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Review

Phytocannabinoid Pharmacology: Medicinal Properties of *Cannabis sativa* Constituents Aside from the "Big Two"

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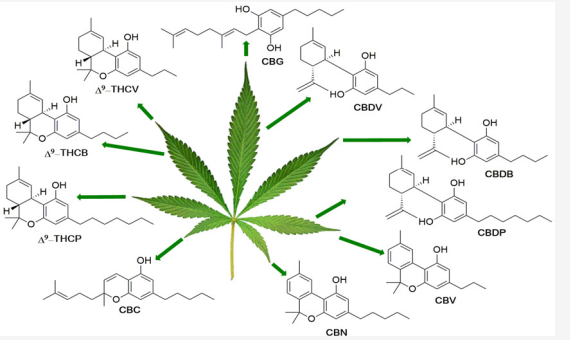
ABSTRACT: Plant-based therapies date back centuries. *Cannabis sativa* is one such plant that was used medicinally up until the early part of the 20th century. Although rich in diverse and interesting phytochemicals, cannabis was largely ignored by the modern scientific community due to its designation as a schedule 1 narcotic and restrictions on access for research purposes. There was renewed interest in the early 1990s when the endocannabinoid system (ECS) was discovered, a complex network of signaling pathways responsible for physiological homeostasis. Two key components of the ECS, cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2), were identified as the molecular targets of the phytocannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC). Restrictions on access to cannabis have eased worldwide, leading to a resurgence in interest in the therapeutic potential of cannabis. Much of the focus has been on the two major constituents, Δ^9-THC and cannabidiol (CBD). Cannabis contains over 140 phytocannabinoids, although only a handful have been tested for pharmacological activity. Many of these minor cannabinoids potently modulate receptors, ionotropic channels, and enzymes associated with the ECS and show therapeutic potential individually or synergistically with other phytocannabinoids. The following review will focus on the pharmacological developments of the next generation of phytocannabinoid therapeutics.



INTRODUCTION

Cannabis sativa was classified globally as a Schedule 1 controlled substance for most of the 20th century. This restriction resulted in scientific research focus on the harms associated with cannabis use, despite its purported therapeutic benefit, which dates back centuries.[1,2] Aside from Δ^9-tetrahydrocannabinol (**1**, Δ^9-THC), which was approved for chemotherapy-induced nausea and vomiting under the trade name Marinol (dronabinol), little research on the therapeutic benefit of cannabis, or any of its unique chemical components, was conducted. Although illegal, people resorted to cannabis to treat symptoms of a multitude of ailments as a last resort when conventional standard-of-care therapeutics proved ineffective. Patients who chose to use cannabis for medical purposes did so in secrecy and often accessed product from illicit, uncontrolled sources. Products from the illicit market have been reported to be inconsistent and potentially adulterated with harmful impurities such as pesticides.[3,4] Recently, illicit vaporizable cannabis products containing a synthetic *O*-acylated tocopherol derivative (vitamin E acetate) have been linked to "popcorn lung" and, in some cases, death.[5] Vitamin E acetate is a common excipient in dietary supplements and cosmetics; however, it has the potential to undergo pyrolysis to toxic byproducts such as ketene when exposed to the high temperatures produced by vaping devices.[6]

In the past 20 years, laws and restrictions for cannabis have loosened. Many countries and individual states have decriminalized cannabis and instituted programs for access to cannabis for medical purposes. These programs include increased analytical testing and quality controls to ensure cannabis-based medical products are safe for human consumption. In 2018, Canada became the first G-7 country to completely legalize cannabis for adult recreational use.[7] At the federal level in the United States, cannabis (marihuana) is classified as a Schedule 1 narcotic under the Controlled Substances Act (CSA). However, in 2018 the Agriculture Improvement Act of 2018 (Farm Bill) was signed into law, allowing for cannabis varieties described as "hemp" to be removed from the CSA. Hemp is defined in the Farm Bill as the cannabis plant, or any part thereof, including its extracts and cannabinoids, having a THC concentration of not more than 0.3% on a dry weight basis.[8] The Farm Bill has allowed for commercialization of hemp products, including hemp-derived extracts and cannabinoids,

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resulting in hemp varieties becoming a significant source of cannabidiol (**2**, CBD).[8] The global cannabis market is expected to reach $73 billion in the next 7 years.[9]

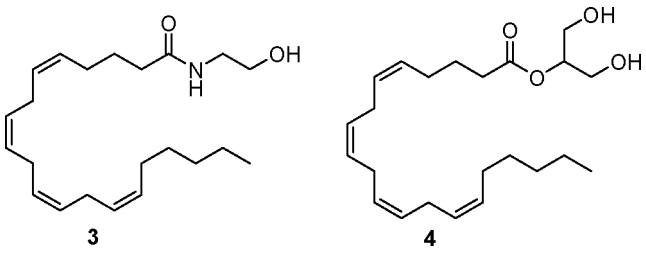
The recent changes in regulations have sparked a renewed interested in cannabis for medical purposes. Numerous clinical trials are registered or underway to examine the therapeutic potential of cannabis. Physicians are now constantly fielding questions and requests from patients who are either currently using cannabis medically or want to try cannabis for a particular disease.[10] Unfortunately, much of the scientific data to date has been underwhelming, inconsistent, and based more on anecdotal reports than randomized controlled trials.[11,12] Many products on the market have unreliable quality and consistency,[13,14] leaving physicians to practice a personalized dosage approach.[15] There is a need for better quality products that are consistent batch to batch and have reliable, standardized measures for the cannabinoid content, particularly for Δ^9-THC and CBD.[16] In addition, there are many examples of cannabis products for sale in dispensaries or online that advertise medical claims of CBD that have not been approved by the FDA.[8] This has resulted in a number of CBD product manufacturers receiving warning letters.[17] Only two new cannabis-based medicines have recently undergone the rigorous testing required for regulatory drug approval, Sativex (a 1:1 mixture of Δ^9-THC and CBD) in 2005 and Epidiolex (CBD) in 2018. Both of these new drugs were developed by GW Pharma and were derived from botanical sources.[18,19]

The rise of the medical cannabis market is a unique scenario in modern medicine. The patient has been the main advocate for the product as opposed to the traditional drug development path of discovery, clinical trials, and regulatory approval.[12] The demand and use of new cannabis-based medicines has far outpaced the scientific research into efficacy and safety.[20,21] There is a significant lack of knowledge around the potential benefits, risks, dosing, and mode of action of cannabis derivatives among both patients and health care professionals. Research and education have been identified as high priorities for cannabis-based medicine. This review will focus on minor phytocannabinoids and provide a perspective on recent developments.

THE ENDOCANNABINOID SYSTEM

In large part, the research to date has focused on the two main cannabinoids present in cannabis, Δ^9-THC and CBD. Traditionally, drug development of natural products has involved the isolation of individual components to determine the mode of action of single chemicals. This rational approach has been applied to cannabis and resulted in the discovery of two receptors as the primary pharmacological targets of Δ^9-THC, cannabinoid receptor 1 (CB1)[22,23] and cannabinoid receptor 2 (CB2).[24] In turn, this led to the identification of the endogenous cannabinoid ligands anandamide (**3**)[25] and 2-arachidonoylglycerol (**4**, 2-AG).[26] The cannabinoid receptors and endogenous ligands, referred to as endocannabinoids (eCBs), are part of what is collectively known as the endocannabinoid system (ECS). The ECS is involved in numerous cellular and systemic pathways and is widely regarded as a master regulator of homeostasis in the body.[27,28]

CB1 receptors are G-protein-coupled receptors (GPCRs) primarily expressed in the central nervous system (CNS). The receptors are located on the axon terminal of GABAergic, glutamatergic, serotonergic, noradrenergic, and dopaminergic synapses. eCBs are not stored but rather produced on demand in the postsynaptic membrane as a response to physiological



stimuli. The eCBs are subsequently released from the postsynaptic neuron, functioning as retrograde messengers to activate CB1 receptors on the presynaptic terminal. This feedback mechanism results in suppression of excitatory or inhibitory neurotransmitter release into the synapse.[29,30]

