Synthetic cannabinoid receptor agonists and antagonists: implication in CNS disorders

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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 Δ^9-tetrahydrocannabinol (Δ^9-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 Ca^{2+} 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 (arachidonylethanolamide; 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].

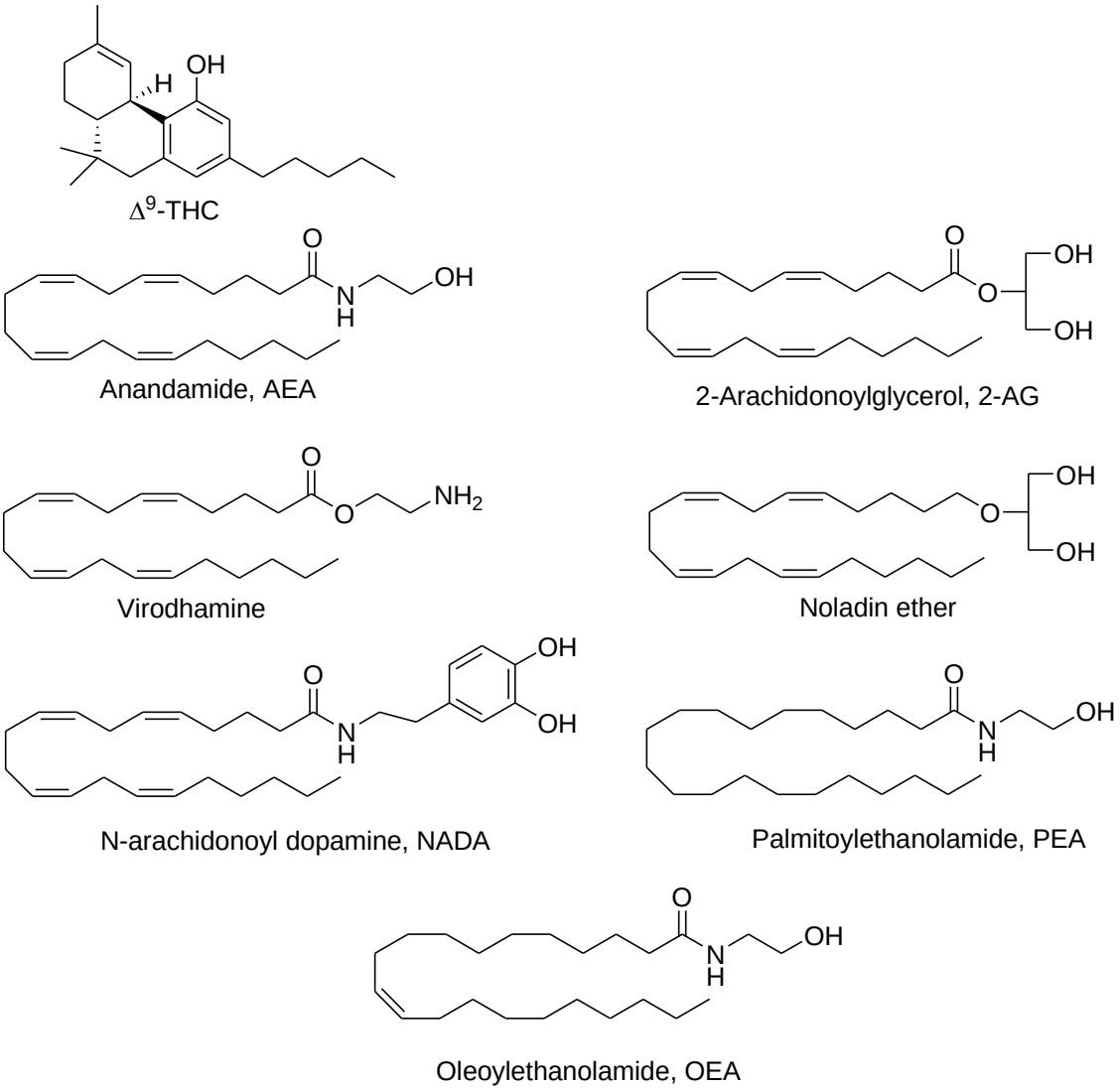


Figure 1. Chemical structures of Δ^9-THC and endocannabinoids.

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 Δ^9-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 Δ^9-THC, such as n-hexyl- Δ^{6a}-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.

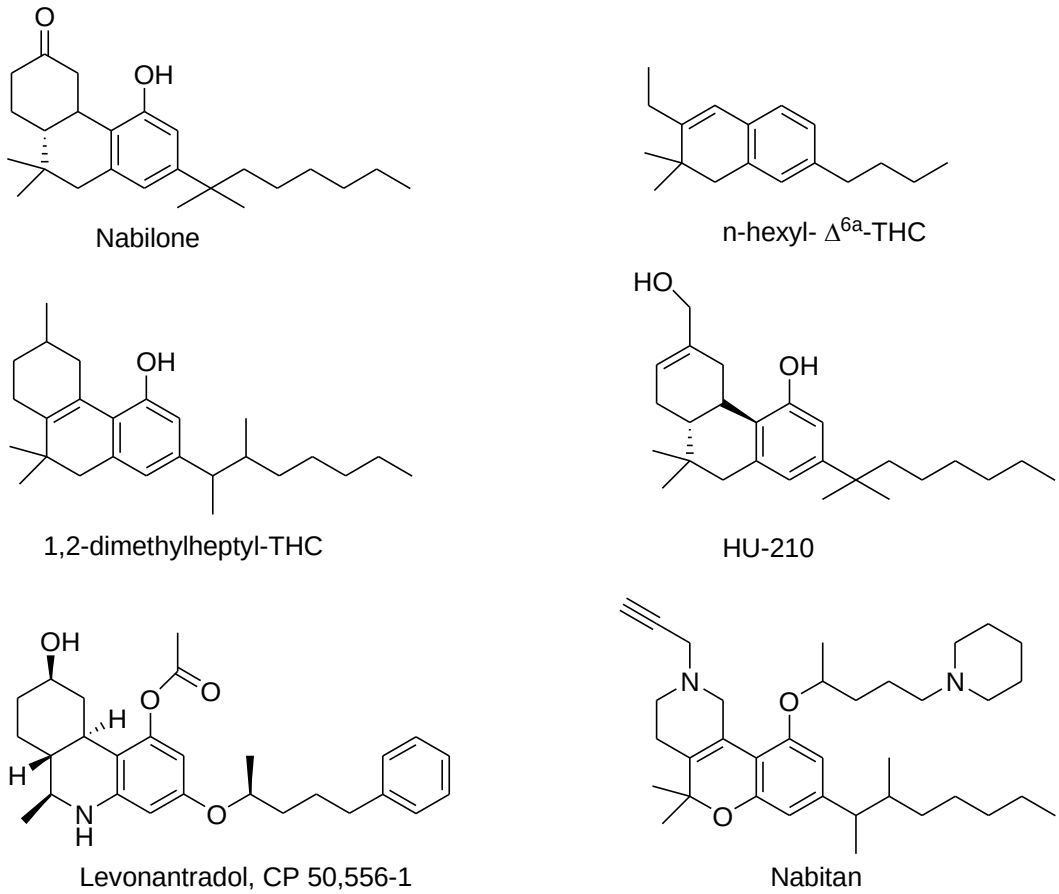
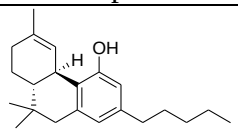
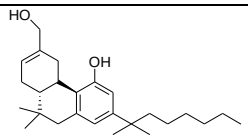
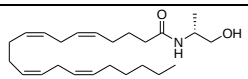
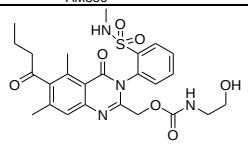
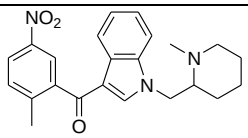
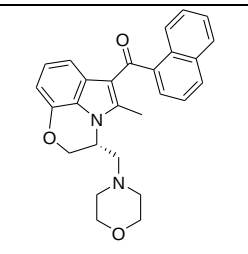
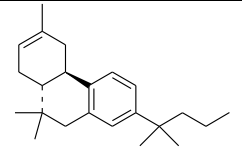
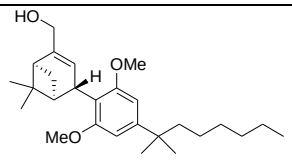
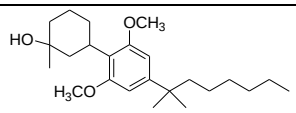
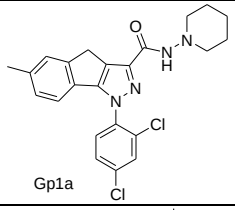
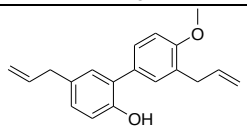
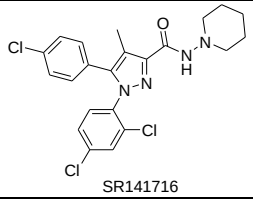


