Mitochondrial dysfunction

Mitochondria are essential organelles responsible for energy production, calcium homeostasis, and the regulation of apoptotic pathways within neurons. In both C-fibers and myelinated axons, paclitaxel induces significant changes in mitochondrial structure [112]. These changes include swelling and abnormal morphology, such as the enlargement of the mitochondrial matrix and disruption of the cristae, the inner membrane folds critical for electron transport and ATP synthesis [113]. The swelling of mitochondria and the associated structural abnormalities are likely attributed to the opening of the mitochondrial permeability transition pore (mPTP), a large, non-specific channel that spans the inner mitochondrial membrane . Upon exposure to paclitaxel, the mPTP opens, leading to the loss of mitochondrial membrane potential, disruption of ATP synthesis, and the efflux of calcium ions ($Ca^{2+}$) from the mitochondria into the cytosol. The opening of the mPTP is a well-known contributor to mitochondrial dysfunction and is particularly harmful in neurons, where calcium homeostasis is crucial for normal function [112,114]. In rat models of paclitaxel-induced neuropathic pain, the efflux of $Ca^{2+}$ from mitochondria has been demonstrated to play a significant role in neuronal damage. Calcium ions are vital secondary messengers in many cellular processes, including neurotransmitter release, synaptic plasticity, and activation of enzymatic pathways. However, dysregulation of calcium levels, as seen with mPTP opening, leads to a cascade of deleterious effects, including the activation of calcium-dependent proteases, increased production of reactive oxygen species (ROS), and

initiation of apoptotic pathways. The abnormal release of $Ca^{2+}$ from mitochondria not only disrupts cellular signaling but also exacerbates oxidative stress and contributes to the progressive degeneration of sensory neurons [67,114]. This mitochondrial dysfunction is a critical factor in the development and persistence of paclitaxel-induced neuropathic pain [15,115]. Further compounding the neurotoxic effects of paclitaxel is its impact on the mitochondrial respiratory chain, the series of protein complexes responsible for oxidative phosphorylation and ATP production. An in vitro study involving the rat sciatic nerve has shown that paclitaxel treatment diminishes the activity of mitochondrial respiratory chain complexes I and II [116,117]. Mitochondrial dysfunction, characterized by structural abnormalities, dysregulated calcium homeostasis, and impaired respiratory chain activity, plays a central role in the pathogenesis of paclitaxel induced neuropathy. The resulting energy deficits, increased oxidative stress, and activation of apoptotic pathways contribute to the degeneration of sensory neurons leading to the persistent pain experienced by CINP patients [118].

#### 1.3.6.3 Increased ion channel activity

The pathogenesis of paclitaxel-induced neuropathy is multifaceted, with the dysregulation of ion channels being one of the most significant contributors to the development and maintenance of neuropathic pain [59,119–121].

- **Voltage-Gated Sodium Channels (VGSCs):** Voltage-gated sodium channels (Nav) are crucial for the initiation and propagation of action potentials in neurons. In normal physiological conditions, Nav channels open in response to membrane depolarization, allowing sodium ions ($Na^+$) to enter the neuron and generate an action potential. However, in the context of CIPN, paclitaxel alters

the expression and functional dynamics of these channels, leading to hyperexcitability of sensory neurons [122]. In rodent models of paclitaxel-induced neuropathy, there is evidence of increased expression and activity of specific Nav channel subtypes, particularly Nav1.7, Nav1.8, and Nav1.9, which are predominantly expressed in peripheral sensory neurons [123,124]. The heightened activity of these channels contributes to the generation of ectopic action potentials, leading to spontaneous pain, allodynia, and hyperalgesia [125]. The involvement of Nav channels in paclitaxel-induced neuropathy has been further confirmed by pharmacological studies. The administration of tetrodotoxin (TTX), a potent inhibitor of voltage-gated sodium channels, has been shown to reduce neuropathic pain in rodent models of CIPN [126]. TTX-sensitive channels, particularly Nav1.7, have been implicated in the pathophysiology of paclitaxel-induced neuropathy, suggesting that targeting these channels could be a potential therapeutic strategy for managing CIPN.

- **Voltage-Gated Potassium Channels (Kv)**: They play a critical role in repolarizing the neuronal membrane following an action potential, thereby regulating neuronal excitability. Kv channels are responsible for the outward flow of potassium ions (K+), which helps to restore the resting membrane potential and terminate the action potential [127]. In CIPN, including paclitaxel-induced neuropathy, there is often a downregulation or functional impairment of Kv channels, particularly Kv1.2 and Kv7.2/7.3 (KCNQ channels) [128,129]. The reduced activity of these channels leads to prolonged depolarization and increased excitability of sensory neurons, contributing to the development of neuropathic pain [130]. The loss of Kv channel function disrupts the balance of

ion flux across the neuronal membrane, making it easier for neurons to fire repetitively, which is associated with spontaneous pain and heightened pain sensitivity.

- **Voltage-Gated Calcium Channels (VGCCs):** Voltage-gated calcium channels (Cav) are essential for the release of neurotransmitters at synaptic terminals and for various intracellular signaling pathways [121]. Cav channels, particularly Cav2.2 & Cav3.2, are highly expressed in dorsal root ganglion (DRG) neurons and play a pivotal role in pain transmission [131]. Paclitaxel treatment has been shown to increase the expression and activity of Cav channels in sensory neurons, leading to enhanced calcium influx [128,132]. This increased calcium entry can activate intracellular signaling cascades that promote the release of excitatory neurotransmitters, such as glutamate and substance P, in the spinal cord, thereby amplifying pain signals [133]. The dysregulation of Cav channels in paclitaxel-induced neuropathy contributes to the overall hyperexcitability of the pain pathway [134]. Pharmacological blockade of Cav channels, particularly with selective inhibitors like ziconotide (a Cav2.2 channel blocker), has been demonstrated to alleviate neuropathic pain in preclinical models of CIPN [135]. This highlights the potential of targeting Cav channels as a therapeutic approach for managing paclitaxel-induced neuropathy.
- **Transient Receptor Potential (TRP) Channels:** The transient receptor potential (TRP) family of ion channels, including TRPV1, TRPA1, and TRPM8, are non-selective cation channels that are activated by various physical and chemical stimuli, including temperature, mechanical stress, and inflammatory mediators [136]. TRP channels are involved in the detection of

noxious stimuli and play a crucial role in pain sensation. In paclitaxel-induced neuropathy, TRP channels are upregulated and sensitized, leading to increased responsiveness to painful stimuli. For instance, TRPV1, also known as the capsaicin receptor, is a well-known mediator of heat pain and is sensitized by chemotherapeutic agents, contributing to heat hyperalgesia in CIPN [120,137]. Similarly, TRPA1 & TRPM8, which is activated by cold and chemical irritants, is implicated in the cold allodynia observed in paclitaxel-treated animals [121,138]. The involvement of TRP channels in CIPN is supported by studies demonstrating that pharmacological inhibition of these channels can reduce neuropathic pain [59]. For example, blocking TRPV1 or TRPA1 channels has been shown to attenuate mechanical allodynia and thermal hyperalgesia in rodent models of paclitaxel-induced neuropathy. This mechanism is further supported by studies showing that inhibiting transient-opening (T-type) CaV channels with ethosuximide led to a decrease in PINP in rats [139,140]. Selective inhibition of TRPV1 with capsazepine, TRPA1 with HC-030031, or TRPV4 with HC-067047 has demonstrated a reduction in PINP, particularly alleviating mechanical allodynia in rodent models [25].