CB1 is major component of the ECS and has been extensively evaluated as a drug target. Activation of CB1 has been considered a potential treatment for conditions such as neuropathic and inflammatory pain, multiple sclerosis, anxiety, and depression. Conversely, inhibition of CB1 has been considered for the treatment of symptoms associated with type II diabetes and Alzheimer's disease.[31] CB1 receptor agonists and antagonists have progressed into clinical development with mixed results. Unfortunately, as CB1 is widely expressed on a number of different cells types, compounds with very high affinity for CB1 can interfere with normal CB1 function on nontarget cells, resulting in unwanted side effects. An example is the antiobesity drug rimonabant (**5**), a potent CB1 inverse agonist, which was withdrawn due to serious psychiatric side effects.[32] High-affinity CB1 ligands that act as full agonists may induce hyperactivity that overrides regulatory checkpoints, resulting in negative side effects.[33] Some of these highly potent synthetic cannabinoids have ended up as adulterants in recreational cannabis known as "spice" or K2.[34] Potential strategies to fine-tune CB1 activity include designing ligands that are directed to the desired site of action, focusing on the pharmacodynamics of the receptor or using structure-based drug design. CB1 compounds with low blood brain barrier permeability may reduce unwanted psychoactive side effects.[31] Designing an inverse agonist can direct the receptor to operate in an opposite manner. As well, inverse agonists modulate receptor activity regardless of the level of endogenous ligands present.[33] A neutral antagonist can tone down hyperactivity resulting from high levels of endogenous ligands. A partial agonist can serve to both enhance receptor activity and reduce hyperactivity due to an excess of endogenous ligand.[33] A compound that binds to an allosteric site can serve to modulate the activity of the receptor. Allosteric modulators lack intrinsic activity, yet can adjust the cellular response without altering the natural physiological function of endogenous ligand signaling.[35] The structure of the CB1 receptor has recently been elucidated, providing insight into the binding mode of CB1 ligands.[36] Structures of agonists,[37,38] antagonists,[39] inverse agonists,[36] and negative allosteric modulators[37] bound to CB1 may serve as templates for structure-based drug design of new orthosteric or allosteric ligands that modulate CB1 activity.

CB2 receptors are GPCRs primarily expressed in immune cells, highlighting the contribution of the ECS to immunomodulation. Activation of CB2 receptors modulates release of proinflammatory cytokines and migration of leucocytes.[30,40,41] CB2 is viewed as a drug target for autoimmune and inflammatory diseases, and a number of CB2 agonists have entered clinical trials.[31] The crystal structure of an antagonist bound to CB2 has recently been elucidated, which provides

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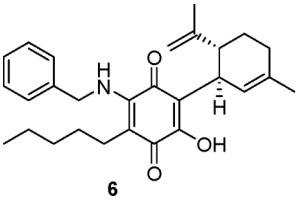
further insight into the CB2 binding pocket and facilitates structure-based design of ligands targeting the orthosteric site of CB2.[42]

Following the discovery of lipid-signaling pathways involving the two main cannabinoid receptors, the characterization of the ECS has evolved to include enzymes and receptors involved in the biosynthesis, transport, and catabolism of **3**, **4**, and additional endocannabinoid congeners. The expanded view of the ECS, also referred to as the "endocannabinoidome", underscores the complexity and promiscuity of this system and the role it plays in regulation of many physiological and pathological conditions.[31,43,44]

A class of receptors that are included in the extended endocannabinoidome are the transient receptor potential (TRP) ion channels. TRP channels are expressed in a wide array of cell types and are critical to sensory physiology.[45] There are six subfamilies of TRP channels, of which three, TRPA (ankyrin), TRPM (melastatin), and TRPV (vanilloid), are known to be activated by endocannabinoids,[46,47] synthetic cannabinoids, and phytocannabinoids.[48] These cannabinoid-susceptible TRP channels are referred to as "ionotropic cannabinoid receptors".[48] Within the three subfamilies of cannabinoid-modulating TRP channels, eight ion channels are known to be activated or inhibited by phytocannabinoids.[49,50] TRP1A is associated with hyperalgia, itching, and cold sensation and is often coexpressed with TRPV1 on sensory neurons.[51] TRP1A is a potential target for chronic pain and inflammatory diseases.[52] TRPM8 is found primarily in afferent neurons and is activated at cool temperatures (<27 °C) or by compounds such as menthol that induce a cooling sensation.[48] TRPV1 is a nonselective cation channel associated with the detection of noxious stimuli and is found in all major types of nociceptive neurons. The channel is activated by heat and chemical agents such as capsaicin that illicit a burning sensation. TPRV1 is viewed as a target for inflammatory and neuropathic pain intervention.[52] TRPV2 is a nonselective cation channel present in many cell types including immune cells and sensory neurons. TRPV2 is activated by inflammation and high temperatures and is viewed as a potential drug target for inflammation and chronic pain.[48] TRPV3 is a nonselective cation channel expressed primarily in skin keratinocytes as well as sensory neurons in the brain and ganglia. TRPV3 is activated by warm temperatures, plays a role in sensitivity to pain and itch, and is a potential therapeutic target for chronic pain and inflammatory skin conditions.[48,53] TRPV4 is a nonselective cation channel commonly found in various cell types throughout the body including epithelial, endothelial, and skin keratinocytes along with tissues such as the kidney and liver. TRPV4 plays a role in many physiological processes, is activated by warm temperatures, and is sensitive to changes in osmotic concentration.[51] TRPV5 and TRPV6 are unique vanilloid receptors that are highly selective for calcium over monovalent cations under physiological ionic conditions and are not overly sensitive to temperature.[50] Both ion channels are highly expressed in epithelial cells and are believed to regulate transcellular calcium transport.[54] TRPV5 is a potential drug target for osteoarthritis.[55] TRPV6 is classified as an oncogene and considered a target for cancer treatment.[54]

The peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors is associated with regulation of a large number of physiological functions such as cell differentiation, metabolic processes, and inflammation. These nuclear receptors, in particular PPARα and PPARγ, are members of the endocannabinoidome that are known to be activated by a variety of eCBs, phytocannabinoids, and synthetic cannabinoids.[31] Activation of PPARα and PPARγ contributes to the analgesic, neuroprotective, and anti-inflammatory properties imparted by some cannabinoids.[56,57] Synthetic cannabinoids such as VCE-004.8 (**6**) and ajulemic acid (**7**), which are dual CB2/PPARγ agonists, are currently under clinical investigation for the treatment of rare inflammatory disease systemic sclerosis.[57]



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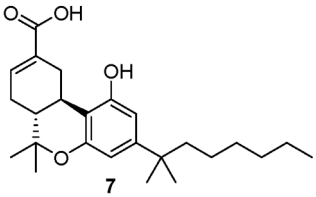

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Dysregulation of the ECS is known to disrupt physiological homeostasis[58] and has been linked to both the progression and suppression of disease symptoms associated with neurological disorders,[59] stress-related psychiatric conditions,[30] pain, and inflammation.[60,61] The system is highly complex, which makes targeting individual enzymes or receptors challenging for drug development.[31,62]

CBD and Δ^9-THC have been shown to act on multiple receptors and targets associated with the ECS.[63,64] Many patients report better efficacy when using cannabis as a whole rather than individually purified cannabinoids.[65] The promiscuous nature of phytocannabinoids appears to offer the advantage of counterbalancing the various pathways involved in endocannabinoid signaling. In addition, this advantage appears to be further enhanced when multiple cannabinoids are involved as opposed to a single chemical entity.[1,49] With respect to CBD and Δ^9-THC, a possible explanation for the synergistic benefit is the different mechanism of how they modulate the CB1 receptor. Δ^9-THC binds with high affinity to CB1 and acts as a partial agonist, whereas CBD is a low-affinity ligand. CBD has long been reported to antagonize the pharmacodynamic effects of Δ^9-THC and **4**, but this antagonism is not a result of competition for the CB1 orthosteric site.[66] CBD is known to bind to an allosteric site of CB1 at nanomolar levels. The ability of CBD to reduce the efficacy of potency of CB1 agonists is a result CBD acting as a negative allosteric modulator of CB1.[67,68] The polypharmacology of individual cannabinoids taken together results in an apparent synergism that has been termed the "entourage effect".[1,69] The entourage concept with respect to cannabinoid-based medicine is still not clearly defined, particularly in view of the complexity of the entire endocannabinoidome. As more and more CBD and Δ^9-THC dominant cannabis-based products become commercially available, it is important to examine the potential contribution of other cannabis constituents aside from the "Big Two".

