Chapter 9

Bioligands Acting on the Cannabinoid Receptor CB1 for the Treatment of Withdrawal Syndrome Caused by *Cannabis sativa*

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Abstract

Every day, the questions about *Cannabis sativa* ability to cause chemical dependence are closed with the considerable increase in the demand for treatment of addicts to this plant. Most drug addicts submitted to treatment have difficulty in achieving and maintaining abstinence from *Cannabis* due to the appearance of symptoms as irritability, anxiety, desire to consume marijuana, decreased quality and quantity of sleep, and change in appetite, weight loss, and physical discomfort, besides emotional and behavioral symptoms. The neurobiological basis for the withdrawal syndrome, that is, withdrawal of *Cannabis,* was established after the discovery of the endogenous cannabinoid system, identification of CB1 and CB2 cannabinoid receptors, and demonstrations of precipitated removal with antagonists of these receptors. The chapter discusses the main studies currently conducted for the treatment of withdrawal syndrome based on bioligands that act directly on the CB1 cannabinoid receptor.

Keywords: receptor cannabinoid CB1, withdrawal syndrome, *Cannabis sativa*, drugs computer aided

1. Introduction

Cannabis sativa, commonly known as marijuana, is the illicit drug most consumed in many countries [1]. The form of *Cannabis* abuse is predominantly smoked, although it can be found

in paste form called hashish, mixed with crack, or as *skunk*, which is a polymorphic form of marijuana [2] cultivated in special appearance and 7–25 times stronger than common marijuana causing greater psychotropic effects, as well as adverse effects such as triggering of schizophrenia [3].

Studies have found moderate evidence that there is a link between *Cannabis* use and in relation to the development of dependence and substance abuse such as alcohol and tobacco among other illicit drugs [4], and after a long discussion about the relevance of recent *Cannabis* withdrawal syndrome, this condition was added to the fifth version of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) [5]. This syndrome appears within 24 h after cessation of *Cannabis* use, reaches a peak in about days 2–6, and can last from 1 or 2 weeks. It affects 55–89% of regular *Cannabis* users. The *Cannabis* withdrawal syndrome is clinically defined by irritability, anger, nervousness, anxiety, sleep difficulties, decreased appetite or weight loss, restlessness, and mood depression, in addition to various physical symptoms such as abdominal pain, tremor, or sweating [6–8].

The evidence of *Cannabis* withdrawal syndrome is based on behavioral observations in animal studies [9], clinical observation of patients [10], or epidemiological surveys [11, 12]. However, the biological correlates of this phenomenon remain obscure, challenging the validity of the syndrome. This lack of knowledge is partly explained by the interindividual variability of delta 9-tetrahydrocannabinol (THC) metabolism [13] and the complexity of plasma-tissue exchanges [14].

In the last decades, many studies have been dedicated to discover and understand the diverse effects of cannabinoids on the organism whether therapeutic (with the relief of chronic pains and muscle spasms related to multiple sclerosis) [15, 16] or derived from the psychoactivity of *C. sativa*, originating the dependence and consequently the withdrawal syndrome [17]. These symptoms include physical discomforts such as headaches and stomach psychological symptoms accompanied by irritability, anxiety, sleep disturbances, decreased appetite/weight loss, restlessness, or depressed mood [18]. The chapter discusses the main studies currently conducted for the treatment of *Cannabis* withdrawal syndrome, that is, molecules which have their activity associated with some kind of interaction by structural complementarity beside the CB1 cannabinoid receptor.

2. Physiology

2.1. Cannabinoid receptor type 1 (CB1)

After the use of *Cannabis*, THC interacts with the CB1 cannabinoid receptor, inducing conformational changes in this receptor, the interaction with the residue of amino acid TRP356 and its surroundings being the activation trigger for the signaling [19]. Also, the binding site of the CB1 receptor comprises the amino acid residues Phenylalanine 174 (PHE174), Leucine 193 (LEU193), and Serine 383 (SER383) (**Figure 1**) that must be in contact or proximity to the preferred THC docking position [20].

The morphological differences between CB1 and CB2 cannabinoid receptors indicate that most cannabinoid compounds interact differently in both receptors [21], and the location of

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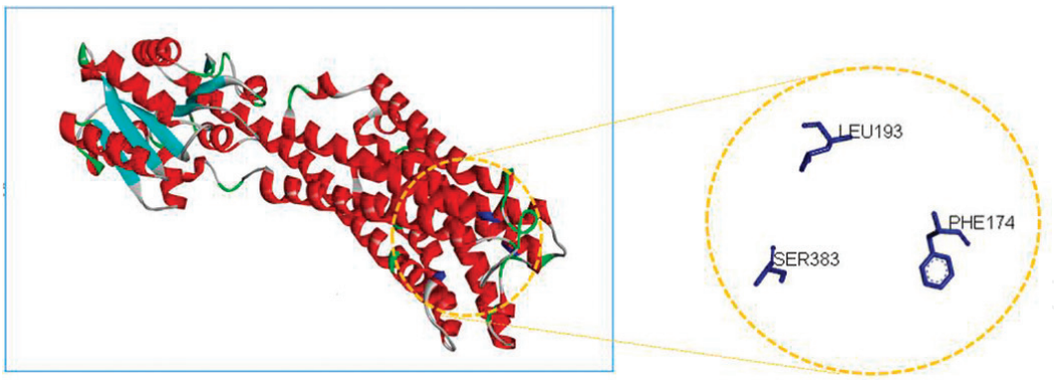

Figure 1. Amino acid residues present at the cannabinoid receptor binding site CB1.

CB1 receptors in the central nervous system is directly associated with the behavioral effects produced by cannabinoids [22, 23]. CB1 gene polymorphisms have been observed and their importance is still unknown, but it is suggested that they are linked to increased susceptibility to *C. sativa* dependence and neuropsychiatric disorders [24].

