Review

Medicinal Chemistry approach, pharmacology and neuroprotective benefits of CB_2R modulators in neurodegenerative diseases

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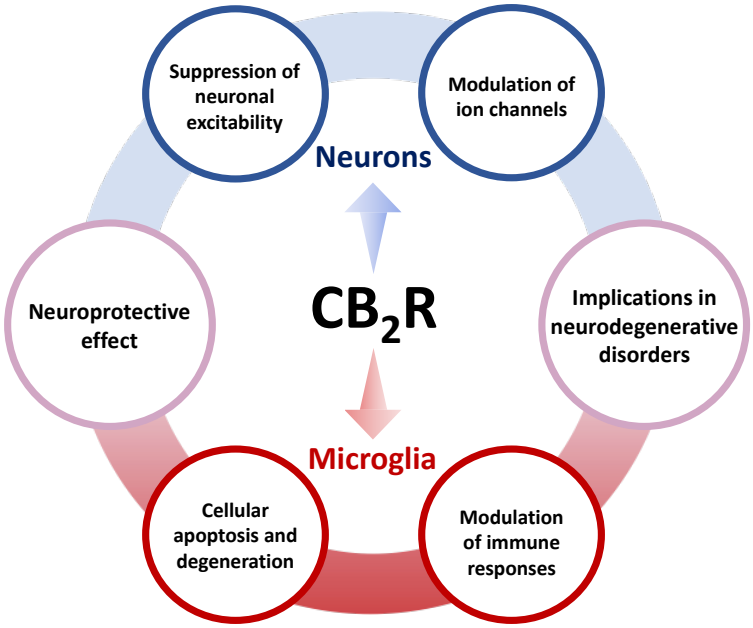
Abstract: In the last decades, cannabinoid receptor 2 (CB_2R) has continued to receive attention as a key therapeutic target in neuroprotection. Indeed, several findings highlight the neuroprotective effects of CB_2R through suppression of both neuronal excitability and reactive microglia. Additionally, CB_2R seems to be a more promising target than cannabinoid receptor 1 (CB_1R) thanks to the lack of central side effects, its lower expression levels in the central nervous system (CNS), and its inducibility, since its expression enhances quickly in the brain following pathological conditions. This review aims to provide a thorough overview of the main natural and synthetic selective CB_2R modulators, their chemical classification and their potential therapeutic usefulness in neuroprotection, a crucial aspect for the treatment of neurodegenerative diseases.

Graphical abstract:



Keywords: CB2 cannabinoid receptor; Endocannabinoid System; neuroprotection; neurodegenerative disorders; CB2 receptor ligands; phytocannabinoids; CB2 receptor allosteric modulators.

1. Introduction

The cannabinoid receptor 2 (CB_2R) together with the cannabinoid receptor 1 (CB_1R) are the two main receptors (CBRs) of the endocannabinoid system (ECS) which includes also its endogenous ligands (endocannabinoids, ECs) such as anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) and the enzymes responsible for ECs synthesis, degradation and transport [1]. Since the discovery and cloning of the CBRs in the 90's, there has been a high interest in the understanding of their pharmacology and in the studying of their expression and function especially in the brain [2]. Initially, the CB_2R was considered the "peripheral" receptor of the ECS for its localization mainly in the cells of the immune system, conversely to the CB_1R which was found predominately in the central nervous system (CNS). Indeed, at the beginning, reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) and *in situ* hybridization (ISH) analysis failed to detect CB_2R mRNA in the brain [3][4] because of the presence of different isoforms of the CB_2R [5] and for the low expression of neural CB_2R mRNA transcripts in physiological conditions (about 100÷300- fold lower than CB_1R mRNA in the brain) [6]. As a matter of fact, several years later, more advanced techniques, such as the use of isoform-specific probes in RT-qPCR, RNAscope-ISH, fluorescence-activated cell sorting (FACS) and immunoblot and immunohistochemical (IHC) assays, allowed the identification of the CB_2R in various regions of the brain, more specifically in the retina, cortex, striatum [7], NeuN + neuronal cells hippocampus, hippocampal glutamate neurons [8], amygdala, brainstem [9], cerebellum [10], postsynaptic somatodendritic areas, in the dopamine (DA) neurons of the ventral tegmental area (VTA) [11], and in NeuN negative (glia) cells (including microglia) [12]. Many studies followed this discovery and led to the assessment of the neuroprotective role played by CB_2R in the CNS [13][14]. The CB_2R neuroprotection is achieved fundamentally through the modulation of the neuronal excitability and the involvement of glia cells (figure 1).

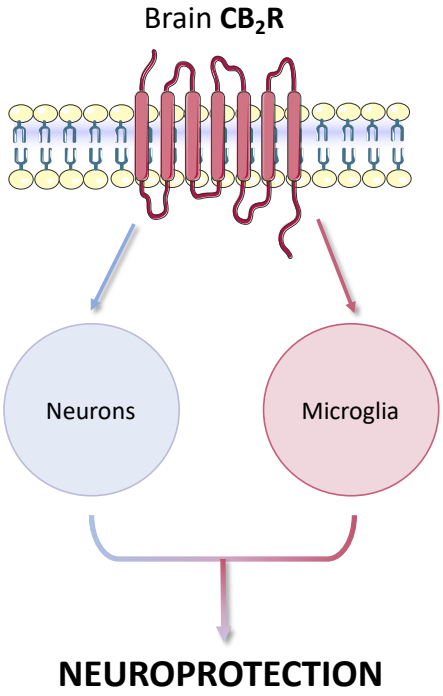


Figure 1. Diagram summarizing brain CB_2R involvement in neuroprotection.

It was reported that the activation of CB_2R reduces neuronal excitability in a cell type-specific manner through different intracellular signaling mechanisms. After the binding of a CB_2R ligand, there is the activation of the $G_{\alpha i/o}$-mediated signaling cascade which results in the inhibition of adenyl cyclase, but also in the activation of intracellular (PI3K-Akt pathway included) and extracellular signal-regulated (ERK) kinases [15]. Moreover, CB_2R activation is also able to modulate the MAP kinase pathway [16]. Finally, the common result is the suppression of the neuronal activity [17]. CB_2R activation also displays region- and cell type-specific modulation of neuronal activity. In particular, the decrease of neuron excitability is obtained, in VTA DA neurons through the modulation of M-type K^+ channel (KCNQ7.4) function [18], in cortical neurons through inwardly-rectifying potassium channels (GIRKs) [19], in pyramidal cells and in prefrontal cortical neurons

through the induction of IP3R activation and the consequently opening of calcium-dependent chloride channels and, more specifically, in hippocampal CA3/CA2 pyramidal neurons through the Na^+/ bicarbonate co-transporter [20][21]. Regarding glia cells, depending on the CNS region considered, they are constituted by microglia cells in a percentage which varies from 5 to 20 %. Microglia cells has a primary role in potentially harmful conditions which could cause neuronal loss such as injury and infection. They are activated as a consequence of neuroinflammatory conditions in order to moderate any potential damage to the CNS and to favor tissue repair. In response to external signals from neuropathological conditions, homeostatic (M0) microglia can adopt one of the two phenotypic variants: M1 and M2 [22]. The classical M1 state is characterized by the release of proinflammatory factors such as interleukins (IL-1beta, IL-18, and IL-6), prostanoids and inducible nitric oxide synthase (NOS2)-derived nitric oxide (NO). Conversely, the neuroprotective M2 state, known as "alternative activation", is associated with the release of anti-inflammatory factors, such as IL- 10, IL-4, and NGF [23]. In physiological conditions, in order to eliminate a pathogen or restrain an injury, the M1 state is the one which prevails. After the onset of the classical releasing proinflammatory factors (M1 state), microglia shift to the alternative M2 activation state, whose anti-inflammatory action dampens the previous pro-inflammatory response before it leads to neurotoxicity and chronic neuroinflammation which are correlated with neurodegenerative diseases. CB_2R signaling is able to shift from neuroinflammatory (M1-type) genes, to neuroprotective (M2-type) and homeostatic (M0-type) genes leading microglia to acquire therapeutic functionality. During pathological states CB_2R expression in microglia increased, driving the acquisition of the alternative phenotype M2 [24]. Indeed, during the last decades, a growing evidence of studies has revealed an intricate correlation between neurons and immune cells in the maintaining of brain homeostasis. If this delicate equilibrium is altered by any pathological stimuli, the inflammatory response can be exaggerated in the CNS [25]. Consequently, the role of the peripheral CB_2Rs worth to be mentioned since they may work together with central CB_2Rs reducing the immune response in order to prevent an over-inflammation through both immune and neuronal mechanisms [2]. Many studies have already been made on microglia and many CB_2R modulators have already been reported in the literature assessing CB_2R action on microglia [24].
Concluding, CB_2R seems to be a more promising target in neuroprotection than CB_1R thanks to some important and specific features: a) its lower expression levels in the CNS; b) its inducibility since its expression enhances quickly in the brain following pathological conditions. Point a) and b) explain the lack of central side effects; c) its specific distribution in CNS mainly in the post synaptic cell body resulting in a inhibition of the neuronal excitability, opposite to CB_1R (mainly pre-synaptic) [2]. The neuroprotective potential role of CB_2R ligands is then due to a specific distribution of CB_2R receptor in particular types of cells such as neuronal subsets, activated astrocytes, reactive microglia, perivascular microglia, oligodendrocytes, and neural progenitor cells but also in particular structures, as the blood brain barrier (BBB) for example, which are all important for the maintenance of the CNS integrity [26][27]. Moreover, as a consequence of the CB_2R wide distribution, CB_2R ligands present a selective action for specific functions of these cells regarding degeneration, protection and repair [28].

This review aims to provide a thorough overview of the recent literature regarding the main CB_2R modulators (natural and synthetic) which may be useful for the treatment of neurodegenerative diseases for their neuroprotective potential.

2. Neuroprotective effects of phytocannabinoids mediated by CB_2R

In the framework of the neuroprotective therapeutic strategies, the phytocannabinoids, terpenophenolic constituents representing approximately 24% of the total natural products of the *Cannabis sativa* [29][30], have emerged as a new class of drugs with potential effects on neuroinflammation, neuroprotection and over a broad range of neurological disorders. The investigation of the possible positive impact of the phytocannabinoids in these complex medical conditions is noteworthy, even though the mechanisms underlying their effects are still not entirely clear, they seem to involve multiple pharmacological targets, even outside the ECS.

Among the eleven different chemical classes of phytocannabinoids that have been identified [30], cannabidiol (CBD) and delta-9-tetrahydrocannabinol (Δ^9-THC) (figure 2) are certainly the most studied and they show numerous therapeutic properties for brain function and protection, modulating a variety of physiological targets through direct and indirect actions [31].
Numerous applications for CBD and Δ^9-THC in neurodegenerative diseases are being evaluated (and are currently under investigation), demonstrating that both can protect the brain from several neuronal insults and improve the symptoms of neurodegeneration in animal models of multiple sclerosis (MS), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS) and Alzheimer's disease (AD) [31]. For example, CBD (figure 2) reduced the transcription and the expression of glial pro-inflammatory molecules in the hippocampus of an *in vivo* mouse model of beta-amyloid (Aβ)-induced neuroinflammation, so representing a novel rationale for the development of drugs able to blunt neuronal damage and slow the course of AD [32][33]. Furthermore, it has been shown that CBD decreased spinal microglial activation and T-cell recruitment in an experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG) in C57BL/6 mice, where ameliorated the clinical signs of MS-like disease [34].
Various phytocannabinoid-based medicines have been examined in several clinical studies to assess the efficacy of CBD and Δ^9-THC in the treatment of neuroinflammation and other neurodegenerative disorders in human patients, although their clinical use is limited by their psychotropic effects. An example is represented by Sativex®, whose active principle is nabixiomls, a standardized 1:1 (w/w) mix of CBD and Δ^9-THC, that has produced neuroprotective effects through a CB_1R- and CB_2R-mediated mechanism in several models of MS [35] and that is currently prescribed for neuropathic pain and spasticity associated with MS [36].

Several studies have also encouraged the use of cannabigerol (CBG) against neurodegeneration, since it reduced oxidative stress and inflammatory markers in some *in vitro* models of neuroinflammation, such as lipopolysaccharide (LPS)-stimulated macrophages in NSC-34 motor neurons [37]. The neuroprotective properties of CBG were also studied in some *in vivo* models, for example, of HD, where it has preserved striatal neurons in mice intoxicated with 3-nitropropionate [38].

There is a wide range of cellular mechanisms proposed for the beneficial effects exerted by most of the mentioned phytocannabinoids in some *in vitro* and *in vivo* models of neurodegenerative disorders, by which they can attenuate the brain damage, reduce the microglia activation, modulate the synaptic plasticity, regulate the immune responses and decrease the levels of pro-inflammatory mediators [31]. These mechanisms seem to be attributable only partially to CB_2R [39], on which Δ^9-THC shows partial agonistic effects [40], CBD displays weak CB_2R antagonistic effect [40][41], while CBG has a low affinity for CB_2R but seems to present weak CB_2R partial agonist activity [40].

In this systematic review, all available data on the CB_2R-mediated neuroprotective effects of phytocannabinoids were collected.

In this context, the plant-derived cannabinoid Δ^9-THC (figure 2) reduced the death of striatal projection neurons in the *in vivo* HD model, generated by administration of 3-nitropropionic acid (3NP), and the current study raised the hypothesis that cannabinoid receptors could be involved in HD pathogenesis [42]. Shortly later, the experiments conducted by Fernandez-Ruiz *et al*. revealed an up-regulation in the CB_2R expression in discrete subpopulations of microglia and astroglia at the lesioned striatum, suggesting that neuroprotective properties of Δ^9-THC in Huntington's disease might be mediated by CB_2R [43].

In parallel, CBD's action on the CB_2R is just one of several pathways by which CBD can affect neuroinflammation [31]. Despite CBD exerting a multitude of neuroprotective properties by the activation of a wide range of cellular mechanisms and having negligible activity at the CB_2R, certain activity at the CB_2R

has been documented in an *in vitro* model of newborn hypoxic-ischemic (HI) brain damage [39][44]. This study supports the hypothesis that the CBD protective effects in the HI brain damage in the immature rat rely mostly upon CB_2R and adenosine—mainly A2A—receptors. Despite the direct effect of CBD on adenosine receptors cannot be ruled out, CB_2R has been largely involved especially in anti-inflammatory and iNOS expression modulatory effects. In fact, CB_2R antagonist AM630 fully reversed the inhibitory effect of CBD on iNOS and COX-2 induction and abolished those on TNFα and IL-6 production and the CBD-induced reduction in LDH efflux. Based on several experiments on its neuroprotective effects, CBD lacks a unique mode of action but, surely, the activation of CB_2R appears to be one of the mechanisms by which CBD may afford neuroprotection [44].