Many of the >140 naturally occurring phytocannabinoids have not yet been tested for pharmacological activity. There is growing interest in a number of "minor cannabinoids" that have data to support further investigation as potential therapeutics. Some of these cannabinoids such as Δ^9-tetrahydrocannabivarin (**8**, Δ^9-THCV) and cannabidivarin (**9**, CBDV) have already entered early stage clinical trials for diabetes[70] and autism,[71] respectively. Other phytocannabinoids such as cannabinol (**10b**, CBN), cannabigerol (**11**, CBG), and cannabichromene (**12**, CBC) have compelling preclinical data to support further investigation as antimicrobial[72] and anti-inflammatory agents.[73] Newly discovered phytocannabinoids such as Δ^9-tetrahydro-

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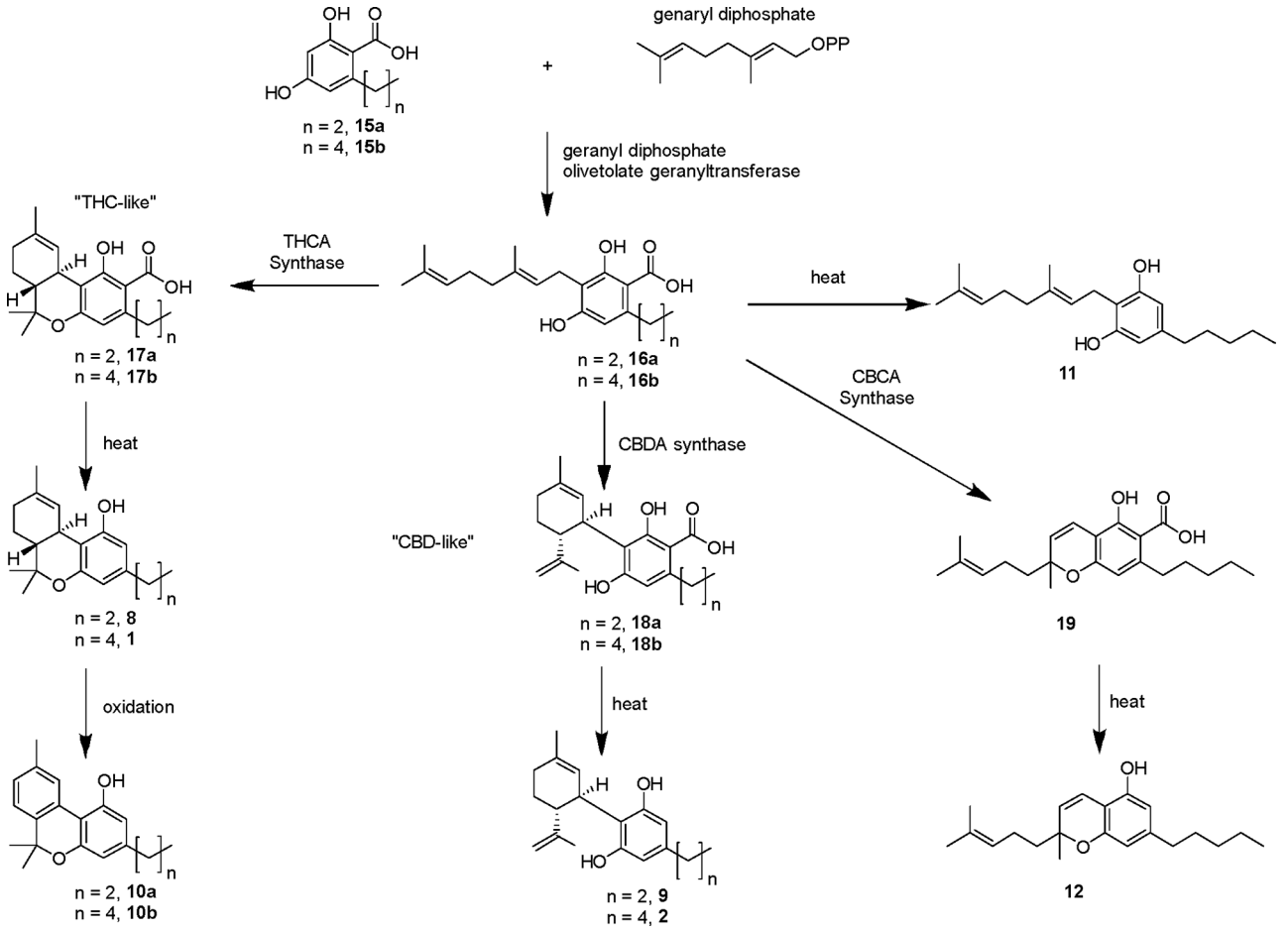
Scheme 1. Biosynthesis and Origin of Various Phytocannabinoids



cannabutol (**13**, Δ^9-THCB)[74] and Δ^9-tetrahydrocannabiphorol (**14**, Δ^9-THCP)[75] are among the highest affinity, naturally occurring, cannabinoid receptor binding agents identified to date.

PHYTOCANNABINOID ORIGINS

The biosynthetic pathways for a number of phytocannabinoids have been elucidated, and many begin from geranyl diphosphate (GPP), a common biosynthetic precursor of terpenoid and sequiterpenoids, as shown in Scheme 1. C3 and C5 phytocannabinoids are derived from GPP prenylation of divarinolic acid (**15a**) or olivetolic acid (**15b**) catalyzed by GPP olivetolate geranyltransferase to generate cannabigerovaric acid (**16a**, CBGVA) or cannabigerolic acid (**16b**, CBGA), respectively.[76,77] CBGVA and CBGA are the main precursors to many of the three main classes of cannabinoid constituents found in *C. sativa*.[78,79] The "THC-like" class of phytocannabinoids is produced through oxidative cyclization of **16a** and **16b** catalyzed by THCA synthase to the tricyclic moieties Δ^9-tetrahydrocannabivaric acid (**17a**, Δ^9-THCVA) and Δ^9-tetrahydrocannabinolic acid (**17b**, Δ^9-THCA).[80] "CBD-like" cannabinoids are produced by cyclization of the geranyl side chain of **16a** and **16b** catalyzed by CBDA synthase to cannabidivarinic acid (**18a**, CBDVA) and cannabidiolic acid (**18b**, CBDA).[76,80] "CBC-like" cannabinoids are produced by stereoselective cyclization of **16b** to the benzopyran heterocycle in the presence of cannabichromenic acid synthase (CBCA synthase) to generate cannabichromenic acid (**19**, CBCA).[81,82]

The non-acid-containing phytocannabinoids **1**, **2**, **8**, **9**, **11**, and **12**, sometimes referred to as "neutral cannabinoids" or "activated cannabinoids", are decarboxylated nonenzymatically by exposure to heat. Phytocannabinoids such as cannabivarin (**10a**, CBV) and **10b** are produced through nonenzymatic dehydrogenation of **8** and **1**, respectively.[83−85]

The biosynthetic pathway to these phytocannabinoids has recently been replicated in yeast through bioengineering of the mevalonate and hexanoyl-CoA biosynthetic pathways. The *C. sativa* genes responsible for the enzymes involved in olivetolic acid biosynthesis were encoded in *S. cerevisiae* as well as GPP:olivetolate geranyltransferase responsible for prenylation of olivetolic acid to CBGA. The cannabis genes for THCA synthase and CBDA synthase were also incorporated into the system to produce Δ^9-THC and CBD.[86]

The promiscuous nature of cannabinoid synthases made it possible to prepare minor cannabinoids Δ^9-THCV and CBDV using a similar process. Divarinolic acid was prepared by substituting butanoyl-CoA into the biosynthetic pathway engineered for hexanoyl-CoA. Both of the desired cannabinoids were detected; however, THCVA was produced in 200× greater concentrations compared to CBDVA.[86] Fermentation holds much promise to prepare such minor cannabinoids with greater efficiency compared to isolation from botanical stock. However, a significant improvement to the isolated yields of cannabinoids is required in order for the process to be viable on an industrial scale.