CB1 cannabinoid receptors are present in areas associated with motor control, emotional response, learning, memory, goal-oriented behaviors, energy homeostasis, and higher cognitive functions, among others [25]. In peripheral organs and tissues, CB1 receptors are expressed in low density and have potential implication to regulate inflammation and autoimmune diseases [26]. Unlike the standard of others neuroreceptor systems, levels of CB1 receptors in rats are increased in the transition from adolescence to adult age, a fact that suggests the propensity to search for cannabinoid compounds at this stage of life [27].

The CB1 receptor is a subfamily member of the G protein-coupled receptors (GPCRs) [28] and is predominantly present in the presynaptic terminal, although small amounts are present in peripheral nerves and its function seems to modulate the release of neurotransmitters such as dopamine, noradrenaline, glutamate, and serotonin in the synaptic cleft [29].

The inhibition of adenylate cyclase by psychoactive cannabinoids in more densely populated regions of CB1 receptors was initially identified in N18TG2 neuroblastoma cells and thereafter in many other preparations [30]. This inhibition causes modulation of intracellular cAMP concentration, thereby regulating protein kinase A (PKA) phosphorylation, fact that may result in large changes on cellular activity, such as regulation of K^+ channels undergoing PKA action in hippocampus [31].

Mitogen-activated protein kinases (MAP kinases) are important signal transduction enzymes involved in cell regulation to physiological functions of gene expression control, proliferation, and programmed cell death (apoptosis) [32]. Studies confirm a positive connection of CB1 receptors with MAP kinase, so that, *in vivo*, acute administration of Δ9-THC and CB1 cannabinoid receptor agonists (CP-55940, WIN 55,212-2, anandamide (AEA), and 2-O-arachidonoylglycerol (2-AG)) stimulates the MAP kinase of guinea pigs. Synaptic plasticity is considered as the capacity of rearrangement by the neural networks, constitutes an important mechanism to recover or adapt in case of injury, and provides the basis for most models of learning, memory, and development in neural circuits [33]. Brain-derived

neurotrophic factor (BDNF) and Krox-24 gene have been recognized for their importance in synaptic plasticity and are prevented by the activation of MAP kinase [34], including studies that indicate that cannabinoid receptors alter this physiological process and may favor the induction of long-term depolarization (LTD) [35].

The voltage-dependent ion channels, mainly K^+ and Ca^{2+}, are modulated by CB1 receptors, suggesting that the release of gamma-aminobutyric acid (GABA), a neurotransmitter responsible for CNS inhibition, is mediated by the opening of these channels [36], thus influencing cognitive processes such as learning and memory [37].

Cerebral cortex neurons expressing the G-protein coupled receptors, called CCK receptors, are responsible for the release of the neuropeptide cholecystokinin (CCK) [38], whose action on the hypothalamus produces the sensation of satiety, and also express the CB1 receptors [39]. Activation of CB1 receptors also activates CCK receptors, thus inhibiting the release of CCK [40] and negatively influencing satiety [41].

Rich areas in CB1 receptors reveal a high expression of N-methyl D-aspartate (NMDA) receptors, a class of receptors involved in glutamate neurotransmission and therefore important in movement control and memory formation [42]. Cannabinoid substances have shown dual effects on NMDA receptor activity, influencing memory acquisition and learning mechanisms [43].

2.2. Cannabinoid receptor type 2 (CB2)

The main and most well-known location of CB2 receptors on human beings is in nonneuronal tissues, mainly in the immune system and hematopoietic cells. The exclusively peripheral location of the CB2 receptors was already questioned when, in 2006, their existence was confirmed in the nervous system, principally in neuronal, glial, and endothelial cells in the brain, although in lower proportions than the CB1 receptors [44]. As CB2 receptors has an important role in neuroinflammatory responses, neurodegenerative diseases such as multiple sclerosis, amyotrophic lateral sclerosis, and Alzheimer's disease become the subject of pharmaceutical studies in this regard [45], where the concentration of these receptors seems to be increased in specific brain regions related to these pathologies [46].

As with CB1 receptors, CB2 receptors are also coupled to G protein although their action seems to be part of a general protection system since its activation has no association with psychoactive effects. Agonist molecules of these receptors are being tested in neuropsychiatric, cardiovascular, and hepatic pathologies [47].

3. Chemical dependence and withdrawal syndrome

THC is a partial agonist of CB1 and CB2 receptors, although it is the interaction with CB1 receptors that is responsible for the psychoactive effects of *C. sativa* [40, 41]. CB1 receptors are found at high densities in the ventral tegmental area, nucleus accumbens, prefrontal cortex, hippocampus, amygdala, and cerebellum, whereas CB2 receptors are primarily located in immune cells [24, 41].

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THC, as well as other CB1 receptor agonists, has inhibitory effects on the release of GABA and glutamate. Excitatory effects on dopamine (DA) are also evident, leading to an increase in the level of extracellular DA [42].

The chemical dependence on *C. sativa* develops in about 10% of the people who experience the plant, being more common and on higher levels of use at an early age [48]. Withdrawal syndrome was recognized and added to DSM-5 in 2014, mainly due to the increase with the number of treatment episodes to chronic users of *Cannabis* in the last years [49]. These treatments involve psychosocial approaches, but only 20% of patients achieve definitive abstinence [50], manifesting a clear need to develop effective treatments for this pathology.

Studies using positron emission tomography revealed a significantly lower availability of CB1 receptors between *Cannabis* smokers and nonsmokers, in which the level of downregulation correlated with the time of *Cannabis* use [46]. Interestingly, after a 30-day abstinence period, there was an increase in CB1 receptor availability to levels comparable to healthy controls [6, 51].