Some findings have demonstrated that the most common phytocannabinoid whose neuroprotective benefits are especially related to CB_2R-mediated biochemical and molecular mechanisms is (−)-β-caryophyllene (BCP) (figure 2). BCP is a volatile bicyclic sesquiterpene lactone compound which represents about 35 % of the essential oil of *Cannabis sativa L*. [45][46]. It is a CB_2R-selective phytocannabinoid and it has been shown to suppresses neuroinflammation in several mouse models. BCP selectively and competitively interact with the CP55,940 binding site (i.e., THC binding site) of the CB_2R [45], with 165-fold selectivity over CB_1R, where it showed a weak partial agonism [46]. (*E*)-BCP is a full CB_2R agonist leading to G_i and G_o signals. As activator of the G_o pathway, (*E*)-BCP concentration-dependently leads to the blockage of various voltage gated Ca^{2+} channels; while, as activator of the G_i pathway, (*E*)-BCP activation of *h*CB_2R inhibits adenylate cyclase, resulting in lower levels of cAMP along with activation of MAPK pathways. BCP causes the inhibition of pro-inflammatory cytokines, the activation of the mitogen-activated kinases Erk1/2 and p38 which further regulates the activation of peroxisome proliferator-activated receptors (PPARs), the stimulation of SIRT1/PGC-1-dependent mechanism, the downregulation of anti-apoptotic genes (Bcl-2, Mdm2, Cox2 and Cmyb) and the up-regulation of pro-apoptotic genes (Bax, Bak1, Caspase-8, Caspase-9 and ATM) [46]. BCP also acts on targets outside the ECS, inhibiting for example pathways triggered by the activation of toll like receptor complex (CD14/TLR4/MD2) or exhibiting synergy with μ-opioid receptor-dependent pathways [46].

Even though the underlying molecular mechanisms of the pharmacological effects of BCP observed in different *in vitro* and *in vivo* studies are multiple, interconnected and still unclear, several data suggest that the specific activation of CB_2R by BCP could offer promising therapeutic applications in numerous pathological disorders, such as neuroinflammatory and neurodegenerative diseases.

The neuroprotective effects of BCP were demonstrated in several studies. Very recently, Askari *et al*. evaluated these effects against LPS-induced cytotoxicity on cell viability of OLN-93, with an estimated IC_{50} of 1 mg/mL. In the same study, BCP has been reported to exert its protective effects at low (0.2 and 1 μM) and high (25 and 50 μM) concentrations, mediated in all cases by CB_2R [47]. Indeed, it has been shown that the application of the CB_2R antagonist AM630 completely eliminated the effects of BCP. On the contrary, the addition of AM251 as a CB_1R antagonist, had no provide a shift in the dose-response curve, demonstrating that BCP may not act through CB_1R [47]. So, BCP is a promising therapeutic strategy in order to combat neurodegenerative and inflammatory diseases such as MS by affecting the oligodendrocytes [47].

The exploration of antioxidant and anti-inflammatory natural agents also led to the identification of BCP as a promising therapeutic agent in the treatment of PD. In fact, an important study was undertaken with the aim to evaluate the neuroprotective effect of BCP against rotenone (ROT)-induced oxidative stress and neuroinflammation in a rat model of PD [48]. In this study, the levels of pro-inflammatory cytokines (IL-1β, IL-6) and tumor necrosis factor-alpha (TNF-α) were measured in the midbrain tissues and they were found significantly ($p < 0.05$) decreased in ROT-injected rats simultaneously treated with BCP at a dose of 50 $mg \cdot kg^{-1}$ body weight. Furthermore, the administration of BCP to rats previously injected with ROT (2.5 $mg \cdot kg^{-1}$ body weight) also attenuated inflammatory mediators, such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), it ameliorated the oxidative stress and inhibited lipid peroxidation. Based on these results, it is possible to conclude that BCP elicits neuroprotective effects through antioxidant and anti-inflammatory activities [48]. Beyond the antioxidant and anti-inflammatory effects, it has been highlighted that the high lipophilicity of BCP, which indicates its ability to cross blood brain barrier, also reinforces its neuroprotective effects [46].

The neuroprotective effects of BCP in PD were evaluated considering another experimental model that simulated a depletion of PD-like dopaminergic neurons. It is an *in vitro* model where 50 μM neurotoxin 1-methyl-4-phenylpyridinium (MPP+) led to a significant decrease in human SH-SY5Y cell viability, which was restored by BCP treatment (1 μM and 2.5 μM). BCP, in fact, reduced MPP+-induced production of reactive oxygen species and reversed reduction of mitochondrial membrane potential. It has also been found that the

neuroprotective effects of BCP upon neurotoxicity induced by MPP+ were CB_2R-mediated, since its effects were blunted by CB_2R antagonist AM630 [49].
The critical role of neuroinflammation for AD pathogenesis led to study the use of BCP as an attractive approach for the treatment of this disorder. The activation of CB_2R, synergistically combined with the peroxisome proliferator-activated receptor-γ (PPARγ) pathway, determined the beneficial effect of reducing neuroinflammatory response in a transgenic APP/PS1 mice as an AD model [50]. The administration of BCP (48 $mg \cdot kg^{-1}$) effectively reduced Aβ burden, astrogliosis and microglial activation, and significantly reversed the elevation of COX-2 protein and the mRNA levels of the proinflammatory cytokines TNF-α and IL-1β [50].
The functionally relevant role of BCP during the inflammatory processes associated with a variety of neuropathies was also demonstrated in relation to the stroke. Stroke is the common form of hypoxia-ischemic brain injury and both oxidative stress and neuroinflammation can contribute to its onset. In this context, it has been proposed that BCP attenuates microglia activation that causes a NF-κB-dependent upregulation of proinflammatory molecules, such as TNF-α and IL-1β, during this pathological condition [51]. The study of Guo K. *et al*. (2014) was carried out on murine BV_2 cell line after hypoxic exposure, where mice were treated with 5 μM of BPC in order to evaluate its hypoxia-induced neuroinflammatory response, which provides beneficial effects in the prevention and treatment of stroke [51].
The effects of BCP on immune inflammatory diseases of the CNS, such as MS, have also been reported. A recent study has endeavored to investigate the therapeutic potential of BCP on EAE, a murine model of MS [52]. Data presented indicate that BCP (at dose of 25 and 50 $mg \cdot kg^{-1}$) is endowed with the ability to inhibit glial activation, downregulate leucocytes proliferation, promote both T cells and macrophages apoptosis and also arrest oxidative damage and demyelination during EAE development. These anti-inflammatory effects of BCP are mediated by modulation of CB_2R in mice, where EAE was induced by the injection with the MOG [52]. This is another neurological disease model in which the CB_2R activation by BPC appears to ameliorate clinical signs or delay syndrome progression.
BCP is the first natural CB_2R agonist which exerted beneficial effects in mouse models of inflammatory and neuropathic pain. In this context, a significant study investigated the BCP analgesic effects whether in a formalin-induced inflammation model and in a partial sciatic nerve injury (SNI)-induced neuropathic model [53][54]. In the first case, in an inflammatory pain model, BCP decreased the pain responses in the late phase of the formalin test in $CB_2R^{+/+}$, but not in $CB_2R^{-/-}$ mice, following the oral administration of BCP at a dose of 5 $mg \cdot kg^{-1}$. Moreover, chronic oral administration of BCP also attenuated thermal hyperalgesia and mechanical allodynia in partial sciatic nerve injury-induced neuropathic mice. These effects were absent in CB_2R knockout mice, proving a CB_2R-mediated action. It has also been observed that BCP treatment (1 $mg \cdot kg^{-1}$) reduced the density of spinal cord glia cell-markers, prevented astrocytosis and reduced microgliosis. It is noteworthy to underline that BCP did not elicit tolerance and CB_1R-specific psychomimetic side effects, such as catalepsy and hypothermia [54].
Chronic oral administration of BCP (25 $mg \cdot kg^{-1}$) also alleviated paclitaxel (PTX)-induced peripheral neuropathy and attenuated the mechanical allodynia in mice models previously treated with 2 $mg \cdot kg^{-1}$ of PTX. By activating CB_2R, BCP inhibited p38 MAPK and NF-κB pathways activation and reduced microglial activation and cytokine release, so preventing neuroinflammation in paclitaxel-treated mice [55].

Other neuroprotective effects due to the activation of CB_2R by phytocannabinoids, apart from (−)-β-caryophyllene, have been reported. Accordingly, some data established that (-)-*trans*-Δ^9–tetrahydrocannabivarin (Δ^9-THCV) (figure 2) might be a promising therapy for alleviating symptoms and delaying neurodegeneration in PD, thanks to the CB_2R activation and CB_1R antagonism. The ability of Δ^9-THCV to reduce motor inhibition and provide neuroprotection was investigated in rats lesioned with 6-hydroxydopamine (6-OHDA) and in mice lesioned with LPS [56][57]. It is important to specify that while the neuroprotective effects of Δ^9-THCV do not seem to be related to the CB_2R activation in the model of 6-hydroxydopamine, as supported by the observation of a poor CB_2R up-regulation in the substantia nigra of parkinsonian animals, by contrast, it is possible to evidence a great up-regulation of CB_2R in the substantia nigra of mice injected with LPS. In fact, in the latter model, Δ^9-THCV (at a dose of 2 $mg \cdot kg^{-1}$) rescued tyrosine hydroxylase positive neurons. This neuroprotective effect was mirrored by the selective CB_2R agonist HU-308 and a different response was observed in the CB_2R-deficient mice, compared to the wild-type animal, revealing an involvement of CB_2R in this PD model [56].

In addition, the interesting phytocannabinoid cannabichromene (CBC) (figure 2) may contribute as potential therapeutic agent through CB_2R-mediated modulation of inflammation. In fact, CBC is a selective cannabinoid

CB_2R agonist, and it can also recruit CB_2R regulatory mechanisms, as demonstrated by Udoh M. *et al.* in their work on AtT20 FlpIn cells transfected with haemagglutinin (HA)-tagged human CB_2R, on which CBC induced a hyperpolarization that was blocked by the selective CB_2R antagonist AM630 [58]. Is well documented that CBC possesses anti-inflammatory properties. For example, a 2010 study tested its pharmacological effects in the LPS-induced paw edema model, although its mechanism of action did not involve CB_1R and CB_2R. Since either the CB_1R antagonist, rimonabant, or the CB_2R antagonist, SR144528, failed to block the activity of CBC, the mechanism by which CBC produced its effects remained to be established in future studies [59]. Another study, conducted by Shinjyo and Di Marzo (2013), placed particular attention on the neuroprotective impact of 1 μM CBC on the viability and differentiation of mouse adult neural stem progenitor cells (NSPCs). CBC augmented cell viability in the B27 medium, in which NSPCs stop proliferating and start differentiating, so proving a positive influence of CBC on adult neurogenesis. Although the involvement of the CB_2R receptor seems to be unlikely, given its moderate affinity for CB_2R (K_i ~ 100 nM) and the lack of expression of this receptor in the NPSC preparation used for these experiments, the pharmacological basis for its neuroprotective actions needs to be investigated [60]. Nonetheless, given its agonism towards CB_2R [58], there are promising signals of the possible contribution of this receptor to the potential therapeutic effectiveness of CBC.

In conclusion, more research is still needed to clarify the CB_2R activation mediated by phytocannabinoids in the treatment of neuroinflammation and neurodegenerative diseases, in order to improve the potential therapeutic effectiveness of some cannabis preparations.

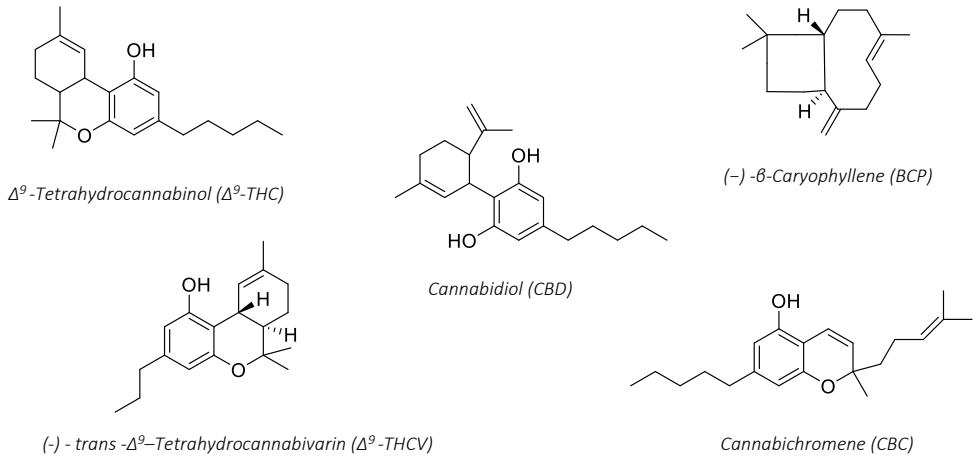


Figure 2. Structure of main phytocannabinoids with promising CB_2R-mediated neuroprotective potential.

3. Selective orthosteric ligands of CB_2R and neuroprotective properties

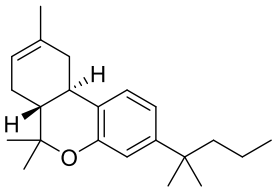
As previously discussed, the modulation of CB_2R may represent a novel therapeutic target in the treatment of neuroinflammation and neurodegenerative disorders, with minimal psychotropic effects [61]. Thus, the development of CB_2R selective modulators is becoming more and more interesting. For convenience, in this review we will divided these selective CB_2R ligands into several classes, which will be discussed separately, based on their chemical structures and on their activities.

3.1 CB_2R selective agonists

The analgesic properties of CB_2R agonists have been known for a long time, since the discovery of HU-308, a specific CB_2R agonist, by the research group of Hanus L. *et al.*, reporting for the first time the evidence of a peripheral analgesic activity of this compound, induced by a CB_2R selective activation [62]. The interest on CB_2R as a potential target for neuroinflammation and neurodegenerative diseases was triggered by the article published in 2000 by the research group of Baker D. *et al.*, reporting the antispastic effects of JHW-133, a CB_2R selective agonist that will be discuss in details below, in a multiple sclerosis model [63]. Since then, more and more pharmacological evidences suggested the essential role of CB_2R in the modulation of microglia activation and in the maintenance of neuronal homeostasis, and different CB_2R agonists were tested to evaluate their neuroprotective effects.