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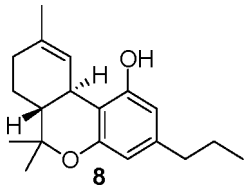
TETRAHYDROCANNABIVARIN

Tetrahydrocannabivarin (**8**, Δ^9-THCV) is an analogue of Δ^9-THC carrying an *n*-propyl (C_3) side chain. The cannabinoid was



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isolated by column chromatography from a tincture of *C. sativa* in 1970. The structure was elucidated by NMR and mass spectrometry and confirmed by chemical synthesis.[87,88]

Small structural changes can impact the way in which a molecule interacts with a receptor, resulting in significant pharmacological changes.[89] Such is the case with Δ^9-THCV and Δ^9-THC, differing only in the length of the aliphatic side chain. Early pharmacological reports show that the propyl analogue **8** was 4−8 times less active in a mouse catalepsy experiment compared to a 5 mg/kg dose of Δ^9-THC.[88] The affinity of Δ^9-THCV for CB1 and CB2 receptors is 10× lower compared to Δ^9-THC and elicits different pharmacological responses both *in vitro* and *in vivo*.[66,89,90] The binding affinities of Δ^9-THCV for CB1 and CB2 are shown in Table 1. The reported K_i values of

Table 1. Cellular Receptor Activity of Δ^9-THCV

receptor	EC_{50} (μM)	efficacy (%)	IC_{50} (μM)
CB1[91]	12.7[a]	n/a	n/a
CB2[91,96]	0.098[b]/0.038[b]	40	0.143
GPR55[97]	0.88[c]	61	n/a
5-HT_{1A}[98]	0.0054[d]/0.028[e]	35[d]/134[e]	2 x10^{-6}[d]/2.1[e]

[a]Inhibition of electronically invoked contractions of the mouse isolated vas deferens. [b][^{35}S]-GTPγS binding assay with **20** in human CB2 transfected in CHO cells. [c]LPI-mediated stimulation of ERK1/2 phosphorylation in GPR55-expressing HEK293 cells. [d]Δ^9-THCV (100 nM) on [^{35}S]-GTPγS binding to Sprague−Dawley rat brainstem membrane. [e]Δ^9-THCV (100 nM) on [^{35}S]-GTPγS binding to human 5-HT_{1A} CHO cell membrane.

Δ^9-THCV for mouse brain membrane CB1 and CHO cells transfected with human CB2 receptors were 0.075 and 0.063 μM in the CP55,940 (**20**) binding assay, respectively. K_b values of 0.093 and 0.01 μM for Δ^9-THCV were reported in the [^{35}S]GTPγS CB1 and CB2 receptor binding assay to measure antagonism of **20**.[91] Δ^9-THC has been well characterized as a partial agonist of the CB1 receptor, whereas the pharmacology of Δ^9-THCV is somewhat more complex.[92] *In vitro*, Δ^9-THCV displays varying affinity to the CB1 receptor depending on the dose and source species. At high concentrations, Δ^9-THCV was reported to act as an agonist with effects similar to Δ^9-THC.[93] Binding affinities for CB1 derived from rat and mouse brain membranes and the humanized receptor transfected into CHO cells are shown in Table 1. Δ^9-THCV displays antagonism/inverse agonism with respect to CB1 activation by endogenous cannabinoids **3** and **4** and the receptor agonists **20** and *R*-(+)-WIN55,212−2 (**21**) in a [^{35}S]-GTPγS binding assay.[91,94] Δ^9-THCV exhibited significantly reduced [^{35}S]-GTPγS binding to CB1 receptors derived from mouse cerebellum and piriform cortex with potency similar to the known CB1 antagonist AM251 (**22**), indicating the compound was acting as an antagonist rather than an inverse agonist.[95] When tested in the presence of Δ^9-THC in mice, Δ^9-THCV was shown to inhibit the effects of Δ^9-THC but did not appear to be acting as an inverse agonist of CB1. The combined data suggest that Δ^9-THCV is a neutral antagonist based on high binding affinity to CB1 but lack of functional efficacy.[66] The structures of common CB1 and CB2 receptor modulators are shown in Figure 1.

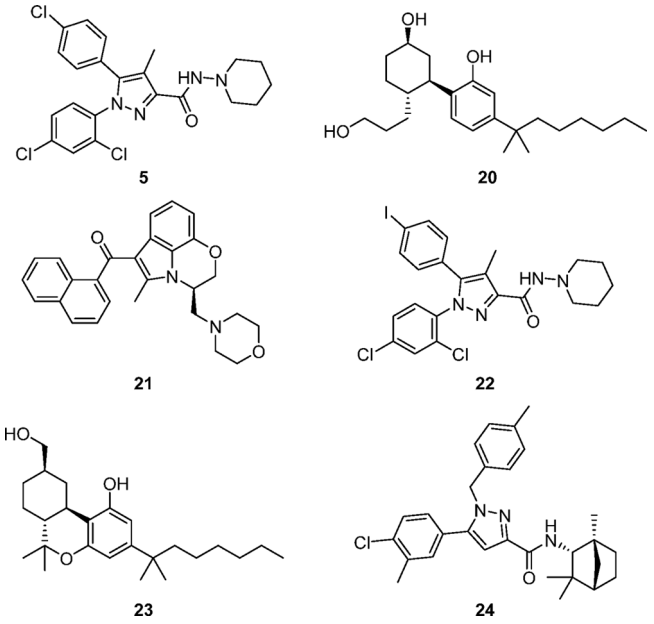

Figure 1. Synthetically derived CB1 and CB2 receptor modulators. Rimonabant **5**, CB1 inverse agonist; CP55,940 **20**, CB1 and CB2 agonist; WIN55,212-2 **21**, CB1 and CB2 agonist; AM251 **22**, CB1 inverse agonist; HU-243 **23**, CB1 and CB2 agonist; SR144258 **24**, CB2 inverse agonist.

Δ^9-THCV appears to act as a partial agonist of the CB2 receptor, and activity is highly influenced by the receptor expression level.[96] In one report Δ^9-THCV displayed behavior consistent with antagonism of the CB2 receptor.[91] However, in the same [^{35}S]-GTPγS binding assay, in which the expression level of CB2 was three times higher, agonism was observed.[96]

A basal reduction of [^{35}S]GTPγS binding and G-protein activity was observed in CB1 and CB2 null CHO-D_2 cells treated with Δ^9-THCV, indicating the pharmacological activity related to Δ^9-THCV is complex and involves other biochemical pathways aside from CB1 and CB2 receptors.[95] Δ^9-THCV also binds to other G-protein couple receptors such as GPR55[97] and the serotonin receptor 5-HT_{1A}[98] as shown in Table 1. GPR55, considered the third cannabinoid receptor, is one of the upstream regulators of ERK1/2 phosphorylation. GPR55 knockout mice have abolished hyperalgesia in nerve ligation and adjuvant-induced models for neuropathic and inflammatory pain,[97,99] and increased phosphorylation of ERK1/2 has been linked to a pro-inflammatory response to persistent inflammatory pain.[100,101] ERK1/2 phosphorylation was shown to be stimulated in the presence of Δ^9-THCV, resulting in a non-concentration-associated response curve. The response was compared to the maximal response of L-α-lysophosphatidylinositol (LPI)-induced inflammation. The data show that Δ^9-THCV is a weak agonist of GPR55, yet exhibits significant inhibition of LPI-induced pERK stimulation.[97] When 1 μM Δ^9-THCV was incubated in the presence of LPI, Δ^9-THCV was shown to be a significant inhibitor of LPI-associated stimulation

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of ERK1/2 phosphorylation, resulting in a downward shift in the dose−response curve and lower levels of pERK. The down-regulation of pERK modulation suggests agonism of GPR55 may contribute to the analgesic properties of Δ^9-THCV.[97]

The potential use of Δ^9-THCV as an antipsychotic was shown in rat models of schizophrenia due in part to upregulation of 5-HT_{1A} activity. *In vitro* assays of both humanized 5-HT_{1A} receptor CHO cells and rat brain stem membranes showed Δ^9-THCV enhanced the 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT)-induced activation of the 5-HT_{1A} in [^{35}S]-GTPγS and [^{3}H]-8-OH-DPAT binding assays.[98] The results suggest that Δ^9-THCV does not bind to the orthosteric site and likely acts as a positive allosteric modulator of 5-HT_{1A}. *In vivo* experiments in a PCP-induced rat model of schizophrenia revealed that Δ^9-THCV administration reduced hyperlocomotion, restored social behaviors, and normalized cognitive performance. As it has been reported that CB1 antagonism is a potential mechanism for pro-cognitive effects, the combined ability of Δ^9-THCV to activate 5-HT_{1A} and diminish CB1 activity is worthy of further investigation as a therapeutic for treatment of schizophrenic-related symptoms.[98]