For the diagnosis of *C. sativa* withdrawal, it is necessary to have criteria such as (1) the development in specific syndrome of the substance due to cessation or reduction in use; (2) the syndrome causes clinically significant distress or impairment in social, occupational, or other important areas of functioning; and (3) the symptoms are not due to a general medical condition and are not better accounted for by another mental disorder [52].

Following the recognition by the ICD-10 (International Statistical Classification of Diseases and Related Health Problems) system of *Cannabis* withdrawal, the demand for treatment of *Cannabis* abuse has grown in several countries and a large proportion of adults and adolescents who participate in the outpatient treatments have difficulty in achieving and maintaining *Cannabis* withdrawal [53].

3.1. Symptoms of withdrawal syndrome

Experimental studies on *Cannabis* withdrawal in humans began in the 1970s and showed moderate withdrawal symptoms (such as transient nervousness) following cessation of marijuana use and more robust symptoms (restlessness, sleep problems, poor appetite, and disorientation). In the 1980s, new withdrawal symptoms were reported as decreased appetite/weight loss, hostility, irritability, mild nausea, lack of cooperation, restlessness, sleep EEG changes (increased REM sleep), and sleep/insomnia difficulties. These symptoms started within 5–6 h of the last dose and decreased by 96 h with a reduction in weight and sleep. Changes in EEG (i.e., increase in REM) are also observed [54, 55].

More recent studies have demonstrated *Cannabis* withdrawal syndrome associated with significantly increased outcomes of anxiety, depression, and irritability; decrease in sleep quality and quantity indices; and decreased food intake [56]. Symptoms such as stomach pain and decreased assessments of contentment, friendliness, language, sociability, and energy were also reported. Most of the mood symptoms begin within 48 h after cessation and appear to peak at day 3 or 4 of the withdrawal phases, and it is interesting to note that studies of oral THC use have not reported sleep disturbances during the withdrawal phases [57].

The symptoms reached the highest levels of aggression on days 3 and 7 of abstinence and lasted until day 28, being reported for up to 6 months after cessation of use, showing an effect transient. Among chronic and daily users, the appetite decreased after day 9 of abstinence, anxiety occurred between days 1 and 11, irritability was greatest on days 1–14, and the mood was lower on days 3–9 and was higher on days 1–10. Daily users have higher levels of anxiety, irritability, nervousness, restlessness, tremors, difficulty sleeping, stomach pain, strange dreams, excessive sweating, negative mood, physical symptoms, and decreased appetite during the abstinence period, suggesting reliable studies of *Cannabis* abstinence [58, 59].

4. Treatment of withdrawal syndrome

Although there are more than 160 million *Cannabis* users in the world, no pharmacological therapy currently available is considered adequate for the treatment of symptoms caused during the withdrawal syndrome. The known effects of withdrawal syndrome, which occur when drug use is deprived and disappear with the reintroduction of Δ9-THC [60], favor the recurrence of use by users attempting to stop. The main compounds that have activity on the cannabinoid receptor and mechanisms related to *Cannabis* withdrawal syndrome are as follows.

4.1. Agonist compounds

The involvement of the CB1 receptor with the development of dependence, as well as the expression of withdrawal symptoms, has already been evidenced in several animal experiments. Therefore, it is suggested that treatment with low doses of CB1 receptor agonists could reduce the severity of withdrawal symptoms [61]. Low doses of Δ9-THC were tested to improve withdrawal symptoms; however, these doses exhibited reinforcing properties in chronic *Cannabis* users, eliminating THC as a viable treatment [62].

The endocannabinoids AEA and 2-AG, which are low and high efficiency agonists for the CB1 receptor, respectively, as well as fatty acid amide hydrolase (FAAH) enzymes responsible for the degradation of AEA and monoacylglycerol lipase (MAGL) responsible for the degradation of 2-AG were proposed as mediating mechanisms of *Cannabis* withdrawal but lack further enlightening studies [6, 63].

4.1.1. Synthetic cannabinoids

These synthetic cannabinoid agonists present themselves as promising molecules, providing ample reduction in *Cannabis* withdrawal symptoms (mood, sleep, and food intake), both in the laboratory and in clinical settings. Unlike the isomer of THC and derived from the *Cannabis* plant, dronabinol, the synthetic cannabinoid nabilone (**Figure 2**) has potential to reduce self-administration of *Cannabis,* presenting as more promising for treatment [64].

Nabilone has more predictable side effects, and it is well tolerated among *Cannabis* users, better bioavailability, and longer duration of action than dronabinol, allowing the end of abstinence with a single daily dose [65]. In addition, nabilone produces non-*Cannabis* urinary

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biomarkers that allow monitoring of abstinence through the use of standard urine toxicology during nabilone maintenance, but this consistently decreases *Cannabis* self-administration in the laboratory, ensuring that testing occurs in a clinical setting [66].

4.1.2. α2a adrenergic receptor agonist

Preclinical data have demonstrated that abstinence of cannabinoid is associated with adrenergic hyperactivity [67], and that α2 receptors agonists decrease the withdrawal symptoms of THC. Therefore, the α2a adrenergic receptor agonist, lofexidine (**Figure 3**), has been tested, and its use has improved sleep during the abstinence period and decreased *Cannabis* relapse [68] but is poorly tolerated even at less frequent doses and at lower target dose (0.6 mg three times a day), with 40% of patients presenting dizziness and fatigue [69]. Another α2-adrenergic agonist, guanfacine hydrochloride (**Figure 3**), which improves memory performance in humans, was tested on the hypothesis that nocturnal administration of this drug would reduce *Cannabis* withdrawal while producing little evidence of sedation or hypotension. Daily administration of the compound significantly reduced irritability, produced small but significant decreases in blood pressure and heart rate, however was well tolerated, producing no sedation, dizziness, or altered food intake observed with lofexidine. Due to these results, guanfacine hydrochloride stands out as one of the first non-cannabinoid agonists to reduce *cannabis* abstinence-related irritability [64, 70].