Figure 2. Chemical structures of synthetic analogs of Δ^9-THC

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

Compound	ES Target	Action	Study type	CNS Disorders
 Δ^9-THC	CB1R CB2R	Partial agonist Partial agonist	Preclinical and Clinical	Multiple sclerosis Parkinson's disease Alzheimer's disease Epilepsy
 HU-210	CB1R CB2R	Partial agonist Partial agonist	Preclinical	Alzheimer's disease
 AM356	CB1R	Agonist	Preclinical	Traumatic brain injuries
 SAD448	CB1R	Agonist	Preclinical	Multiple sclerosis
 AM1241	CB2R	Agonist	Preclinical	Amyotrophic lateral sclerosis
 WIN 55212-2	CB1R CB2R	Agonist Agonist	Preclinical	Multiple sclerosis Parkinson's disease Alzheimer's disease
 JWH-133	CB2R	Agonist	Preclinical	Multiple sclerosis Alzheimer's disease
 HU-308	CB2R	Agonist	Preclinical	Parkinson's disease

 O-1966	CB2R	Agonist	Preclinical	BBB protection
 Gp1a	CB2R	Agonist	Preclinical	Multiple sclerosis
 4'-O-methylhokiol	CB2R	Agonist	Preclinical	Traumatic brain injuries
 SR141716	CB1R	Antagonist	Preclinical	Parkinson's disease

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 Δ^9-THC and CBD showed to protect nigrostriatal dopaminergic neurons from the neurotoxin 6-hydroxydopamine exerting antioxidant action [39]. Therefore the CBD/ Δ^9-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 Δ^9-THC can counteract β-amyloid aggregation and the consequent plaque formation by inhibiting acetylcholinesterase activity [42]. In addition, Δ^9-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 Δ^9-THC, is used as antiemetic and appetite-stimulator in AD patients [44]. Furthermore in patients with severe AD, Δ^9-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 Δ^9-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 Δ^9-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 on-going for the evaluation of Epidiolex® in two forms of severe, childhood-onset epilepsy [51,52].

CANNABINOID RECEPTOR AGONIST

CB1 receptor agonists

Δ^9-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, tetrahydrocannabivarin (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 cannabidivarin (Fig. 3) without psychotropic activity, exhibit very low affinities for both cannabinoid receptors, and the mechanism through which cannabidiol acts is not clear. Δ^9-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 Δ^9-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].

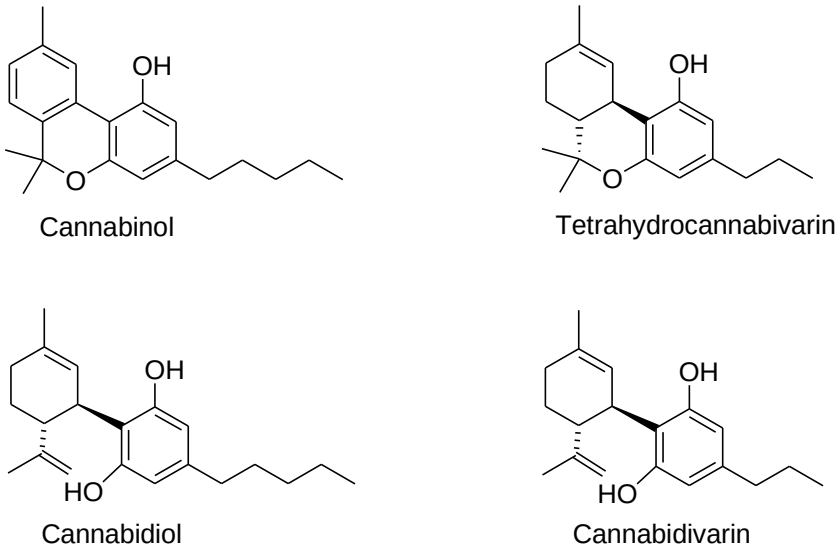


Figure 3. Chemical structures of phytocannabinoids

(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 Δ^9-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].

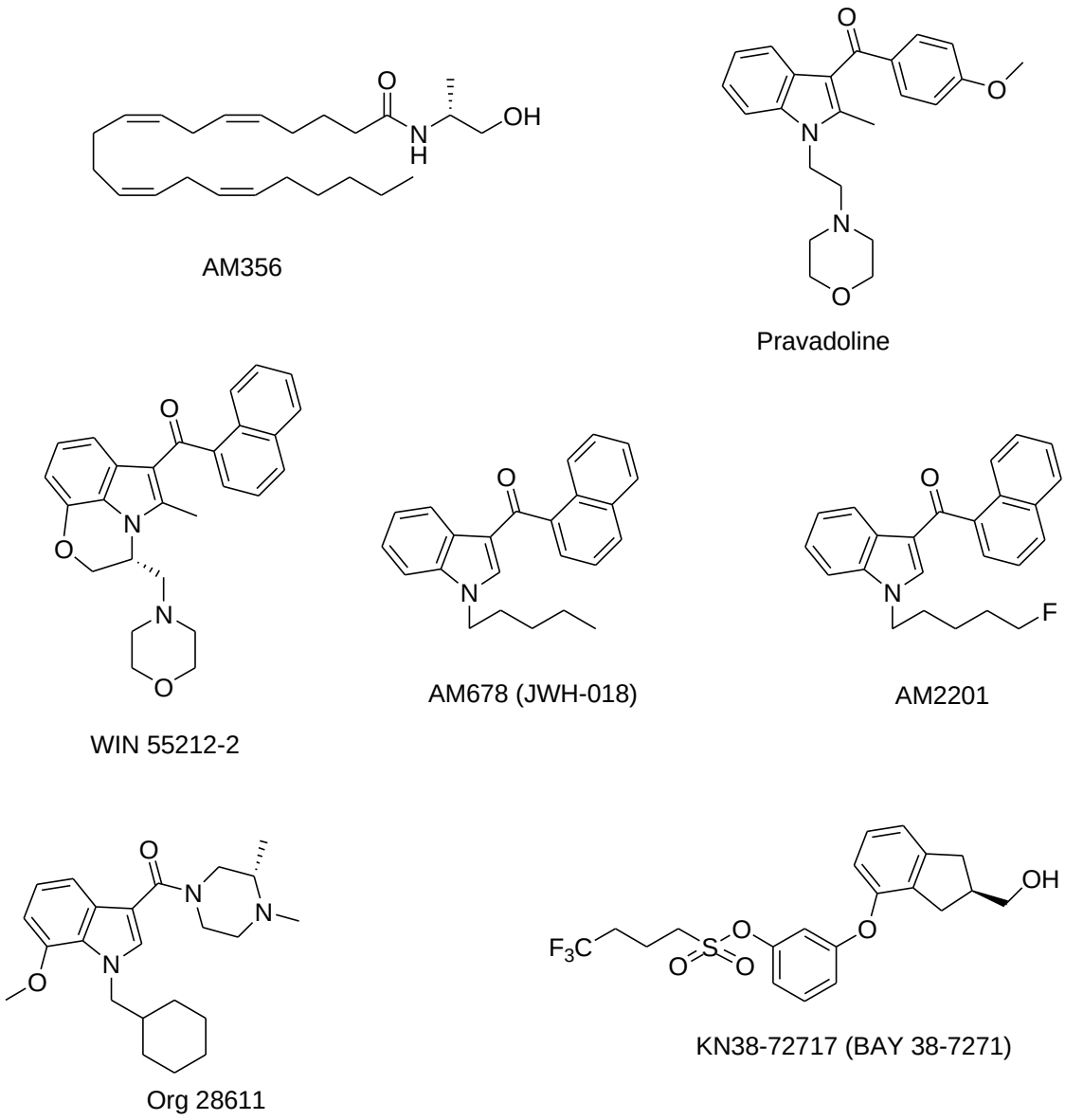


Figure 4. Chemical structures of CB1 receptor agonists

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].