3.1.1 8-THC derivatives

Compounds belonging to this class have a chemical structure which resembles the delta-9-tetrahydrocannabinol (Δ^9-THC). In particular the research group of Huffman *et al.* discovered that the removal of the phenolic OH group from HU-210, non-selective CBRs agonist [64], to obtain JWH-051, did not significantly influence affinity for CB_1R, but greatly enhanced CB_2R affinity and selectivity [65]. The additional deletion of alcoholic group and further modifications of the alkyl chain led to more CB_2R-selective compounds. Among them, JWH-133, (6aR,10aR)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethy-6H-dibenzo[b,d]pyran, (figure 3) is noteworthy: it is a potent CB_2R agonist, with a K_i of 3.4 nM and a very high selectivity for CB_2R (around 200 folds over CB_1R) [66].



JWH-133

Figure 3. Structure of JWH-133.

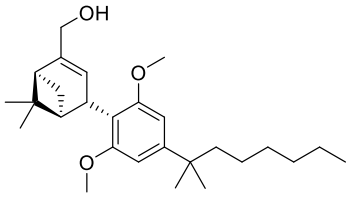
JWH-133

As previously discussed, the research group of Baker D. *et al.* first discovered its antispasticity effects in an autoimmune model of MS. In particular, using the CB_2R-selective agonist JWH-133 (1.5 $mg \cdot kg^{-1}$ i.v.) (figure 3), spasticity was reduced both 10 min ($P < 0.05$) and 30 min ($P < 0.001$) after injection at a time when 0.05 $mg \cdot kg^{-1}$ i.v. (dose selected to exhibit similar CB_1 activity to JWH-133) of methanandamide (AM-356, a potent CBRs-agonist, especially on CB_1R) was not active [63]. Later, Elmes S. JR. *et al.* evaluated the neuroprotective activity of JWH-133 in a model of neuropathic pain. In fact, its local administration (5 and 15 μg ⁄ 50 μL) mechanically evoked responses of spinal wide dynamic range (WDR) neurons in a models of spinal nerve-ligated (SNL) rats, attenuated by the selective CB_2R antagonist, SR144528 (10 mg ⁄ 50 mL), but not by the

CB_1R antagonist SR141716A [67][68]. Ramìrez B.G. *et al.* demonstrated that microglial activation induced *in vivo* by Aβ peptide in Aβ-treated rats was completely reversed by cannabinoid administration, including JWH-133. In addition, CB_2R agonist also attenuated the loss of neuronal markers induced by Aβ and prevented cognitive deficits which occur in Aβ-treated rats [69]. More recently, Gomez O. *et al.* reported that the treatment of purified oligodendrocyte progenitor cells (OPC), obtained from primary mixed glial cell cultures with JWH-133 (0.5 μM), with or without the CBRs antagonists AM281 or AM630, both at 1 μM, markedly accelerated differentiation and increased level of myelin basic protein (MBP), a marker of oligodendrocyte maturity, as soon as 48 h after the differentiation process starts [70]. This work was complemented and extended by following studies, which have reported that the markedly raised oligodendrocyte progenitor proliferation, promoted by JWH-133, appear to be mediated by the activation of the PI3K/Akt/mTOR signaling pathways. In addition, antagonistic binding to CBRs seemed to induce cell cycle arrest, as evidenced by the downregulation of Ki67 or by the increase in the cell cycle inhibitor p27Kip1. These results further suggest the importance of CB_2R in the progression of demyelinating diseases, such as MS, and highlights the therapeutic potential of the CB_2R signaling in the emerging field of brain repair [71].
In studies carried out by Moreno A.M. *et al.*, JWH-133 (400 μM) decreased intracellular Ca^{2+} level, after the addition of ATP, in cultured N13 microglial cell (2.5-fold compared with controls), promoting also their migration. This effect was counteracted by the CB_2R-selective antagonist SR144528, but completely unchanged by the CB_1R-selective antagonist SR141716, as expected. In addition, JWH-133 concentration-dependently decreased ATP-induced (400 μM) increase in intracellular calcium ($[Ca^{2+}]_i$) in cultured N13 microglial cells and in rat primary microglia [72]. Moreover, the oral administration of JWH-133 (0.2 $mg \cdot kg^{-1}$ /day during 4 months) reduced the enhancement of COX-2 protein levels and TNF-α mRNA expression and increased Aβ clearance in mouse model of AD [73].
Later, Zoppi S. *et al.* demonstrated the neuroprotective effect of JWH-133, which increased control levels of glutamate uptake (reduced by stress back to control levels) and prevented the stress-induced increase in proinflammatory cytokines (TNF-α and CCL2), in NF-kB, and in NOS-2 and COX-2, preventing the consequent cellular oxidative and nitrosative damage after daily administration (2 $mg \cdot kg^{-1}$, i.p.). To confirm that these effects were CB_2R-mediated, mice overexpressing CB_2R exhibited anti-inflammatory or neuroprotective actions similar to those in JWH-133 pre-treated animals, while $CB_2^{-/-}$ mice showed stress-induced neuroinflammatory responses [74]. Furthermore, the research group of Zarruk J.G. *et al.* reported that the administration of JWH-133 significantly reduced the brain infarction and neurological impairment by blocking the release of pro-inflammatory cytokine and by inhibiting the activation of different subpopulations of microglia/macrophages. These effects were reversed by the CB_2R antagonist SR144528 and were absent in $CB_2^{-/-}$. In particular, these were the first results able to demonstrate that a unique dose of JWH-133 (1.5 $mg \cdot kg^{-1}$), administered after the onset of ischemic injury, decreased the expression of proinflammatory molecules in the cortical peri-infarct tissue [75].
Finally, Aso E. *et al.* reported positive results of JWH-133 in a double AβPP/PS1 transgenic mice, a genetic model of AD that mimics the progressive cognitive deficiency and neurodegenerative process occurring in this disease. AβPP/PS1 transgenic mice were treated with JWH-133 (0.2 $mg \cdot kg^{-1}$) in the pre-symptomatic stage (3 months), and they exhibited an improvement of the long-term recognition memory, manifested as a reduction of microglia reactivity and as reduction of expression of cytokines (IL-1β, IL-6, TNF-α, and IFN-γ). Additionally, they also shown reduced levels of tau hyperphosphorylation at Thr181 in dystrophic neurites surrounding Aβ plaques, revealing a maintaining of tau functionality and a protection against effects of Aβ by CB_2R stimulation [76].

3.1.2 Bicyclic derivatives

This class was designed, synthesized and designated as nonclassical cannabinoids (NCCs), which differ from classical cannabinoids by the absence of the tetrahydropyran ring**.** The best known compound of this series is (-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol, CP55,940, full agonist of both CBRs [77]. Among them, HU-308 ((1R,2R,5R)-2-(2,6-Dimethoxy-4-(2-methyloctan-2-yl) phenyl)-7,7-dimethyl-4-bicyclo (3.1.1) hept-3-enyl) methanol (figure 4), synthetized by the research group of Hanus L. *et al.* [62], is distinguished by a high affinity and selectivity for CB_2R ($K_i = 22.7 \pm 3.9$ nM for CB_2R, $K_i > 10$ mM for CB_1R).



HU308

Figure 4. Structure of HU-308.

HU-308

As previously mentioned, its peripheral analgesic and anti-inflammatory activities were reported for the first time in 1999. In particular Hanus L. *et al.* demonstrated that the administration of HU-308 (figure 4) (at a dose of 50 and 20 mg·kg^{-1}, respectively, and injected between 30 and 90 min before the application of arachidonic acid) significantly decreased the arachidonic acid-induced ear swelling and reduced peripheral pain during the late phase of pain behavior, without psychotropic effects. Both the analgesic and the anti-inflammatory effects were reversed by CB_2R antagonist SR144528, though not by SR141716A [62].
In studies carried out by research group of García-Arencibia M. *et al.*, the daily intraperitoneal administration of HU-308 (5 mg·kg^{-1}) to 6-hydroxydopamine-lesioned rats (rat model of PD) produced a small recovery in DA depletion, while the administration of ACEA (a selective CB_1R agonist) or WIN55,212-2 (a non-selective CBRs agonist) did not reverse the DA depletion caused by the toxin in the lesioned striatum, supporting a possible involvement of CB_2R, but not CB_1R [78]. Moreover, Sagredo O. *et al.* reported that the administration of HU-308 (5 mg·kg^{-1}) in a rat model of HD (obtained through intra-striatal injection of the mitochondrial complex II inhibitor malonate) attenuated the reduction in mRNA levels for NSE, caused by the administration of malonate and partially reduced malonate-induced GABA deficit in the striatum and the globus pallidus, changing GABA contents only in the lesioned side. These effects were reversed by CB_2R antagonist, confirming the neuroprotective properties of this CB_2R agonist in this model of HD [79]. Supporting this, in the same year, Palazuelos J. *et al.* evaluated the direct stimulation of CB_2R using HU-308 (5 mg·kg^{-1}). Analysis of neurological damage evidenced a significantly decrease of microglial activation and astroglial reactivity in the striatum after excitotoxicity. These results were further confirmed by real-time PCR analyses, which evaluated different markers of glial activation and inflammation, as well as by the determination of NO levels. In addition, cannabinoid administration reduced the loss of striatal GABA levels and improved motor performance [80]. Moreno A.M. *et al.* reported that the intraperitoneal treatment with HU-308 (0.5 mg·kg^{-1}) promoted the migration of primary microglial cells, only inhibited by the CB_2R antagonist, as previously reported for JWH-133. However, in this case, the administration of HU-308 did not affect the intracellular calcium ($[Ca^{2+}]_i$) concentration, as seen above for JWH-133 [72]. Later, the research group of Gómez-Gálvez Y. *et al.* demonstrated that the activation of CB_2R with HU-308 (5 mg·kg^{-1} for 14 days) reversed the LPS-induced elevation of CD68 immunofluorescence in the striatum, as well as the reduction in TH immunofluorescence in the substantia nigra [81], associated with an increase of proinflammatory mediators, such as proinflammatory cytokines and enzymes [82], as also observed in experimental parkinsonism [83][84]. Additionally, they reported a decrease of gene expression for iNOS and other proinflammatory factors, promoted by selective CB_2R activation in the striatum, but not in the substantia nigra, and showed for the first time an up-regulation of CB_2Rs in glial elements in postmortem tissues of PD [81].

3.1.3 Aminoalkylindoles

Derivatives belonging to this class have a distinctly different structure to those of the compounds previously seen. The best known of this class is WIN 55212-2, 5-methyl-3-(morpholin-4-yl-methyl) -2,3-dihydro [1,4] oxazino[2,3,4-hi] indole substituted at position 6 by a 1-naphthylcarbonyl group, which presents good affinity for both CBRs, but higher for CB_2R than CB_1R [85].
Among these compounds, (2-Methyl-1-propyl-1*H*-indol-3-yl)-1-naphthalenylmethanone (JWH-015, figure 5), (2-iodo-5-nitrophenyl)-[1-[(1-methylpiperidin-2-yl)methyl]indol-3-yl]methanone (AM1241, figure 5) and (2,3-dichlorophenyl)-[5-methoxy-2-methyl-3-(2-morpholin-4-ylethyl)indol-1-yl]methanone (GW 405833,

figure 5), resulting from structural modifications of WIN 55212-2, are cannabimimetic indoles characterized by high affinity for CB_2R and low affinity for CB_1R [86][87][88].

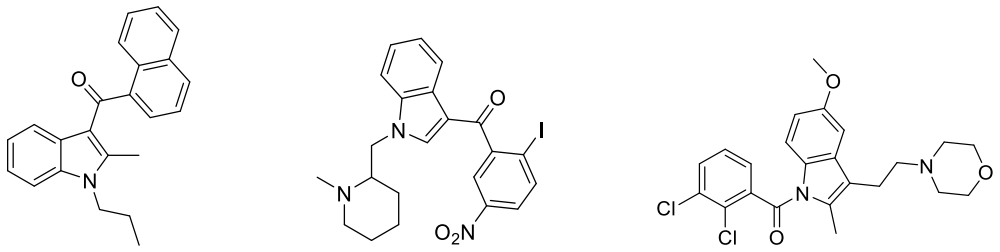


Figure 5. Structures of JWH-015, AM1241 and GW405833 (L 768242).

JWH-015

The first evidence of a neuroprotective effect of JWH-015 (figure 5) was reported by Arévalo-Martìn A. *et al.* in a murine model of MS: the administration of JWH-015 to female SJL/J mice, susceptible to TMEV-induced demyelinating disease (TMEV-IDD) development (0.6 $mg \cdot kg^{-1}$ for 3 d, 0.9 $mg \cdot kg^{-1}$ on days 4–6, and 1.2 $mg \cdot kg^{-1}$ on the last 4 d), reduced microglial activation and major histocompatibility complex class II antigen expression. In addition, it has been demonstrated for the first time that cannabinoid treatment promoted remyelination in the spinal cord, decreasing the number of $CD4^+$ infiltrating T cells [89]. In the same year, Klegeris A. *et al.* showed the neuroprotective activity of JHW-015 (5-10 μg) in human microglia, blocked by SR144528 but not by SR141716A, decreasing TNF-α and IL-1β secretion. The CB_2R agonist was also able to reduce toxicity of their culture THP-1 cell supernatants, before the stimulation with LPS and IFN-γ. Indeed, at a concentration of 10 μM, cell death was reduced by 25–50% according to the LDH assay and cell survival increased by 200–300% in the MTT assay) [90]. These results were later supported by research group of Ehrhart J. *et al.* that investigated the neuroprotective effect of JWH-015 and highlighted the ability to significantly suppress IFN-γ-induced phosphorylation of JAK/STAT1 and to inhibit microglial TNF-α and NO production, both induced by Aβ peptide or by IFN-γ microglial CD40 ligation. In addition, CB_2R activation considerably reduced CD40-mediated impairment of microglial phagocytosis of $A\beta_{1-42}$ peptide [91].
Moreover, in studies carried out by Tolón R.M. *et al.* incubation of THP-1 human macrophages with low levels of JWH-013 (1nM) dramatically reduced *in vitro* and in situ Aβ plaque, suggesting a CB_2R-mediated action of this compound, corroborated by the fact that SR144528, but not SR141716A, was able to reverse the neuroprotective effect of CB_2R agonist. Higher concentrations of JWH-015 were also effective, with a maximum effect at 5 nM and 10 nM, showing for the first time *in situ* effect of a CB_2R agonist on Aβ reduction in human samples [92].
In the same year, the research group of Price D. A. *et al.* showed, in a model of PD, that the treatment of tyrosine hydroxylase positive (TH+) neurons in the substantia nigra pars compacta (SNc) with JWH015 (4 $mg \cdot kg^{-1}$, i.p.) decreased MPTP-induced microglial activation, while genetic CB_2R ablation aggravated MPTP systemic toxicity [93].
Later, Ribeiro R. *et al.* reported the neuroprotective effects of CBRs agonists, including JWH-015 (100 nM), being able to reduce iNOS induction and ROS generation in LPS-activated BV-2 cells and in primary microglia, inhibiting also NF-κB activity in LPS-activated BV-2 cells. But surprisingly, the inhibitory effect was not reversed either by AM630 (CB_2R antagonist), or by AM281 (CB_1R antagonist) and the antagonists themselves also suppressed microglia activation by inhibiting ERK1/2 and cPLA2 phosphorylation and NF-kB activation, as well as CB_2R agonist, suggesting that both agonists and antagonists are able to reduce peroxynitrite formation in activated microglia [94]. Results obtained by Avraham H.K. *et al.* highlighted the neuroprotective effects of CB_2R agonists, by showing the ability of JWH-015 (10 μM) and AM1241 (another CB_2R selective agonist, that will be discussed below) to suppress Gp120-mediated toxicity both in *in vitro* cultures of murine and human NPC cell [95].
Finally, more recently, in a work carried out by Li C. *et al.*, the CB_2R activation by JWH-015 (0.5 $mg \cdot kg^{-1}$ i.p. for 8 weeks) decreased deficit of the cortex-dependent novel object recognition memory in transgenic APP/PS1 mice (mouse model of AD), but it was ineffective for hippocampus-dependent spatial cognitive dysfunction.