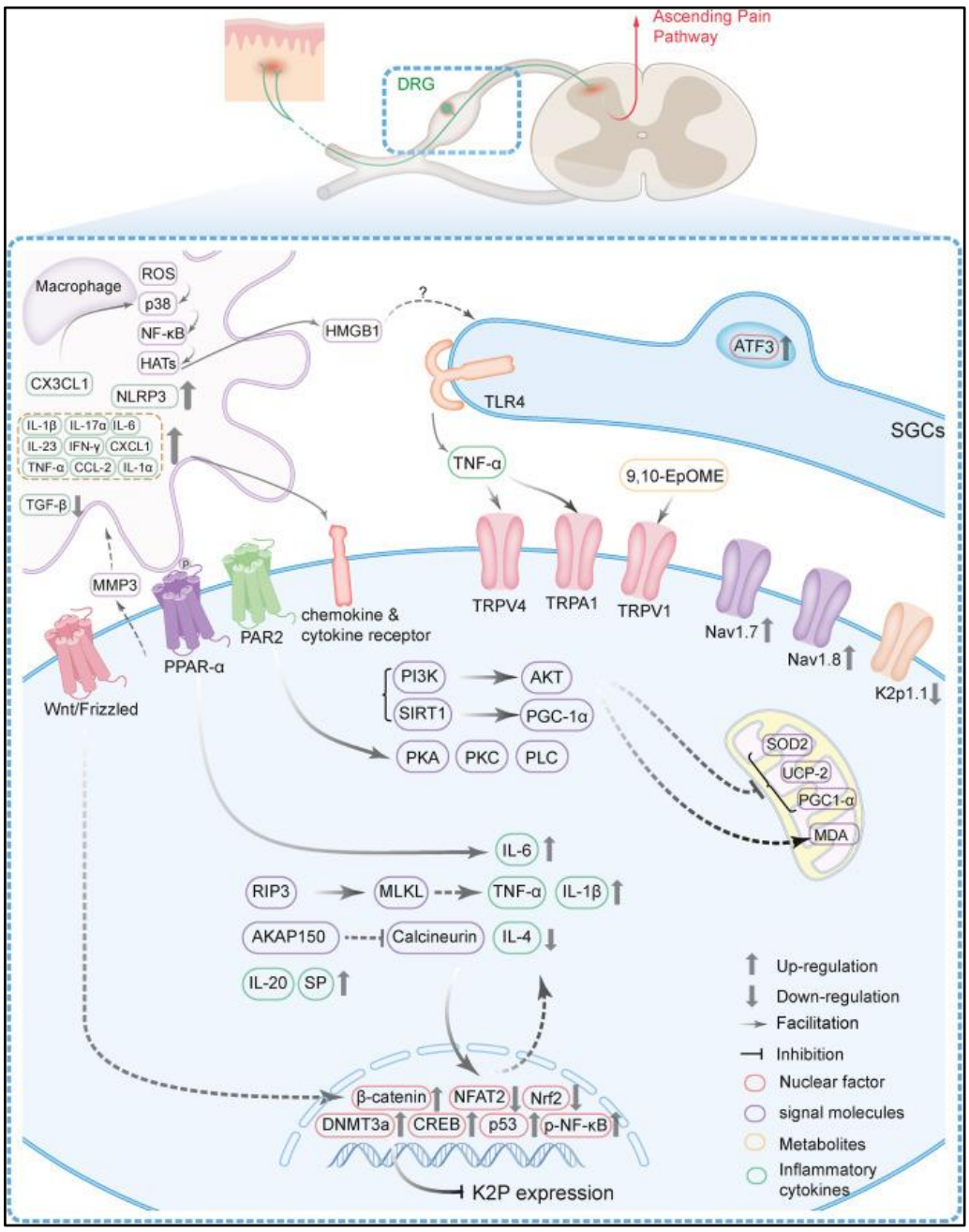


**Figure. 1.11 The mechanism of paclitaxel-induced pain on a peripheral level** [141]

### 1.3.6.4 Role of NMDA receptor system in the neurobiology of CINP

NMDA receptors are ionotropic, excitatory, heteromeric glutamate receptors. In the CNS of mammals, fast synaptic transmission is mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors and kainite receptors while the

slow synaptic transmission is mediated by NMDA receptors. NMDA receptors have been widely implicated in the transmission of both nociceptive and neuropathic pain [146]. The NMDA receptors are abundantly distributed in the pain pathway and are responsible for synaptic plasticity corresponding to chronic pain [147]. NR2B subunit is very essential for making the NMDA receptors functional after getting assembled into a working system. This subunit is highly distinct from other subunits of NMDARs as it consists of a larger intracellular c-terminal tail. The NR2B subunit of the NMDA receptor system is highly expressed across the descending and ascending pain pathways and several animal models of chronic pain have demonstrated a strong reproducibility to this pipeline. A recent study has reported that the NR2B subunit phosphorylation regulates synaptic plasticity under migraine condition [148]. Furthermore, the animal model of peripheral nerve injury, cancer pain, and chemotherapy-induced neuropathic pain have also suggested the critical involvement of this subunit in the progression of chronic pain [149,150]. This highlights the potential of targeting NR2B as a therapeutic approach for managing paclitaxel-induced neuropathy.

#### 1.3.6.5 Neuronal injury and inflammation

Neuroinflammation emerges as a critical contributor to the pathogenesis of PINP. Following paclitaxel administration, a cascade of inflammatory events emerges within the peripheral nerves. Pro-inflammatory cytokines, notably tumor necrosis factor-alpha (TNF-α) and interleukins, are released, creating a microenvironment that sensitizes nerves and amplifies pain signaling [142,143]. Immune cells, particularly macrophages, are recruited to the site of injury, intensifying the inflammatory response. Their activation leads to the release of additional inflammatory mediators, perpetuating the neuroinflammatory cascade. Concurrently, glial cells, such as microglia in the central

nervous system (CNS) and satellite glial cells in the peripheral nervous system, become activated [144]. This glial cell activation further contributes to the release of pro-inflammatory substances, exacerbating the overall inflammatory milieu in the nerve tissue [145]. Paclitaxel treatment further increases production of peroxynitrite by Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase in the spinal cord, indicating that mitochondrial dysfunction and subsequent reactive oxygen species (ROS) production in sensory neurons or glial cells contributes to further progression of neuropathy [54].

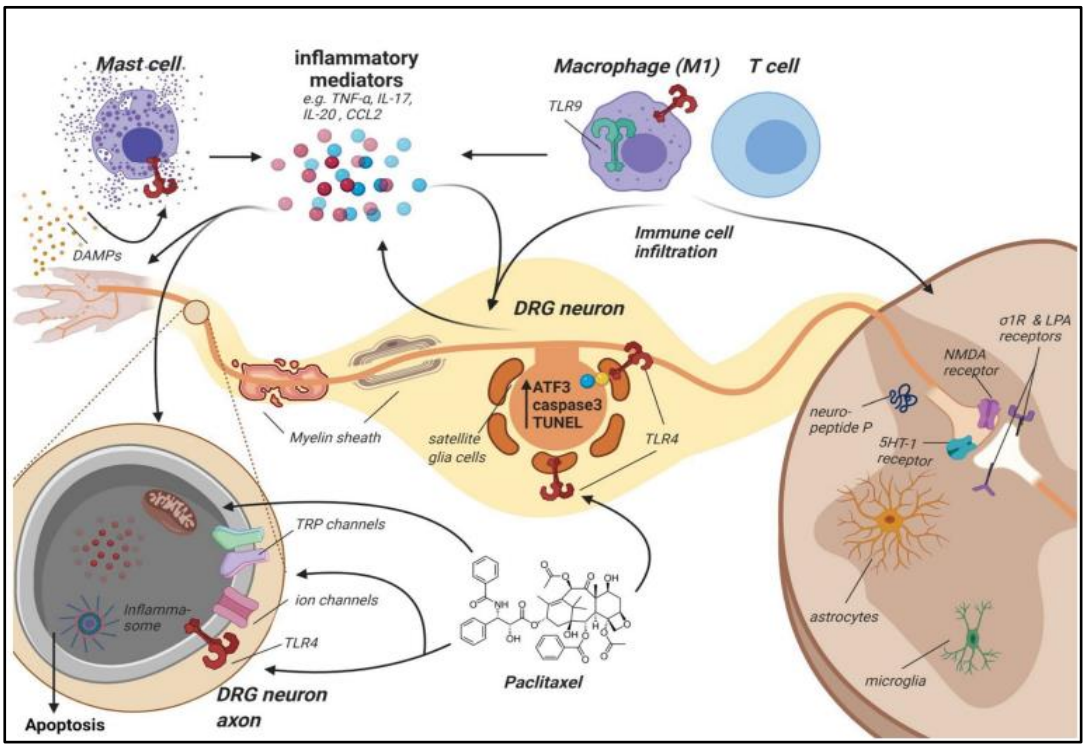


**Figure. 1.12 Overview of dorsal root ganglion and spinal cord to indicate the various molecular mechanisms that contribute to paclitaxel-induced peripheral neuropathy** [144]**.**

## 1.4 Therapeutics for the treatment of CINP and their limitations

### 1.4.1 Preventive Measures for Minimizing Functional Impairment in CINP

Despite extensive clinical studies exploring the efficacy of various compounds, including anticonvulsants, antidepressants, vitamins, minerals, and chemoprotectants, there is currently no robust evidence supporting their consistent and clinically meaningful benefits in preventing CINP [151,152]. The absence of established medical prophylaxis underscores the complexity of managing CIPN. Nevertheless, dose adjustment algorithms have shown promise in reducing the severity of higher-grade CINP while preserving treatment efficacy in individual patients. Additionally, some literature suggests the potential benefits of extremity cooling during the administration of specific chemotherapeutic agents, such as taxanes, as a strategy to mitigate the severity of CIPN when its development or worsening is anticipated [153,154].

