Synthetic cannabinoid receptor agonists and antagonists: implication in CNS disorders

Clementina Manera,\textsuperscript{a} Chiara Arena,\textsuperscript{a,\textsuperscript{b}} Andrea Chicca\textsuperscript{b}

\textsuperscript{a}Department of Pharmacy, University of Pisa, via Bonanno 6, 56126 Pisa, Italy.
\textsuperscript{b}Institute of Biochemistry and Molecular Medicine, National Center of Competence in Research TransCure, University of Bern, CH 3012 Bern, Switzerland

\textsuperscript{*}To whom correspondence should be addressed. C.M.: email address: clementina.manera@farm.unipi.it; telephone: +39(0)502219548; fax: +39(0)502219605
ABSTRACT

Background. Since the discovery of the cannabinoid receptors, numerous studies associate the endocannabinoid system with several physiological and pathological processes including cancer, appetite, fertility, memory, neuropathic and inflammatory pain, obesity, and neurodegenerative diseases. Over the last two decades, several researches have been dedicated extensively on the cannabinoid receptors ligands since the direct activation of cannabinoid receptors results in several beneficial effects, in the brain and in the periphery.

Methods. The cannabinoid CB1 and CB2 receptor synthetic ligands reported in this review have been collected by a wide research of scientific literature in particular in public database for patents and clinical trials. The references for patent numbers, clinical trial registry numbers, websites and scientific articles are reported in the reference section.

Results. During past years, cannabinoid CB1 and CB2 receptor ligands from plants or lab were rapidly developed and then various new structures were reported to be cannabinoids. However the CB1 receptor ligands have had a limited usefulness due to their psychotropic effects, dependence, and cognitive impairment. On the contrary the development of CB2 receptor ligands has been more productive. Furthermore peripherally restricted agonists as well as CB1 receptor positive or negative allosteric modulators were studied with the aim of eliminating the undesirable CB1 receptor central effects.

Conclusions. The CB1 and CB2 receptor ligands offer several therapeutic opportunities for several CNS-related diseases. Based on the scientific literature, this review provides an overview of CB1 and CB2 receptor synthetic ligands obtained from drug research and in particular those synthesized for therapeutic purposes and potential clinical applications for central nervous system disorders.
INTRODUCTION

For many centuries, preparations from Cannabis sativa L have been used as popular recreational drug as well as for their therapeutic effect, however the chemical and biological bases of their pharmacological effects are still not fully understood. Cannabis plants produce large number of related compounds called phytocannabinoids, of which Δ⁹-tetrahydrocannabinol (Δ⁹-THC, Fig.1) is the principal bioactive component. This compound was identified and synthesized in the 1960s [1] and, it was used as model for synthesizing analogues with very potent medical properties. However the new compounds showed psychotropic side effects and in several cases the pharmacological mechanisms of action were not well-identified. Particularly important was the identification of the cannabinoid receptors (CB1 and CB2 receptors) followed by the discovery of their endogenous ligands and of different enzymes involved in their biosynthesis and biotransformation that allowed to deepen the knowledge of endocannabinoid system (ES) and the clinical applicability of cannabis-based treatments [2].

The CB1 and CB2 receptors are members of the G protein-coupled receptor (GPCR) family. They are characterized by an N-terminal extracellular domain that possesses glycosylation sites, a C-terminal intracellular domain coupled to a G protein complex, and seven hydrophobic transmembrane segments. The cannabinoid receptors are expressed in many species, including human. Initially, it was hypothesized that CB1 receptor was localized generally in the brain whereas CB2 receptor was restricted in immune cells such as leucocytes and those of the spleen and tonsils [3]. However, CB1 receptor has recently been found also in peripheral tissues, whereas CB2 receptor was identified also in the central nervous system, e.g. in the microglial cells. The human CB2 receptor has 44% amino acid sequence identity with CB1 receptor for all protein and 68% similarity for the transmembrane domains. The cannabinoid receptors are coupled with Gi or Go protein, positively to mitogen-activated protein (MAP) kinase and negatively to adenylyl cyclase, thus reducing the production of cAMP [3].

CB1 receptor also modulates ion channels, resulting, for example, in the inhibition of P/Q-type voltage-sensitive Ca²⁺ channels. Inhibition of presynaptic calcium channels by cannabinoids likely reduces neurotransmitter release from CB1 receptor-expressing presynaptic terminals. One of the functions of cannabinoid receptors in the immune system is modulation of cytokine release. Activation of B- and T-cell CB2 receptor by cannabinoids inhibits adenylyl cyclase in these cells and reduces the immune response.

Experiments utilizing CB1 and CB2 receptors knockout mice indicate the presence of further cannabinoid receptors such as GPR18, GPR55, and GPR119 [4,5]. The latter is involved in the regulation of metabolism [6] and body weight [7]. GRP55 [8,9] and GPR18 [10] regulate the activation of microglia and the neuropathic pain. Finally the TRPV1 receptor is involved in pain and inflammation and is regulated by cannabinoids [11]. For these reasons these receptors may be interesting targets for the treatment of neuroinflammation and neuropathic pain.