TRP ion channels are known to be activated by endocannabinoids.[46,47] Phytocannabinoids, due to their lipophilic character, are also able to access and bind to these intracellular ion channels to elicit a significant pharmacological response.[49] Δ^9-THCV has been investigated against a number of TRP ion channels, as shown in Table 2. Agonism of TRPV1, TRPV2, TRPV3, TRPV4, and TRPA1 expressed in HEK-293 cells was observed, whereas antagonism of icilin activation was observed for TRPM8. Low single digit micromolar EC_{50}s and IC_{50}s were reported for purified Δ^9-THCV.[49,102]

Table 2. Ion-Channel Activity of Δ^9-THCV

ion channel	EC_{50} (μM)	efficacy (%)	desensitization IC_{50} (μM)
TRPV1[a]	1.5	68	1.3
TRPV2[b]	4.1	74	0.8
TRPV3[c]	3.8	72	3.0
TRPV4[d]	6.4	60	3.2
TRPV5[50]	NT	NT	4.8
TRPV6[50]	NT	NT	9.4
TRPA1[e]	1.5	243	3.07
TRPM8[f]	n/a	n/a	0.87

[a]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 0.1 μM capsaicin. [b]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 3 μM lysophosphatidylcholine. [c]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 1 mM carvacrol. [d]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 1 μM α-phorbol-12,13-didecanoate (4αPDD). [e]Efficacy as a percentage effect and desensitization in the presence of 100 μM isothiocyanate. [f]Desensitization in the presence of 0.25 μM icilin.

A Δ^9-THCV-dominant botanical extract was tested in the TRP ion channel assay. There was a significant additive effect observed for agonism of TRPV1 and TRPA1 with EC_{50} values of 200 and 70 nM, respectively, for the extract relative to pure Δ^9-THCV.[49] These results indicate that there are additional phytochemicals present in the extract that are enhancing the stimulation of these ionotropic channels. Additionally, the reported potency of the Δ^9-THCV-dominant extract as an antagonist of TRPM8 (IC_{50} = 20 nM) was significantly enhanced compared to pure Δ^9-THCV.[49] Unfortunately, there is little description of the composition of the extract to suggest which additional components in the extract are potentially responsible for these additive effects.

Δ^9-THCV is shown to affect calcium homeostasis through modulation of TRP channels. TRPA1 activation by Δ^9-THCV has been associated with increased calcium levels in human satellite cells and in the promotion of myotube formation, a critical component of muscle regeneration.[103] Conversely, inhibition of TRPV5 and TRPV6 by Δ^9-THCV resulted in a reduction in calcium-dependent axial ossification *in vivo* in zebrafish embryos.[50] Inhibition of TRPV5 was shown to slow the progression of joint destruction in a rat model for osteoarthritis, suggesting TRPV5 as a potential drug target for osteoarthritis.[55]

A large focus of phytocannabinoid pharmacology is based on the concept that these natural products are involved in protection of neurons from degeneration or noxious stimuli. Phytocannabinoids can modulate endocannabinoid levels through cannabinoid receptor binding, modulate immune cell function, and function as antioxidants,.[89] CB2 agonism by Δ^9-THCV in combination with blocking of the CB1 receptor has been suggested as an *in vivo* mechanism of neuroprotection.[104]

Δ^9-THCV acts to increase transmission of inhibitory neurotransmitters through blockage of the CB1 receptor. In mouse acute parasagittal cerebellar brain slice interneuron−Purkinje cell (IN-PC) experiments, micromolar concentrations of Δ^9-THCV were associated with increased GABAergic transmission, leading to a reduction in Purkinje cell firing.[105] Similarly in a multielectrode array method, Δ^9-THCV reduced both the amplitude and frequency of *in vitro* epileptiform bursting activity in rat Purkinje cells.[106] Antiepileptic effects of Δ^9-THCV have been observed *in vivo*. Significant reduction in the frequency of pentylenetetrazole-induced seizures was observed in rats treated with Δ^9-THCV at 0.25 mg/kg.[106] These results support further investigation of Δ^9-THCV alone as a potential anticonvulsant or synergistically in conjunction with other antiepileptic phytocannabinoids such as CBD or standard-of-care epilepsy drugs.

Endocannabinoid signaling in the basal ganglia has been viewed as a potential pharmacological target for the treatment of Parkinson's disease (PD). There are biphasic changes in endocannabinoid signaling that occur during the progression of PD.[107] CB1 receptors are highly expressed in the basal ganglia; thus downregulation of CB1 activity may be effective to reduce bradykinesia associated with PD as well as improve the therapeutic window of L-DOPA.[104] Several synthetic cannabinoids have shown promise in alleviating some PD-related symptoms, such as rimonabant for akinesia[108] and L-DOPA-induced dyskinsea[107] and the CB2 agonist **21** as a neuroprotective agent against nigral degeneration.[109]

Δ^9-THCV was investigated in a 6-hydroxydopamine-induced rat model for PD. Low-dose treatment (2 mg/kg i.p.) was efficacious in reducing motor function similar to rimonabant, likely due to increased glutamate levels in the striatum as a result of CB1 receptor blockage and dampening of excitatory transmission. As well, continual Δ^9-THCV treatment over 14 days was shown to partially reduce nigral degeneration in this rat model.[107] CBD has also been reported to elicit similar results in the same model, although unlikely as a result of CB2 agonism.[110] It is not clear whether the neuroprotective potential of Δ^9-THCV is due to enhanced CB2 activity, antioxidant activity, or a combination of both mechanisms. These results support the investigation of Δ^9-THCV alone or in combination with other

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phytocannabinoids such as CBD in the treatment of PD-related symptoms.

Anti-inflammatory and immunomodulatory effects have long been associated with *C. sativa*. Scientific rationale and evidence now support long held anecdotal reports for use of cannabis to treat chronic pain due to inflammation. In mouse models of acute inflammation and inflammatory pain, Δ^9-THCV (0.3 mg/kg and 1.0 mg/kg i.p.) decreased both carrageenan-induced edema and formalin-induced pain. The inflammation was reversed when mice were treated with the CB2 inverse agonist **24**, indicating that the anti-inflammatory properties of Δ^9-THCV are driven in part by CB2 modulation.[96]

A mechanism of pro-inflammatory response to infection is the production of nitric oxide (NO) by macrophages. Phytocannabinoids such as Δ^9-THC have been shown to downregulate NO production.[111] Similarly, Δ^9-THCV has also been shown to reduce NO production in a lipopolysaccharide (LPS)-induced model for inflammation through activation of the CB2 receptor. Pure Δ^9-THCV was more potent in the model compared to a Δ^9-THCV-dominant botanical extract (65% Δ^9-THCV), suggesting a counteractive effect rather than positive synergism with other phytochemicals in this particular extract. In addition to a reduction in NO levels, Δ^9-THCV was also shown to downregulate the levels of both the pro-inflammatory cytokine IL-1β and prostaglandin synthase COX-2. Il-1β is one of the main cytokines responsible for COX-2 induction in macrophages.[111]

The endocannabinoid system is known to regulate appetite and feeding behavior through modulation of the appetite-suppressing hormone leptin. The hyperphagic effects of Δ^9-THC have long been known and can be reversed through coadministration of a CB1 antagonist. CB1 antagonists, such as Δ^9-THCV, have been investigated as appetite suppressants and as a possible treatment for obesity.[112,113] Preclinical studies of male mice dosed with Δ^9-THCV at 3, 10, and 30 mg/kg (i.p.) resulted in hypophagia and weight loss in the treated mice, similar to the synthetic CB1 inverse agonist **22**. The effect was only observed for pure Δ^9-THCV. A Δ^9-THCV-rich botanical extract did not show hypophagia and suggested residual phytochemicals in the extract, perhaps Δ^9-THC, were counteracting the response.[114] The effect on body weight in obese female mice treated orally with Δ^9-THCV (0.3−12.5 mg/kg BID) was reported in a separate study. The treated mice showed a reduction in body fat content, fasting insulin levels in the oral glucose tolerance test, and liver triglyceride levels. An increase in energy expenditure was also observed.[115] These promising preclinical findings served as rationale for the clinical development of Δ^9-THCV.