In the absence of a well-established medical prophylaxis, implementing regular functional exercises, including mobility, sensorimotor, and vibration training, upon initiation of neurotoxic anticancer therapies is recommended [155,156]. This aligns with a growing body of literature suggesting that physical exercise may help alleviate CIPN-induced symptoms and functional impairment [157]. Overall, a multifaceted approach involving dose adjustments, cooling strategies, and physical exercises may offer avenues to address CIPN-related challenges.

## 1.4.2 Pharmacological interventions

The pharmacological management of CINP involves a diverse array of drug classes, each targeting specific aspects of the neuropathic pain pathway. Established neuropathic pain treatments include TCAs, SNRIs, anticonvulsants, and opioid agonists.

### 1.4.2.1 Selective serotonin-noradrenalin reuptake inhibitors

The involvement of both serotonergic and noradrenergic signaling in CINP makes SNRIs attractive candidates for its treatment. Notably, promising outcomes have been reported with venlafaxine and duloxetine in alleviating CINP. Studies indicate that venlafaxine demonstrated efficacy in reducing oxaliplatin-induced NP in a small randomized controlled trial (RCT) [158,159]. A more extensive RCT involving 231 participants showed that duloxetine effectively ameliorated CINP, representing the only phase III trial with over 100 treated patients demonstrating such improvement [160].

Interestingly, when patient groups were categorized based on the chemotherapeutic agent they received, duloxetine exhibited greater efficacy in ameliorating oxaliplatin-induced CINP compared to taxane-induced CINP. The varying mechanisms underlying CINP induced by oxaliplatin and taxane might explain this observation. Duloxetine's impact on glial activation and cytokine release in the spinal cord is limited; instead, it appears to balance neurotransmitter concentrations, alleviating the pathophysiological neuronal effects of oxaliplatin. Notably, oxaliplatin-induced CINP involves a more pronounced component of neuronal damage, while

taxane-induced CINP is characterized by a strong inflammatory component in the dorsal root ganglia (DRG) and spinal cord.

The differences in the efficacy of duloxetine and amitriptyline in reducing CINP could stem from their distinct affinities for noradrenaline transporters, leading to diverse concentrations of synaptic noradrenaline. Moreover, the additional effects of amitriptyline on muscarinic, histaminergic, and adrenergic receptors may contribute to its differential impact [161].

#### 1.4.2.2 Tricyclic antidepressants

Despite the proven efficacy of TCAs in managing various types of painful neuropathy and polyneuropathy, the evidence supporting their effectiveness in alleviating CINP is limited. Nortriptyline, a TCA, was found to be ineffective in reducing pain associated with cisplatin-induced neuropathic pain [162]. Similarly, amitriptyline, a leading compound in the TCA class, did not show a significant difference from a placebo in ameliorating neuropathic pain induced by taxane, platinum, or vinca alkaloids in double-blind randomized controlled trials (RCTs). Despite these findings, considering the limited options available for CINP therapy and the established efficacy of TCAs in managing other neuropathic pain conditions, there is a rationale for exploring TCAs as a potential treatment option. However, the use of TCAs should be approached with caution, taking into consideration potential side effects, drug interactions, and the risk of cardiac toxicity. A thorough risk-benefit analysis is essential before considering TCAs as part of the treatment regimen for CIPN

### 1.4.2.3 Anticonvulsants:

Gabapentin and pregabalin have exhibited efficacy in addressing polyneuropathies through their modulation of voltage-gated calcium channels (VGCCs), leading to a reduction in neuronal excitability. However, the scientific evidence supporting their application specifically for CIPN is presently limited. In comparison, pregabalin was found to be superior to amitriptyline, gabapentin, and placebo in a randomized cancer study, particularly in addressing neuropathic pain and minimizing side effects [163]. Common adverse reactions to both pregabalin and gabapentin include dizziness, drowsiness, and somnolence, with pregabalin more frequently associated with weight gain. Despite the limited scientific evidence for CIPN treatment specifically, gabapentin and pregabalin might be considered due to the restricted therapeutic options available and their demonstrated efficacy in managing other neuropathic pain conditions [164]. Similar to TCAs, the use of gabapentin and pregabalin in CIPN should be approached cautiously, taking into account potential side effects and individual patient considerations.

### 1.4.2.4 Opioids:

Opioids are commonly used in the management of severe pain, including neuropathic pain associated with chemotherapy [165,166]. Despite their widespread use, the efficacy of opioids in treating neuropathic pain, particularly CINP, remains a topic of ongoing research and debate [167–169]. These opioids have been shown to provide significant pain relief; however, their effectiveness may be limited by the need for noradrenaline uptake inhibition to achieve a more pronounced reduction in CIPN symptoms [170]. Tramadol, a weak μ-opioid receptor (MOR) agonist and

serotonin/noradrenaline reuptake inhibitor, has demonstrated moderate efficacy in reducing neuropathic pain [171]. Controlled-release oxycodone, a stronger MOR agonist, has also been used effectively in treating CINP [172]. However, the risk CNS adverse effects, including sedation, cognitive impairment, and the potential for addiction, often limits the long-term use of these opioids [173]. Tapentadol, which acts as a selective MOR agonist and noradrenaline reuptake inhibitor, represents a promising alternative for the treatment of CINP [174]. By combining opioid receptor agonism with enhanced noradrenaline reuptake inhibition, tapentadol may offer more effective pain relief with a potentially lower risk of opioid-related adverse effects compared to traditional opioids. This dual mechanism of action is particularly beneficial in the context of CINP, where noradrenaline uptake inhibition appears to be critical for reducing neuropathic pain symptoms [174]. Buprenorphine is another opioid that holds potential for CINP management. Its unique pharmacological profile, which includes partial agonism at MORs, antagonism at κ-opioid receptors, inhibition of N-methyl-D-aspartate receptors (NMDARs), and enhancement of voltage-gated potassium channel activity in neurons, suggests that it could be particularly effective in alleviating CINP [175]. Buprenorphine's ceiling effect on respiratory depression and lower risk of physical dependence make it an attractive option for managing chronic pain, including CINP [176]. While opioids can be effective in managing severe pain, their use in CINP is often restricted due to concerns about side effects, including sedation, cognitive dysfunction, potential for misuse, and the development of tolerance. Additionally, opioids are associated with a significant risk of addiction, which can complicate long-term management strategies. Due to these concerns, opioids are

typically reserved for patients with refractory CINP who have not responded to other treatments.

**1.4.2.5 Topical Agents:**

Topical therapies for CINP encompass patches and gel/cream formulations. Lidocaine patches (700 mg) are suggested for localized neuropathic pain, including CINP, though conclusive clinical trials are lacking [177]. Capsaicin patches (179 mg) are approved in Europe for peripheral neuropathic pain, with evidence supporting their efficacy in CINP, particularly in high-dose formulations [178]. Gel formulations with baclofen, amitriptyline, and ketamine demonstrated modest benefit in a trial with 208 CINP patients, but details on availability and composition remain unknown. Additionally, a combination of topical amitriptyline (4%) and ketamine (2%) showed no significant effect on pain in a study with 462 cancer survivors with CINP [179]. Menthol (1%) displayed a significant reduction in pain in a proof-of-concept study with 51 patients, but the study was small and non-blinded [180]. Overall, these topical interventions present varying levels of evidence and effectiveness in managing CINP symptoms.