Cannabinoid receptors are localized in the presynaptic junction and are activated by lipid mediators called endocannabinoids. At the present moment, the most bioactive and best characterized
endocannabinoids are anandamide (arachidonylthanolamide; AEA) (Fig.1) and 2-arachidonoylglycerol (2-AG) (Fig. 1), however the family of endocannabinoids comprises also noladin ether virodhamine, N-arachidonoyl dopamine (NADA), and other compounds such as palmitoylethanolamide (PEA) and oleoylethanolamide (OEA) (Fig.1). Unlike many other neurotransmitters, endocannabinoids are produced upon demand, are biosynthesized from integral constituents of cellular membrane by action of some enzymes and have a moderately slow time frame of action. The endocannabinoid signaling is terminated by cellular reuptake and their enzymatic hydrolysis. There is only pharmacological evidence of the existence of reuptake transporter of endocannabinoids [12]. On the contrary, the enzymes involved in the metabolism of endocannabinoids are better known. 2-AG is degraded by the monoacylglycerol lipase (MAGL) giving arachidonic acid and glycerol [13] and fatty acid amide hydrolase (FAAH) degrades AEA to arachidonic acid and ethanolamine [14].

![Chemical structures of Δ⁹-THC and endocannabinoids.](image)

**Figure 1.** Chemical structures of Δ⁹-THC and endocannabinoids.

During past years, cannabinoid compounds from plants or lab were rapidly developed and then various new structures were reported to be cannabinoids. Cannabinoids receptors have an important role
in controlling of cell fate and then they represent an attractive for novel drug development [15,16]. Recently various researches reported that natural and synthetic cannabinoids showed an important antitumor activity in preclinical trials, they could inhibit cell proliferation, induce apoptosis and block angiogenesis [17]. Nabilone (Cesamet) (Fig. 2), synthetic analog of Δ⁹-THC, is an example of cannabinoid-based drugs; this compound is a potent CB1/CB2 agonist, and at this time is used for the treatment of chemotherapy-induced nausea and vomiting in humans. Synthetic analogs of Δ⁹-THC, such as n-hexyl- Δ⁶α-THC (Fig. 2), 1,2-dimethylheptyl-THC (Fig. 2), HU-210 (Fig. 2) levonantradol (CP 50,556-1) (Fig. 2) and nabitan (Fig. 2), were initially tested, but none of these were introduced in the market. Furthermore, in different research model cannabinoid ligands showed to inhibit pain via the CB1 or/and CB2 receptor. For example the phase II studies of NIH (http://clinicaltrials.gov) regarding the CB2 receptor agonist GW842166X (Fig. 6) [18], indicated that this compound possesses analgesic effect in dental surgery and in the treatment of osteoarthritis pain of the knee. Besides cannabinoids are related to hepatic pathological conditions and to many other diseases such as osteoporosis and atopic dermatitis [19,20]. Finally some researches on neurological diseases demonstrated that cannabinoids should be used to slow the progression of neurodegenerative disorders [21] such as Huntington’s disease [22], Alzheimer’s disease [23], Parkinson’s disease [24] and multiple sclerosis [25] (Table 1). For these reasons cannabinoid receptor could be considered new targets for neuropsychiatric and neurodegenerative disorders.

Figure 2. Chemical structures of synthetic analogs of Δ⁹-THC
The CB1 and CB2 ligands could be classified as agonists, antagonists and inverse agonist. The agonist produce an increase in the basal level of signaling after binding to the receptor; the inverse agonists down regulate the signaling while the antagonists stop the agonists or the inverse agonists modulating the receptors. Furthermore ligands able to modulate ES by binding to allosteric sites (positive, negative, and silent allosteric modulators) were developed [26].

Table 1. Examples of cannabinoid receptor ligands and their potential use for specific CNS disorders

<table>
<thead>
<tr>
<th>Compound</th>
<th>ES Target</th>
<th>Action</th>
<th>Study type</th>
<th>CNS Disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB1R CB2R</td>
<td>Partial agonist</td>
<td>Partial agonist</td>
<td>Preclinical and Clinical</td>
<td>Multiple sclerosis Parkinson’s disease Alzheimer’s disease Epilepsy</td>
</tr>
<tr>
<td>CB1R CB2R</td>
<td>Partial agonist</td>
<td>Partial agonist</td>
<td>Preclinical</td>
<td>Alzheimer’s disease</td>
</tr>
<tr>
<td>CB1R</td>
<td>Agonist</td>
<td>Preclinical</td>
<td>Multiple sclerosis</td>
<td></td>
</tr>
<tr>
<td>CB2R</td>
<td>Agonist</td>
<td>Preclinical</td>
<td>Amyotrophic lateral sclerosis</td>
<td></td>
</tr>
<tr>
<td>CB1R CB2R</td>
<td>Agonist</td>
<td>Preclinical</td>
<td>Multiple sclerosis Parkinson’s disease Alzheimer’s disease</td>
<td></td>
</tr>
<tr>
<td>CB2R</td>
<td>Agonist</td>
<td>Preclinical</td>
<td>Multiple sclerosis Alzheimer's disease</td>
<td></td>
</tr>
<tr>
<td>CB2R</td>
<td>Agonist</td>
<td>Preclinical</td>
<td>Parkinson’s disease</td>
<td></td>
</tr>
<tr>
<td>CB2R</td>
<td>Agonist</td>
<td>Preclinical</td>
<td>Parkinson’s disease</td>
<td></td>
</tr>
<tr>
<td>CB2R</td>
<td>Agonist</td>
<td>Preclinical</td>
<td>Parkinson’s disease</td>
<td></td>
</tr>
</tbody>
</table>
ROLE OF THE ENDOCANNABINOID SYSTEM IN THE PATHOPHYSIOLOGY OF CNS DISORDERS