Despite reductions in certain withdrawal symptoms, guanfacine did not reduce self-administration of *Cannabis* and did not worsen abstinence-related anorexia and weight loss but did not

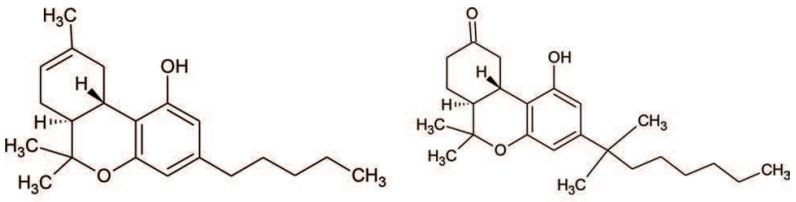

Figure 2. Chemical structure of isomer of THC, dronabinol and synthetic cannabinoid, and nabilone.

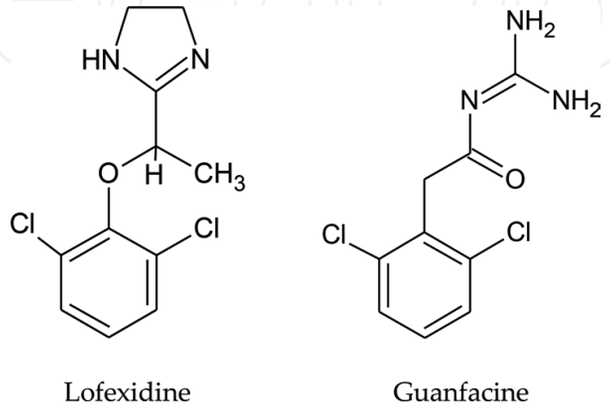

Figure 3. Chemical structure of α2a adrenergic receptor agonist.

improve both. In contrast, lofexidine decreased self-administration of *Cannabis* in the laboratory after abstinence but worsened the performance of psychomotor tasks [68].

4.1.3. Nabiximols

Nabiximols are used to treat muscle spasticity associated with multiple sclerosis. These produce little intoxication, tolerance, or abstinence. They are oral spray medications containing THC, cannabidiol (CBD), and various terpenoids (**Figure 4**) derived from *C. sativa* plants. Once CBD attenuated the paranoia and euphoria associated with THC studies, nabiximols were used to treat *Cannabis* withdrawal and observed that they attenuated abstinence symptoms and improved patient compliance to treatment, as well as reducing irritability and depression of the users [71].

The indirect CBD agonist, which has a relatively low affinity for CB1 and CB2 receptors, inhibits AEA reuptake and hydrolysis while maintaining CB1 receptor stimulation, thus potentiating endocannabinoid transmission and emerging as an alternative treatment for the abstinence syndrome of *C. sativa* [72]. It is a compound with no significant adverse effects even with chronic

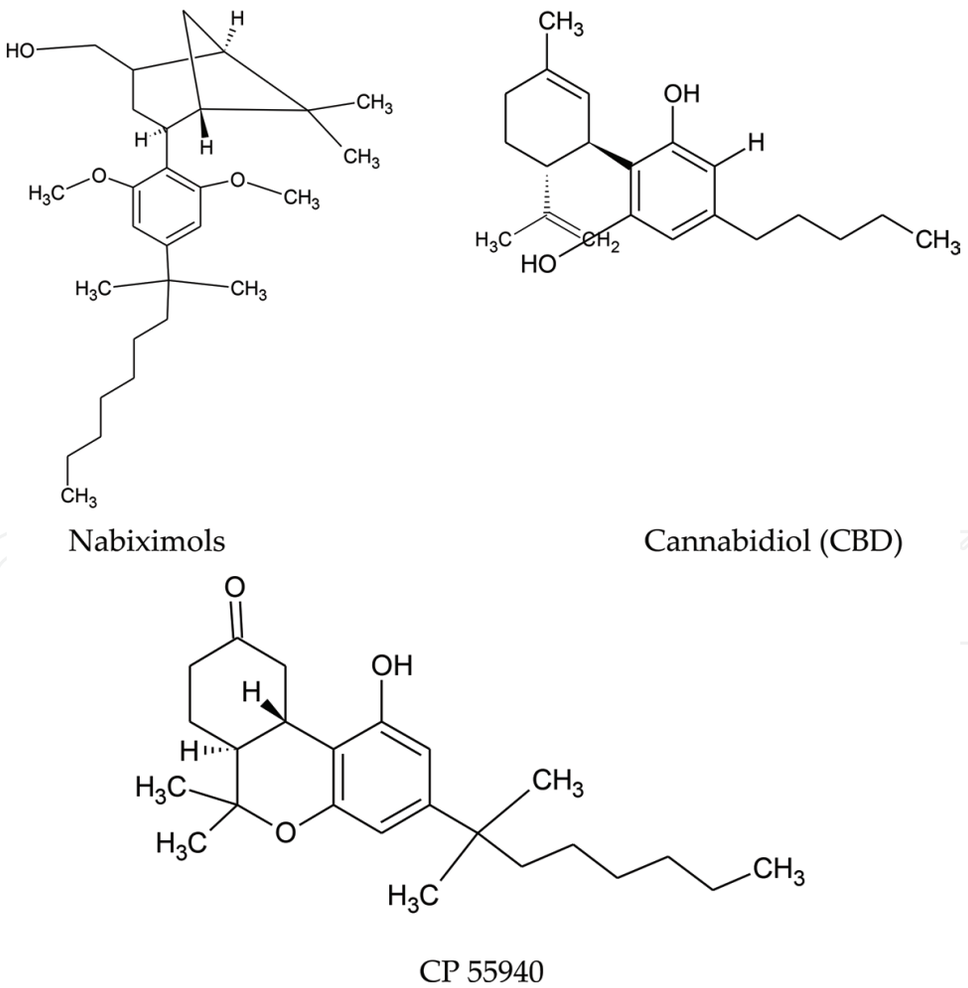

Figure 4. Chemical structure of nabiximols and terpenoids derived from *C. sativa* plants.