**1.4.2.6 Cannabinoids:**

The endocannabinoid signaling system has emerged as a promising target for the management of CIPN, with both exogenous cannabinoid agonists and inhibitors of endocannabinoid degradation showing potential in preclinical and early clinical studies [181]. This system plays a critical role in modulating pain and inflammation, making it an attractive therapeutic avenue for alleviating the neuropathic pain associated with chemotherapy. Cannabinoid receptor agonists, which activate peripheral cannabinoid

receptors (CB1 and CB2), have shown significant promise in preclinical studies. One such compound, WIN 55,212-2, a potent synthetic cannabinoid agonist, has demonstrated efficacy in reducing paclitaxel-induced heat hyperalgesia and mechanical allodynia in rat models [182]. This suggests that cannabinoid receptor activation can effectively mitigate the sensory disturbances commonly seen in CIPN [182]. In addition to synthetic agonists like WIN 55,212-2, natural cannabinoids such as cannabidiol (CBD) have also been explored for their potential in CIPN management [183,184]. In a mouse model, CBD administration was found to alleviate paclitaxel-induced CIPN, likely through a mechanism involving the 5-HT1A receptor. This interaction between CBD and the serotonergic system suggests a broader mechanism of action, potentially enhancing its efficacy in neuropathic pain management [185]. CB2 receptors, primarily expressed in the immune system and peripheral tissues, have been a focal point in CIPN research due to their role in modulating inflammation and immune response. Activation of CB2 receptors has been shown to reduce CINP induced by cisplatin and paclitaxel in animal models, indicating a potential pathway for therapeutic intervention. The selective activation of CB2 receptors could provide pain relief without the psychoactive effects typically associated with CB1 receptor activation, making it a more favorable option for long-term management [186,187]. Another promising approach involves inhibiting the degradation of endogenous cannabinoids (endocannabinoids) to enhance their natural antinociceptive effects. Inhibitors of fatty acid amide hydrolase (FAAH), the enzyme responsible for the breakdown of the endocannabinoid anandamide, have shown efficacy in reducing CIPN symptoms. For instance, the FAAH inhibitor ST4070 was found to increase endocannabinoid levels in the brain and reduce vincristine-induced neuropathic pain in rats [188]. This suggests that maintaining higher levels of

endocannabinoids can effectively counteract the development of CIPN. In a small placebo-controlled clinical trial, the cannabinoid nabiximols, which contains both THC (tetrahydrocannabinol) and CBD, demonstrated beneficial effects in 16 patients with CIPN. Although the sample size was limited, these findings provide a foundation for further exploration into the clinical efficacy of cannabinoid-based therapies in managing chemotherapy-induced neuropathic pain [186,189].

While the preclinical data on cannabinoid receptor agonists and endocannabinoid deactivation inhibitors are promising, translating these findings into clinical practice requires further investigation. The psychoactive effects associated with CB1 receptor activation, the variability in individual responses to cannabinoids, and the potential for drug interactions present significant challenges that need to be addressed. Moreover, the long-term safety and efficacy of these therapies in cancer patients, who may already be vulnerable to various side effects, must be rigorously evaluated. Nonetheless, the modulation of the endocannabinoid system remains a highly promising avenue for the development of new treatments for CIPN.

### 1.4.3 Combination Therapies:

Combining medications from different classes or incorporating non-pharmacological interventions, such as physical therapy or psychological support, may enhance overall efficacy while minimizing side effects.

| Drug | NNT (95% CI) | Strength of recommendation for use |
|---|---|---|
| SNRIs (mainly Duloxetine) | 6.4 (5.2-8.4) | Strong, first line |
| Pregabalin | 7.7 (6.5-9.4) | |
| Gabapentin | 7.2 (5.9-9.21) | |
| Tri-cyclic anti-depressants | 3.6 (3.0-4.4) | |
| Lidocaine patches 5% | Low quality evidence | Weak, second line |
| Capsaicin patch 8% | 10.6 (7.4-19.0) | |
| Tramadol | 4.7 (3.6-6.7) | |
| Strong opioids | 4.3 (3.4-5.8) | Weak, third line |
| Botulinum toxin A | Only very small studies | Weak, third line, specialist use only |

**Table. 1.1 Pharmacological agents for symptomatic treatment of CINP** [100]

### 1.4.4 Limitations associated with current therapeutics

Pharmacological approaches for CINP, encompassing TCAs, SNRIs, opioids, cannabinoids, and anti-convulsants, come with inherent limitations and potential side effects. TCAs and SNRIs, effective in various neuropathic conditions, may pose challenges related to tolerability, including sedation and anticholinergic effects. Opioids, despite their pain relief efficacy, carry risks of addiction, tolerance, and adverse effects such as constipation and sedation [190]. Cannabinoids, while promising, may induce psychotropic effects and lack standardized formulations. Anti-convulsants like gabapentin and pregabalin may lead to dizziness and somnolence [151]. The

ongoing pursuit of newer, safer, and more precisely targeted analgesic options aims to overcome these limitations and deliver enhanced efficacy with improved tolerability for CIPN treatment.

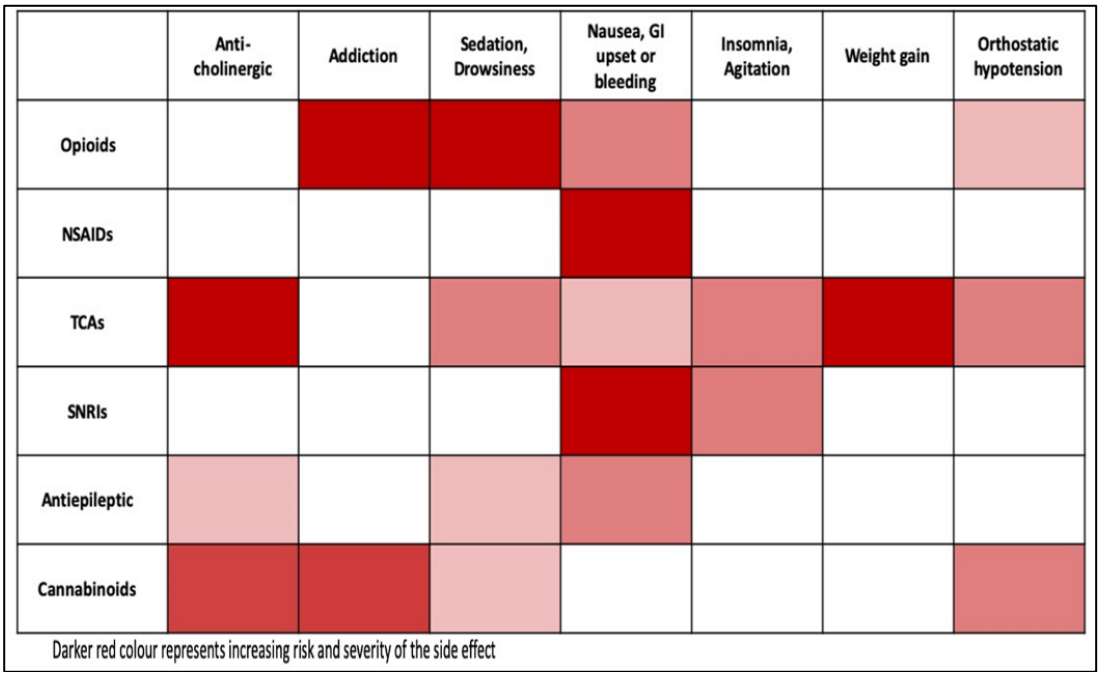


**Table. 1.2 Currently available drugs for the treatment of CINP and their limitations**

## 1.5 G Protein- Coupled Receptors

G protein-coupled receptors (GPCRs) represent a diverse and pivotal family of cell surface receptors that play a fundamental role in cellular signaling. These receptors are integral to the transmission of signals from the external environment to the inside of the cell, thereby regulating a wide array of physiological processes. Given their crucial involvement in cellular communication, GPCRs have emerged as primary targets for therapeutic strategies across various medical disciplines. GPCRs are involved in mediating the effects of numerous neurotransmitters, hormones, and sensory stimuli [191]. Their versatile nature allows them to influence diverse cellular

functions, including neurotransmission, immune response, and metabolic regulation [192]. Consequently, aberrations in GPCR signaling are associated with numerous diseases, making them attractive targets for drug development.