In the last 10–15 years, several preclinical and clinical studies have been reported in the literature, demonstrating that ES-targeting compounds exert neuroprotective effects through an array of different mechanisms [27]. Cannabinoids may achieve better therapeutic outcomes compared to classic neuroprotective agents due to their multi-target modulation. Indeed neurodegenerative disorders are generally characterized by different cytotoxic events such as energy failure, excitotoxicity, mitochondrial dysfunction, inflammation, failures in proteostasis, and oxidative stress. Compounds acting on the ES exhibit a wide spectrum of activity as they can modulate different molecular targets that might lead to a synergistic neuroprotective effects [28]. They comprise not only the activation of cannabinoid receptors but also CB receptor-independent mechanisms, such as the blockade of NMDA receptors, or the activation of nuclear receptors as the PPARs [29].

The CB1-mediated neuroprotective effects apparently occur at two specific neuronal sites: presynaptic glutamatergic neurons where CB1 receptor activation leads to a reduction of glutamate release and postsynaptic CB1 receptor on NMDA-expressing neurons, where CB1 activation prevents an excessive intracellular Ca\(^{2+}\) concentration by closing voltage dependent calcium channels [29]. Another neuroprotective effect mediated by CB1 receptor activation consists in improving the blood circulation in case of injured brain, which is particularly important in stroke or traumatic injuries [30]. In particular for this protective effect, recent evidence indicates the involvement of CB2 receptor [31].

The CB2-mediated effects occur in activated microglial cells, astrocytes, oligodendrocytes and in some restricted neuronal subpopulations [29]. In particular, CB2 receptor show an important role in the proliferation and migration of microglia cells to the lesion sites [32] and reducing the production of neurotoxic factors (e.g. tumor necrosis factor-α (TNF-α)) [29]. The modulation of astrocyte activity for
a damage to brain, involves CB1 receptor [29] and appears to be associated with an increased production of metabolic substrates such as lactate or ketone bodies [33], neurotrophins and anti-inflammatory mediators that can limit the neuronal damage. Furthermore the activation of CB2 receptor could also inhibit the production of pro-inflammatory chemokines (e.g., fractalkine) by astrocytes. Finally, CB2 receptor has been also identified in oligodendrocytes which play a crucial role in modulating neuronal activity [34].

**Cannabinoids in Parkinson’s disease.** Parkinson’s disease (PD) is a neurodegenerative condition that affects dopaminergic neurotransmission in the basal ganglia resulting in hypokinesia. The ES regulates neurotransmitter release [35] and motor activity [36], thus representing a promising target for treatment of motor dysfunction. The neuroprotective effects can be associated either to the activation of CB2 receptor which exert anti-inflammatory effects or the blockade of CB1 receptor which improve motor activity. In addition, the combination of antioxidant agents with agonists for alternative AEA targets, such as PPARs, showed beneficial effects in animal models of PD [37]. In particular, preclinical studies proved that low doses of the CB1 receptor antagonist rimonabant, decrease hypokinesia in an animal model of PD [38]. Moreover Δ⁹-THC and CBD showed to protect nigrostriatal dopaminergic neurons from the neurotoxin 6-hydroxydopamine exerting antioxidant action [39]. Therefore the CBD/ Δ⁹-THC-based medicine Sativex® may deserve further clinical investigation in PD.

**Cannabinoids in Alzheimer’s disease.** Alzheimer’s disease (AD) is an aging-related neurodegenerative disease characterized by the progressive deterioration of cognition and memory. It was reported that changes in the endocannabinoid signaling occur during the progression of AD, particularly in the hippocampus and cerebral cortex. The increase of 2-AG levels and upregulation of CB2 receptors in microglial cells may exert some protective effects against β-amyloid-induced neuroinflammation and neuronal injury [40] especially in plaque-bearing areas [41]. On the other hand, the downregulation of CB1 receptor expression in hippocampus and basal ganglia may contribute to the destructive inflammatory process which accompanies AD progression [41]. In addition, the increased FAAH activity, especially in astrocytes, can lead to the formation of more arachidonic acid eventually leading to pro-inflammatory effects. However, it was shown that Δ⁹-THC can counteract β-amyloid aggregation and the consequent plaque formation by inhibiting acetylcholinesterase activity [42]. In addition, Δ⁹-THC can reduce the plaque density by increasing the expression of neprilysin, which is one of the key enzymes involved in the β-amyloid degradation cascade. Sativex® was in recent times investigated in a preclinical model of AD. The results showed a reduced gliosis, oxidative stress, tau- and β-amyloid aggregation and the induction of autophagy [43]. Dronabinol, a synthetic preparation of Δ⁹-THC, is used as antiemetic and appetite-stimulator in AD patients [44]. Furthermore in patients with severe AD, Δ⁹-THC also showed to reduce agitation which is a hallmark of disease progression [45].

**Cannabinoids in Huntington’s disease.** Huntington’s disease (HD) is a chronic progressive disorder caused by the dysfunction of huntingtin. In HD, the gene encoding for this protein has an excessive number of cytosine-adenine-guanine triplet repeats (CAG) leading to the biosynthesis of a non-functional protein. In animal models of HD, it was demonstrated that the activation of a restricted CB1
receptor population in cortical glutamatergic neurons protects these cells from death [46]. Furthermore, CB2 receptor activation showed positive effects by reducing chronic inflammation and activation of microglia cells in different rodent models of HD [47]. Recently, the combination of CBD and Δ⁹-THC (Sativex®) was tested on HD patients in a phase II clinical trial [48] to assess the therapeutic potential of activating CB receptors in this chronic inflammatory disease. The results showed that Sativex® was safe and well tolerated in HD patients, but unfortunately, the drug did not show any significant reduction of disease progression [48].