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and high dose use. Due to this property on the endocannabinoid system, CBD has several pharmacological effects, including anxiolytic, antipsychotic, neuroprotective, antiinflammatory, and antiemetic actions, favoring its use in the treatment of *Cannabis* withdrawal syndrome [73, 74].

Comparative studies of the use of nabiximols and dronabinol concluded that they did not produce significant cognitive or psychomotor adverse effects and showed a similar or lower reinforcement potential than dronabinol at lower doses [71, 75]. However, high doses of both drugs exhibited some potential for a booster. This fact highlights the need for careful monitoring related to drug administration during future studies and clinical practice for treatment of dependence and abstinence from *Cannabis* with nabiximols.

4.2. Antagonists

The use of CB1 cannabinoid receptor antagonists is more related to the treatment of *C. sativa* dependence than to the treatment of withdrawal syndrome triggered by the withdrawal of this use in chronic users, as much as characteristic symptoms of withdrawal syndrome such as insomnia, dysphoria, and anxiety manifesting with the use of the CB1 receptor antagonist, rimonabant (also known as SR 141716A) (**Figure 5**) [8]. For this reason, the rimonabant, previously used in the treatment of obesity, was removed from the market in 2008, but it is useful in inducing signs of withdrawal in *Cannabis*-dependent individuals. One of the explanations is that the neural circuits involved with the serotonergic, noradrenergic, and dopaminergic systems have been shown to be sensitive to CB1 receptor antagonists [76, 77].

It is important to mention that the endogenous opioid system also contributes to the dependence of *Cannabis* because it also has G protein-coupled membrane receptors [78], and users of opioid-dependent *Cannabis* are less likely to experience withdrawal symptoms. Opioid receptor antagonists, such as naltrexone, reduce self-administration of *C. sativa* and their subjective positive effects in chronic plant users [79].

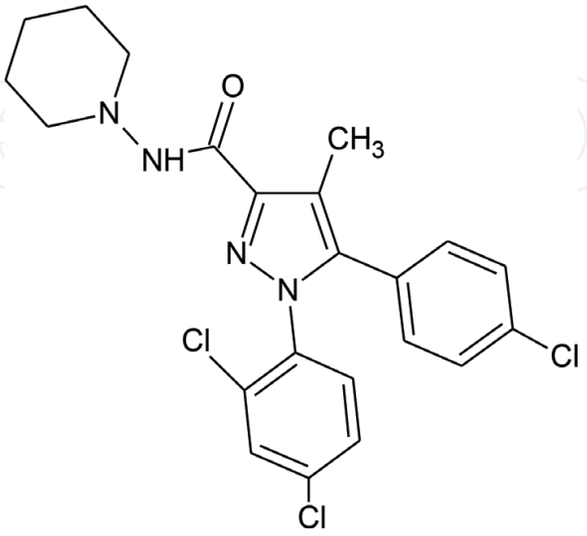

Figure 5. Chemical structure of SR 141716A.

5. New studies on the treatment of withdrawal syndrome

There are no drugs approved for the treatment of addiction or withdrawal syndrome of *Cannabis*. Pharmacotherapy in these cases is focused exclusively on symptoms such as increased anxiety, insomnia, loss of appetite, migraine, and irritability. We disclose these symptoms being a result of desensitization of CB1 receptors by THC studies advancing toward the development of compounds that act selectively at this receptor. There are four main chemical classes of exogenous cannabinoid ligands under study: (a) classical cannabinoids such as Δ9-THC, AM2389, cannabinol, nabilone, HU-210, and other tricyclic terpenoid derivatives, such as Δ9-tetrahydrocannabivarin (Δ9-THCV) (**Figure 6**), which contains a polar benzopyran moiety attached to a hydrophilic (*n*-pentyl) alkyl terminus [80]; (b) the nonclassical cannabinoids CP 55,940, HU-308 (**Figure 7**) and other bicyclic and tricyclic analogs of Δ9-THC without the pyran ring of classical cannabinoids [81]; (c) the aminoalkylindoles WIN55,212-2, JWH-018, JWH-073, and AM1241 (**Figure 8**), which differ in structure, lipophilicity, and binding activity at cannabinoid receptors compared to nonclassical cannabinoids [82]; and (d) biarylpyrazole ligands such as rimonabant and AM251 antagonists, which are selective for the CB1 receptor, and SR144528 (**Figure 9**), which is selective for the CB2 receptor [83].

5.1. *In vivo* and *in vitro*

It is known that because cannabinoid receptors, when bound by agonists or antagonists, have the potential to treat a variety of pathologies such as pain, neurodegeneration, obesity, tumors,