The modular structure of GPCRs, with an extracellular N-terminus, seven transmembrane helices, and an intracellular C-terminus, offers multiple potential points of intervention for drug design [193]. Therapeutic strategies often involve modulating GPCR activity through ligands in the form of agonists, antagonists, or allosteric modulators, influencing downstream signaling pathways. The appeal of targeting GPCRs lies in their wide-ranging impact on physiological processes and their druggability. A significant percentage of currently marketed drugs target GPCRs, underscoring their importance in pharmaceutical development [194,195].

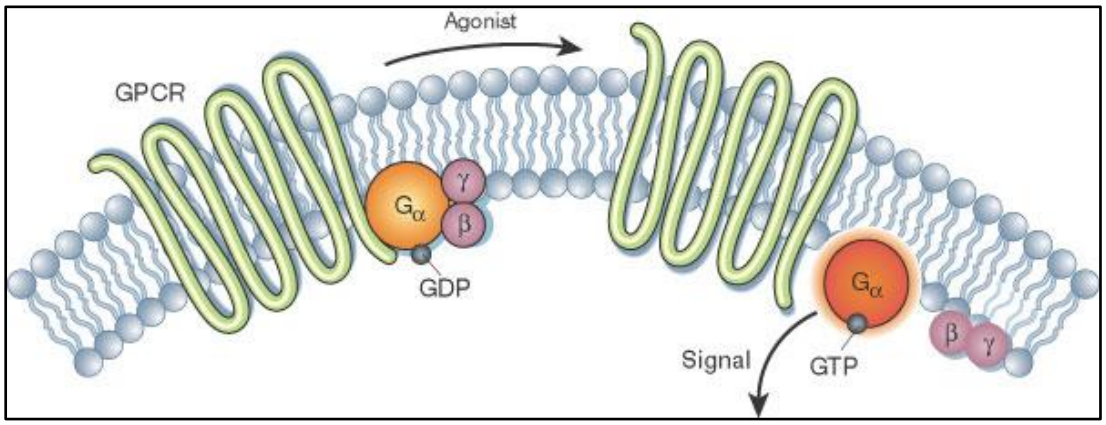


**Figure. 1.13 GPCR Structure and activation of the G alpha subunit** [196]**.**

## 1.5.1 GPCRs and Pain modulation

GPCRs make up a superfamily of seven-transmembrane proteins that sense neuronal or extracellular stimuli. They are the targets of more than one-third of marketed drugs and play important roles in virtually every aspect of physiological activity, including acute and chronic pain sensation [197]. Some GPCRs expressed in the nociceptive

neurons mediate pro-nociceptive effects and increase sensitivity to external stimuli, while others act as antinociceptive entities to attenuate nerve firing and reduce pain sensation. The GPCRs are expressed on presynaptic, postsynaptic terminals, and soma of somatosensory neurons, which binds to various types of ligands to modulate neuronal activity and thus pain sensation in both directions. GPCRs can block pain by interacting with various receptor types, including opioid, cannabinoid, α2-adrenergic, muscarinic acetylcholine and several others. They regulate the pathways and mechanisms during pain progression. Almost all GPCR agonists that have an analgesic action are coupled to Gi/o proteins [198]. Hence, they emerge as novel targets for pain inhibition.

- The earliest breakthrough in GPCR-based pain relief came with the discovery of opioid receptors. Endorphins, the body's endogenous opioids, bind to these receptors to naturally modulate pain perception. The isolation and synthesis of opioids like morphine, derived from the opium poppy, marked a major milestone in pain management history [199].
- Another major player in pain relief is the endocannabinoid system. Cannabinoid receptors, primarily CB1 and CB2, respond to endogenous cannabinoids as well as external compounds like THC and CBD from the cannabis plant. The use of cannabis for pain relief has been documented for centuries and gained scientific validation as understanding of the endocannabinoid system deepened [200].
- α2-Adrenergic receptors, part of the adrenergic system, have been targeted for their role in pain modulation. Medications like clonidine, which activates α2 receptors, have been used to manage certain types of pain, especially neuropathic pain [201].

- The Muscarinic Acetylcholine Receptors (mAChRs), activated by acetylcholine, are another GPCR target. One of the physiological functions of the mAChRs is to tonically regulate nociceptive transmission in the spinal cord [200]. While traditionally associated with the parasympathetic nervous system, these receptors play a role in pain modulation.
- Gamma-aminobutyric acid (GABA) is a major inhibitory neurotransmitter widely distributed throughout the CNS, and activation of GABA-B receptors has been explored for its analgesic effects. The GABA-B receptor agonist baclofen has long been known to produce an antinociceptive effect in animal models of acute pain upon intrathecal administration [202]
- Group II and III metabotropic glutamate receptors are involved in the modulation of glutamate, a key excitatory neurotransmitter. Modulating these receptors has been investigated for its potential in pain management [203].
- Somatostatin (SST) Receptors are involved in the regulation of somatostatin, a peptide that inhibits the release of various hormones. Targeting these receptors has been explored for their role in pain control [204]. Peripheral (local) application of SST and SST analogues also produces analgesic effects. Intraplantar injection of the SST analogue octreotide reduces formalin induced nociceptive behaviors and the responses of C-fibers to noxious stimulation [205].

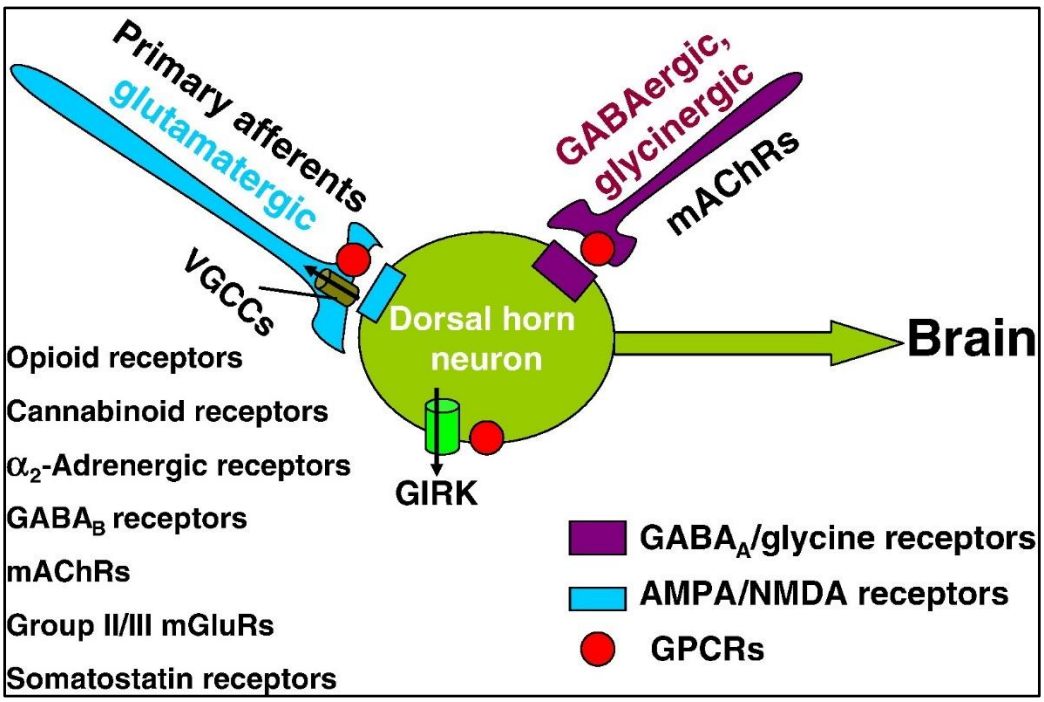


**Figure. 1.14. Schematic drawing showing the site and effectors of GPCRs in the modulation of pain transmission. Reprinted with permission from source reference** [200]

## 1.6 Peripheral GPCRs: A Potential Target for Chronic Pain

In pain research, the exploration of peripheral GPCR targeting has gained considerable attention as a strategic avenue to alleviate pain while circumventing CNS toxicities. GPCRs, abundant on the surface of peripheral nerves, play a crucial role in transmitting pain signals [200]. The appeal of targeting peripheral GPCRs lies in their potential to modulate pain perception without inducing the unwanted side effects associated with centrally acting drugs. Traditional analgesics, particularly opioids and cannabinoids, though potent in pain relief, often pose challenges due to CNS-related adverse effects, including sedation, addiction, and cognitive impairment [206]. By focusing on peripheral GPCRs, researchers aim to specifically influence pain pathways at the site of injury or inflammation, mitigating the risk of systemic side effects.