Cannabinoids in Epilepsy. Cannabis has been used as a folk medicine for millennia such as for the treatment of neurological disorders (e.g. epilepsy). The exact targets that mediate the anti-seizure effects of cannabinoids are still unknown. Several cannabinoids bind and modulate multiple brain targets beside cannabinoid receptors such as the transient receptor potential cation channel (TRPV1-3), glycine receptor alpha (α3GlyR), peroxisome proliferator-activated receptor gamma (PPAR-γ), calcium-gated ion channel (Cav3 ion channel). Several studies have examined the effects of cannabis in epilepsy but generally they had methodological problems such as the lack of placebo-controlled studies [49]. A clinical trial, initiated in 2013 on children and young adults with severe epilepsy, assessed the therapeutic potential of a pharmaceutical preparation, named Epidiolex®, composed by purified cannabis extracts containing 99% of CBD and less than 0.1% of Δ⁹-THC. A preliminary report showed that 54% of patients (74 out of 137) who had received the treatment for 12 weeks, experienced a significant reduction in the number of seizures [50]. Two additional clinical trials are currently ongoing for the evaluation of Epidiolex® in two forms of severe, childhood-onset epilepsy [51,52].

CANNABINOID RECEPTOR AGONIST

CB1 receptor agonists

Δ⁹-THC (Fig. 1), the psychotropic component of cannabis, acts as a CB1 receptor partial agonist with modest affinities for both cannabinoid receptors. Instead the nonpsychotropic compounds of cannabis, tetrahydrocannabivarain (Fig. 3) and cannabinol (Fig. 3), behave as weak antagonist and agonist for CB1 receptor respectively, whereas show modest agonist activity for CB2 receptor. Cannabidiol (Fig. 3) and cannabidivarain (Fig. 3) without psychotropic activity, exhibit very low affinities for both cannabinoid receptors, and the mechanism through which cannabidiol acts is not clear. Δ⁹-THC (Dronabinol, Marinol) in the United States is approved for treatment of nausea and vomiting in patients receiving chemotherapy and for treating anorexia related to weight loss in patients with AIDS [53]. Furthermore it decreases neuropathic pain associated with multiple sclerosis [54] and is useful as an antiemetic agent. The oromucosal spray combination of Δ⁹-THC and cannabidiol (Sativex, Nabiximols) is used in treating multiple sclerosis-related spasticity [55] and pain in patients with advanced cancer [56]. The same combination showed to protect nigrostriatal dopaminergic neurones from the neurotoxin 6-hydroxydopamine via an antioxidant action indicating to be a possible candidate for clinical investigation in Parkinson’s disease [39].
The CB1/CB2 agonist, HU-210 (Fig. 2) prevents amyloid-beta (Aβ)-induced increases in microglia activation and TNF-α release [41]. In Alzheimer’s disease the activation of CB1 receptor induces the modulation of several inflammatory cytokines [57], whereas the stimulation of CB2 receptor suppresses microglial activation and subsequent production of TNF-α and nitric oxide [58]. The synthetic cannabinoid, HU-211 (Dexanabinol) is the enantiomer of HU-210; it does not act as a cannabinoid receptor agonist, but instead has NMDA receptors antagonist effects and thus it protects cells from NMDA induced neurotoxicity mimicking the results of the current drug memantine, a noncompetitive antagonist of the N-methyl D-aspartate (NMDA) receptor approved by the Food and Drug Administration (FDA), and improving symptoms [59].

![Chemical structures of phytocannabinoids](image)

**Figure 3.** Chemical structures of phytocannabinoids

(R)-Methanandamide also known as AM356 (Fig. 4) is an anandamide analog substituted with a chiral methyl group. It showed a significantly higher metabolic stability and CB1 receptor potency [60]. AM356 inhibited the LPS-induced release of interleukin-1 beta and tumor necrosis factor-alpha from microglia and could be useful for patients with severe traumatic brain injuries as well as neurodegenerative diseases which are associated with neuroinflammation [61].

Aminoalkylindole derivatives represent the first distinct class of compounds that differ from the natural cannabinoids. This class contains pravadoline (Fig. 4) and its very potent CB1/CB2 receptors agonist analog WIN 55212-2 (Fig. 4), which is usually used as pharmacological probe. WIN 55212-2 showed to reduce neuronal death and infarct volumes in a CB1 receptor-dependant manner [62]. Other important aminoalkylindole derivatives are AM678 (JWH-018) (Fig. 4) and AM2201 (Fig. 4) that exhibited antinociceptive properties, and Org 28611 (Fig. 4) which was tested in humans both as an analgesic and a sedative [63]. In particular AM678 is a CB1 and CB2 receptors agonist with a higher affinity than Δ⁹-THC and it shares with the active ingredient of Marijuana, CB1-dependent reinforcing and dopamine stimulant actions [64]. However many aminoalkylindole derivatives have been classified...
as Schedule 1 Controlled Substances and has been detected in several samples of a smokable herbal mixture termed "Spice", marring their therapeutic value [65].