In addition, in the cortex of APP/PS1 mice, it reduced immunofluorescence intensity of Iba1 and, preventing also the switch from the M1 to the M2 microglial phenotype and restoring dendritic homeostasis after a long treatment [96].

AM1241

In 2003, the research group of Ibrahim M. M. *et al.*, on the basis of the previous demonstration of the inhibitory activity of AM1241 (figure 5) on acute thermal nociception [97], showed that AM1241 (1 $mg \cdot kg^{-1}$ i.p. injection) reduced in a dose-dependently manner tactile and thermal hypersensitivity, obtained through SNL of the L5 and L6 nerves in rat. This effect was selectively antagonized by AM630 but not by AM251, corroborated by the evidence that AM1241 was also active in blocking SNL-induced tactile and thermal hypersensitivity in $CB_1^{-/-}$ mice [87]. The anti-inflammatory effect of AM1241 was also confirmed by Quartilho A. *et al.*, proving that injection (intrapaw 50 µl and intraperitoneally 0.5 ml) into the inflame paw fully reversed carrageenan-induced inflammatory thermal hyperalgesia, as well as the local edema caused by hind paw carrageenan injection. Also in this case the anti-inflammatory effect of the CB_2R agonist was reversed by AM630 (100 $\mu g \cdot kg^{-1}$, intraperitoneal) but not by AM251 (300 $\mu g \cdot kg^{-1}$, intraperitoneal) [98].
Moreover, in a work carried out by the research group of Beltramo M. *et al.*, (+) AM1241 (3 and 6 $mg \cdot kg^{-1}$ i.v.) reduced allodynia produced by SNL of L5–L6 nerves in a rat model. The effect was reversed by SR144528, supporting a CB_2R-mediated effect. In addition, the CB_2R agonist also decreased capsaicin-induced calcitonin gene related peptide (CGRP) release in a dose dependent manner (4.5 nM, respectively) and the coadministration of SR144528 produced a right-forward shift (pKB 8.1) of the dose–response curve [99]. In the same year, Kim K. *et al.* showed, in $hSOD1^{G93A}$ transgenic mice (mouse model of ALS), that AM1241 (1 $mg \cdot kg^{-1}$) slowed progression of disease in male mice when administered after onset of signs, highlighting for the first time beneficial effects of CB2 agonist in chronic neurodegenerative disease model [100].
Later, the research group of Ribeiro R. *et al.* demonstrated a consistent decrease of iNOS induction and NO production and a reduction of ERK1/2 and cPLA2 phosphorylation in LPS-activated BV2 microglial cells as a consequence of the treatment with AM1241 (10 nM, 100 nM and 1 µM). As previously seen for JWH-015, neither AM630 or AM281 reversed the effects of CB_2R agonist and surprisingly, both CBRs antagonists (100 nM) alone or in combination also significantly blocked iNOS induction [94].
Finally, as previously seen for the CB_2R agonist JWH-015, H. K. Avraham *et al.* also reported the neuroprotective action of AM1241 (the optimal concentration was 100 nM) in a model of HIV-associated dementia (HAD), being able to inhibit Gp120-mediated toxicity. In addition, it enhanced survival and differentiation of murine NPCs to neuronal cells, demonstrating a neuroprotective effect on neurogenesis in GFAP/Gp120 Tg mice *in vivo*, indicated also by a significant increase in the number of $BrdU^+$, $PCNA^+$, neuroblasts and neuronal cells. These effects were mediated by CB_2R, in fact pretreatment of NPCs with the CB_2R antagonist AM630 abolished these AM1241-mediated effects on survival and differentiation of NPCs [95].

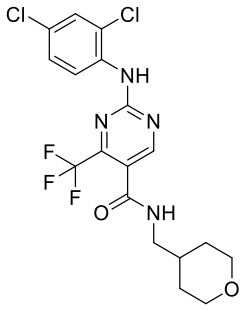
GW405833 (L 768242)

In 2002 Clayton N. *et al.* reported the anti-inflammatory effect of GW405833 (0.3 -10 $mg \cdot kg^{-1}$ i.p.) (figure 5), blocked by SR144528, being able to inhibit the hypersensitivity in a *in vivo* rat model of nociceptive pain [101]. However, the first evidence of the neuroprotective action of GW405833 was reported by Valenzano K. J. *et al.*, who demonstrated potent and efficacious dose-dependent anti-hyperalgesic effects (up to 30 $mg \cdot kg^{-1}$) in a model of partial ligation of the sciatic nerve (Seltzer model). In addition, in the same study, the intraperitoneal administration of GW405833 (0.3-100 $mg \cdot kg^{-1}$) to rats was found to have a dose-dependently linear increase in plasma levels and a substantial penetration into the CNS [102]. To corroborate these results, the neuroprotective effect was subsequently confirmed in the same mouse model of neuropathic pain, showing that its intraperitoneal administration (30 $mg \cdot kg^{-1}$) reduced tactile allodynia produced by nerve injury and inflammation. However, high-dose of GW405833 (100 $mg \cdot kg^{-1}$) showed both sedative and analgesic effects in wild-type mice, observed in cannabinoid $CB_2^{-/-}$ mice too [103]. Moreover, as previously reported for AM1241, GW405833 (30 $mg \cdot kg^{-1}$ i.p. and 1 and 3 $mg \cdot kg^{-1}$ i.v.) reduced the second phase of nocifensive

behaviors, produced by formalin intraplantar injection, and reduced the release of CGRP (EC_{50} 3.6 nM) in a dose-dependent manner [99].
More recently, the research group of Bouchard J. *et al.* demonstrated in a mouse model of HD that the treatment with GW405833 deleted neuroinflammation, motor deficits and synapse loss, extending also life span, and a peripheral treatment with SR144528 blocked these effects. In particular, the administration of CB_2R agonist (20 $mg \cdot kg^{-1}$/d, i.p. starting at about 4 weeks of age, an early symptomatic stage in these mice) reduced behavioral and neuropathological deficits in R6/2 mice and improved mice performance on balance beam, as well as rotarod, highlighting also the importance of CB_2R signaling in peripheral immune cells at early disease stages. Both these effects were reversed when the CB_2R agonist was co-administered with the peripherally restricted SR144528 [104].

3.1.4 Pyrimidine derivatives

Compounds belonging to this class are characterized by a novel chemical structure based on a pyrimidine core and were obtained by structural modifications, starting from (6a*R*,-10a*R*)-3-(1,1-dimethylheptyl)-9-(hydroxymethyl)-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (HU210), a non-selective CBRs agonist [105] synthetized by Mechoulam R. *et al.* at the Hebrew University [64]. Among them, GW842166X (figure 6) is notable: it is a potent CB_2R selective agonist, with a similar potency and efficacy for rat and human recombinant CBRs (for rat, CB_2R EC_{50} = 91 nM, E = 100%, n = 6; for human, CB_2R EC_{50} = 63nM, E = 95%, n = 20), with no relevant agonist activity for CB_1R and promising pharmacokinetic profile in rats [106]. It also completed phase II for pain therapy [107].



GW842166X

Figure 6. Structure of GW842166X.

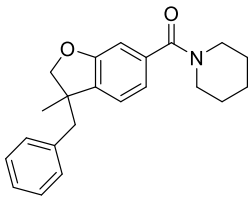
GW842166X

The research group of Giblin G.M.P. *et al.* reported the analgesic properties of GW842166X (figure 6), showing to be able to fully reverse hyperalgesia at 0.3 $mg \cdot kg^{-1}$, determined by a weight bearing protocol in a Freund's Complete Adjuvant (FCA) model of inflammatory pain. This activity was reversed by administration of AM630, corroborating the conclusion that this anti-inflammatory effect was mediated by CB_2R [106].
Later, Ribeiro R. *et al.* displayed the involvement of GW842166X in attenuating ERK1/2 and cPLA2 phosphorylation and iNOS induction in activated BV2 cells, as previously reported in details for JWH-015 and AM1241 [94].

3.1.5 Benzofuran derivatives

It is a more recent class whose derivatives are marked by a benzofuran scaffold, to increase bioavailability compared with the isatin series [108]. Among them, (1-[(3-benzyl-3-methyl-2,3-dihydro-1-benzofuran-6-yl) carbonyl] piperidine (MDA7, figure 7) is noteworthy: it was synthetized by the research group of Naguib M. *et al.* and showed good affinity and high selectivity for CB_2R (rat CB_2R K_i = 238 ± 143 nM and human CB_2R = 422 ± 123 nM, while rat CB_1R K_i = 2565 ± 695 nM and human CB_1R K_i > 10,000 nM) [109]. Diaz P. *et al.* evaluated MDA7activity of the separate enantiomers proving that the S enantiomer (MDA 104) was the active

compound. In this study another benzo-furan derivative, MDA 42, was tested in a model of neuropathic pain, and exhibited activity in the same range as that of MDA7 [108].



MDA7

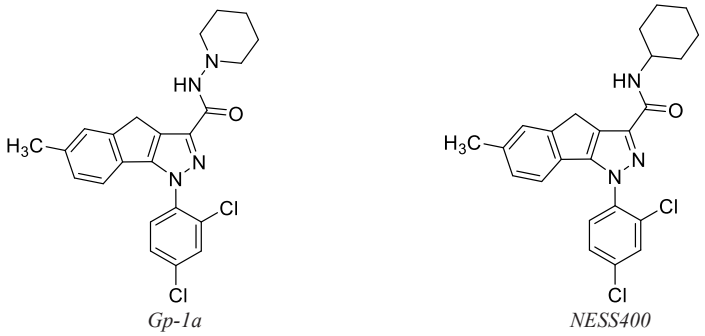
Figure 7. Structure of MDA7.

MDA7

Naguib M. *et al.* first reported the neuroprotective effect of MDA7 (figure 7). In a rat model of neuropathic pain, the treatment with the CB_2R agonist (5, 10 and 15 $mg \cdot kg^{-1}$) attenuated tactile allodynia, produced by SNL or by paclitaxel, in a dose-related manner (ED_{50} of 7.5 $mg \cdot kg^{-1}$ and an efficacy of 75.1 ± 5% after the dose of 15 $mg \cdot kg^{-1}$), without affecting locomotor behavior. These effects were selectively antagonized by AM630 but not by the CB_1R antagonist AM251 or opioid receptor antagonist naloxone [109]. These results were corroborated by subsequent demonstration that MDA7 (15 $mg \cdot kg^{-1}$ i.p. for 14 days) prevented the development of paclitaxel-induced mechanical allodynia in rat and in $CB_2^{+/+}$ rat, but not in $CB_2^{-/-}$ rat, reducing also levels of CD11b in microglia (major indicator of neuroinflammatory process activation) and of glial fibrillary acidic protein (GFAP) in astrocytes. In addition, the CB_2R agonist-mediated an anti-inflammatory effect in LPS-stimulated primary glial cells *in vitro*, reducing release of TNF-α and IL-1β, and decreased level of TLR2 in the spinal cord of paclitaxel-treated rats [110].
Moreover, based on results previously discussed, Wu J. *et al.* corroborated the neuroprotective effect of MDA7 evaluated by Naguib M. *et al.* [110] in Aβ-injected rats (model of AD). Furthermore, the administration of CB_2R agonist (15 $mg \cdot kg^{-1}$ i.p. daily for 14 days) reduced the Aβ-mediated suppression of glutamatergic transmission in the hippocampus, improving also spatial memory performance using the Morris water maze test [111]. These results were then validated by the evidence that activation of microglial CB_2R by MDA7 (14 $mg \cdot kg^{-1}$ i.p. daily for 5 months) reduced the intensity of Iba1 in the hippocampal CA1 and in dentate gyrus (DG) areas in APP/PS1 mice (transgenic mouse model of AD). In addition, a substantially reduction of Aβ plaques was observed in the hippocampal DG, together with a restore of Sox2 expression and an improvement of the cognitive decline [112].

3.1.6 Pyrazole derivatives

The compounds of this class present a tricyclic pyrazole scaffold and represent a rigid analogue of SR141716A, a CB_1R selective antagonist. Among them, Gp-1a, N-(piperidin-1-yl)-1-(2,4-dichlorophenyl)-1,4-dihydro-6 methylindeno[1,2-c]pyrazole-3-carboxamide (figure 8) and later NESS400, 1-(2′,4′-dichlorophenyl)-6-methyl-N-cyclohexylamine- 1,4-dihydroindeno[1,2-c]pyrazole-3 carboxamide (figure 8) showed very high affinity for CB_2R, resulted as two of the most selective and potent CB_2R agonists of this class [113] [114].



Gp-1a *NESS400*

Figure 8. Structures of Gp-1a and NESS400.