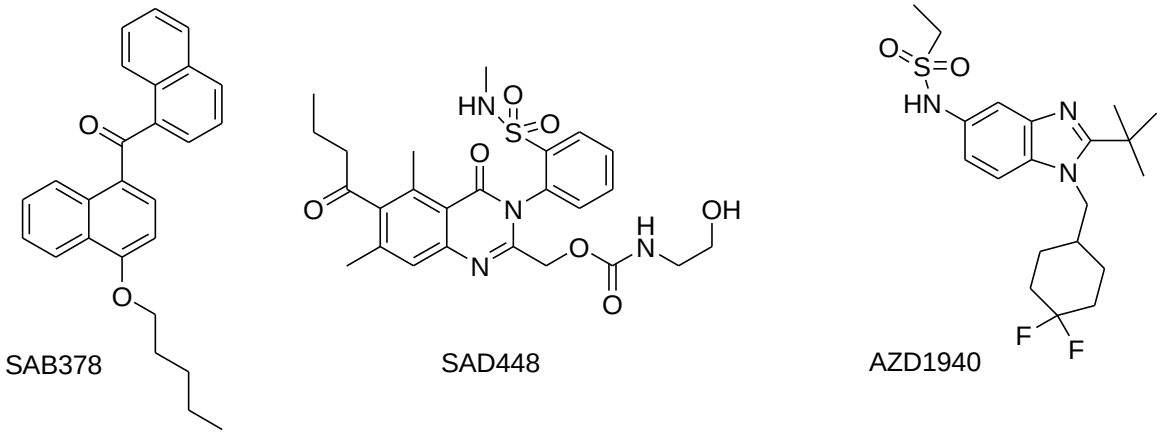


Figure 5. Chemical structures of peripherally restricted CB1 receptor agonists

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 *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. *In vitro* and *in vivo*, pregnenolone partially counteracted some of the Δ^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 Δ^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]. Pepcans (pepcan endocannabinoids) are a family of *N*-terminal extended endogenous peptides which spans from 12 to 23 aminoacids and derive from the α-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 *in vitro* and *in vivo*. Furthermore, lipoxinA4 showed protective effects in mice against β-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.

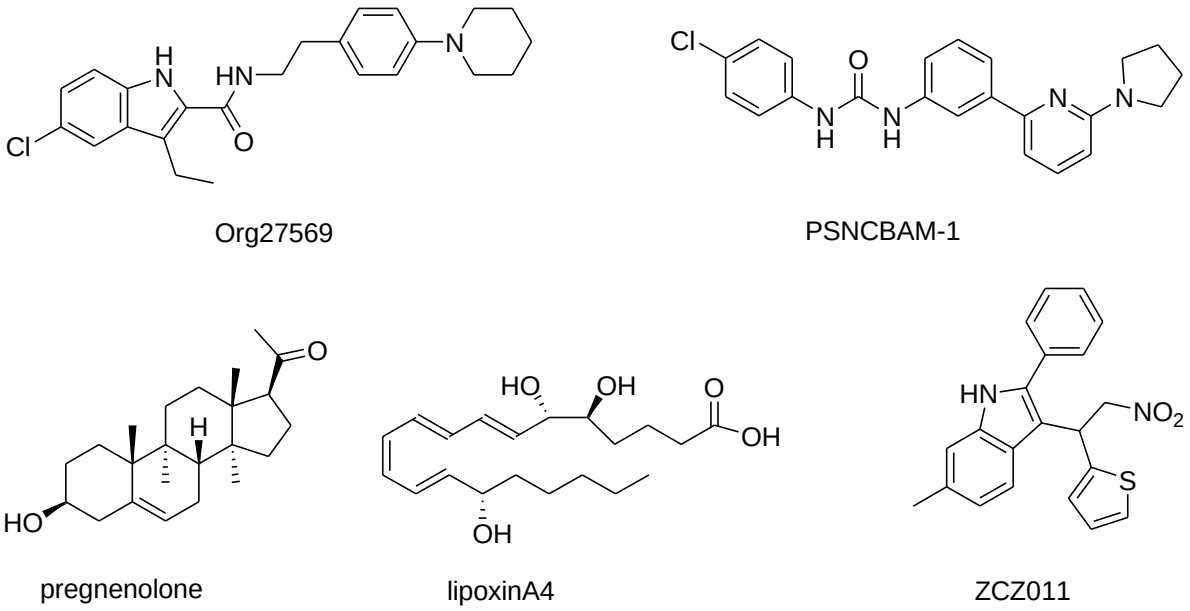


Figure 6. Chemical structures of CB1 receptor allosteric modulators

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

[92]. 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 Δ^9-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(1*H*)-on-3-carboxamide derivatives were reported as novel cannabinoid ligands [96]. Successively 1,8-naphthyridin-2(1*H*)-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(1*H*)-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(1*H*)-on-3-carboxamide agonists from antagonists/inverse agonists [100].