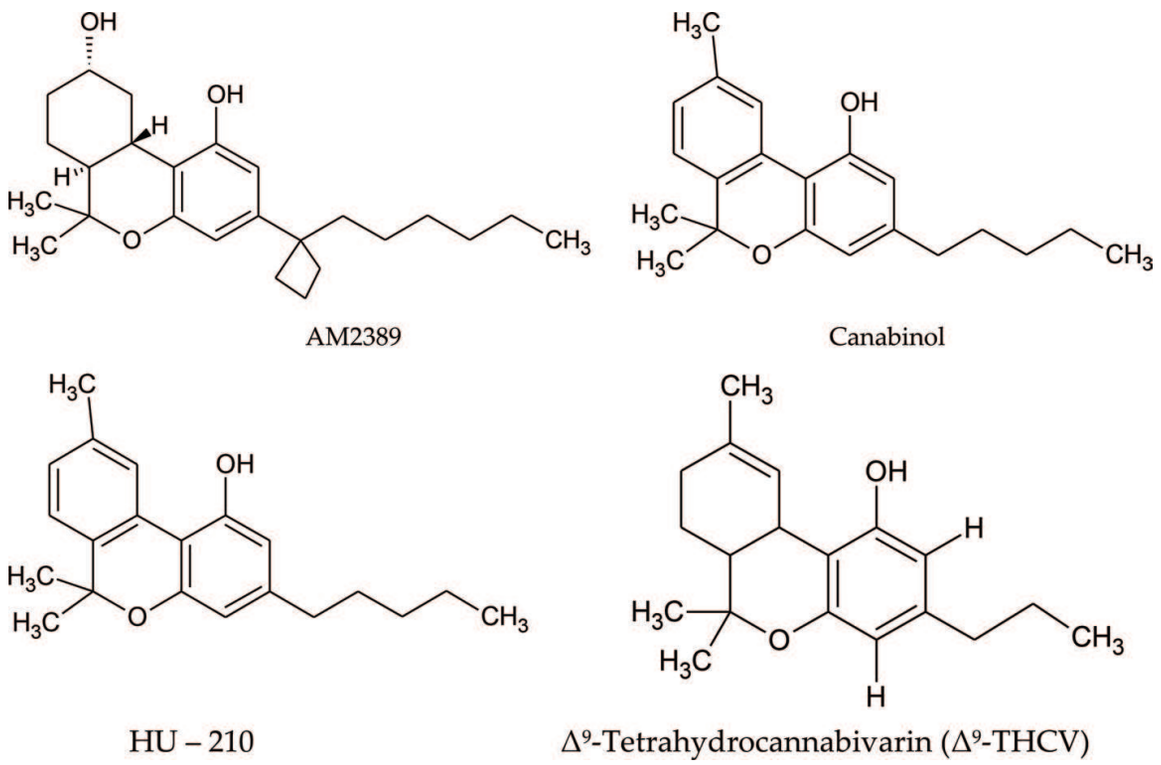

Figure 6. Chemical structure of classical cannabinoids.

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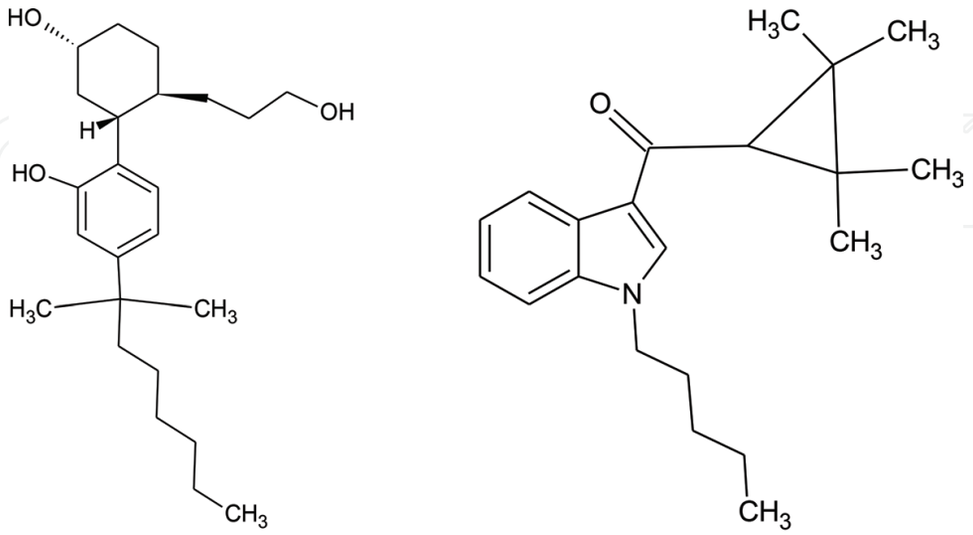
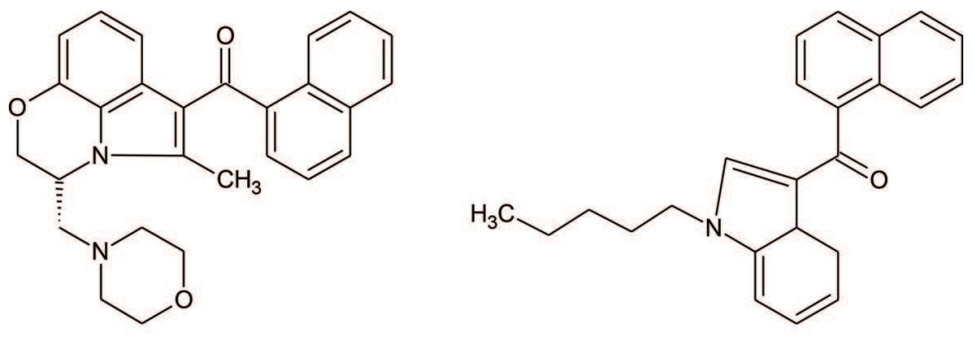
chemical dependency, and immune function, it is important to develop in vitro bioassays activity determination and the function of these receptors [84]. The *in vitro* assays established in the studies related to CB1 and CB2 receptors involve the use of membranes or tissues containing

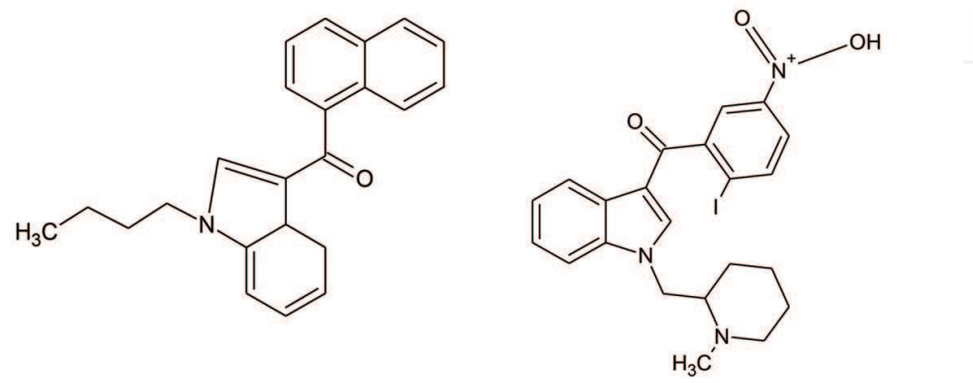


CP55,940 HU 308

Figure 7. Chemical structure of the non-classical cannabinoids.