Gp-1a

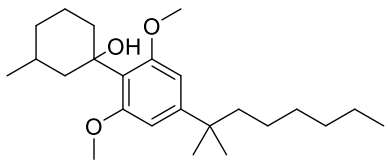
Kong W. *et al.* first reported *in vitro* and *in vivo* CB_2R-mediated Gp1a (figure 8) inhibition of Th17/Th1 differentiation, emphasizing the relevance of this CB_2R agonist as a potential therapeutic agent in neuroinflammation. In particular the administration of two different concentration of Gp1a (5 and 10 μM) positively affected EAE through two different mechanisms: the first one in immune organs on Th1/Th17 differentiation, and a later one in the CNS, associated with reduction of adhesion molecule, cytokines and chemokine release. In addition, GP-1a ameliorated recovery in EAE in conjunction with long term reduction in demyelination and axonal loss [115].
Moreover, in a work carried out by Braun M. *et al.*, administration of Gp-1a (1-5 $mg \cdot kg^{-1}$), in a murine controlled cortical impact (CCI) model of traumatic brain injury (TBI), increased polarization of anti-inflammatory M2 polarization, reducing at the same time proinflammatory M1 macrophage polarization. Additionally, it improved cerebral blood flow, and ameliorated neurobehavioral outcomes, reducing also edema development. In this case the CB_2R selective antagonist AM630 worsened the outcomes [116].
Recently, Sheng W.S. *et al.* evaluated the neuroprotective activity of Gp-1a on a retrovirus infection-induced distal symmetric polyneuropathy (DSP) murine model, showing that i.p. injections of CB_2R agonists such as Gp1a (5 $mg \cdot kg^{-1}$ starting at 5 wk i.p.), JWH-015 and JWH-133, but not HU-308, significantly ameliorated allodynia when administrated 2 h after ligand injection, but not 24 h after ligand injection. Stimulation of CB_2R also blocked IFN-γ-induced phosphorylation of STAT1 and STAT3 in primary murine microglia, but did not influenced *in vivo* leukocyte migration [117].

NESS400

To date, few neuroprotective evidences were reported by Luongo L. *et al.* They evaluated pain thresholds in a mouse model of spared nerve injury (SNI), as well as the distribution and activation of spinal microglia, following chronic treatment with NESS400 (figure 8), demonstrating that CB_2R agonist (4 $mg \cdot kg^{-1}$) consistently reduced thermal hyperalgesia and neuropathic mechanical allodynia. In particular, NESS400 reduced astrocyte upregulation and pro-inflammatory cytokine mRNA expression (IL-1β, IFN-γ), and increased anti-inflammatory cytokine IL-10. Even if these data evidenced an involvement of peripheral mechanism, the up-regulation of CB_2R in non-neuronal cells in the spinal cord, as well as the ability of NESS400 to influence phenotypical changes of microglial and glial cells, suggested also an involvement of central mechanism [118].

3.1.7 Resorcinol derivatives

These are analogues of bicyclic resorcinol, a chemical compound structurally similar to cannabidiol. In particular, 1-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-3-methylcyclohexan-1-ol (0-1966, figure 9), described by Wiley J.L. *et al.*, showed high affinity and selectivity for CB_2R (K_i CB_2R= 23 ± 2.1 nM, K_i CB_1R= 5055 ± 984 nM) [119].



0-1966

Figure 9 Structure of O-1966.

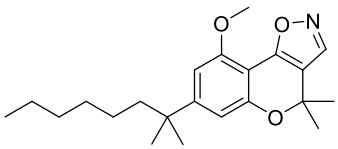
0-1966

In studies carried out by Zhang M. *et al.*, administration of 0-1966 (0.1, 1 and 10 $mg \cdot kg^{-1}$ with 1 $mg \cdot kg^{-1}$ as the optimal dose) (figure 9) after the onset of symptoms attenuated disease progression in the remitting–

relapsing model (SJL/J/PLP mice), and ameliorated motor function both in chronic autoimmune encephalomyelitis EAE mice (C57BL/6/MOG mice) and in SJL/J/PLP mice. In addition, in C57BL/6/MOG mice, treatment with CB_2R agonist shifted cytokine response from IFNγ to IL-4/IL-13/IL-10 and reduced the proliferation of T-cells [120]. Elliott M.B. *et al.* subsequently reported that mice treated with 0-1966 (5 $mg \cdot kg^{-1}$ i.p. at 1 h and 24 h post injury) showed in cortical contusion impact injury (CCI) mice (model of TBI) a decrease of macrophage/microglial activation in the injured brains at 48 h post-injury and of perivascular substance P immunoreactivity, with the following reduction of cerebral edema and the improvement of locomotor function and exploratory activity [121]. Furthermore, in order to confirm these results, 0-1966 (5 $mg \cdot kg^{-1}$ i.p.) was tested in the same model of TBI showing that it significantly reduced neuron degeneration and NaF uptake, improving also rotarod and open-field testing compared to vehicle-treated mice. These results were also associated with reduction in microglia/macrophage cell counts [26]. These data were later confirmed by other studies. In particular 0-1966 was found to reduce iNOS (expressed by macrophage/microglia in the injured cortex) mRNA expression, while genetic deletion of the CB_2R significantly increased iNOS expression. Moreover, treatment with CB_2R agonist also reduced intracellular adhesion molecule (ICAM-1) mRNA and TNF-α levels. The same effects were also observed for JWH-133 [122].
Finally, Adhikary S. *et al.* evaluated the neuroprotective effect of 0-1966 in a mouse model of Spinal Cord Injury (SPI): the treatment with CB_2R agonist (5 $mg \cdot kg^{-1}$ i.p.) led to a decrease of chemokines/cytokines, resulting in reductions in CXCL-9, CXCL-11 and IL-23p19 expression, and in a reduction of its receptor IL-23R. Treatment with O-1966 also caused inhibition of toll-like receptor expression (TLR1, TLR4, TLR6 and TLR7) and therefore improved motor function [123].

3.1.8 Chromenpyrazole derivatives

This series derives from chromeno-isoxazoles and –pyrazoles, which seems to have a promising profile related to the CB_2R. Among them, PM226, 7-(1,1-dimethylheptyl)-4,4-dimethyl-9-methoxychromeno[3,4-d]isoxazole (figure 10), has shown very high affinity, selectivity (K_i CB_2R= 12.8 ± 2.4 nM and K_i CB_1R> 40000 nM) and agonist activity (EC_{50} = 38.67 ± 6.70 nM) for CB_2R. Furthermore, *in silico* analysis demonstrated a good pharmacokinetic profile and a predicted ability to cross the blood-brain barrier [124].



PM-226

Figure 10. Structure of PM226.

PM226

The neuroprotective properties of PM226 (figure 10) were first investigated by the research group of Gómez-Cañas M. *et al*., in an *in vitro* model of neuronal death, using mouse microglial cell line BV2 and the striatal neuronal cell line M213-20. The conditioned media were collected from LPS-stimulated cultures of BV2 microglial cell line and then added to cultured M213-2O neuronal cells, with the consequent reduction of cell viability. As expected, the presence in cellular media of PM226 (0.1, 1 and 10 μM), significantly increased cell viability in a dose-dependent manner. Moreover, the co-incubation with the CB_2R antagonist SR144528, added only for the high concentration of PM226, reversed the effect, underling the specific involvement of CB_2R. Moreover, in *in vivo* model of striatal damage with the mitochondrial neurotoxin malonate, MRI analysis showed that PM226 administration (1 $mg \cdot kg^{-1}$ as lower active dose) reduced edema volume caused by malonate. Even in this second case, the neuroprotective effect was reversed by CB_2R antagonist AM630 [124]. In the same year, Morales P. *et al.* corroborated these results, reporting that injection of PM226 (5 $mg \cdot kg^{-1}$, i.p.) reduced microglia activation in the acute phase of Theiler's murine encephalomyelitis virus-induced demyelinating disease (TMEV-IDD), a model of MS [125].

3.1.9 1,8-Naphthyridin-2(1*H*)-one and quinolin-4(1*H*)-one derivatives

Various 1,8-Naphthyridin-2(1H)-one and quinolin-4(1H)-one derivatives showing high CB_2R receptor selectivity and affinity versus the CB_1R receptor were reported in literature [126] [127] [128] [129]. It is noteworthy to evidence two studies on peripheral blood mononuclear cells (PBMC) conducted with N-(4-methylcyclohexyl)-1- benzyl-1,8-naphthyridin-2(1*H*)-on-3-carboxamide (CB74) and N-(4-methylcyclohexyl)-1-(*p*-fluorobenzyl)-1,8-naphthyridin-2(1H)-on-3-carboxamide (CB91) (figure 11), two highly selective 1,8-naphthyridine derivatives, and N-cycloheptyl-1-(2-morpholin-4-yl-ethyl)-quinolin-2(1*H*)-on-3-carboxamide (VL23), as representative compound of quinoline derivatives (figure 11) [130] [131]. In particular, the immune-modulatory effects in activated lymphocytes isolated from MS patients with respect to healthy controls were evaluated. These compounds blocked cell proliferation through a mechanism partially reverted by the CB2R antagonist SR144528, down-regulated TNF-a production and did not induce cell death. They also down-regulated the expression of Akt, Erk and NF-kB phosphorylation and of the enzyme COX-2. Finally, they inhibited some T cell activation markers, such as CD69 and the adhesion molecule CD54, in MS patient derived lymphocytes more efficiently than in healthy control derived cells [130] [131]. The described immunomodulatory and anti-inflammatory effects of the 1,8-naphthyridine and quinoline derivatives, suggest these classes of compounds as good candidates for further studies with the aim to establish their potential in the therapy of MS.
The interesting therapeutic potential of the quinoline derivatives had been already explored by Pasquini *et al*. in the formalin test of acute peripheral and inflammatory pain in mice [126]. One of the most potent and CB_2R-selective ligands of the reported set of compounds in this work, compound 11c (figure 11), at a dose of 1 or 3 $mg \cdot kg^{-1}$, dose-dependently inhibited the second phase of the formalin-induced nocifensive response in mice. The involvement of CB_2R was demonstrated by using the CB_2R antagonist AM630, suggesting that quinolone-3-carboxamides possess agonist properties at the CB_2R and might be considered as potential analgesic agents [126]. The therapeutic potential of compound 11c, named COR167 (figure 11) by Annunziata P. *et al*. in 2017, was carefully described in an interesting study on both PBMC and myelin basic protein-reactive T cell lines from patients with multiple sclerosis, compared to healthy subjects. Notably, COR167 was able to markedly inhibit the PBMC as well as the myelin basic protein (MBP)-reactive T cell lines proliferation and partial shift from Th1 phenotype to anti-inflammatory Th2 phenotype, coupled with a reduction of IL-4, IL-5, and Th17-related cytokines levels, demonstrating its immunomodulatory and anti-inflammatory properties [132].

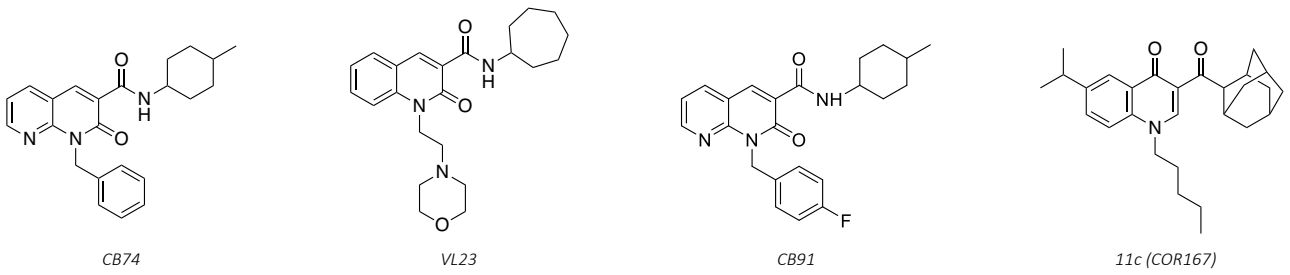


Figure 11. Structures of CB74, VL23, CB91 and 11c (COR167).

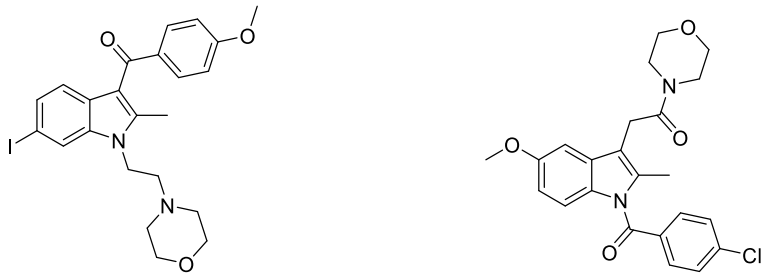
3.2 CB_2R selective antagonists/inverse agonists

The neuroprotective activity of this class has been discovered more recently, compared to CB_2R agonists. In 2003, in fact, the research group of Klegeris A. *et al.* reported the anti-inflammatory effect of BML-190, an inverse agonist discovered by New D.C. *et al.* [133] in human activated microglia [90]. Since then, several CB_2R antagonists/inverse agonists have been studied and have showed positive effects in models of neuroprotection, as reported below.
Even if additional studies are needed, the results collected to date suggest a possible use of these compounds as innovative therapies in the treatment of neuroinflammation and neurodegenerative diseases.

3.2.1 Aminoalkylindole derivatives

AM-630, [6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone] (figure 12), was originally described as a CBRs antagonist in the mouse isolated vas deferens, as reported by Pertwee

R. *et al.* [134], but it also possessed the properties of a weak cannabinoid CB_1R agonist in the myenteric plexus-longitudinal muscle preparation of guinea-pig small intestine [135]. Furthermore, two reports classified AM-630 as a CBRs antagonist in mouse and guinea-pig brain membrane preparations [136] and as an inverse agonist in chinese hamster ovary (CHO) cells stably transfected with CB_1R [137]. However, the research group of Ross R. A. *et al*. found AM-630 to behave also as an inverse agonist at CB_2R in CB_2R-transfected cells, showing to be CB_2R-selective with a CB_1/CB_2 K_i ratio of 165 in transfected CHO cell membranes [138].
Another aminoalkylindole derivative, 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-1-morpholinoethan-1-one (BML-190, figure 12) was originally described as a CB_2R agonist, with a K_i value for CB_2R of 435 nM with 50-fold selectivity over CB_1R, and also used as a CB_2R agonist by Melck D. *et al.*, to evaluate the effect of cannabinoids on the proliferation of cancer cells [139]. Later, New D. C. *et al.* demonstrated that it was a potent CB_2R inverse agonist [133].



AM-630 *BML-190*

Figure 12. Structures of AM-630 and BML-190.