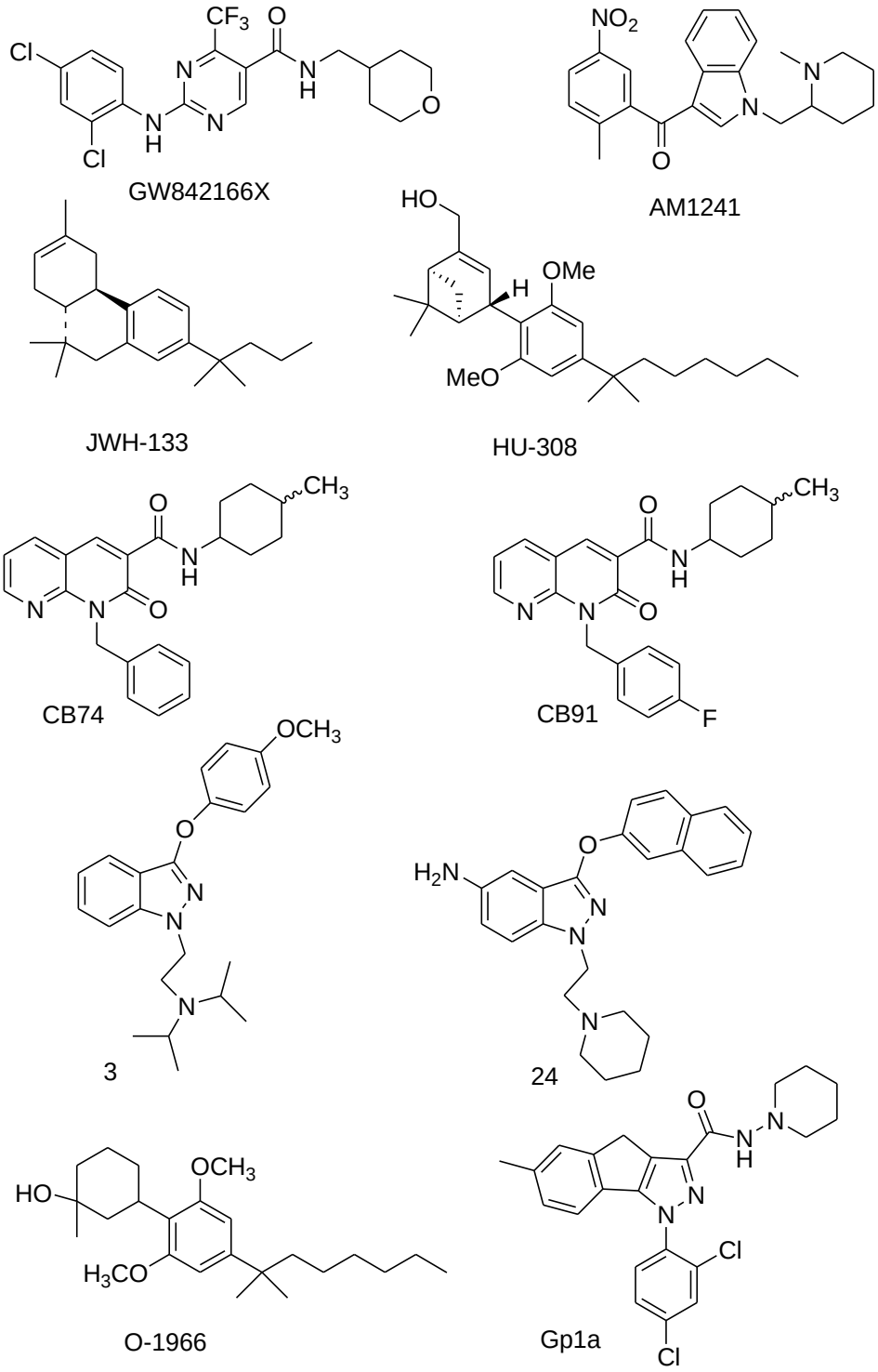


Figure 7. Chemical structures of CB2 receptor agonists

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 prophilaxis for upper respiratory tract infections in children and acute respiratory illness [124].

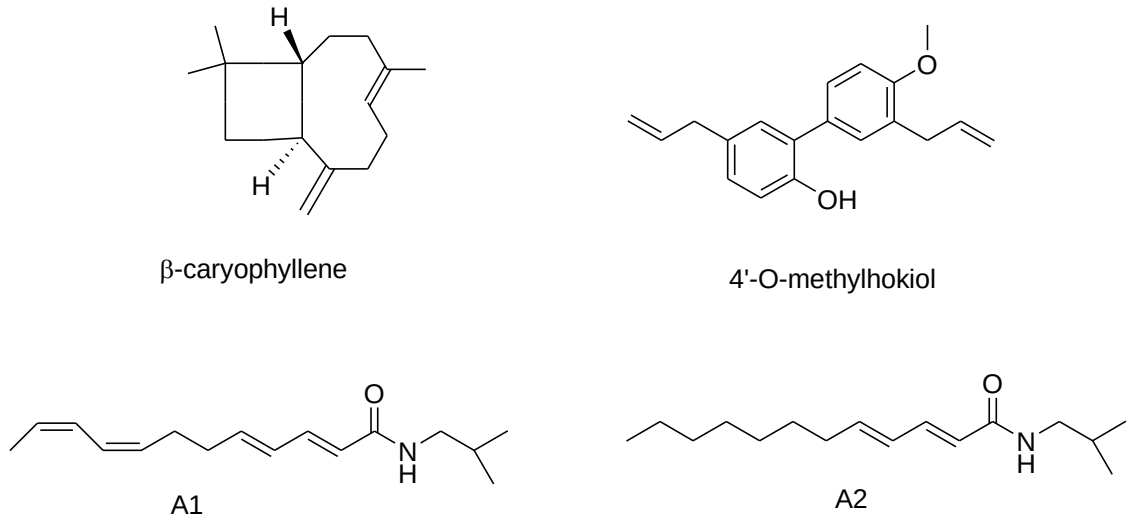


Figure 8. Chemical structures of CB2 receptor agonists isolated from different natural sources

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].

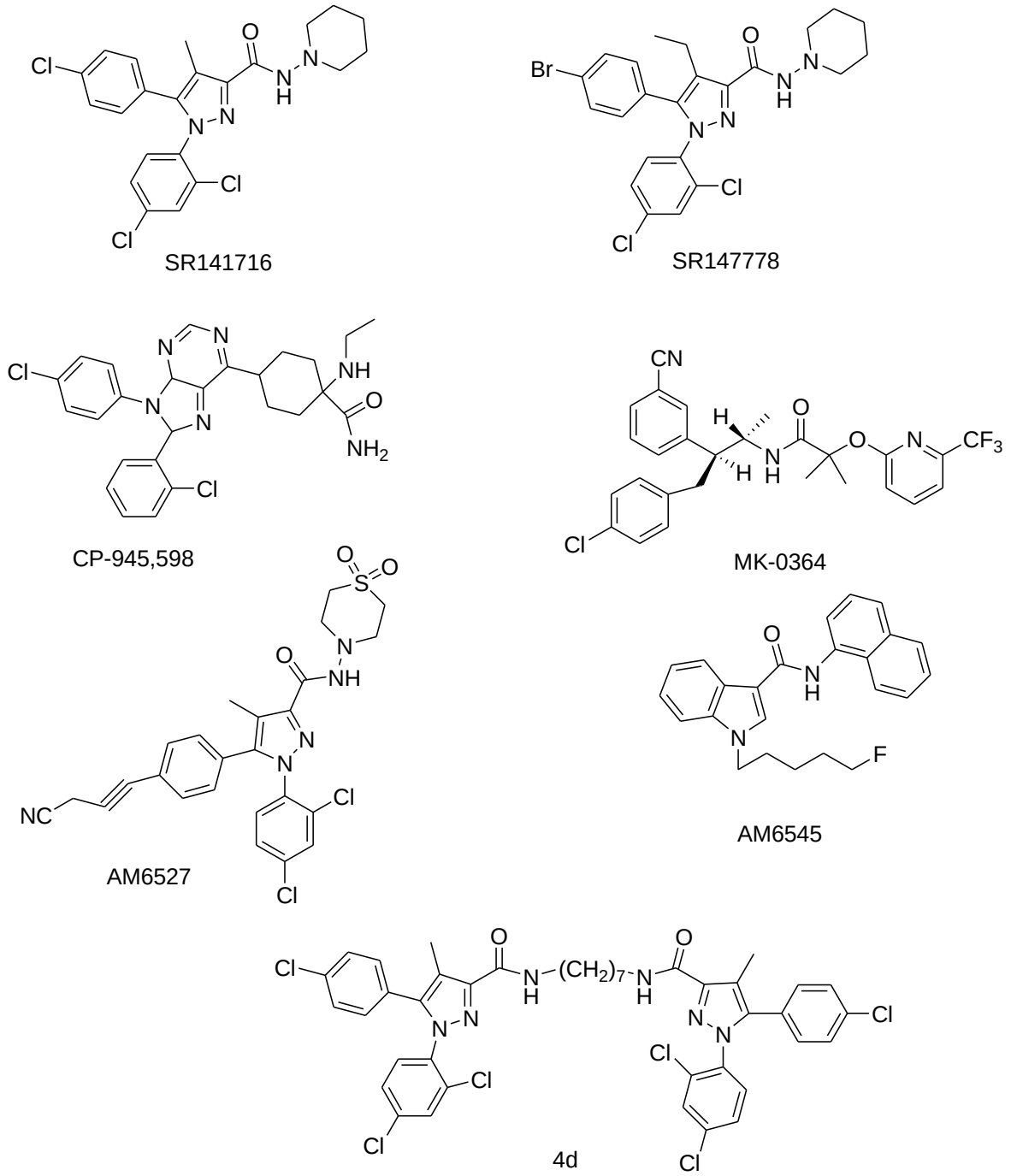


Figure 9. Chemical structures of CB1 receptor antagonists

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].