This approach involves the development of drugs that selectively activate or inhibit peripheral GPCRs, providing a more nuanced and localized impact on pain signaling. The endeavor to target peripheral GPCRs in pain research represents a promising paradigm shift, offering the potential for improved pain management strategies with enhanced efficacy and a reduced risk of CNS-related complications [207]. As research in this field progresses, the prospect of developing novel analgesics specifically designed to address peripheral pain mechanisms holds significant promise for advancing the field of pain therapeutics [208].

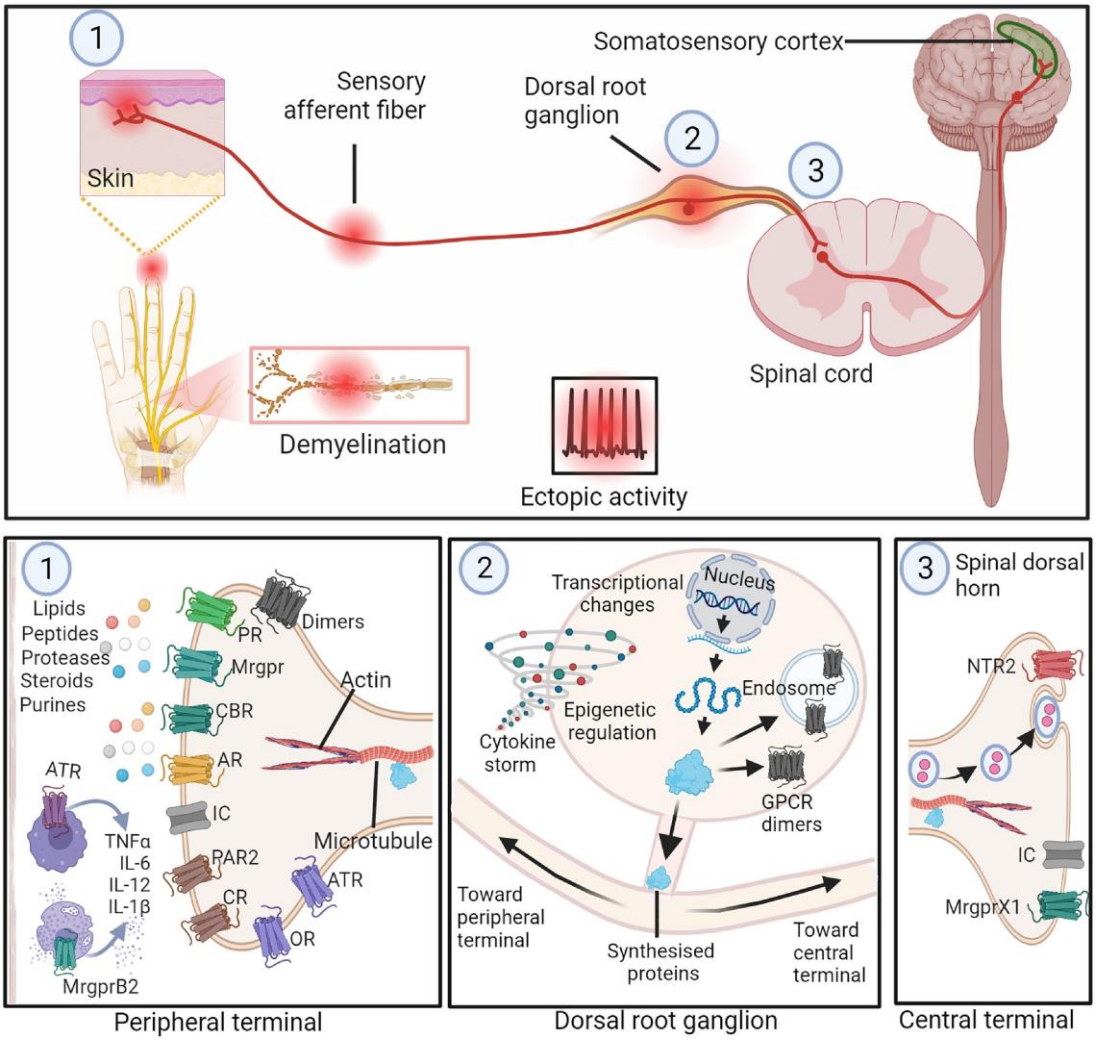


**Figure. 1.15. Possible sites on primary sensory neurons for G protein- coupled receptor (GPCR)-mediated inhibition of peripheral neuropathic pain (NP)** [209]**.**

## 1.6.1 Mu-opioid receptors

The investigation into peripheral MORs offers a promising avenue for refining analgesic therapies, presenting an opportunity to design medications that maintain efficacy while avoiding the drawbacks associated with centrally acting opioids. As our understanding of the molecular and cellular intricacies of peripheral MOR signaling advances, the prospect of developing targeted and safer analgesics for managing pain without undue CNS impact becomes increasingly viable. This focused approach represents a significant stride toward enhancing the precision and safety of pain management strategies.

### 1.6.1.1 Mechanism of peripheral Opioid Analgesia

MORs are predominantly expressed in peptidergic primary sensory neurons (PSNs) and also in immune cells, where their activation can modulate pain signals at the site of injury. In addition to their classical GPCR Gi/o signaling, MORs regulate various cellular processes, such as the inhibition of voltage-sensitive calcium channels, induction of hyperpolarization through modulation of potassium efflux, inhibition of neurotransmitter release, and suppression of neuroinflammatory cytokine signaling [210]. When a ligand binds extracellularly at the receptor, conformational changes allow intracellular coupling of Gi/o proteins to the C-terminus of opioid receptors. Subsequently cyclic adenosine monophosphate (cAMP) production is inhibited or G-protein subunits interact directly with $Ca^{2+}$ and ion channels in the membrane [211]. As a result, opioid agonists can attenuate the excitability of nociceptive neurons and the release of the pronociceptive neuropeptides substance P and calcitonin gene-related

peptide (CGRP) from central and peripheral neuron terminals. Particularly within injured tissue, these events lead to antinociceptive and anti-inflammatory effects.