AM-630

As previously mentioned, evaluating the neuroprotective effect of JWH-015, Ribeiro R. *et al.* discovered that AM-630 (100 nM) (figure 12), together with the CB_1R antagonist/inverse agonist AM-281 (100 nM), was able to block the iNOS induction alone or in combination and significantly reduced ROS and NF-kB generation in LPS activated BV-2 cells, demonstrating that both CB_2R agonists and antagonists/inverse agonists are able to attenuate peroxynitrite formation in activated microglia. In addition, AM-630 suppressed microglia activation also by interfering with ERK1/2 and cPLA2 phosphorylation. All these results suggest that CB_2R and CB_1R agonists and antagonists/inverse agonists may suppress microglia activation by novel CB_2R- and CB_1R-independent mechanisms [94].

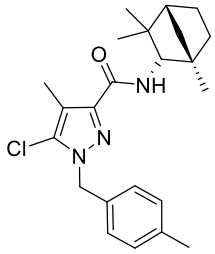
BML-190

To date, the only relevant evidence about a neuroprotective activity of BML-190 (figure 12) was described by Klegeris A. *et al.*, as previously reported for JWH-015. In fact, when BML-190 was added to THP-1 cells before stimulation with LPS and IFN-γ in activated human microglia and microglia-like THP-1 cells, it reduced the toxicity of their culture supernatants to SH-SY5Y cells in a dose-dependent manner. At the concentration of 10 μM, cell survival increased by 200–300% in the MTT assay and cell death declined by 25–50% according to the LDH assay. At the same time, however, BML-190 significantly increased the release of TNF-α and had no effect on IL-1β secretion [90].

3.2.2 Pyrazole derivatives

Derivatives belonging to this class are characterized by a variously substituted pyrazole core. Among them, SR-144,528, 5-chloro-4-methyl-1-(4-methylbenzyl)-N-((1S,2S,4R)-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl)-1H-pyrazole-3-carboxamide (figure 13) was introduced by Rinaldi-Carmona M. *et al.* as the first, highly potent, selective and orally active CB_2R antagonist , with sub-nanomolar affinity (K_i = 0.6 nM) for both rat spleen and cloned human CB_2R and a 700-fold lower affinity (K_i = 400 nM) for both rat brain and cloned human CB_1R [140], further corroborated by *Griffin G. et al.* [141]. Later, Rhee M. H. *et al.* showed in transfected COS cells the inverse agonist activity of SR144528 (stimulation of adenylyl cyclase V and

inhibition of adenylyl cyclase II), depending on the temperature and on the concentration of the cDNA of CB_2R transfected cells [142].



SR144528

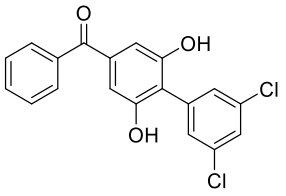
Figure 13. Structure of SR144528.

SR-144528

As mentioned above for AM-630 in studies carried out by Ribeiro R. *et al.*, SR-144528 (figure 13) showed anti-inflammatory and antioxidative effects (100 nM), such as the CB_1R selective antagonist/inverse agonist SR-141716 (100 nM) [94]. In addition, the research group of Presley C. *et al.* reported the ability of SR144528 (added in 50 μL containing 5X of previously determined EC_{50} and 1X of LPS) and SMM-189 (an inverse agonist that will be discussed below), to decrease CD16/32, which is a marker of a proinflammatory state consistent with LPS activation, and to significantly increase CD206, a marker of a pro-wound healing state consistent with alternative activation in a model of TBI. CB_2R agonists (JWH-133 and HU-308) decreased CD16/32, but also CD206, exhibiting at this level the opposite effect of inverse agonists [143].

3.2.3 Tri-aryl derivatives

These compounds, characterized by a tri-aryl core, combine aspects of two distinct cannabinoid series, the resorcinol derivatives and bicyclic HU-308, with the aim of achieving new and potent agonist of CB_2R. In particular, 3',5'-dichloro-2,6-dihydroxy-biphenyl-4-yl)-phenyl-methanone (SMM-189, figure 14) [144], characterized by high affinity for CB_2R (K_i CB_2R= 121.3 ± 20.6 nM and K_i CB_1R= 4778 ± 246 nM) [143], is a CB_2R selective inverse agonist which showed also a relatively high uptakes (>1.2% injected dose/g tissue) in mouse brains [145]. However, Presley C. *et al.* determined that SMM-189 behaves also as a non-competitive CB_2R-antagonist against CP 55,940 [143].



SMM-189

Figure 14. Structure of SMM-189.

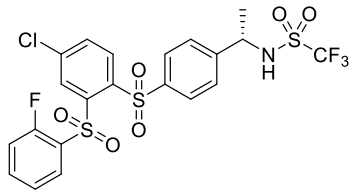
SMM-189

In a research work carried out by Reiner A. *et al.*, *in vitro* analysis indicated that SMM-189 (figure 14) shifted human microglia from the M1 phenotype to the M2 phenotype, associated with nuclear translocation of pCREB. By itself, SMM-189 did not affect the expression of any of the examined chemokines, except for a reducing effect on TARC. However, when SMM-189 (9.8 μM) was combined with LPS, it reduced the expression of several pro-inflammatory factors, including IL-8, MIP-1β, MDC, IFN-γ, IL-6, IL-10, and IL-12p70, underlining both an anti-inflammatory and a pro-repair effect of CB_2R inverse agonists. In an *in vivo* mouse model of TBI, SMM-189 improved the motor, visual, and emotional behavior, associated with CREB

activation in microglia [146]. Furthermore, these results were also confirmed by the remarking ability of SMM-189 to regulate microglial activation in a murine model of TBI. In particular, the administration of SMM-189 to LPS-activated C8B4 murine microglia cells (added in 50 μL containing 5X of previously determined EC_{50} and 1X of LPS) resulted in a decrease of the M1 proinflammatory marker CD16/32 and an increase of M2 anti-inflammatory and pro-healing marker CD206. Additionally, it determined also an increase in rod-shaped cells [143]. Considering that rod-shaped cells have been suggested to be highly beneficial in repairing neurodegenerative areas of the CNS [147], the CB_2R inverse agonist could be potentially useful in the treatment of neurodegenerative disorders. The later work extended previous findings, showing that SMM-189 rescued TBI-related neuronal loss in several brain regions, indicating that M1 microglial activation during the aftermath of mild TBI worsened the outcome, while biasing toward M2 microglial activation during this same time period allowed the survival of many neurons. Treatment with a CB_2R agonist instead would reduce M1 activation but not promote M2 activation and it could explain why CB_2R agonists have not shown significant positive effects on TBI [148].

3.2.4 Triaryl bis-sulfone derivatives

Triaryl bis-sulfones are CB_2R specific inverse agonists, which in general showed high affinity and selectivity for CB_2R. In particular Sch.414319, (S)-N-(1-(4-((4-chloro-2-((2-fluorophenyl) sulfonyl) phenyl) sulfonyl) phenyl) ethyl) -1,1,1-trifluoromethanesulfonamide (figure 15, K_i CB_2R = 2 nM and K_i CB_1R = 6785 nM) provided encouraging results in blocking experimental autoimmune encephalomyelitis in the rat [149].



Sch.414319

Figure 15. Structure of Sch.414319.

Sch.414319

As previously mentioned, Sch.414319 (figure 15) provided promising results in a mouse model of EAE. The research group of Lunn C.nA. *et al.* first investigated the role of the CB_2R in inflammatory diseases of the central nervous system, using the cannabinoid CB_2R-deficient mice, showing that the capability of a peptide derived from myelin oligodendrocyte glycoprotein (MOG35–55) to elicit EAE was reduced in a cannabinoid CB_2R-deficient mouse strain [150]. Subsequently, these initial studies were deepened, proving the ability of Sch.414319 to modulate EAE in the rat. In particular, oral administration of the CB_2R inverse agonist (2 and 20 $mg \cdot kg^{-1}$) significantly reduced the clinical signs of EAE, converting complete hind-limb paralysis to a flaccid tail phenotype [149].

4. Potential role of CB_2R allosteric ligands as neuroprotective agents

Several evidences have established the key role of endocannabinoid-CB_2R signaling in neuroinflammation, neuroprotection, and in neurodegenerative disorders, as reviewed in detail in previous paragraphs. The mechanism of neuroprotection, which includes a whole of excitotoxic and immunological processes of the CB_2R, led to the understanding that also allosteric modulation of CB_2R may successfully offer an interesting, novel, and promising therapeutic approach for a range of neurodegenerative disorders. CB_2R agonists show great therapeutic promise that, however, could be outshined by immune suppression due to the long-term elevations in endocannabinoid levels following the chronic use and pro-inflammatory actions in certain disease-specific contexts [151].

Generally, allosteric modulators (AMs) offer several advantages: greater subtype selectivity, because the allosteric binding sites present a higher sequence divergence across receptor subtypes, compared to the orthosteric domains; tissue selectivity and saturable responses, because their effects are closely related to the presence of endogenous ligands; biased signaling, because they can handle certain signaling pathways rather than other [152]. All these features of allosteric modulation can minimize side-effect profile, observed with CBRs orthosteric ligands that are associated with the possibility of the over-stimulation or over-inhibition of ECS [151]. Allosteric mechanism complexity at CBRs is also due to the opportunity to modulate receptor function in different ways. AMs can be classified as positive (PAM), negative (NAM), or neutral (NAL), depending on how the functional effects of orthosteric ligands vary. Upon binding, PAM alters the conformation of the receptor and increases the likelihood that an agonist will bind to a receptor (i.e. affinity) and/or its ability to activate the receptor (i.e. efficacy). On the contrary, NAM inhibits the receptor signaling, resulting in a net decrease of the affinity and/or efficacy of the orthosteric ligands. Finally, NAL does not affect orthosteric ligands activity but competes with other modulators from binding to an allosteric site [152][153].

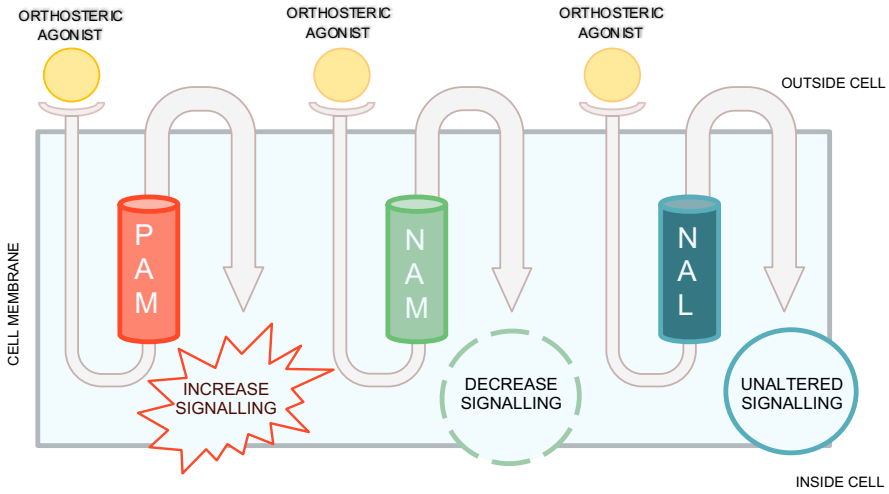


Figure 16. General classification of AMs.

The complex regulation pattern of CB_2R ligands, as well as the high variability of aminoacidic sequence of the allosteric binding site, are slowing the discovery of CB_2R AMs and made this ongoing challenge very difficult [154]. To date, only a limited number of strategies involving the allosteric modulation of CB_2R have been proposed and, even more, pharmacological studies are required to fully characterize a CB_2R allosteric ligand as neuroprotective agents.

In previous reviews (Gado F. *et al*., 2019) [152][155], it has been proposed an accurate overview of CBRs AMs and, therefore, all currently known CB_2R AMs were described. Among them, we can find the pepcan-12 (RVD-hemopressin) (figure 17), an α-hemoglobin-derived dodecapeptide, which initially exhibited negative allosteric activity toward CB_1R [156], but later it has been reported to be also a PAM for CB_2R (K_i value about 50 nM), by Petrucci *et al*. in 2017 [157]. In this study, Petrucci *et al.* tested pepcan-12 (PC-12) in competitive radioligand *h*CB_2R binding assays by using both [^{3}H] CP55940 and [^{3}H] WIN55212-2 as orthosteric radioligands on *h*CB_2R transfected CHO cell membranes [16]. In this context PC-12, at the concentrations of

100 nM and 1 μM, enhanced CB_2R specific binding of both radioligands and it has been quantified the binding cooperativity factor ($\alpha > 1$) in order to confirm the positive cooperation between PC-12 and both orthosteric ligands. PC-12 at nanomolar concentrations (10 nM, 30 nM and 100 nM) also potentiated CP55,940 and 2-AG induced *h*CB_2R signaling, through a significant increase of cAMP inhibition in a concentration-dependent manner and [^{35}S] GTPγS binding in CHO-K1-*h*CB_2R cell membranes, and without affecting β-arrestin2 recruitment and receptor internalization [157].
Given the proven overexpression of CB_2R in microglia in response to neuroinflammation and the great number of evidences that have correlated the ECS to neuroprotection, it cannot be excluded that this peptide mediates neuromodulatory effects *via* CB_2R allosteric modulation, although actually there is no direct *in vitro* or *in vivo* proof of CB_2R positive allosteric modulating role of PC-12 in neuroinflammation disorders. In addition, Hofer C. *et al*. in 2015 carried out an important study highlighting that peptide endocannabinoids with the *N*-terminal extended hemopressin sequence, such as PC-12, are expressed in some areas of the CNS and are primarily released by noradrenergic neurons [158]. These findings might encourage the neuromodulatory pharmacological potential of PC-12 that could be a useful strategy to face neuroinflammation in neurodegeneration. Despite all these promising findings, on the other hand, reproducibility of bioactivity studies and work with PC-12 in drug development could be hampered due to its self-aggregation propensity. This last feature was investigated by Emendato *et al.* in 2018, so contributing to the research on structure-driven design of endocannabinoid peptides [159].