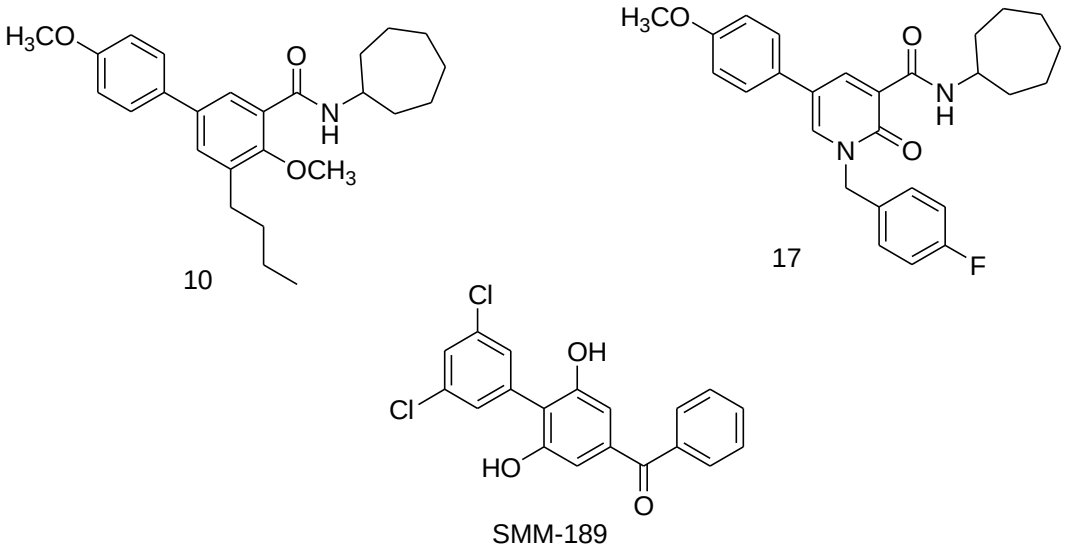


Figure 10. Chemical structures of CB2 receptor antagonists/inverse agonists

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.

References

1) Gaoni Y, Mechoulam R, Isolation, structure and partial synthesis of an active constituent of hashish. J Am Chem Soc 1964; 86: 1646-47.
2) Battista N, Di Tommaso M, Bari M, Maccarrone M, The endocannabinoid system: an overview. Front Behav Neurosci 2012; 6: 9.
3) Svíženská I, Dubový P, Sulcová A, Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structure. Pharmacol Biochem Behav 2008; 90: 501-11.
4) Brown AJ, Novel cannabinoid receptors. Br J Pharmacol 2007; 152: 567−75.
5) Mackie K, Stella N, Cannabinoid receptors and endocannabinoids: evidence for new players. AAPS J 2006; 8: E298−E306.
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.
7) Overton HA, Babbs AJ, Doel SM, Fyfe MC, Gardner LS, Griffin G, *et al*., Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. Cell Metab 2006; 3: 167−75.
8) Sharir H, Abood ME, Pharmacological characterization of GPR55, a putative cannabinoid receptor. Pharmacol Ther 2010; 126: 301–13.
9) Nevalainen T, Irving AJ, GPR55, a lysophosphatidylinositol receptor with cannabinoid sensitivity? Curr Top Med Chem 2010; 10: 799–813.
10) McHugh D, Hu SS, Rimmerman N, Juknat A, Vogel Z, Walker JM, *et al.* N-arachidononyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. BMC Neurosci 2010; 11: 44.
11) Costa B, Bettoni I, Petrosino S, Comelli F, Giagnoni G, Di Marzo V. The dual fatty acid amide hydrolase/TRPV1 blocker, arachidonoyl-serotonin, relieves carrageenan-induced inflammation and hyperalgesia in mice. Pharmacol Res 2010; 61: 537–46.
12) Bermudez-Silva FJ, Viveros MP, McPartland JM, Rodriguez de Fonseca F. The endocannabinoid system, eating behavior and energy homeostasis: The end or a new beginning? Pharmacol Biochem Behav 2010; 95: 375-82.
13) Nomura DK, Long JZ, Niessen S, Hoover HS, Ng SW, Cravatt BF. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell 2010; 140: 49-61.
14) Long Z, LaCava M, Jin X, Cravatt BF. An anatomical and temporal portrait of physiological substrates for fatty acid amide hydrolase. J Lipid Res 2011; 52: 337-44.
15) Pisanti S, Picardi P, D'Alessandro A, Laezza C, Bifulco M. The endocannabinoid signaling system in cancer. Trends Pharmacol Sci 2013; 34: 273-82.
16) Oesch S, Gertsch J. Cannabinoid receptor ligands as potential anticancer agents--high hopes for new therapies? J Pharm Pharmacol 2009; 6: 839-53.

17) Velasco G, Sánchez C, Guzmán M. Towards the use of cannabinoids as antitumour agents. Nat Rev Cancer 2012; 12: 436-44.
18) Giblin GM, O'Shaughnessy CT, Naylor A, Mitchell WL, Eatherton AJ, Slingsby BP, *et al.* Discovery of 2-[(2,4-dichlorophenyl)amino]-N-[(tetrahydro- 2H-pyran-4-yl)methyl]-4-(trifluoromethyl)- 5-pyrimidinecarboxamide, a selective CB2 receptor agonist for the treatment of inflammatory pain. J Med Chem 2007; 50: 2597-600.
19) Odan M, Ishizuka N, Hiramatsu Y, Inagaki M, Hashizume H, Fujii Y, *et al.* Discovery of S-777469: an orally available CB2 agonist as an antipruritic agent. Bioorg Med Chem Lett 2012; 22: 2803-806.
20) Idris AI, Ralston SH. Role of cannabinoids in the regulation of bone remodeling. Front Endocrinol 2012; 3: 136.
21) Skaper SD, Di Marzo V. Endocannabinoids in nervous system health and disease: the big picture in a nutshell. Philos Trans R Soc Lond B Biol Sci 2012; 367: 3193-200.
22) Sagredo O, Pazos MR, Valdeolivas S, Fernandez-Ruiz J. Cannabinoids: novel medicines for the treatment of Huntington's disease. Recent Pat CNS Drug Discov 2012; 7: 41-8.
23) Pazos MR, Núñez E, Benito C, Tolón RM, Romero J. Role of the endocannabinoid system in Alzheimer's disease: new perspectives. Life Sci 2004; 75: 1907-15.
24) García-Arencibia M, García C, Fernández-Ruiz J. Cannabinoids and Parkinson's disease. *CNS* Neurol Disord Drug Targets 2009; 8: 432-9.
25) Pryce G, Baker D. Endocannabinoids in Multiple Sclerosis and Amyotrophic Lateral Sclerosis. Handb Exp Pharmacol 2015; 231: 213-31.
26) Makriyannis, A. Division of medicinal chemistry award address. Trekking the cannabinoid road: a personal perspective. J Med Chem 2012; 57: 3891–911.
27) Fernández-Ruiz J, Romero J, Ramos JA. Endocannabinoids and Neurodegenerative Disorders: Parkinson's Disease, Huntington's Chorea, Alzheimer's Disease, and Others. Handb Exp Pharmacol 2015; 231: 233-59.
28) Fernández-Ruiz J, de Lago E, Gómez-Ruiz M *et al*. Neurodegenerative disorders other than multiple sclerosis. In: Pertwee RG Eds. Handbook of cannabis. Oxford University Press, Oxford 2014: 505–25.
29) Fernández-Ruiz J, García C, Sagredo O, Gómez-Ruiz M, de Lago E. The endocannabinoid system as a target for the treatment of neuronal damage. Expert Opin Ther Targets 2010; 14: 387–404.
30) Fernández-Ruiz J, González S, Romero J, Ramos JA. Cannabinoids in neurodegeneration and neuroprotection. In: Mechoulam R Eds. Cannabinoids as therapeutics (MDT). Birkhäuser Verlag, Basel 2005: 79–109.
31) Choi IY, Ju C, Anthony Jalin AM Lee da I, Prather PL, Kim WK. Activation of cannabinoid CB2 receptor-mediated AMPK/CREB pathway reduces cerebral ischemic injury. Am J Pathol 2013; 182: 928–39.

32) Carrier EJ, Kearn CS, Barkmeier AJ, Breese NM, Yang W, Nithipatikom K, Pfister SL, *et al*. Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonylglycerol, which increases proliferation via a CB2 receptor dependent mechanism. Mol Pharmacol 2004; 65: 999-1007.