Clinical trials with pure Δ^9-THCV have been conducted for safety and tolerability and in type II diabetes as shown in Table 3. A placebo-controlled, double-blind, crossover pilot study of 10 male subjects was conducted to measure safety and tolerability of Δ^9-THCV administration as well as the effect on coadministration with Δ^9-THC. Patients were given 10 mg of pure Δ^9-THCV or placebo in capsule form for 5 days and 1 mg of Δ^9-THC intravenously on the fifth day. Generally, Δ^9-THCV was subjectively indistinguishable from placebo and well tolerated with no reported serious adverse effects. On the fifth day, the low dose of Δ^9-THC did show some expected intoxicating effects. Patients who were treated with Δ^9-THCV scored better in the delayed recall experiment and Δ^9-THC-associated heart rate increase was inhibited, suggesting that Δ^9-THCV can counteract some typical Δ^9-THC physiological effects. There was no change in the plasma concentration of Δ^9-THC observed, making it unlikely the effect is a due to a change in Δ^9-THC pharmacokinetics.[116]

Table 3. Clinical Trials with Δ^9-THCV

indication	dose regimen	phase/type of trial
effect on Δ^9-THC-induced symptoms	A: Δ^9-THCV (10 mg QD) B: placebo Δ^9-THC (1 mg QD on 5th day)	pilot study/safety and tolerability
diabetes mellitus, type 2	A: Δ^9-THCV (2 mg BID) B: Δ^9-THCV (5 mg BID) C: Δ^9-THCV (15 mg BID) D: placebo	II/RCT, interventional
diabetes mellitus, type 2	A: CBD (100 mg BID) B: Δ^9-THCV (5 mg BID) C: 1:1 CBD/Δ^9-THCV (5 mg/5 mg, BID) D: 20:1 CBD/Δ^9-THCV (100 mg/5 mg, BID) E: placebo	II/RCT, interventional

A phase IIa randomized, double-blind, placebo-controlled, parallel-group pilot study was conducted in 62 non-insulin-treated type 2 diabetic patients. The 13-week trial was composed of five treatment arms. The lowering of high-density lipoprotein cholesterol as the primary end point was not met; however, there were positive results in secondary clinical markers. Compared to placebo, Δ^9-THCV was shown to significantly reduce fasting plasma glucose levels, improve HOMA2 pancreatic b-cell function, and increase levels of both adiponectin and apolipoprotein A.[70]

In summary, preclinical data in rodents support that Δ^9-THCV may be beneficial in the treatment of inflammatory pain. Δ^9-THCV demonstrated significant seizure reduction in rodent models, supporting further investigation in preclinical models of epilepsy. Δ^9-THCV also showed promising neuroprotective properties and could serve to treat symptoms associated with neurological disorders such as Parkinson's disease. Δ^9-THCV was shown to regulate appetite and thus could potentially be used as a treatment for obesity. Of interest is the apparent lack of synergy with other phytocannabinoids for particular indications. Δ^9-THCV alone was shown to be more effective compared to a THCV-dominant extract in rodent models for inflammation and hyperphagia, suggesting a negative aspect of cannabinoid combination therapy in these models. Conversely, Δ^9-THCV was shown to clinically potentiate some negative physiological effects of Δ^9-THC. Clinical evidence shows that Δ^9-THCV is well tolerated and shows promise as a treatment for regulating blood glucose levels in type II diabetes. The metabolism of Δ^9-THCV and how it relates to potential drug−drug interactions should also be investigated.

CANNABIDIVARIN

Cannabidivarin (**9**, CBDV) is an analogue of CBD carrying an *n*-propyl (C_3) side chain. The phytocannabinoid was first isolated from *C. sativa* in 1969.[117]

CBDV has low affinity for the CB1 receptor but does have strong affinity for CB2 (K_i = 0.57 μM) in the [^{3}H]-**20** binding assay in human Sf9 cells as shown in Table 4.[118] The apparent CB1 receptor affinity was enhanced when CBDV-dominant botanical extracts were tested in the [^{3}H]-**20** binding assay in CB1-expressing mouse MF1 brain membranes. Two CBDV-dominant botanical extracts (A: 47.4% CBDV, 13.9% CBD, 1%

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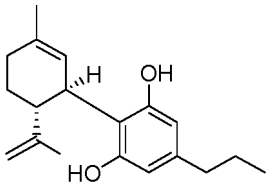

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Table 4. Cellular Receptor Activity of CBDV

receptor	EC_{50} (μM)	efficacy (%)	IC_{50} (μM)	K_i (μM)
CB1	>10[a]	n/a	13.8[b]	14.7[b]/4.0[c]
CB2	0.003[a]	n/a	3.45[b]	0.57[b]/0.5[c]
GPR6	2.4[d]	90	n/a	n/a
GPR55	0.40[e]	49	n/a	n/a

[a][^{35}S]-GTPγS binding assay.[121] [b]Binding assay with [^{3}H]-**20** in CB1- and CB2-expressing Sf9 cells.[118] [c][^{3}H]-**20** binding assay in HEK293 cells.[121] [d]cAMP inGPR6-expressing HEK293 cells.[120] [e]LPI-mediated stimulation of ERK1/2 phosphorylation in GPR55-expressing HEK293 cells.[97]

Δ^9-THC, and 2.5% Δ^9-THCV and B: 57.8% CBDV and 13.7% CBD) were tested compared to pure CBDV. Minimal affinity was observed for 10 μM pure CBDV, the highest concentration tested. However, extract A, which contained both Δ^9-THC and Δ^9-THCV, significantly enhanced the CB1 potency (K_i = 0.13 μM). In comparison, extract B, void of Δ^9-THC and Δ^9-THCV, had lower CB1 affinity (K_i = 1.0 μM) and lower efficiency of [^{3}H]-**20** displacement at the highest concentration tested in the assay.[119] This result shows that low levels of either Δ^9-THC and Δ^9-THCV are responsible for CB1 modulation in a CBDV-dominant extract.

Some of the pharmacological activity of CBDV may be the result of indirect regulation of these two key endocannabinoid receptors through modulation of targets involved in endocannabinoid processing. CBDV is a weak inhibitor (IC_{50} = 16.6 μM) of diacylglycerol lipase a (DAGLα), an enzyme involved in the biosynthetic pathway of the endocannabinoid **4**.[49] CBDV has high affinity for other G-protein coupled receptors such as GPR55, a potential therapeutic target for neuropathic and inflammatory pain. CBDV was associated with a 56% reduction in LPI-associated ERK1/2 activation. The functional assay showed a downward shift in the dose−response curve and lower levels of pERK, similar to the effect observed for Δ^9-THCV in the same assay.[97] CBDV acts as an inverse agonist of the orphan G-protein coupled receptor GPR6, a potential target for neurological disorders. In the GPR6 functional assay, CBDV was shown to block the β-arrestin signaling pathway.[120]

CBDV has been shown to activate the ionotropic channels TRPV1, TRPV2, and TRPA1 at low micromolar concentrations, and the channels are desensitized to subsequent application, as shown in Table 5. Agonism of TRP channels expressed in HEK-293 cells was observed for TRPV1, TRPV2, TRPV3, TRPV4, and TRPA1, whereas antagonism of icilin activation was observed for TRPM8. Low single digit micromolar EC_{50}s and IC_{50}s were reported for purified CBDV.[49,102] Unlike Δ^9-THCV-dominant extracts, a CBDV-dominant botanical extract did not show any significant difference in TRP ion-channel modulation.[49] These results indicate that in this particular extract there are no additional phytochemicals present at a level capable of synergistically enhancing the stimulation of these ionotropic channels.[49]

Table 5. Ion-Channel Activity of CBDV

ion channel	EC_{50} (μM)	efficacy (%)	desensitization IC_{50} (μM)
TRPV1[a]	3.6	21	10
TRPV2[b]	7.3	50	31
TRPV3[c]	1.7	16	25
TRPV4[d]	0.9	30	2.9
TRPA1[e]	0.42	105	1.3
TRPM8[f]	n/a	n/a	0.9

[a]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 0.1 μM capsaicin. [b]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 3 μM lysophosphatidylcholine. [c]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 1 mM carvacrol. [d]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 1 μM 4αPDD. [e]Efficacy as a percentage effect and desensitization in the presence of 100 μM isothiocyanate. [f]Desensitization in the presence of 0.25 μM icilin.