According to the previous systematic review [152], it has been reported by Martinez-Panilla *et al.* in 2017 that CBD (figure 17), which has many molecular targets and presents an antagonistic interaction on CB_2R, also behaves as a NAM of CB_2R [152] [16]. Accordingly, in a classical radioligand-binding assay CBD was not able to bind with high affinity to the orthosteric site in the presence of [^{3}H]-WIN 55,212-2 as orthosteric probe, and its effect on binding to CB_2R was different compared to that exerted by the orthosteric antagonist, SR144528. In addition, CBD at nanomolar concentrations blocked the action of the selective CB_2R agonist JWH133 in a dose-dependent manner, on forskolin-induced intracellular cAMP levels and on activation of the MAP kinase pathway [16]. More recently, Laprairie R. and coworkers [160] have reported that also the synthetic CBD derivative bearing a 1,1-dimethylheptyl lipophilic side chain [(−)-CBD-DMH] (figure 17) behaves as an CB_2R AM. In the same study, cAMP inhibition was quantified relatively to CP55,940 in HEK-CRE cells expressing CB_2R-GFP^2 treated with 1 nM–10 μM of CBD-DMH. The same concentrations of CBD-DMH were also used in bioluminescence resonance energy transfer (BRET) assay for measuring β-arrestin 1 recruitment in HEK293A cells expressing CB_2R-GFP^2 and βarrestin1-*Renilla* luciferase. Interestingly, CBD-DMH enhanced CP55,940-dependent cAMP signaling consistent with a PAM activity, but it reduced orthosteric probe binding and β-arrestin 1 recruitment to CB_2R consistent with NAM activity [152] [160]. Several studies established that DMH-CBD has similar anti-inflammatory properties to those of CBD, these last described in the previous paragraph. For example, it has been shown that DMH-CBD downregulates the mRNA expression of LPS-upregulated pro-inflammatory genes (IL-1β, IL-6, and TNF-α) in BV-2 microglial cells [161]. In similarity to CBD, DMH-CBD could be of high therapeutic value in neuroinflammatory diseases and related syndromes, but the possible involvement of allosteric modulation of CB_2R behind these effects has not yet demonstrated.

Extensive *in vitro* and *in vivo* experiments revealed the potential allosteric modulatory properties of *trans*-β-caryophyllene (TBC) (figure 17) and dihydro-gambogic acid (DHGA) (figure 17) at CB_2R. It has been demonstrated that both TBC and DHGA produce an incomplete but saturable reduction in the binding of the orthosteric cannabinoid radioligand [^{3}H] CP55,940 at CB_2Rs, acting as NAMs [151]. In particular, both TBC, which acts also as an agonist as suggested in the previous paragraph, and DHGA, alter the dissociation kinetics of two different radiolabeled CB_2R orthosteric ligands, [^{3}H] WIN-55,212-2 and [^{3}H] CP55,940, from *h*CB_2R in a probe-dependent manner. Additionally, functional studies were performed demonstrating that both TBC and DHGA also modulate the ability of CB_2R agonists and inverse agonists to regulate the intracellular effector adenylyl cyclase in CHO-*h*CB_2 whole cells [151]. Moreover, these two known CB_2 NAMs were used by Pandey and coworkers in order to characterize allosteric sites within the complex of the CB_2R, composed of CP55,940, DHGA and TBC, so giving an important contribute on the discovery of novel classes of CB_2R AMs [162].
Rajasekaran in his PhD thesis in 2011 [151] carried out a preliminary study to determine whether the allosteric modulatory properties of TBC at CB_2R, would be reflected in a SOD1-G93A mouse model of ALS. Similarly, also anti-inflammatory properties of DHGA were investigated in order to understand whether the consistent

reduction by DHGA of IL-1β and IL-6 levels in a mouse model of sepsis were due to the allosteric modulation at CB_2R [151]. However, the results of activity profile of these compounds have not yet been published and future studies will be required to fully characterize the allosteric modulatory properties of TBC and DHGA at CB_2Rs in *in vivo* models of CNS inflammation.

Thus, it is difficult to understand which of many molecular mechanisms mostly contributes to the beneficial effects that the aforementioned compounds display in animal models of neurodegenerative disorders and this issue remains to be fully investigated. However, the concept that the CB_2R allosteric modulation may represent a key mechanism to exert the neuroprotective effects is supported by the study of antinociceptive activity of the first synthetic CB_2R PAM in a mouse model of neuropathic pain. Tested in competitive radioligand *h*CB_2R binding assays using [^{3}H] CP55,940, N-[5-Bromo-1,2-dihydro-1-(4'-fluorobenzyl) -4-methyl-2-oxo-pyridin-3-yl] cycloheptane carboxamide, compound C2 (EC21a) (figure 17), at 1 nM to 1 μM, enhanced CB_2R-specific binding of orthosteric radioligand. Regarding its functional activity, the CP55,940- and 2-AG-induced stimulation of [^{35}S] GTPγS CB_2R binding was significantly enhanced by 100 nM of C2 (EC21a), but no enhancement induced by AEA was reported. Moreover, C2 (EC21a) significantly decreased [^{3}H]CP55,940 kinetic dissociation from CB_2R,supporting its allosteric nature [163] [155] [152].
Also *in vivo,* C2 (EC21a) acts as a CB_2R PAM as it has been shown in cold allodynia assays, where it demonstrated antinociceptive effects, at a dose of 1, 5, 10, 20 $mg \cdot kg^{-1}$, in oxaliplatin-treated mice as neuropathic pain model. In a cold plate test, C2 (EC21a) reversed the hypersensitivity to cold stimuli induced by oxaliplatin in mice by measuring their pain-related behaviors [163].
Very recently, another important step was accomplished towards the understanding of the C2 compound (EC21a) endowed with CB_2R-mediated neuroprotective effects. Accordingly, it has been demonstrated that C2 (EC21a) can modulate the microglia-mediated neuroinflammation by the potent orthosteric CB_1R/CB_2R-agonist B2 [164]. B2 has been already shown activity on neuroinflammation *in vitro* assays, modulating the balance between pro-and anti-inflammatory cytokines *via* CB_2R activation [165]. So, it has been demonstrated that 0.1 μM of C2 (EC21a), *per se* ineffective, potentiated the ability of 1 μM B2 to modulate IL release in LPS-activated BV2 cells. In detail, the release of the pro-inflammatory IL-1β and IL-6 was significantly decreased; by contrast, the levels of the anti-inflammatory IL-10 was further increased, compared to the B2 treatment alone. In parallel, the co-treatment of C2 (EC-21a) and B2 induced an anti-inflammatory effect, which was totally counteracted by the CB_2R antagonist SR144528, proving the involvement of the CB_2R for the C2 (EC21a)-mediated effect [164]. C2 (EC21a) represent an intriguing candidate for future development of library of 2-oxo-pyridin-3-cycloheptanecarboxamide derivatives as efficacious anti-neuroinflammatory drug. However, further studies need to be undertaken with the aim to confirm the allosteric functionality on CB_2R *in vivo*, similarly to that observed *in vitro*, without any interference from the other protein targets.
Concluding, in the last years, several evidences have been collected in support of the therapeutic potential of compounds allosterically targeting the CB_2R. Therefore, progress must be made in this research field in order to achieve new formulations of selective CB_2R ligands active at the allosteric binding site to prevent devastating neurological disorders.

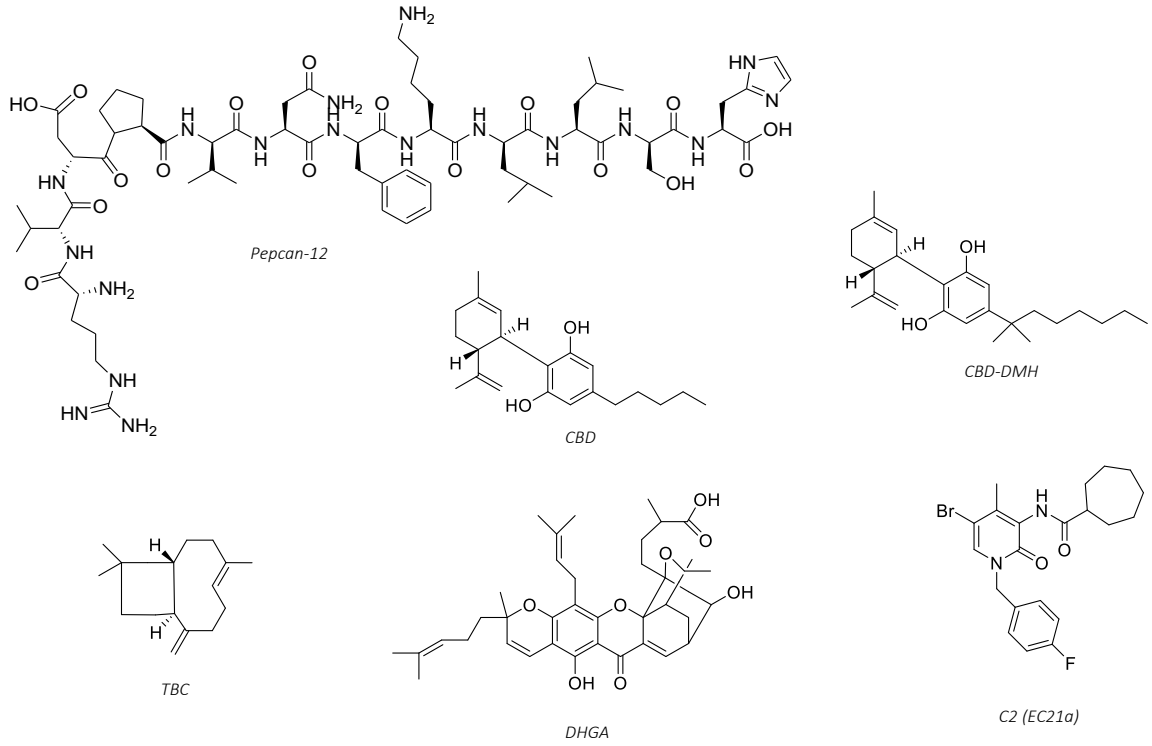


Figure 17. Chemical structure of CB_2R AMs.

5. Conclusions and Future Perspectives

Neuroprotection represents one of the most desirable therapeutic response in many neurodegenerative diseases and it usually aims at preventing neuronal damage over short (acute) or longer (chronic) time periods [166]. Over the years, it has been generally accepted that neuroprotection could be achieved directly, by acting on neurons (primary neuroprotection), and/or indirectly, by interfering with processes that lead secondarily to cell death, such as inflammation [167]. CB_2R has been proposed as a potential target for a neuroprotective outcome since, thanks to its distribution, drugs binding to CB_2R might be able to achieve neuroprotection through both direct and indirect actions without the central side effects typical of CB_1R ligands. Indeed, CB_2R compounds may have effects on neurons, by modulating their excitability through different signaling pathways, and on the neuroinflammatory process. For this reason, CB_2R has been studied and many ligands have been synthesized and developed in the years. In this review, the main natural and synthetic CB_2R ligands with demonstrated neuroprotective properties have been reported and described. However, despite their objective beneficial effects on neuroprotection, CB_2R ligands could present some limitations in their clinical development due to an immune suppression upon chronic use and pro-inflammatory actions as a consequence of a reduction of the immune response [168]. Allosteric modulators might represent a new strategy to maintain the therapeutic benefits due to the stimulation of the CB_2R without the limitations of a direct activation. Only few CB_2R allosteric ligands have been reported in the literature and extensive preclinical studies are still needed in order to assess their potential clinical development. This review highlights the important implications in neuroprotection of CB_2R which, thanks to its localization and its demonstrated actions, represents one of the most promising targets to achieve a neuroprotective effect.

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Conflicts of Interest:

The authors declare no conflict of interest, including financial and material support for the research and work in this manuscript.

	Compound	Activity	Experimental model	CB_2R-mediated neuroprotective effects	References
PHYTOCANNABINOIDS	**Cannabidiol (CBD)**	not fully defined on CB_2R	newborn hypoxic-ischemic (HI) brain damage	▪ ↓ TNF-α and IL-6 release ▪ ↓ iNOS and COX-2 levels ▪ ↓ LDH efflux	[44]
	(−) -β-caryophyllene (BCP)	CB_2R agonist	LPS-induced cytotoxicity on cell viability of OLN-93 (MS model)	▪ ↓ TNF-α, ROS, and NO release ▪ ↑ Nrf2, PPAR-γ, and heme oxygenase enzyme-1 (HO-1) expression	[47]
			ROT-injected rats (PD model)	▪ prevents ROT-induced loss of DA neurons in SNc and striatal DA fibers ▪ ↓ malondialdehyde (MDA) and glutathione (GSH) levels, as lipid peroxidation markers ▪ ↑ activities of antioxidant enzymes SOD and CAT ▪ ↓ GFAP and Iba-1, inhibiting the activation of microglia and astrocytes. ▪ ↓IL-1β, IL-6, and TNF-α release ▪ ↓ iNOS and COX-2 levels	[48]
			MPP+-induced SH-SY5Y cells (PD model)	▪ ↑ cell viability of human SH-SY5Y cells against MPP+-induced cytotoxicity at the concentration of 1 μM and 2.5 μM. ▪ ↓ the release of LDH in a dose dependent manner ▪ prevents the reduction in mitochondrial membrane potential (MMP) after 24h treatment ▪ ↓ MPP+-induced production of ROS ▪ ↑ GSH levels and GPx activity ▪ inhibits MPP+-induced apoptosis in human SH-SY5Y cells and regulates the expression of apoptosis-related molecules (Caspase-3, Bax and Bcl-2) ▪ ↓ HO-1 and p-JNK ▪ ↑ Nrf2	[49]
			transgenic APP/PS1 mice (AD model)	▪ markedly restores the memory function of APP/PS1 mice ▪ ↓ β-amyloid burden in the hippocampus and cerebral cortex ▪ ↓ astrogliosis in the cerebral cortex but not in the hippocampus. ▪ ↓ microglial activation as well as the levels of COX-2 protein and the mRNA levels of the proinflammatory cytokines, TNF-α and IL-1β, in the cerebral cortex	[50]
			murine BV2 cell line after hypoxic exposure (1 % O_2, 24 h) (stroke model)	▪ ↓ hypoxia-induced cytotoxicity ▪ ↓ IL-1β, TNF-α, and IL-6 ▪ ↓ hypoxia-induced generation of ROS in mitochondria ▪ ↓ phosphorylation of IκBα in microglia and suppresses the NF-κB luciferase activity	[51]
			EAE induced in C57BL/6 mice with myelin MOG35-55 peptide (MS model)	▪ BCP (1–100 μM) ↑ IL-10 levels and ↓ IFN-γ production after administration of MOG35-55 peptide (10 mg/mL) in the lymphocytes culture. Moreover, AM630 blocks the immunomodulatory effects of BCP ▪ BCP (25 or 50 mg/kg, oral route—p.o.—daily, twice/day) attenuates disease progression diminishing its severity and ameliorating weight loss after immunization in the chronic EAE mice model ▪ ↓ mechanical hyperalgesia induced by EAE, during pre-motor phase of the disease ▪ ↓ the glial activation marker Iba-1 ▪ ↓ oxidative damage (iNOS) ▪ ↓ the expression of neurofilament-H, a demyelination marker, compared to the EAE-control group ▪ ↓ both CD4+ and CD8+ T cells lymphocytes in peripheral lymphoid tissue during EAE pathology ▪ ↑ infiltration/differentiation of Treg and ↓ inhibits Th1 myelin-specific cells via CB2R activation	[52]
			1. formalin test: $CB2^{+/+}$ and $CB2^{-/-}$ mice injected with	▪ ↓ the pain responses in the late phase (5–30 min after formalin injection) in $CB2^{+/+}$ mice, after oral administration of 5 mg kg^{-1} BCP, which is instead ineffective at the dose of 1 mg kg^{-1}.	[54]