WIN55,212-2 JWH-018



JWH-073 AM1241

Figure 8. Chemical structure of the aminoalkylindoles.

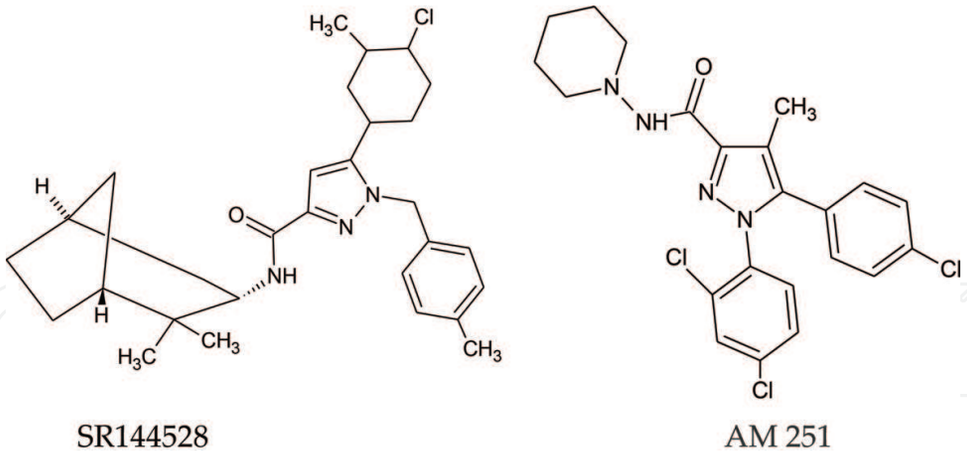


Figure 9. Chemical structure of the biarylpyrazole ligands such as rimonabant and AM251 antagonists.

these receptors [85]. Of particular note is the assay using radiolabeled CB1 or CB2 receptors with [^{3}H] CP55940 (**Figure 10**) and bioassays with preparations of nerve-smooth muscle where the ability of the molecule under study to produce inhibition or excitation of cannabinoid receptors is verified [86].

In vitro functional bioassays measure the effects of synthetic cannabinoids and their metabolites in relation to cannabinoid receptor signaling CB1/CB2, evaluating the production of cyclic ATP and elevation of intracellular calcium. In the middle of the last century, initial studies on the effects of cannabinoids used Gayer's tests (found at the time as a useful test for the effects of THC [87]), where corneal areflexia was measured in rabbits, catatonia in mice, and increased defecation and aggressiveness in rats stressed by REM sleep deprivation [88]. In mice, high-dose catalepsy with Δ9-THC was also observed [95]. In rodents, the main bioassay is the measurement of locomotor activity, rectal temperature, and analgesia (in the tail or hot plate test) [89].

The sum of the various symptoms observed in the initial studies originated characteristic effects in laboratory animals called cannabinoid tetrad and being characterized by hypothermia, analgesia, catalepsy, and locomotor suppression [90]. This tetrad is widely used nowadays because, since the data obtained through its observation are qualitatively consistent, it is common to evaluate the dose-dependence relation of cannabinoids quickly and without any specific training of the animals, a fact that is configured as an advantage [89].

The Δ9-THC dependency/withdrawal modeling studies are based on the cannabinoid tetrad in which The triggered effects are verified with the administration of cannabinoid antagonist (usually rimonabant), and precipitation withdrawal symptoms, being, in general, the synthetic cannabinoids such as UR-144 (**Figure 11**), responsible to promote effects greater than that of Δ9-THC [91].

Studies in rats revealed that individual enzyme activity mainly related to the genetic polymorphisms of cytochrome P450 enzymes in the phase I metabolism of cannabinoids has an important role in determining the response of an individual on the use of cannabinoids [92]. Thus, an individual may experience attenuated effects and other individual effects exacerbated by

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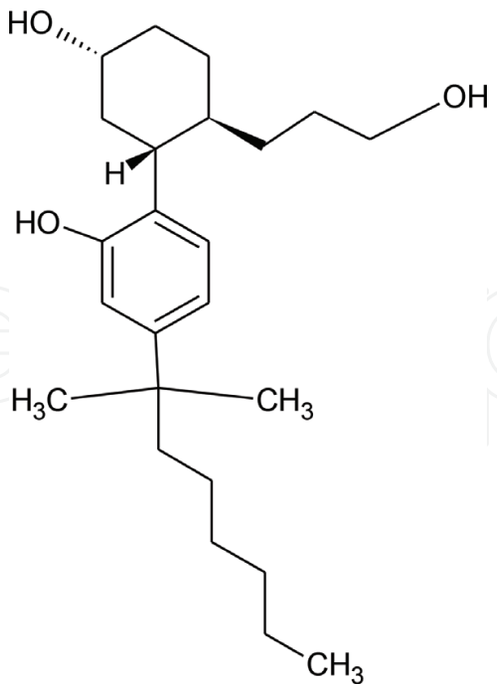

Figure 10. Chemical structure of the CP55940.