CBDV was investigated in both *in vitro* and *in vivo* models for Duchenne muscular dystrophy (DMD), a condition that results in skeletal muscle degeneration and chronic inflammation, due to its potent TRPA1 activity. CBDV was shown to promote myotube formation in murine C2C12 myoblasts at 1 μM. Mice dosed with CBDV (60 mg/kg, i.p.) showed reduced levels of inflammation and muscle cell degeneration, and locomotor function was restored. These results suggest potential for CBDV as an add-on therapy for the treatment of DMD.[103]

The antiepileptic properties of CBD are now well established with the recent approval of Epidiolex for the treatment of Lennox-Gastaut and Dravet syndrome, two rare subtypes of childhood epilepsy.[122,123] CBDV, a close structural analogue of CBD, also displays anticonvulsive properties in preclinical rodent models for epilepsy.[124−126] Mortality-associated seizures and seizure severity were reduced when CBDV was administered 1 h before maximal electroshock-induced seizures in mice or PTZ-induced seizures in rats. The effect was observed for both i.p. (50−200 mg/kg) and oral gavage (400 mg/kg, 3.5 h prior to seizure induction) dose regimens.[127] By comparison, CBDV was equally effective as CBD at 100 mg/kg in improving mortality. However, CBDV required a higher dose relative to CBD (200 mg/kg vs 100 mg/kg) to reduce seizure severity. CBDV also delayed seizure onset in a dose-dependent manner, which was not observed with CBD.[127] CBDV was also investigated in combination with clinically relevant antiepileptic medications. CBDV (200 mg/kg, i.p.) was well tolerated and contributed to a notable improvement in the severity, mortality, and latency compared to CBD when coadministered with the antiseizure medications valproate (50−200 mg/kg, i.p.) or ethosuximide (60−175 mg/kg, i.p.) in a pilocarpine-induced seizure model. CBDV was not effective on its own in this model, whereas CBD reduced tonic-clonic seizures. The study shows that while both CBD and CBDV have significant antiseizure activity, there are subtle differences in the mechanism of action of these two similar cannabinoids.[127]

Additionally, CBDV-dominant botanical extracts have been reported to significantly reduce seizure activity and mortality in three murine models of epilepsy.[119] Two botanical extracts were evaluated (A: 47.4% CBDV, 13.9% CBD, 1% Δ^9-THC, and 2.5% Δ^9-THCV and B: 57.8% CBDV and 13.7% CBD). Extract B (100 mg/kg) suppressed PTZ-induced seizure activity similarly to purified CBDV (100 mg/kg), even though the extract contained a lower amount of CBDV. Extract A displayed comparable antiseizure activity to extract B; however, motor

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function was impaired due to the presence of Δ^9-THC. Purified CBDV was not effective in the pilocarpine-induced mouse seizure model.[127] However, extract B significantly reduced seizure severity in this model, which supports further investigation of CBDV/CBD combinations for treatment of temporal lobe seizures.[119]

The physiological mechanism by which CBDV imparts antiepileptic effects is likely independent of CB1 or CB2 modulation given the low cannabinoid receptor affinity. The activity may derive from the activation and desensitization of ionotropic TRP channels. Modulation of ion-gated channel activity can reduce neuronal activity during states of neuronal hyperexcitability.[128] CBDV reduced epileptiform burst amplitude and duration in rat hippocampal slices measured using microelectrode arrays. This effect may be related to CBDV-induced dephosphorylation of channels such as TRPV1, resulting in desensitization.[128] A significant decrease, but not full mitigation, in the anticonvulsant effect of CBDV was observed in TRPV1 knockout mice, suggesting TRPV1 activation is not the only mechanism related to the antiepileptic properties of CBDV.[126]

Another potential mechanism of action for the antiepileptic properties of CBDV is to counteract the suppression of $GABA_A$ receptor activity. Some patients with drug-resistant temporal lobe epilepsy show a pattern of $GABA_A$ receptor dysfunction. This results in an usual run-down of GABA current.[129,130] Morano and co-workers reported an improvement in $GABA_A$ current run-down in hippocampal tissues obtained from patients with treatment-resistant temporal lobe epilepsy following prolonged exposure to CBDV. A case report of a single patient showed improvement in seizure frequency following oral administration of a cannabis extract containing a high concentration of CBDV in conjunction with standard therapy. The improvement cannot be solely attributed to a single cannabinoid from this empirical report. However, the data suggest CBDV may play a role in contributing to the anticonvulsive effect,[131] and it is under clinical investigation for epilepsy as shown in Table 6.[132]

Table 6. Clinical Trials with CBDV

indication	dose regimen	phase/type of trial
epilepsy, focal seizures	A: CBDV (400 mg BID) B: placebo	II/RCT, interventional
epilepsy, focal seizures	A: CBDV (400−800 mg BID) B: placebo	II/RCT, interventional
autism spectrum disorder	A: CBD (600 mg QD) B: CBDV (600 mg, QD) C: placebo	II/RCT, interventional
autism spectrum disorder	A: CBDV (10 mg/kg, QD) B: placebo	II/RCT, interventional

CBDV was shown to modulate excitatory−inhibitory systems in the brain. Glutamate and GABA level regulation related to autism spectrum disorder (ASD) was further investigated as part of two clinical trials in children and adults as shown in Table 6.[71] CBDV induced changes in GABA+ (GABA + macromolecules) and Glx (glutamate + glutamine) concentrations. These neurotransmitter markers were measured in both "typical" and autistic brains. The study design included 34 healthy male subjects with (n = 17) and without (n = 17) ASD. Neurotransmitter levels were measured by magnetic resonance spectroscopy 2 h postdose of CBDV (600 mg, p.o.), focusing on the left basal ganglia (BG) and dorsomedial prefrontal cortex, two regions in the brain linked to phenotypes of ASD. CBDV was shown to significantly increase levels of Glx in the BG but not the frontal region in both test cohorts. There were noteworthy response variances within the ASD arm of the study group relative to the baseline measures. In subjects who had a high level of baseline Glx, decrease in Glx was observed, whereas individuals with a low baseline Glx experienced an increase in Glx following treatment with CBDV.[71] The reason for elevated neurotransmitter levels and activity localized in the BG is not entirely clear. This clinical outcome is potentially related to the high expression of TRP channels in the BG, such as TRPV1, TRPV2, and TRPA1, each of which has high affinity for CBDV.[49]

TRP channels are associated with modulating proinflammatory response to noxious stimuli. In particular, TRPA1 is known to be dysregulated in intestinal inflammatory diseases. CBDV has been investigated for anti-inflammatory properties in models for irritable bowel disease (IBD) and ulcerative colitis (UC) due to its potent TRPA1 activity.[133] CBDV was administered to mice orally and intraperitoneally at doses in the range of 0.3−10 mg/kg, both prior to and after colonic inflammation induced by dinitrobenzenesulfonic acid (DNBS). CBDV was shown to significantly reverse the colon weight/length ratio due to inflammation and counteract upregulation of the TRP1A channel. No physiological changes were observed for TPRV1 or TRPV2 expression. Neutrophil infiltration into the tissue as related to myeloperoxidase (MPO) activity and a reduction in pro-inflammatory cytokine IL-1β and IL-6 levels was also observed. TRP1A levels were also elevated in tissue samples obtained from pediatric patients with UC. *In vitro* experiments with human colonic tissue obtained from biopsies also showed that CDBV reduced the production of proinflammatory cytokines such as IL-6.[133]

The metabolism and safety profile of the Δ^9-THC and CBD prescription medications Marinol, Sativex, and Epidiolex are known.[18,19] There is little known about the mechanism by which whole cannabis or individual minor cannabinoids are metabolized *in vivo* and whether there are concerns for potential drug−drug interactions when cannabis is consumed concurrently with pharmaceuticals. The metabolism of CBDV is similar to its structural analogue CBD. CBD is a known inhibitor of many CYP450 isoforms; thus CBDV has potential for drug−drug interactions related to cytochrome P450 clearance mechanisms. CBDV is a potent inhibitor of CYP1A1,[134] CYP2C19,[135] and CYP2B6 as shown in Table 7.[136] It is a weak inhibitor of CYP2D6.[137] Generally CBDV is a weaker inhibitor of the CYP enzymes with reported data compared to CBD.