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.

34) Gómez O, Sanchez-Rodriguez A, Le M Sanchez-Caro C, Molina-Holgado F, Molina-Holgado E. Cannabinoid receptor agonists modulate oligodendrocyte differentiation by activating PI3K/Akt and the mammalian target of rapamycin (mTOR) pathways. Br J Pharmacol 2011; 163:1520–32.

35) Köfalvi A, Rodrigues RJ, Ledent C, Mackie K, Vizi ES, Cunha RA *et al.* Involvement of cannabinoid receptors in the regulation of neurotransmitter release in the rodent striatum: A combined immunochemical and pharmacological analysis. J Neurochem 2005; 25: 2874–84.

36) Fernandez-Ruiz J. The endocannabinoid system as a target for the treatment of motor dysfunction. Br J Pharmacol 2009; 156:1029–40.

37) Carta AR, Simuni T. Thiazolidinediones under preclinical and early clinical development for the treatment of Parkinson's disease. Expert Opin Investig Drugs 2014; 17: 1–9.

38) González S, Scorticati C, García-Arencibia M, de Miguel R, Ramos JA, Fernández-Ruiz J. Effects of rimonabant, a selective cannabinoid CB1 receptor antagonist, in a rat model of Parkinson's disease. Brain Res 2006; 1073: 209-19.

39) Lastres-Becker I, Molina-Holgado F, Ramos JA, Mechoulam R, Fernandez-Ruiz J. Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity in vivo and in vitro: Relevance to Parkinson's disease. Neurobiol Dis 2005; 19: 97–107.

40) van der Stelt M, Veldhuis WB, Bar PR, Veldink GA, Vliegenthart JF, Nicolay K. Neuroprotection by Δ9-tetrahydrocannabinol, the main active compound in marijuana, against ouabain-induced in vivo excitotoxicity. J Neurosci 2001; 21: 6475–79.

41) Ramírez BG, Blázquez C, Gómez del Pulgar T, Guzmán M, de Ceballos ML. Prevention of Alzheimer's disease pathology by cannabinoids: Neuroprotection mediated by blockade of microglial activation. J Neurosci 2005; 25: 1904–13.

42) Eubanks LM, Rogers CJ, Beuscher AE, Koob GF, Olson AJ, Dickerson TJ *et al.* A molecular link between the active component of marijuana and Alzheimer's disease pathology. Mol Pharm 2006; 3: 773–77.

43) Casarejos MJ, Perucho J, Gomez A, Muñoz MP, Fernandez-Estevez M, Sagredo O *et al*. Natural cannabinoids improve dopamine neurotransmission and tau and amyloid pathology in a mouse model of tauopathy. J Alzheimers Dis 2013; 35: 525–39.

44) Volicer L, Stelly M, Morris J, McLaughlin J, Volicer BJ. Effects of dronabinol on anorexia and disturbed behavior in patients with Alzheimer's disease. Int J Geriatr Psychiatry 1997; 12: 913–19.

45) Walther S, Mahlberg R, Eichmann U, Kunz D. Δ9-tetrahydrocannabinol for nighttime agitation in severe dementia. Psychopharmacology (Berl) 2006; 185: 524–28.

46) Chiarlone A, Bellocchio L, Blázquez C, Resel E, Soria-Gómez E, Cannich A, *et al*. A restricted population of CB1 cannabinoid receptors with neuroprotective activity. Proc Natl Acad Sci U S A 2014; 111: 8257–62.

47) Bouchard J, Truong J, Bouchard K, Dunkelberger D, Desrayaud S, Moussaoui S, *et al*. Cannabinoid receptor 2 signaling in peripheral immune cells modulates disease onset and severity in mouse models of Huntington's disease. J Neurosci 2012; 32: 18259–68.

48) Neuroprotection by Cannabinoids in Huntington's Disease https://clinicaltrials.gov/ct2/show/NCT01502046.

49) Friedman D, Devinsky O. Cannabinoids in the Treatment of Epilepsy. N Engl J Med. 2015; 373: 1048-58.

50) Devinsky O, Sullivan J, Friedman D, et al. Epidiolex (cannabidiol) in treatment resistant epilepsy. *American Academy of Neurology, Annual Meeting* Washington, DC, April 18‑25, 2015.

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

52) A Study to Investigate the Efficacy and Safety of Cannabidiol (GWP42003-P; CBD) as Adjunctive Treatment for Seizures Associated With Lennox-Gastaut Syndrome in Children and Adults. https://clinicaltrials.gov/ct2/show/NCT02224690.

53) United States Drug Enforcement Administration http://www.justice.gov/dea/index.shtml.

54) Wade DT, Makela P, Robson P, House H, Bateman C. Do cannabis-based medicinal extracts have general or specific effects on symptoms in multiple sclerosis? A double-blind, randomized, placebo-controlled study on 160 patients. Mult Scler 2004; 10: 434-41.

55) Zajicek J, Fox P, Sanders H, Wright DE, Vickery PJ, Nunn AJ, *et al*. Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): Multicentre randomised placebo-controlled trial. Lancet 2003; 362: 1517–26.

56) Guzman M, Duarte MJ, Blazquez C, et al. A pilot clinical study of Δ^9-tetrahydrocannabinol in patients with recurrent glioblasoma multiforme. Br J Cancer 2006; 95: 197–203.

57) Molina-Holgado F, Pinteaux E, Moore JD, Molina-Holgado E, Guaza C, Ribson RM, *et al.* Endogenous interleukin-1 receptor antagonist mediates anti-inflammatory and neuroprotective actions of cannabinoids in neurons and glia. J Neurosci 2003; 23: 6470–74.

58) Ehrhart J, Obergon D, Mori T, Hou H, Sun N, Bai Y *et al.* Stimulation of CB2 suppresses microglial activation. J Neuroinflamm 2005; 12: 22–29.

59) Nadler V, Mechoulam R, Sokolovsky M. Blockade of 45Ca2+ influx through the N-methyl-D-aspartate receptor ion channel by the non-psychoactive cannabinoid HU-211. Brain Res 1993; 622: 79–85.

60) Abadji V, Lin S, Taha G, Griffin G, Stevenson LA, Pertwee RG, *et al.* (R)-methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. J Med Chem 1994; 37: 1889–93.

61) Froger N, Orellana JA, Cohen-Salmon M, Ezan P, Amigou E, Sáez JC, *et al.* Cannabinoids prevent the opposite regulation of astroglial connexin43 hemichannels and gap junction channels induced by pro-inflammatory treatments. J Neurochem 2009; 111: 1383-97.

62) Hu B, Wang Q, Chen Y, Du J, Zhu X, Lu Y, *et al.* Neuroprotective effect of WIN 55,212-2 pretreatment against focal cerebral ischemia through activation of extracellular signal-regulated kinases in rats. Eur J Pharmacol 2010; 645: 102-7.

63) Zuurman L, Passier PC, de Kam Ml, Kleijn HJ, Cohen AF, van Gerven JM. Pharmacodynamic and pharmacokinetic effects of the intravenously administered CB1 receptor agonist Org28611 in healthy male volunteers. J Psychopharmacol 2009; 23: 633-44.

64) De Luca MA, Bimpisidis Z, Melis M, Marti M, Caboni P, Valentini V, *et al.* Stimulation of in vivo dopamine transmission and intravenous self-administration in rats and mice by JWH-018, a Spice cannabinoid. Neuropharmacology 2015; 99: 705-14.

65) Castaneto MS, Gorelick DA, Desrosiers NA, Hartman RL, Pirard, S. Huestis M. Synthetic cannabinoids: epidemiology, pharmacodynamics, and clinical implications. Drug Alcohol Depend 2014; 144, 12–41.

66) Mauler F, Horváth E, De Vry J, Jäger R, Schwarz T, Sandmann S, *et al.* BAY 38-7271: a novel highly selective and highly potent cannabinoid receptor agonist for the treatment of traumatic brain injury. CNS Drug Rev 2003; 9: 343-58.