			20ml of 5% formalin (inflammatory pain model) 2. Partial sciatic nerve ligation (PNL) injury in mice (neuropathic pain model)	▪ ↑ mechanical withdrawal thresholds of ipsilateral hind paws in a PNL-induced mechanical allodynia model at different doses of BCP (1, 5 and 10 mg kg^{-1}) ▪ ↑ thermal withdrawal thresholds in the ipsilateral side in a PNL-induced thermal allodynia model at dose of 1 mg kg^{-1} BCP (the other doses were ineffective) ▪ ↓ the density of microglia and astrocytes in the dorsal horn of the spinal cord at a dose of 1 mg kg^{-1}.	
			Paclitaxel-induced neuropathy in mice (peripheral neuropathy model)	▪ ↓ mechanical allodynia induced by PTX, while BCP did not alter the thermal threshold of paclitaxel- treated mice (at the same dose of 25 mg kg^{-1}) ▪ ↑ the levels of CB2 receptor and its transcript in the spinal cord ▪ ↓ p38 MAPK and NF-kB activation ▪ Effect in the expression of inflammation markers in the spinal cord: ↓ Iba-1, IL-1β and MCP-1 levels	[55]
	(-) - *trans* -Δ ^{9} – tetrahydrocannabivarin (Δ (9) -THCV)	CB_2R agonist	LPS-lesioned mice (PD model)	▪ ↓ the loss of tyrosine hydroxylase–positive neurones	[56]
ORTHOSTERIC LIGANDS	**JWH-133**	CB_2R agonist	WDR murine neurons (SNL models)	▪ ↓ innocuous and noxious evoked responses	[67] [68]
			βA-treated rat (AD model)	▪ ↓ microglial activation ▪ ↓loss of neuronal markers ▪ Prevention of cognitive deficits	[69]
			OPC from primary mixed glial cell cultures	▪ ↑ oligodendrocyte differentiation ▪ ↑ level of MBP ▪ Activation of the PI3K/Akt/mTOR signaling pathways	[70] [71]
			Cultured N13 microglial cell line rat primary microglia (AD model)	▪ ↓ATP-induced increase in intracellular Ca2+ ▪ ↑N13 microglia cells migration	[72]
			APP transgenic mice (AD model)	▪ ↓COX-2; TNF-α ▪ ↑ βA clearance	[73]
			transgenic overexpressing CB2 receptors mice (stress-related neuropathologies model)	▪ ↓ TNF-α; CCL2; NF-kB; NOS-2; COX-2 ▪ Preventing cellular oxidative and nitrosative damage	[74]
			Wild-type and CB2R knockout male mice (stroke model)	▪ ↓TNF-α; IL-6; IL-12/IL-23p40; MCP-1; MIP-1α; RANTES; iNOS ▪ ↓IL-10; TGF-β; Ym1	[75]
			AβPP/PS1 transgenic mice (AD model)	▪ ↓IL-1β, IL-6, TNF-α, and IFN-γ ▪ ↓tau hyperphosphorylation ▪ Protection against effects of Aβ	[76]
	HU-308	CB_2R agonist	6-hydroxydopamine-lesioned rats (PD model)	▪ Recovery in DA depletion	[78]
			intrastriatal injection of the mitochondrial complex II inhibitor malonate (HD rat model)	▪ ↑mRNA levels forNSE ▪ ↓Malonate-induced GABA deficit	[79]

			Hemizigous transgenic male mice (HD model)	▪ ↓microglial activation and astroglial reactivity ▪ ↑striatal GABA levels ▪ Improved motor performance	[80]
			Cultured N13 microglial cell line rat primary microglia (AD model)	▪ ↑N13 microglia cells migration	[72]
			LPS-activated striatum (PD model)	▪ ↓LPS-induced elevation of CD68 immunofluorescence in the striatum ▪ ↑TH immunofluorescence in the substantia nigra ▪ gene expression for iNOS promoted by selective CB_2R activation in the striatum ▪ gene expression for iNOS and other ↓proinflammatory factors ▪ Up-regulation of CB2Rs in glial elements in postmortem tissues of PD	[81]
	JWH-015	CB_2R agonist	Female SJL/J mice, susceptible to TMEV-IDD development (MD model)	▪ ↓microglial activation ▪ ↓major histocompatibility complex class II antigen expression ▪ ↓CD4+ infiltrating T cells	[89]
			Human monocytic THP-1 cells and human microglial cells	▪ ↓TNF-α; IL-1β ▪ ↓cell death by 25–50% ▪ ↑ cell survival by 200–300%	[90]
			Cultured microglial cells activated by IFN-γ	▪ ↓IFN-γ-induced phosphorylation of JAK/STAT1 ▪ Inhibit microglial TNF-α and NO ▪ ↓CD40-mediated impairment of microglial phagocytosis of Aβ1–42 peptide	[91]
			THP-1 human macrophages	▪ ↓Aβ plaque in vitro ▪ ↓Aβ plaque in situ	[92]
			TH+ neurons in SNc (PD model)	▪ ↓MPTP-induced microglial activation ▪ ↑ MPTP systemic toxicity following genetic CB2R ablation	[93]
			LPS-activated BV-2 cells and primary microglia	▪ ↓iNOS; ROS ▪ ↓ NF-ϰB activity ▪ ↓ ERK1/2 and cPLA2 phosphorylation	[94]
			murine and human NPC cell (neurodegenerative model)	▪ Inhibition of Gp120-induced decrease proliferation	[95]
			Transgenic APP/PS1 mice (AD model)	▪ ↓deficit of the cortex-dependent novel object recognition memory ▪ ↓immunofluorescence intensity of Iba1 ▪ ↓ M1 to M2 microglial conversion ▪ Restoring of dendritic homeostasis	[96]
	AM1241	CB_2R agonist	SNL mice and $CB_1^{-/-}$ SNL mice	▪ ↓acute thermal nociception ▪ ↓ tactile and thermal hypersensitivity both in wild and CB1-/- mice	[87]
			Rats injected with carrageenan or capsaicin in hind paw	▪ ↓carrageenan-induced inflammatory thermal hyperalgesia ▪ ↓carrageenan-induced edema	[98]
			SNL male mice	▪ ↓ allodynia ▪ ↓ CGRP	[99]

			hSOD1^{G93A} transgenic mice (ALS model)	▪ ↓ progression of disease	[100]
			LPS-activated BV2 microglial cells	▪ ↓ iNOS, NO ▪ ↓ ERK1/2 and cPLA2 phosphorylation	[94]
			GFAP/Gp120 Tg mice (HAD model)	▪ ↓Gp120-mediated toxicity ▪ ↑ BrdU+, PCNA+, neuroblasts and neuronal cells	[95]
	GW405833 (L 768242)	CB_2R agonist	Seltzer model (neuropathic pain model)	▪ Antihyperalgesic effects ▪ Linear penetration into the CNS	[102]
			Seltzer model (neuropathic pain model)	▪ ↓tactile allodynia	[103]
			SNL male mice	▪ ↓nocifensive behaviors ▪ ↓ CGRP	[99]
			R6/2 mice (HD model)	▪ ↓neuroinflammation ▪ ↓ synapse loss ▪ ↑ life span ▪ ↑performance on balance beam and rotarod	[104]
	GW842166X	CB_2R agonist	FCA model (inflammatory pain model)	▪ Complete resolution of hyperalgesia	[106]
			LPS-activated BV2 microglial cells	▪ ↓ iNOS, NO ▪ ↓ ERK1/2 and cPLA2 phosphorylation	[94]
	MDA7	CB_2R agonist	SNL and paclitaxel-induced tactile allodynia (neuropathic pain model)	▪ ↓ tactile allodynia without affecting locomotor behavior	[109]
			SNL and paclitaxel-induced mechanical allodynia (neuropathic pain model)	▪ Prevention of paclitaxel-induced mechanical allodynia ▪ ↓ CD11b, GFAP ▪ ↓ TLR2 ▪ ↓ TNF-α, IL-1β	[110]
			Aβ-injected rats (AD model)	▪ ↓ CD11b, GFAP ▪ ↓IL-1β ▪ ↓Aβ-mediated suppression of glutamatergic transmission ▪ ↑spatial memory performance	[111]
			APP/PS1 mice (AD model)	▪ ↓Iba1 ▪ ↓Aβ plaques ▪ ↑ Sox2 ▪ ↑ cognitive functions	[112]
	Gp-1a	CB_2R agonist	EAE-induced mice (EAE model)	▪ ↓ Th17/Th1 differentiation ▪ ↓IL-12p40, IL23p19, ▪ TNFα, IL1β, IFNγ, IL17 ▪ ↓CCL2, CCL5, CXCL10 ▪ ↓VCAM-1 ▪ ↓iNOS	[115]

				▪ ↓demyelination and axonal loss	
			CCI mice (TBI model)	▪ ↑M2 polarization ▪ ↓M1 polarization ▪ ↑cerebral blood flow ▪ ↑neurobehavioral outcomes ▪ ↓edema	[116]
			DSP mice (neuropathic pain model)	▪ Improvement of allodynia ▪ ↓IFN-γ-induced phosphorylation of STAT1 and STAT3	[117]
	NESS400	CB_2R agonist	SNI mice and spinal microglia (neuropathic pain model)	▪ ↓thermal hyperalgesia ▪ ↓neuropathic mechanical allodynia ▪ ↓ astrocyte upregulation ▪ ↓IL-1β, IFN-γ ▪ ↑ IL-10	[118]
	0-1966	CB_2R agonist	C57BL/6/MOG and SJL/J/PLP mice (MD models)	▪ ↓disease progression ▪ Improvement of motor function ▪ ↓ T-cell proliferation ▪ Shift from IFNγ to IL-4/IL-13/IL-10	[120]
			CCI mice (TBI model)	▪ ↓macrophage/microglial activation ▪ ↓ perivascular substance P immunoreactivity ▪ ↓cerebral edema ▪ Improvement of locomotor function and exploratory activity	[121]
			CCI mice (TBI model)	▪ ↓neuron degeneration ▪ ↓ NaF uptake ▪ Improvement of rota-rod and open-field testing ▪ ↓microglia/macrophage cell counts	[26]
			CCI mice (TBI model)	▪ ↓iNOS ▪ ↓ICAM-1 ▪ ↓ TNF-α	[122]
			SCI-induced mice (SCI model)	▪ ↓CXCL-9, CXCL-11 ▪ ↓ IL-23p19, IL-23R ▪ ↓TLR1, TLR4, TLR6, TLR7 ▪ Improvement of motor function	[123]
	PM226	CB_2R agonist	LPS-stimulated BV2 microglial cells and M213-20 neuronal cells *In vivo* malonate-mediated striatal damage model	▪ ↑cell viability ▪ ↓edema volume	[124]
			TMEV-IDD mice (MS model)	▪ ↓microglia activation	[125]
	CB74 **CB91**	CB_2R agonist	Peripheral Blood Mononuclear Cells (PBMC)	▪ ↓ PBMC proliferation ▪ ↓ T cell activation markers, CD69 and CD54 adhesion molecule	[130] [131]

	VL23		(MS model)	▪ ↓ Akt, NF-kB, Erk and Cox-2 expression	
	11c (COR167)	CB_2R agonist	Formalin test (acute inflammatory pain model)	▪ ↓ the second phase of the formalin-induced nocifensive response in mice in a dose-dependent manner	[126]
			Peripheral Blood Mononuclear Cells (PBMC) and Myelin Basic Protein (MBP)-reactive T cells (MS model)	▪ ↓ PBMC and MBP-reactive T cells proliferation ▪ ↑incomplete shift of Th1 phenotype towards Th2 phenotype ▪ ↓ Th1-related cytokines (IL-2, IFN- γ and TNF-α), Th2-related cytokines (IL-4, IL-5 and slightly for IL-13), and Th17-related cytokines (IL-6, IL-17A, G- CSF and TGF-β1) ▪ ↑ IL-10 and IL-12 ▪ ↓ of several chemokine such as IL- 8, MCP-1, RANTES, MIPa, MIPb, I-TAC and GROa ▪ ↓ migration of MBP-reactive T cell lines and PBMC through human brain endothelium	[132]
	AM-630	CB_2R antagonist /inverse agonist	LPS-activated BV-2 cells and primary microglia	▪ ↓iNOS; ROS ▪ ↓ NF-ϰB activity ▪ ↓ ERK1/2 and cPLA2 phosphorylation	[94]
	BML-190	CB_2R inverse agonist	Human monocytic THP-1 cells and human microglial cells	▪ ↓cell death by 25–50% ▪ ↑ cell survival by 200–300%	[90]
	SR-144528	CB_2R antagonist /inverse agonist	LPS-activated BV-2 cells and primary microglia	▪ ↓iNOS ▪ ↓ ERK1/2 and cPLA2 phosphorylation	[94]
			LPS-activated C8B4 murine microglia (TBI model)	▪ ↓CD16/32 ▪ ↑ CD206	[143]
	SMM-189	CB_2R antagonist /inverse agonist	LPS-activated murine microglia (*in vitro* TBI model) and *in vivo* TBI model	▪ Shift from M1 to M2 ▪ ↓IL-8, MIP-1β, MDC, IFN-γ, IL-6, IL-10, IL-12p70 ▪ Improvement of motor, visual, and emotional behaviour	[146]
			LPS-activated C8B4 murine microglia (TBI model)	▪ ↓CD16/32 ▪ ↑ CD206 ▪ ↑rod-shaped cells	[143]
			LPS-treated murine cortex,striatum,and basolateral amygdala (TBI model)	▪ ↓ neuronal loss	[148]
	Sch.414319	CB_2R inverse agonist	EAE-induced mice (EAE model)	▪ Conversion of complete hind-limb paralysis to a flaccid tail phenotype	[149]
AMs	**C2 (EC21a)**	CB_2R PAM	Oxaliplatin-treated mice (neuropathic pain model)	▪ ↓ pain threshold of mice evaluated as hypersensitivity to a cold non-noxious stimulus	[163]

Table 1. Summary of main natural and synthetic CB_2R modulators as neuroprotective agents.

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