Among the indanyl-4-oxy derivatives reported in literature as cannabinoid receptors ligands, KN38-72717 (BAY 38–7271) (Fig. 4) showed to be selective and highly potent cannabinoid CB1/CB2 receptors agonist with neuroprotective efficacy in traumatic brain injury animal model [66]. Recently this compound showed a significant, dose-dependent and long-lasting reduction of cortical lesion sizes due to occlusion of the middle cerebral artery (eMCAO) and appeared beneficial in the acute early phase of the comatose patient after a head injury [67].

![Chemical structures of CB1 receptor agonists](image)

**Figure 4.** Chemical structures of CB1 receptor agonists

Many authors reported that the activation of CB1 receptor has potential to serve for therapeutic attenuation of degeneration in select CNS disorders [68-70]. Unfortunately the activation of this receptor also engenders psychotropic effects, dependence, and cognitive impairment [71-73]. Recently with the aim to eliminate the undesirable CB1 central effects, peripherally restricted agonists were
developed. This study has led to the discovery of naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone, SAB378 (Fig. 5), which shows good oral bioavailability, potent antihyperalgesic activity, and limited brain penetration [74]. In addition, the quinazoline derivative SAD448 (Fig. 5) was identified as highly polar CB1 agonist that showed to be analgesic in inflammatory and neuropathic pain models. These compounds demonstrated to control spasticity in a multiple sclerosis model via action on the peripheral nerve CB1 receptor [75]. Furthermore the imidazol derivatives AZD1940, AZD1704 and AZ11713908 when administered orally have produced analgesia effect in rodent models of acute, inflammatory and/or neuropathic pain through the activation of peripheral CB1 receptor [76]. In particular AZD1940 (Fig. 5) was studied as candidate drug for treatment of neuropathic pain and it has undergone evaluation in humans [77].

![Chemical structures of peripherally restricted CB1 receptor agonists](image)

**Figure 5.** Chemical structures of peripherally restricted CB1 receptor agonists

**CB1 receptor allosteric modulators**

CB1 positive or negative allosteric modulators represent interesting prospects for developing CB1 receptor ligands lacking CB1-related undesirable side effects. Allosteric modulators offer several advantages, in fact they show greater subtype selectivity for the higher sequence divergence at extracellular allosteric binding sites, respect to the conserved orthosteric domains. Furthermore allosteric modulators have tissue selectivity, because they exert effects only where endogenous ligands are present. Among allosteric modulators recently reported, the compounds Org27569 (Fig. 6) [78] and PSNCBAM-1 (Fig. 6) [79] have been more widely studied. Org27569 enhances CB1 receptor binding of CP55,940, but on the contrary it behaves as an negative modulator for receptor activation in several biochemical assays. In vivo, Org27569 did not produce any cannabimimetic effects alone. Similar to the blockage of the orthosteric binding site, Org27569 reduced food intake, but unlike SR141716A, its effects resulted CB1-independent [80]. In another study, the anorexic effect of Org27569 developed tolerance after 4 days of daily administration at the dose of 5.6 mg/kg. The effect on body weight gain lasted for 10 days after the cessation of drug treatment [81]. Another study showed that Org27569 induced a dose-dependent (1-5.6 mg/kg) diminution of both cue- and drug-induced reinstatement of
cocaine- and methamphetamine-seeking behaviour. In the same model, SR141716A exhibited similar inhibitory actions on reinstatement of drug-seeking, suggesting a CB1-mediated mechanism [82]. Altogether, these data suggest that Org27569 may not function as an efficient CB1 receptor allosteric modulator \textit{in vivo}, although the negative allosteric modulation might deserve further investigations as a potentially treatment for drug addiction.

Recently it was shown that pregnenolone levels (Fig. 6) increase in the brain upon CB1 receptor activation. \textit{In vitro} and \textit{in vivo}, pregnenolone partially counteracted some of the \( \Delta^9 \)-THC-mediated central effects, including hypothermia, catalepsy, hypomotility, analgesia and food intake. Despite pregnenolone exhibited a mild negative allosteric modulation of CB1 receptors, it rather behaved as a signaling-specific inhibitor for CB1-mediated signaling, reducing several effects of \( \Delta^9 \)-THC. This negative feedback mechanism suggests that pregnenolone might act as an endogenous protector against excessive CB1 receptor activation in the brain, thus offering a potential novel therapeutic approach for the treatment of cannabis addiction [83]. Pregnenolone is not a druggable molecule because of its conversion into other steroids and the very poor bioavailability, therefore Aelis Farma is developing a new pharmacological class of pregnenolone derivatives C3-17,NMPDs (Non Metabolized Pregnenolone Derivatives) which have a good bioavailability and are not converted into other steroids [84]. Pepsans (pepcan endocannabinoids) are a family of N-terminal extended endogenous peptides which spans from 12 to 23 aminoacids and derive from the \( \alpha \)-chain of hemoglobin. The shortest member of the family, pepcan-12 is the most potent and efficacious NAM (negative allosteric modulator) at CB1 receptors and showed to non-competitively reduce the binding and functional activity of endogenous and synthetic CB1 orthosteric agonists [85]. Localization studies identified distinct biosynthetic/releasing sites for pepcan-12 in the periphery (adrenal medulla) and in the brain (locus ceruleus) [86]. Striker et al. investigated the different CB1 allosteric modulators for the ability to modulate the 2-AG-mediated depolarization-induced suppression of excitation (DSE) as a model to test CB1 modulators in a neuronal model of endogenous cannabinoid signaling. Interestingly, PSNCBAM-1, Org27569 and pepcan-12 were the only compounds to confirm a negative allosteric modulation of the 2-AG-induced DSE [87]. The first pharmacological evidence demonstrating the effectiveness of a positive allosteric modulator (PAM) at CB1 receptor was shown for the endogenous anti-inflammatory mediator, lipoxinA4 (Fig. 6), which was found to enhance the pharmacological effects of AEA at the CB1 receptors both \textit{in vitro} and \textit{in vivo}. Furthermore, lipoxinA4 showed protective effects in mice against \( \beta \)-amyloid (1-40)-induced impairment in learning and memory [88]. Ignatowska-Jankowska et al. recently described a new allosteric modulator for CB1 receptors, ZCZ011 (Fig. 6) which enhances orthosteric agonist effects, thus behaving as a positive modulator (PAM) [89]. In mice, ZCZ011 improved the potency of different orthosteric agonists in behavioural tests indicative of cannabimimetic activity, including antinociception, hypothermia, catalepsy, locomotor activity, and in the drug discrimination paradigm. Administration of ZCZ011 alone devoid any activity, but elicited CB1-mediated antinociceptive effects in a model of neuropathic and inflammatory pain, thus suggesting that CB1 PAMs might be used to treat pain without producing unwanted cannabimimetic side effects.
Altogether, the pharmacological profile of CB1 allosteric modulators is very complex and further investigations are warranted to clearly identify the potential therapeutic exploitation for this new class of compounds.