67) Firsching R, Piek J, Skalej M, Rohde V, Schmidt U, Striggow F; KN38-7271. Study Group. Early survival of comatose patients after severe traumatic brain injury with the dual cannabinoid CB1/CB2 receptor agonist KN38-7271: a randomized, double-blind, placebo-controlled phase II trial. J Neurol Surg A Cent Eur Neurosurg 2012; 73: 204-16.

68) Baker D, Jackson SJ, Pryce G. Cannabinoid control of neuroinflammation related to multiple sclerosis. Br J Pharmacol 2007; 152: 649-54.

69) Shen M, Thayer SA. Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. Mol Pharmacol 1998; 54: 459–62.

70) Pryce G, Riddall DR, Selwood DL, Giovannoni G, Baker D. Neuroprotection in experimental autoimmune encephalomyelitis and progressive multiple sclerosis by cannabis-based cannabinoids. J Neuroimmune Pharmacol 2015; 10: 281-92.

71) Skosnik PD, D'Souza DC, Steinmetz AB, Edwards CR, Vollmer JM, Hetrick WP. The effect of chronic cannabinoids on broad band EEG neural oscillations in humans. Neuropsychopharmacology 2012; 37: 2184–93.

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.

73) Vandrey R, Haney M. Pharmacotherapy for cannabis dependence: how close are we? CNS Drugs 2009; 23: 543–53.

74) Dziadulewicz EK, Bevan SJ, Brain CT, Coote PR, Culshaw AJ, Davis AJ, *et al.* Naphthalen-1-yl-(4-pentyloxynaphthalen-1-yl)methanone: a potent, orally bioavailable human CB1/CB2 dual agonist with antihyperalgesic properties and restricted central nervous system penetration. J Med Chem 2007; 50: 3851-6.

75) Pryce G, Visintin C, Ramagopalan SV, Al-Izki S, De Faveri LE, Nuamah RA, *et al.* Control of spasticity in a multiple sclerosis model using central nervous system-excluded CB1 cannabinoid receptor agonists. FASEB J 2014; 28: 117-30.

76) Yu XH, Cao CQ, Martino G, Puma C, Morinville A, St-Onge S, *et al.* A peripherally restricted cannabinoid receptor agonist produces robust antinociceptive effects in rodent models of inflammatory and neuropathic pain. Pain 2010; 151: 337–44.

77) Kalliomäki J, Annas P, Huizar K, Clarke C, Zettergren A, Karlsten R, *et al.* Evaluation of the analgesic efficacy and psychoactive effects of AZD1940, a novel peripherally acting cannabinoid agonist, in human capsaicin-induced pain and hyperalgesia. Clin Exp Pharmacol Physiol 2013; 40: 212-8.

78) Price MR, Baillie GL, Thomas A, Stevenson LA, Easson M, Goodwin R, *et al.* Allosteric modulation of the cannabinoid CB1 receptor. Mol. Pharmacol 2005; 68: 1484−95.

79) Horswill JG, Bali U, Shaaban S, Keily JF, Jeevaratnam P, Babbs AJ, *et al.* PSNCBAM-1, a novel allosteric antagonist at cannabinoid CB1 receptors with hypophagic effects in rats. Br J Pharmacol 2007; 152: 805−14.

80) Gamage TF, Ignatowska-Jankowska BM, Wiley JL, Abdelrahman M, Trembleau L, Greig IR, *et al.* In-vivo pharmacological evaluation of the CB1-receptor allosteric modulator Org-27569. Behav Pharmacol 2014; 25: 182-5.

81) Ding Y, Qiu Y, Jing L, Thorn DA, Zhang Y, Li JX. Behavioral effects of the cannabinoid CB1 receptor allosteric modulator ORG27569 in rats. Pharmacol Res Perspect 2014; 2: e00069.

82) Jing L, Qiu Y, Zhang Y, Li JX. Effects of the cannabinoid CB_1 receptor allosteric modulator ORG 27569 on reinstatement of cocaine- and methamphetamine-seeking behavior in rats. Drug Alcohol Depend 2014; 143: 251-6.

83) Vallée M, Vitiello S, Bellocchio L, Hébert-Chatelain E, Monlezun S, Martin-Garcia E, *et al.* Pregnenolone can protect the brain from cannabis intoxication. Science 2014; 343: 94-8.

84) Rose JE, Marx CE. Neuroactive Steroid Compositions And Methods Of Use Therefor Patent WO2010107815A1 (**2010**).

85) Bauer M, Chicca A, Tamborrini M, Eisen D, Lerner R, Lutz B, *et al.* Identification and quantification of a new family of peptide endocanabinoids (Pepcans) showing negative allosteric modulation at CB1 receptors. J Biol Chem 2012; 287: 36944-67.

86) Hofer SC, Ralvenius WT, Gachet MS, Fritschy JM, Zeilhofer HU, Gertsch J. Localization and production of peptide endocannabinoids in the rodent CNS and adrenal medulla. Neuropharmacology 2015; 98: 78-89.

87) Straiker A, Mitjavila J, Yin D, Gibson A, Mackie K. Aiming for allosterism: Evaluation of allosteric modulators of CB1 in a neuronal model. Pharmacol Res 2015; 99: 370-6.

88) Pamplona FA, Ferreira J, Menezes de Lima O Jr, Duarte FS, Bento AF, Forner S, *et al.* Anti-inflammatory lipoxin A4 is an endogenous allosteric enhancer of CB1 cannabinoid receptor. Proc Natl Acad Sci U S A. 2012; 109: 21134-9.

89) Ignatowska-Jankowska BM, Baillie GL, Kinsey S, Crowe M, Ghosh S, Owens RA, *et al.* A Cannabinoid CB1 Receptor-Positive Allosteric Modulator Reduces Neuropathic Pain in the Mouse with No Psychoactive Effects. Neuropsychopharmacology 2015; 40: 2948-59.

90) Shoemaker JL, Seely KA, Reed RL, Crow JP, Prather PL. The CB2 cannabinoid agonist AM-1241 prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis when initiated at symptom onset. J Neurochem 2007; 101: 87-98.

91) Price DA, Martinez AA, Seillier A, Koek W, Acosta Y, Fernandez E, *et al.* WIN55,212-2, a cannabinoid receptor agonist, protects against nigrostriatal cell loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. Eur J Neurosci 2009; 29: 2177–86.

92) Croxford JL, Miller SD. Immunoregulation of a viral model of multiple sclerosis using the synthetic cannabinoid R+WIN55,212. J Clin Invest 2003; 111: 1231-40.

93) Fu W, Taylor BK. Activation of cannabinoid CB2 receptors reduces hyperalgesia in an experimental autoimmune encephalomyelitis mouse model of multiple sclerosis. Neurosci Lett 2015; 595:1-6.

94) Aso E, Juvés S, Maldonado R, Ferrer I. CB2 cannabinoid receptor agonist ameliorates Alzheimer-like phenotype in AβPP/PS1 mice. J Alzheimers Dis 2013; 35: 847-58.

95) Gómez-Gálvez Y, Palomo-Garo C, Fernández-Ruiz J, García C. Potential of the cannabinoid CB2 receptor as a pharmacological target against inflammation in Parkinson's disease. Prog Neuropsychopharmacol Biol Psychiatry 2016; 64: 200-8.

96) Manera C, Benetti V, Castelli MP, Cavallini T, Lazzarotti S, Pibiri F, *et al.* Design, synthesis, and biological evaluation of new 1,8-naphthyridin-4(1H)-on-3-carboxamide and quinolin-4(1H)-on-3-carboxamide derivatives as CB2 selective agonists. J Med Chem 2006; 49: 5947-57.

97) Manera C, Saccomanni G, Adinolfi B, Benetti V, Ligresti A, Cascio MG, *et al.* Rational design, synthesis, and pharmacological properties of new 1,8-naphthyridin-2(1H)-on-3-carboxamide derivatives as highly selective cannabinoid-2 receptor agonists. J Med Chem 2009; 52: 3644-51.