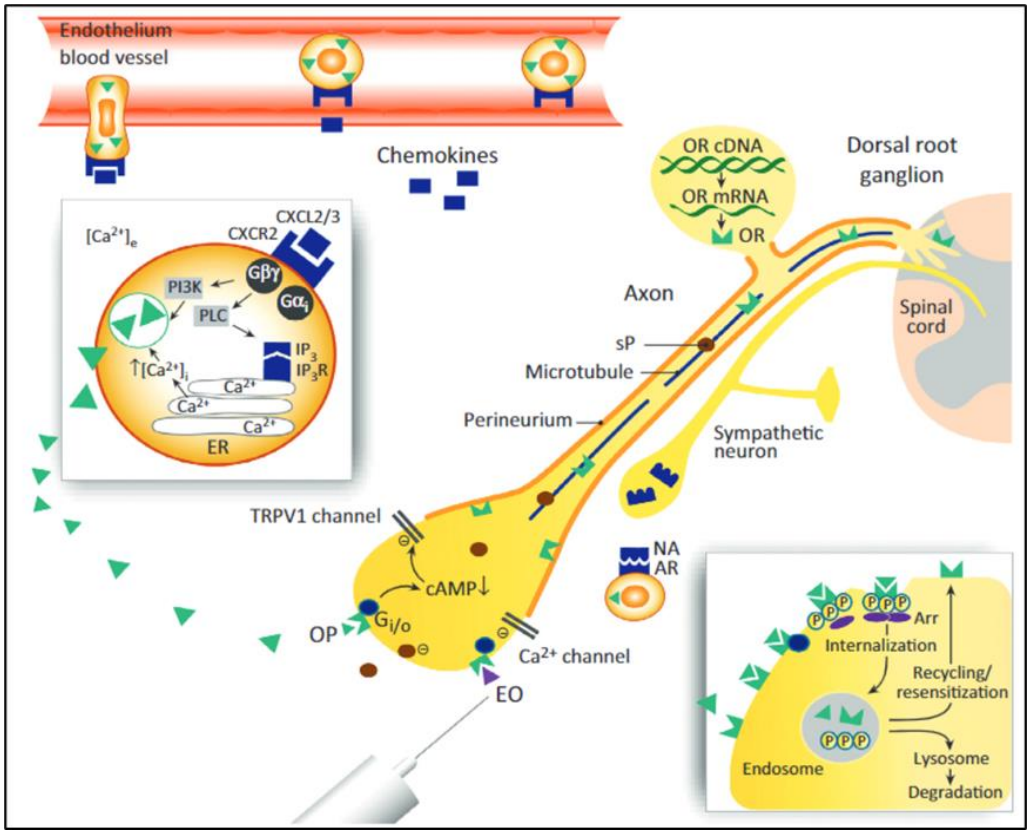


**Figure. 1.16. Structures and molecules involved in peripheral opioid-mediated analgesia** [211]**.**

### 1.6.1.2 Loperamide, a peripheral mu opioid agonist

Loperamide is an over-the-counter oral antidiarrheal agent made in 1969, first used medically in 1976, and became available without a prescription in 1988 [212]. Initially, due to its opioid-like abuse potential, it was categorized as a Schedule V drug by the Federal Drug Administration (FDA). Currently, loperamide has been FDA approved to treat various forms of diarrhea and has also been used off-label to treat the adverse effects of chemotherapy resulting in diarrhea. The peak plasma time is 4 to 5 hours, with a half-life of 7 to 19 hours. It has a low bioavailability of < 1% due to the

first-pass metabolism. It has high protein binding and a large volume of distribution [213]. The limited CNS penetration of loperamide is due to the efflux by P-glycoprotein that prevents circulating loperamide from effectively crossing the blood–brain barrier[214].

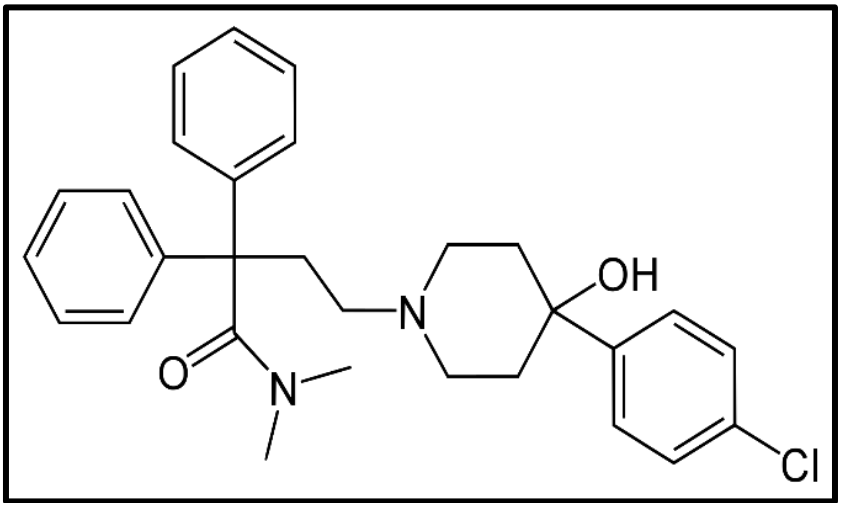


**Figure. 1.17 Chemical Structure of Loperamide**

- **IUPAC Name:** 4-[4-(4-chlorophenyl)-4-hydroxypiperidin-1-yl]-N,N-dimethyl- 2,2-diphenylbutanamide
- **Chemical Formula:** C29H33ClN2O2
- **Molecular Weight:** 477.0 g/mol

Loperamide has previously been evaluated for the attenuation of inflammatory pain, neuropathic pain and bone cancer pain. In a study on nerve injured rats systemic loperamide demonstrated varied anti-allodynic potency over different time points, with consistent effectiveness at days 7, 28, and 42 post-Spinal Nerve Ligation (SNL), but reduced potency at day 14 post-SNL. Moreover, the intraplantar injection of loperamide also demonstrated a dose-dependent reversal of mechanical allodynia on day 7 post-SNL. These findings suggest that loperamide has the potential to effectively alleviate

neuropathic pain, primarily by activating peripheral MORs in local tissue [215]. This evidence contributes to the existing literature on the therapeutic role of loperamide in mitigating neuropathic pain, emphasizing its potential as a relevant subject for further research and exploration within the scientific community. The analgesic effects of loperamide were studied in a mice model of bone cancer pain in which they observed inhibition of both thermal and mechanical hyperalgesia when injected s.c., locally over the tibial tumoral mass (7.5–75μg) or distantly, under the fur of the neck (4 mg/kg). These analgesic effects were proven to be peripherally mediated since they are reverted by the administration of naloxone methiodide (10 mg/kg) and because the withdrawal latencies of the contralateral, non-affected, paws remained unaltered [216]. Another report suggested that loperamide attenuated inflammatory hyperalgesia by inhibiting the sodium channels (Nav1.8) in the DRG neurons [217]. The selection of loperamide for evaluating the analgesic potential of peripheral MOR activation in mitigating CINP is based on these evidences from previous reports.

#### 1.6.1.3 Dermorphin [d-Arg2, Lys4] (1–4) amide (DALDA), a preferential peripheral MOR agonist

Dermorphin, a natural heptapeptide found in amphibian skin is a MOR agonist [218]. Degradation of dermorphin by peptidases produces the N-terminal tetrapeptide H-Tyr-d-Ala-Phe-Gly-OH. Additional amino acid substitutions to this tetrapeptide lead to the development of Dermorphin [d-Arg2, Lys4] (1–4) amide (DALDA), a highly selective MOR agonist [219]. DALDA is highly polar and metabolically stable, with limited penetration of the blood–brain barrier and the placental barrier. At physiological pH, it has three net positive charges. Furthermore, it is more metabolically stable than its other analogues and has a more limited penetration into the CNS following systemic

drug administration. Its incapacity to cross these barriers, together with its metabolic stability, suggests that it might be useful as a peripheral analgesic with reduced risk of central side effects that occurs with opioids [220].

These characteristics make DALDA a good candidate for the treatment of chronic pain. Numerous studies have substantiated its analgesic efficacy in pain management. Vaidya et al. reported that DALDA effectively mitigates mechanical and thermal hypersensitivity in nerve-injured rats, concurrently inhibiting serum and spinal inflammatory markers [221]. Furthermore, Shimoyama et al. observed that DALDA exhibits superior potency compared to morphine in treating neuropathic pain in nerve-injured rats [222,223]. Additional reports indicate that DALDA not only attenuates spontaneous ongoing pain but also effectively reduces spontaneous neuronal activity in nerve-injured rats. It further demonstrates a modality-preferred inhibition of neuropathic pain associated with SNL [224,225]. These findings collectively underscore DALDA's potential as an effective agent for managing chronic pain.

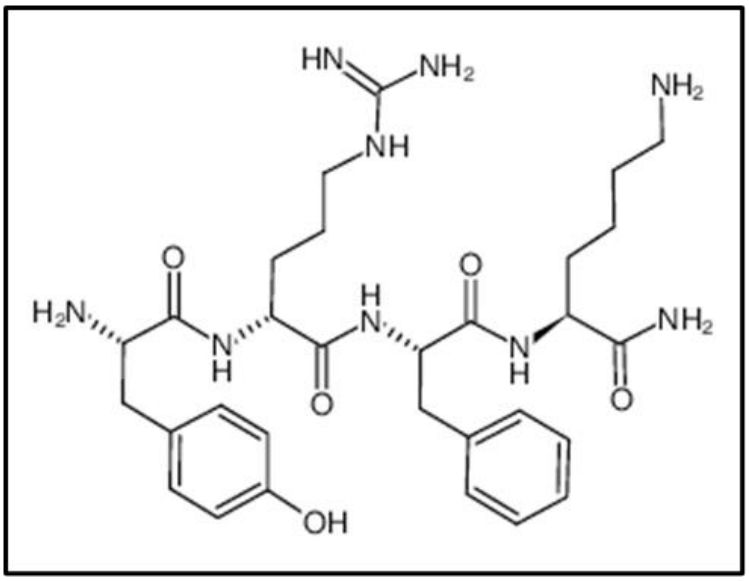


**Figure 1.18 Chemical Structure of DALDA[H-TyrD-Arg-Phe-Lys-NH2] amide**

- **Chemical Formula:** C30H45N9O5
- **Molecular weight:** 611.7356 g/mol
- **Nature:** Hydrophilic

## 1.6.2 Cannabinoid receptors

Traditional cannabinoids, such as those found in cannabis, exert their effects by activating both central and peripheral cannabinoid receptors. The targeted modulation of peripheral cannabinoid receptors has gained prominence as a promising strategy for managing pain while minimizing CNS side effects. Cannabinoid receptors, particularly CB1 and CB2 receptors, are distributed widely in the peripheral nervous system and immune cells, offering potential targets for therapeutic interventions [226]. As our understanding of the distinctive roles of peripheral cannabinoid receptors advances, the potential for designing targeted and efficacious analgesics continues to grow, contributing to the evolution of safer and more precise pain therapeutics.