**Figure 6.** Chemical structures of CB1 receptor allosteric modulators

### CB2 receptor agonists
In the last years the development of CB2 receptor ligands as potential drugs is increased since they are devoid of the psychotropic side-effects associated with the activation of CB1 receptor. Under normal homeostatic conditions the CB2 receptor is almost entirely peripheral where exhibits antinociceptive and anti-inflammatory activity. Moreover CB2 receptor is up-regulated in inflamed tissues associated with CNS disorders and, when activated, it is important in regulating neural inflammation and neurogenesis properties. These considerations caused a great development of CB2 receptor selective agonists and initially some aminoalkylindoles resulted very effective. One of them AM1241 (Fig 7) showed important results when tested in rodent models for neuropathic and inflammatory pain [26]. More importantly, it was reported that daily injections of AM-1241 in animal models of ALS increase the survival interval after disease onset by slowing motor neuron degeneration [90].

The non-selective cannabinoid receptor agonist WIN55,212-2 (Fig. 4) protects mouse nigrostriatal neurons from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity and neuroinflammation by inhibiting microglial activation/infiltration suggesting that CB2 receptor might be a new therapeutic target to slow the degenerative process in Parkinson disease [91]. Furthermore WIN 55212-2 showed to reduce the differentiation of T cells into Th1 effector cells, thereby reducing the production of inflammatory mediators and disease severity in a viral model of multiple sclerosis.
Finally the same compound in an animal model of Alzheimer’s disease, prevents Aβ-induced cognitive impairment and neuronal loss [41].

Regarding synthetic analogs of Δ⁹-THC, JWH-133 (Fig. 7), a CB2-specific agonist, showed to reduce dose-dependently hyperalgesia in an autoimmune encephalomyelitis mouse model of multiple sclerosis promoting CB2 receptor as a possible target for the treatment of central pain in multiple sclerosis [93]. The same compound demonstrated to ameliorate several parameters in Alzheimer's disease such as decreased memory and learning, neuroinflammation, oxidative stress damage and oxidative stress responses, selected tau kinases, and tau hyperphosphorylation around plaques; on the contrary, the chronic treatment with JWH-133 was ineffective for the amyloid-β production or deposition in cortex and hippocampus [94]. Finally the selective agonist HU-308 (Fig. 7) was used to show the benefic effects of CB2 receptor activation for stimulation of microglial cells, infiltration of macrophages and also certain capability of these cells to generate proinflammatory factors in animal model of Parkinson inflammation [95].

During the early 2000s 1,8-naphthyridin-4(1H)-on-3-carboxamide derivatives were reported as novel cannabinoid ligands [96]. Successively 1,8-naphthyridin-2(1H)-on-3-carboxamide derivatives were reported as potent and selective CB2 receptor ligands. The concentration–dependent inhibitory action on human basophils activation and the concentration-dependent decrease of cell viability in Jurkat cells shown by N-(4-methylcyclohexyl)-1-benzyl-1,8-naphthyridin-2(1H)-on-3-carboxamide (CB74) (Fig. 7) indicate that this compound possess agonist properties on CB2 receptor [97]. Recently the same compound showed to inhibit cell activation markers in multiple sclerosis patient derived lymphocytes more efficiently than in healthy control derived cells [98]. Indeed, this derivative reduced the levels of Cox-2 in lymphocytes from patients whereas no effect was observed in control cells [98]. The structural analogue CB91 (Fig. 7) showed to modulate the immune response of peripheral blood mononuclear cells (PBMC) acting with a CB2 receptor mediated mechanism [99]. Furthermore CB91 showed medium level of BBB permeability that may be looked-for to maintain its beneficial effects on infiltrating lymphocytes at the levels of the CNS [99]. The obtained findings suggest potential application of these compounds in neuroinflammation, suggesting the necessity of further investigations of their effects in the therapy of multiple sclerosis. Very interesting, recently it was demonstrated that the substituent at C-6 position of the central nucleus of this class of compounds is crucial for the functionality identifying it as the key molecular feature that discriminates 1,8-naphthyridin-2(1H)-on-3-carboxamide agonists from antagonists/inverse agonists [100].
Recently a new class of indazoles was developed with a specific multi-target profile that is a promising approach against multifactorial illnesses as Alzheimer’s disease. In particular 1-(2-diisopropylaminoethyl)-3-(4-methoxybenzyloxy)indazole (3) (Fig. 7) and 5-Amino-3-(2-naphthylmethoxy)-1-(2-piperidinoethyl)indazole (24) (Fig. 7) behave as CB2 receptor agonists and simultaneously show inhibition of butyrylcholinesterase (BuChE) activity, related to the loss of episodic memory, with antioxidant properties suggesting that these compounds can be regarded as a potential agent useful for Alzheimer’s disease [101].