98) Malfitano AM, Laezza C, D'Alessandro A, Procaccini C, Saccomanni G, Tuccinardi T, *et al.* Effects on immune cells of a new 1,8-naphthyridin-2-one derivative and its analogues as selective CB2 agonists: implications in multiple sclerosis. PLoS One 2013; 8: e62511.

99) Malfitano AM, Laezza C, Saccomanni G, Tuccinardi T, Manera C, Martinelli A, *et al.* Immune-modulation and properties of absorption and blood brain barrier permeability of 1,8-naphthyridine derivatives. J Neuroimmune Pharmacol 2013; 8: 1077-86.

100) Lucchesi V, Hurst DP, Shore DM, Bertini S, Ehrmann BM, Allarà M, *et al.* CB2-selective cannabinoid receptor ligands: synthesis, pharmacological evaluation, and molecular modeling investigation of 1,8-Naphthyridin-2(1H)-one-3-carboxamides. J Med Chem 2014; 57: 8777-91.

101) González-Naranjo P, Pérez-Macias N, Campillo NE, Pérez C, Arán VJ, Girón R, et al. Cannabinoid agonists showing BuChE inhibition as potential therapeutic agents for Alzheimer's disease. Eur J Med Chem 2014; 73: 56-72.

102) Amenta PS, Jallo JI, Tuma RF, Elliott MB. A cannabinoid type 2 receptor agonist attenuates blood-brain barrier damage and neurodegeneration in a murine model of traumatic brain injury. J Neurosci Res. 2012; 90: 2293-305.

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.

104) Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie XQ, *et al*. Beta-caryophyllene is a dietary cannabinoid. Proc Natl Acad Sci U S A 2008; 105: 9099-104.

105) Cheng Y, Dong Z, Liu S. β-Caryophyllene ameliorates the Alzheimer-like phenotype in APP/PS1 Mice through CB2 receptor activation and the PPARγ pathway. Pharmacology 2014; 94: 1-12.

106) Bahi A, Al Mansouri S, Al Memari E, Al Ameri M, Nurulain SM, Ojha S. β-Caryophyllene, a CB2 receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice. Physiol Behav 2014; 135: 119-24.

107) Guo K, Mou X, Huang J, Xiong N, Li H. Trans-caryophyllene suppresses hypoxia-induced neuroinflammatory responses by inhibiting NF-κB activation in microglia. J Mol Neurosci 2014; 54: 41-8.

108) Anavi-Goffer S, Gertsch J. Treatment of schizophrenia using beta-caryophyllene and CB2 receptor agonists. WO 2013140342 A1 (**2013**).

109) Chicca A, Caprioglio D, Minassi A, Petrucci V, Appendino G, Taglialatela-Scafati O, Gertsch J. Functionalization of β-caryophyllene generates novel polypharmacology in the endocannabinoid system. *ACS Chem Biol* 2014; 9:1499-507.

110) Chicca A, Gachet MS, Petrucci V, Schuehly W, Charles RP, Gertsch J. 4'-O-methylhonokiol increases levels of 2-arachidonoyl glycerol in mouse brain via selective inhibition of its COX-2-mediated oxygenation. J Neuroinflammation 2015: 13; 12:89.

111) Schuehly W, Paredes JM, Kleyer J, Huefner A, Anavi-Goffer S, Raduner S, *et al.* Mechanisms of osteoclastogenesis inhibition by a novel class of biphenyl-type cannabinoid CB (2) receptor inverse agonists. Chem Biol 2011; 18: 1053–64.

112) Lee YJ, Choi DY, Choi IS, Kim KH, Kim YH, Kim HM, *et al.* Inhibitory effect of 4-O-methylhonokiol on lipopolysaccharide-induced neuroinflammation, amyloidogenesis and memory impairment via inhibition of nuclear factor-kappaB in vitro and in vivo models. J Neuroinflammation 2012; 9:35.

113) Jung YY, Lee YJ, Choi DY, Hong JT. Amelioration of cognitive dysfunction in APP/ps1 double transgenic mice by long-term treatment of 4-O-Methylhonokiol. Biomol Ther (Seoul) 2014; 22: 232–8.

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**).

116) Kim KH, Kim KS, Kim YH, Kim JG, ; Kim KT, Han CS, Lee SI. Composition containing 4-O'-methylhonokiol for treating or preventing amyloid- related diseases EP 2327402 B1 (**2011**).

117) Raduner S, Majewska A, Chen JZ, Xie XQ, Hamon J, Faller B, *et al.* Alkylamides from Echinacea are a new class of cannabinomimetics. Cannabinoid type 2 receptor-dependent and -independent immunomodulatory effects. J Biol Chem 2006; 281: 14192-206.

118) Chicca A, Raduner S, Pellati F, Strompen T, Altmann KH, Schoop R, Gertsch J. Synergistic immunomopharmacological effects of N-alkylamides in Echinacea purpurea herbal extracts. Int Immunopharmacol 2009; 9: 850-8.

119) Anelli A, Nelli A, Giori A, Morazzoni P, Di Pierro F. Echinacea angustifolia extracts. CA 2494088 C (**2004**).

120) Lehmann R, Bone K, Penman K, Matthias A. An echinacea formulation. WO 2006002493 A1 (**2006**).

121) Three Arm Trial of Immune Effects of Echinacea, https://clinicaltrials.gov/ct2/show/NCT01129128.

122) Immunologic Effects of Echinacea, https://clinicaltrials.gov/ct2/show/NCT00860795.

123) Echinacea Purpurea and Osteopathy in Children With Recurrent Otitis Media, https://clinicaltrials.gov/ct2/show/NCT00689468

124) A Trial of Echinacea in Children https://clinicaltrials.gov/ct2/show/NCT00029211

125) Gelfand EV, Cannon CP. Rimonabant: a selective blocker of the cannabinoid CB1 receptors for the management of obesity, smoking cessation and cardiometabolic risk factors. Expert Opin Investig Drugs 2006; 15: 307-15.

126) Christensen R, Kristensen PK, Bartels EM, Bliddal H, Astrup A. Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. Lancet 2007; 370:1706–13.

127) Sink KS, Vemuri VK, Wood J, Makriyannis A, Salamone JD. Oral bioavailability of the novel cannabinoid CB1 antagonist AM6527: effects on food-reinforced behavior and comparisons with AM4113. Pharmacol Biochem Behav 2009; 91: 303–6.

128) Janero DR, Lindsley L, Vemuri VK, Makriyannis A. Cannabinoid 1 G protein-coupled receptor (periphero-)neutral antagonists: emerging therapeutics for treating obesity-driven metabolic disease and reducing cardiovascular risk. Expert Opin Drug Discov 2011; 6: 995–1025.

129) Fernández-Fernández C, Decara J, Bermúdez-Silva FJ, Sánchez E, Morales P, Gómez-Cañas M, *et al.* Description of a bivalent cannabinoid ligand with hypophagic properties. Arch Pharm (Weinheim) 2013; 346: 171-9.

130) Bertini S, Parkkari T, Savinainen JR, Arena C, Saccomanni G, Saguto S, *et al* Synthesis, biological activity and molecular modeling of new biphenylic carboxamides as potent and selective CB2 receptor ligands. Eur J Med Chem 2015; 90: 526-36.

131) Lucchesi V, Parkkari T, Savinainen JR, Malfitano AM, Allarà M, Bertini S, *et al.*. 1,2-Dihydro-2-oxopyridine-3-carboxamides: the C-5 substituent is responsible for functionality switch at CB2 cannabinoid receptor. Eur J Med Chem 2014; 74: 524-32.

132) Han S, Thatte J, Buzard DJ, Jones RM. Therapeutic utility of cannabinoid receptor type 2 (CB2) selective agonists. J Med Chem 2013; 56: 8224-56.

133) Presley C, Abidi A, Suryawanshi S, Mustafa S, Meibohm B, Moore BM. Preclinical evaluation of SMM-189, a cannabinoid receptor 2-specific inverse agonist. Pharmacol Res Perspect 2015; 3: e00159.