### 1.6.2.1 Mechanism of peripheral cannabinoid analgesia

Cannabinoid receptors are a class of GPCRs that mediate the effects of endocannabinoids, the body's naturally occurring cannabinoids, as well as exogenous ligands. The two primary types of cannabinoid receptors are CB1 and CB2 [227]. CB1 and CB2 are coupled mainly with the Gαi/o subunit, which inhibits adenylate cyclase activity and therefore lowers intracellular $Ca^{2+}$ concentration. This leads to protein kinase A (PKA) inhibition and dephosphorylation of potassium channel type A. The increase in potassium conductivity results in cell hyperpolarization and the inhibition of neurotransmitter release [228]. Gαi/o activation leads to the inhibition of various

types of voltage-gated calcium channels including L-type [229] N-type [230] and T-type [231] therefore limits $Ca^{2+}$ influx.

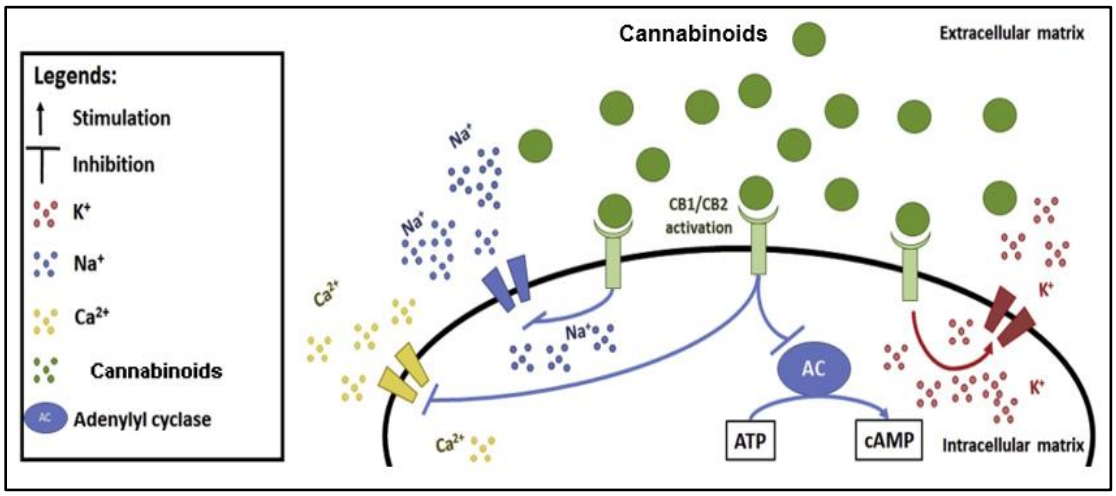


**Figure. 1.19. Structures and molecules involved in peripheral cannabinoid-mediated analgesia** [232]

Cannabinoid 1 receptors (CB1Rs) are expressed in peripheral and central terminals of small nociceptive as well as large neurons. In mouse and human DRG, CB1R is expressed in peptidergic and nonpeptidergic neurons [233,234]. Studies using cKO mice lacking CB1Rs in PSNs and the peripherally restricted dual cannabinoid receptor agonist, CB13, revealed the role of peripheral CB1Rs in attenuating inflammatory hypersensitivity. CB13 inhibited high voltage-activated calcium currents in cultured DRG neurons [235]. Peripherally restricted CB1R-selective agonists (LBP1, AZ11713908, and PRNMI) exhibit significant analgesic effects in animal models of SNL, bone cancer pain, and CIPN, without CNS side effects [236].

Although cannabinoid 2 receptors (CB2Rs) are considered to be localized mainly in non-neuronal cells, studies with human DRG demonstrated the colocalization of CB2Rs with TRPV1 in small/medium neurons [237]. A peripheral CB2 agonist,

AM1710, inhibited CIPN in mice, an effect that was blocked by co-administration of a peripherally acting CB2R antagonist [238].

#### 1.6.2.2 CB13 (CRA13 or SAB-378): peripherally restricted dual cannabinoid receptor agonist

CB-13, a potent CB1/CB2 receptor dual agonist with restricted brain penetration, has demonstrated significant anti-hyperalgesic properties in animal studies, prompting its advancement to preliminary human trials [239]. Notably, its limited blood-brain barrier penetration at low doses ensures primarily peripheral effects. The study by Ford et al., 2022 reported that systemic administration of CB-13, (5 mg/ kg), inhibited nociceptive pain and complete Freund adjuvant induced inflammatory pain [235]. The findings revealed that CB-13 inhibits high voltage-activated calcium currents (HVA-$I_{Ca}$) in cultured DRG neurons, suggesting that HVA-$I_{Ca}$ inhibition is a key ionic mechanism for its pain-inhibitory effects. Both oral administration and local injection of CB13 into the hind paw reversed established mechanical hyperalgesia. At an oral dose of 3 mg/kg, the compound reversed mechanical hyperalgesia with rapid onset of action and long duration but without apparent cardiovascular and CB1 receptor-mediated CNS side effects [240]. A CB1-selective antagonist but not a CB2-selective antagonist inhibited the anti-hyperalgesic effects, indicating that peripheral CB1 receptors are mainly responsible for the anti-hyperalgesic action of the compound [240]. Crucially, the clear analgesic effect observed with acute activation of peripheral CB1 receptors underscores the potential of peripherally restricted cannabinoids as a promising target for the development of novel analgesics. These results provide compelling support for selecting CB-13 for pre-clinical investigation in CINP.

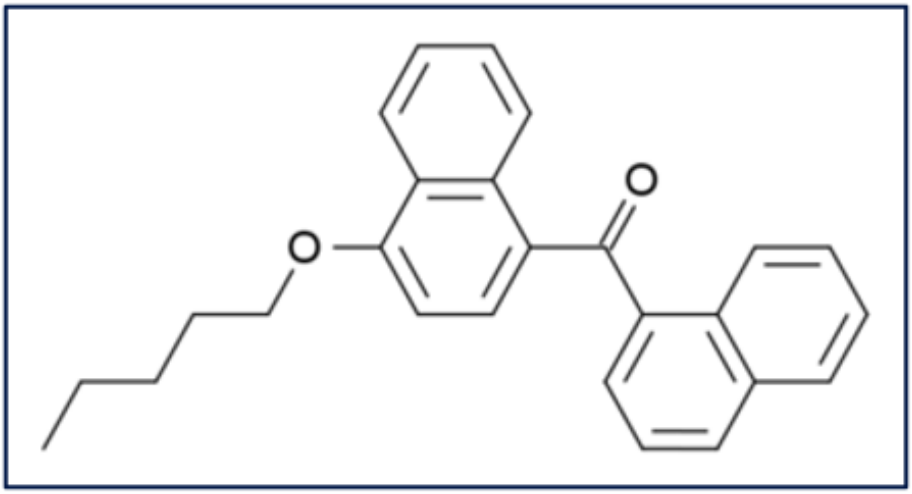

**Figure 1.20 Chemical Structure of CB13**

- **IUPAC Name:** Naphthalen-1-yl-(4-pentoxynaphthalen-1-yl) methanone
- **Chemical Formula:** C26H24O2
- **Molecular weight:** 368.5 g/mol