A novel resorcinol-based compound O-1966 (Fig. 7) was reported as selective CB2 receptor ligand that attenuated both chronic and remitting-relapsing autoimmune encephalomyelitis and reduced rolling and
adhesion of endogenous leukocytes to pial microvasculature in experimental animal model suggesting that pharmacological CB2 receptor ligands offer a new strategy for BBB protection during neuroinflammation [102]. Furthermore the repeated treatment with this CB2 agonist determined positive effects on blood-brain barrier integrity and neuronal degeneration in mice with traumatic brain injury [102].

Tricyclic pyrazoles represents a new class of highly selective CB2 receptor agonist. Among them Gp1a (Fig. 7) showed a positive effect on experimental autoimmune encephalomyelitis animal model that has similarities to human MS, through two different mechanisms: an initial effect on Th1/Th17 differentiation in peripheral immune organs, and a later effect on the accumulation of pathogenic immune cells in the CNS, connected to reduction of chemokines and adhesion molecules [103]. These results indicate the importance of CB2 selective ligands as potential therapeutic agents in neuroinflammation.

Several CB2 ligands of miscellaneous structure isolated from different natural sources have been described. Among them, β-caryophyllene (Fig. 8), which is a major sesquiterpenoid constituent of cloves (Syzygium aromaticum L.) and of the essential oils of numerous plants, including hemp (Cannabis sativa L.), exhibited a nanomolar potency in binding and activating CB2 receptors in vitro and in vivo [104]. β-Caryophyllene showed CB2-mediated beneficial effects in animal models of inflammation, anxiety, depression and neurodegenerative diseases [105-107]. β-caryophyllene has been patented for the treatment of schizophrenia [108]. Several derivatives of β-caryophyllene have been recently shown to retain the binding properties to CB2 receptors and acquiring new interesting pharmacological features which might lead to synergistic effects in vivo [109]. 4′-O-methylhokiol (MH) (Fig. 8) is a relatively simple biphenyl scaffold which is the major bioactive constituent of Magnolia grandiflora L. seed oil. MH showed to potently and selectively activate CB2 receptors [110] and to exert pronounced anti-inflammatory, anti-osteoclastogenic, and neuroprotective effects in vivo [111-113]. MH was patented as a potential treatment for inflammatory diseases [114], dementia [115] and preventing amyloid-related diseases [116]. Finally, the alkylamides dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide (A1) and dodeca-2E,4E-dienoic acid isobutylamide (A2) (Fig. 8) which have been isolated from Echinacea purpurea and Echinacea angustifolia, were shown to bind to and activate CB2 receptors more strongly than the endogenous cannabinoids with Ki values in the low nanomolar range [117]. In cellular systems and in human blood, these alkylamides potently inhibited lipopolysaccharide-induced inflammation and exerted modulatory effects on cytokine expression [117,118]. Echinacea extracts were patented for potential therapeutic uses and several clinical trials testing alkylamide-containing Echinacea extracts were performed for various indications such as immunostimulation [119,120], for the treatment of common cold [121,122], as osteopathy treatment in children with recurrent otitis media [123], as prophylaxis for upper respiratory tract infections in children and acute respiratory illness [124].
CANNABINOID RECEPTOR ANTAGONIST

CB1 receptor antagonists

The CB1 receptor shows a key role in the control of food intake and energy balance. Several CB1 receptor antagonist or inverse agonist were studied for treatment of obesity, diabetes and correlated cardio-metabolic problems. The biarylpyrazole derivative SR141716 (Fig. 9) known also as Rimonabant by Sanofi behaves as CB1 receptor antagonist with inverse agonist functional properties. When tested in humans, this compound showed to reduce body weight and to ameliorate dyslipidemias, diabetes, and metabolic syndrome [125]. Rimonabant was launched in Europe since 2006 but it was not approved by the US Food and Drug Administration. However in 2008 was withdrawn from the European market because of undesirable side effects, which comprised nausea, anxiety, depression, and in some cases, suicidal tendencies [126]. Since then, various inverse CB1 antagonists with similar pharmacological profiles were developed [26], such as SR147778 (Fig. 9), CP-945,598 (Fig. 9) and MK-0364 (Fig. 9) but their studies were stopped in the clinical phase because they exhibit the same typical side effects of Rimonabant. Interestingly, recent preclinical studies have demonstrated that Rimonabant, reduces the hypokinesia in an animal model of Parkinson disease at low dose [38].
In the last years various efforts to develop novel compounds with no or reduced side effect were made following two different approaches. The first was to develop CB1 receptor antagonists with no inverse agonist or weak inverse agonist properties. The best known of these compounds are AM4113 that is a pyrazole analog structurally related to Rimonabant, and AM6527 (Fig. 9) [127]. These compounds did not exhibit any of the undesirable side effects of inverse agonists in a number of animal models whereas they possess therapeutic effects typical of inverse CB1 receptor antagonists such as reduction in food consumption, weight loss, and capacity to antagonize the effects of stimulant and nicotine addiction [127].
The development of peripherally active compounds represents another approach for obtaining CB1 receptor antagonists with reduced undesirable side effects. It was demonstrated that the effects of these compounds involve the modulation in lipid metabolism and energy balance. The compound AM6545 (Fig. 9) induces weight loss and improves lipid profile and insulin sensitivity [128]. Currently, this compound is in preclinical testing for the treatment of nonalcoholic fatty liver disease (NAFLD), as well as liver fibrosis [128].