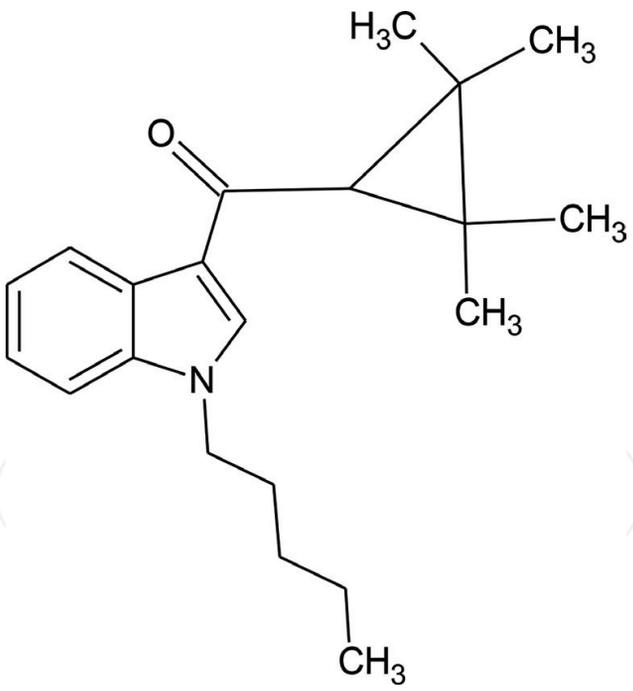

Figure 11. Chemical structure UR-144.

cannabinoids, depending on the liver enzyme profile that favors the formation of antagonistic or agonist metabolites, respectively [93].

Technological refinement has led to the use of new techniques and different experimental models [94] in the studies of compounds in potential for reinforcement, with the search for

new targets and biomarkers [95]. Among the experimental models emerges the *Danio rerio* (*Zebrafish*), a small fish, because it has the facility of genetic manipulation and the biology of its development [96]. *Zebrafish* is particularly useful for measuring changes in the development of the nervous system [97], and its measures of sensorimotor plasticity, emotional function, cognition, and social interaction have been used to characterize the adverse effects of drug abuse such as Δ9-THC [98, 99] due to phylogenetic analyzes, which reveal the endocannabinoid system as highly conserved between *Zebrafish* and mammals [100].

Tolerance and cross-tolerance tests for cannabinoids are also performed *in vivo*, although studies indicate that not all effects of cannabinoids are developed during these tests, for example, adrenocorticotropic hormone (ACTH) secretion is not observed in rodents during these tests, indicating low reliability and the need for greater improvement *in vivo* methods used in this sense [101, 102].

5.2. *In silico*

There are several computational methods; among them, homology modeling is being used in cannabinoid studies [103], considering that the drugs utilized during the withdrawal syndrome of *C. sativa* act at a symptomatic level. The resolution of the crystalline structure of the CB1cannabinoid receptor is recent [19], and this fact favored *in silico* studies that evolve toward the planning of molecules that act as selective agonists of this receptor, mainly studies related to better understanding of the interaction and the relation structure-activity of

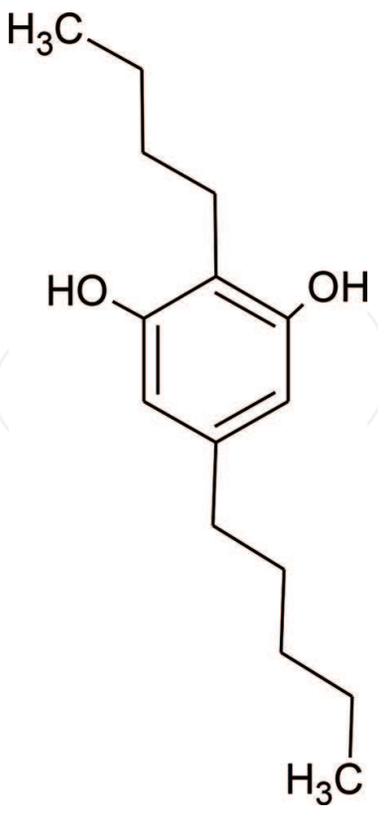

Figure 12. Chemical structure of Stemphol.

synthetic cannabinoids [104]. A study can be mentioned where computational tools were used, with the objective of proposing drug candidates for the treatment of the abstinence syndrome based on the natural ligands of this receptor. A particular compound derived from marine fungi, stemphol (**Figure 12**) [105], presented positive predictions regarding pharmacokinetic and toxicological properties for a human CB1 receptor ligand, in addition to having a relatively simple molecular structure. Due to these computational results and the recent crystallographic elucidation of the cannabinoid CB1 receptor [20], experimental studies are being conducted for the development of candidate pharmacotherapeutic alternatives for the treatment of *C. sativa* withdrawal syndrome [106].

6. Conclusion

Studies on cannabinoids were stimulated after the characterization and structural elucidation of Δ9-THC in the 1960s, and later on, the discovery of the cannabinoid system represented by CB1/CB2 receptors and binding substances to these receptors. Many *in vitro*, *in vivo,* and *in silico* trials have been developed in the last decades, and advances mainly regarding the mechanism of addiction, abuse, and withdrawal syndrome have been achieved. However, with the use of cannabinoid-based drugs and the chemical development of synthetic cannabinoids, further studies into these mechanisms are relevant, especially considering that Δ9-THC is a low-efficacy cannabinoid compared to the "new cannabinoids."

It is expected in the future that the investigations will deepen the knowledge on the mechanisms of the cannabinoids, especially those that cause chemical dependence, both as cannabinoid system and as noncanabinoid physiological systems. In this way, it is possible to increase the knowledge about the different classes of these substances and, therefore, favor the development of new models and improvement of the tests currently used in the studies related to *C. sativa*.

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