Recently a series of double amides based on the Rimonabant structure separated by an alkyl chain of several methylene units was reported. These compounds possess a different pharmacokinetic profile and thus reduced side effects. One of them, the compound N,N’-Heptan-1,7-diylbis[5-(4-chlorophenyl)-1-(2,4- dichlorophenyl)-1H-pyrazole-3-carboxamide] (4d) (Fig. 9), was selected for in vivo pharmacological evaluations. In particular its intraperitoneal administration resulted in a dose-dependent inhibition of feeding indicating that, since this compound possesses a restricted brain-penetrant, the appetite and weight reduction should be mediated by a mechanism involving leptin [129].

**CB2 receptor antagonists**

At the present, very few compounds have been described as CB2-selective “neutral” antagonists, i.e. high-affinity ligands that lack significant inverse agonist action. CB2 neutral antagonist could be useful as a pharmacological “tool” to distinguish between tonic activity arising from endocannabinoid release (which it should oppose) and tonic “constitutive” activity of CB2 receptor (which it should not oppose). Recently biphenylic carboxamide derivative 10 (Fig. 10) [130] was described having such activity. Furthermore the 1,2-dihydro-2-oxopyridine-3-carboxamide 17 (Fig. 10) [131] was reported to show behaviour as CB2R neutral antagonist/weak partial inverse agonist. In particular it was reported that the functionality activity of the series of 1,2-dihydro-2-oxopyridine is controlled by the presence of substituent in position 5 of the heterocyclic nucleus.

Several CB2 ligands with inverse agonist activity were reported in literature for treatment of various indications but often there is discrepancy in the reported data [132].

Very recently the biphenyl-phenyl-methanone derivative SMM-189 (Fig. 10) which is a potent and selective CB2 inverse agonist, showed to regulate microglial activation, in terms of chemokine expression and cell morphology [133]. Furthermore SMM-189 possesses acceptable biopharmaceutical properties indicating that this compound could be useful for the treatment of neurodegenerative disorders and traumatic brain injury [133].
Current & Future Development
As a therapeutic target, the cannabinoid CB1 and CB2 receptors have found to date only modest success. However regarding cannabinoid CB1 receptor the development of CB1 agonists with reduced side effects through peripheralization could represent potential analgesic medications for treatment of pain. Furthermore the new peripherally-acting CB1 antagonist or brain penetrant neutral CB1 antagonists could find usefulness for important disorders such as fat metabolism or those from opioids and alcohol. Allosteric modulators might offer an alternative strategy to pharmacologically modulate CB1 receptors in the brain without eliciting classic unwanted side effects. Finally CB2 agonists continue to be considered as potential medications for inflammatory and neuropathic pain, as well as in neurodegenerative conditions, including ALS, Huntington’s disease, Alzheimer’s disease, Parkinson’s disease, and multiple sclerosis.

Figure 10. Chemical structures of CB2 receptor antagonists/inverse agonists
References
6) Jones RM, Discovery of agonists of the glucose dependent insulinotropic receptor, GPR119, a pancreatic beta-cell oGPCR, for the treatment of NIDDM. Drugs Future 2006; 31 (Suppl. A), No: L48.

33) Duarte JM, Ferreira SG, Carvalho RA, Cunha RA, Köfalvi A. CB1 receptor activation inhibits neuronal and astrocytic intermediary metabolism in the rat hippocampus. Neurochem Int 2012; 60: 1–8.


51) Antiepileptic Efficacy Study of GWP42003-P in Children and Young Adults With Dravet Syndrome https://clinicaltrials.gov/ct2/show/NCT02091375.


72) Radhakrishnan R, Wilkinson ST, D’Souza DC. Gone to pot – a review of the association between cannabis and psychosis. Front Psychiatr 2014; 5: 54.


103) Kong W, Li H, Tuma RF, Ganea D. Selective CB2 receptor activation ameliorates EAE by reducing Th17 differentiation and immune cell accumulation in the CNS. Cell Immunol 2014; 287: 1-17.


114) Han SB, Jung JK, Kwak YS, Seo SY Lee KH, Song SG, Hong JT. Novel 4-O’-methylhonokiol derivative and composition containing same as active ingredient for treatment of inflammatory diseases WO 2012102560 A3 (2012).

115) Han SB, Jung JK, Kwak YS, Seo SY. Lee KH, Song SG, Hong JT. Composition containing novel 4-O’-methylhonokiol derivative as active ingredient for treatment of dementia WO 2012102562 A2 (2012).