In summary, CBDV exhibited encouraging results in a rodent model to offset some pathological indications of DMD; thus, further studies of CBDV in models for DMD and other types of muscular dystrophy are justified. The anti-inflammatory

Table 7. Metabolic Targets of CBDV

CYP isoform	IC_{50} (μM)	K_i (μM)	type of inhibition
CYP1A1[134]	0.06	1.6	mechanism-based, metabolism dependent
CYP2B6[136]	7.4	1.3	mixed inhibition
CYP2D6[137]	10.2	5.8	n/a
CYP2C19[135]	3.7	1.4	mixed inhibition

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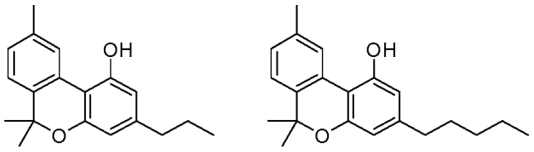
property of CBDV was demonstrated in both rodent models and human tissue models of ulcerative colitis, supporting further investigation for the treatment of inflammatory disorders. In some preclinical rodent models, CBDV showed antiseizure properties on par with CBD. Additionally, there appeared to be an added benefit to using a CBDV/CBD extract in this model. Similar reduction in seizure severity was observed for a lower overall dose of CBDV, which supports further investigation of CBD/CBDV combinations in preclinical seizure models. Of significance is the clinical investigation of CBDV for autism and epilepsy, highlighting that CBDV can be administered safely at doses of up to 800 mg. Given the structural similarity of CBDV and CBD however, potential drug−drug interactions should be further investigated.

CANNABIVARIN AND CANNABINOL

Cannabivarin (**10a**, CBV) and cannabinol (**10b**, CBN) and are naturally occurring phytocannabinoids found in *C. sativa.* CBN



10a **10b**

was first isolated from hashish in the 1930s.[138] The structure was determined by total synthesis, derivatization, and comparison of melting point with an authentic sample.[139−141] There are only a few reports of the pharmacology of CBN since its discovery. However, preclinical studies with CBN as a treatment for ocular disease and epidermolysis bullosa showed an excellent safety profile.[142]

CBV was first isolated in the early 1970s. and the structure was elucidated by mass spectrometry by comparison to the fragmentation pattern of its C-5 homologue, CBN.[143] Despite the similarity to CBN, the pharmacological data available for CBV is sparse.

Receptor activity for CBN and CBV data is shown in Table 8. CBN binds to both the CB1 and CB2 receptors with 10−100-fold lower activity compared to Δ^9-THC depending on the assay and the tissue source of the receptor. CBN is a CB1 agonist and shows affinity in [^{3}H]-**20** binding assays with CB1 receptors expressed in murine-L cells,[144] Sf9 cell membranes,[118] and CHO cells[145,146] as well as in HU-243 CB1 receptor binding assays in rat synaptosomal and COS-7 cells.[147]

Table 8. Cellular Receptor Activity of CBN

cannabinoid	receptor	EC_{50} (μM)	K_i (μM)	IC_{50} (μM)
CBN	CB1	0.12[a]/0.3[b]	0.21[c]/1.1[d]/0.39[e]/ 0.012[f]/0.07[g]/0.07[i]	0.017[f]
	CB2	0.26[a]/ 0.29[b]/ 0.06[i]	0.12[c]/0.016[f]/0.07[g]/ 0.3[h]/0.07[i]	0.055[f]
CBV	CB1	>10[b]	0.56[i]	n/a
	CB2	>10[b]	4.8[i]	n/a

[a]Inhibition of adenylcyclase.[147] [b][^{35}S]-GTPγS binding assay.[121] [c][^{3}H]-**23** binding assay in COS cells.[147] [d][^{3}H]-**20** binding assay in murine-L cells.[144] [e][^{3}H]-**23** binding to CB1 expressed in rat synaptosomal membrane.[147] [f][^{3}H]-**20** binding assay in Sf9 cells.[118] [g][^{3}H]-**20** binding assay in CHO-KT cells.[146] [h][^{3}H]-**20** binding assay in AtT-20 cells.[144] [i][^{3}H]-**20** binding assay in HEK293 cells.[121] [j][^{35}S]-GTPγS binding assay.[146]

CBN binds with high affinity to the CB2 receptor; however, reports differ on the mode of receptor activation. CBN was reported as an agonist of the CB2 receptor in the forskolin-stimulated cAMP accumulation assay in COS-7 cells with the CB2 agonist **23**.[147] In a separate study, CBN was reported to act as an inverse agonist in the [^{35}S]-GTPγS binding assay with **20** in human CB2 receptor transfected CHO cells.[145,146]

CBV binds with 8× and 60× lower affinity for CB1 and CB2 receptors, respectively.[84] No activity was observed in the [^{35}S]-GTPγS functional assay. CBV also shows far less CB1 and CB2 activity compared to CBN, as shown in Table 8, highlighting the importance of alkyl chain length for cannabinoid receptor activity within these classes of phytocannabinoids.

CBN has been evaluated for activity with a number of TRP ion channels as shown in Table 9. CBN is a potent agonist of TRPA1 and a potent inhibitor of TRPM8.[49] CBN shows very low efficacy in activation of TRPV1, TRPV3, and TRPV4.[49,102] It has been suggested that inactivation of these channels at levels lower than required for endogenous stimulation is potentially advantageous for treating pain, itch, and inflammation, as well as metabolic and cardiovascular disorders and cancer that result from upregulated ionotropic channel activity.[102]

Table 9. Ion-Channel Activity of CBN and CBV

	EC_{50} (μM)		efficacy (%)		desensitization IC_{50} (μM)	
ion channel	CBN	CBV	CBN	CBV	CBN	CBV
TRPV1[a]	6.2	6.6	<10	50	82	11.9
TRPV2[b]	19	3.1	74	79	15.7	3.2
TRPV3[c]	5.3	3.5	72	78	9.4	1.6
TRPV4[d]	16.1	7.3	60	37	5.4	4.0
TRPA1[e]	0.18	0.20	243	123	0.4	0.17
TRPM8[f]	n/a	n/a	n/a	n/a	0.21	0.50

[a]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 0.1 μM capsaicin. [b]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 3 μM lysophosphatidylcholine. [c]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 1 mM carvacrol (CBN) and 0.1 mM thymol (CBV). [d]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 1 μM 4αPDD (CBN) and 10 nM GSK1016790A (CBV). [e]Efficacy as a percentage effect and desensitization in the presence of 100 μM isothiocyanate. [f]Desensitization in the presence of 0.25 μM icilin.

CBV has also been evaluated for activity with the same panel of TRP ion channels, shown in Table 9.[148] Moderate activity on par with CBN was observed for TRPV1, TRPV3, and TRPV4. A significant increase in activity was observed for TRPV2 relative to CBN. The increase in activity is opposite to that of other C3−C5 homologue comparators, where a decrease in TRPV2 activity was observed for CBDV and THCV relative to CBD and THC.[49] CBV maintains potent activity for both activation of TRPA1 and inhibition of TRPM8 at nanomolar levels comparable to CBN.[148]

The study of the pharmacological activity of CBN compared to Δ^9-THC dates back to the early 1970s. CBN was known to mimic many of the typical pharmacological effects of Δ^9-THC; however, higher doses of CBN were generally required.[149] In a series of *in vivo* studies, CBN was reported to block the corneal reflex of rabbits in the Gayer test, induce catatonia and analgesia,

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increase pentobarbital sleeping time in mice, and promote aggressiveness and irritability in rats after REM sleep deprivation.[149] In a rat open-field test, CBN did not impair rat gait compared to Δ^9-THC. When both CBN and Δ^9-THC were administered together, there did appear to be a synergistic effect in the depressant mouse models for catalepsy, analgesia, and pentobarbital-induced sleeping time.[149]

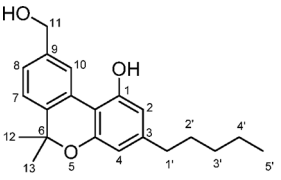
CBN is known to induce immunosuppression by lowering intracellular levels of cyclic AMP through the downregulation cAMP adenylate cyclase.[150] One mechanism by which cAMP levels are controlled is through CB2 receptor modulation. CBN was shown to invoke a concentration-dependent inhibition of mouse lymphocyte proliferation and inhibition of anti-sheep red blood cell IgM antibody forming cell response.[150] The CBN-induced decrease in cAMP levels results in inhibition of interleukin 2 (IL-2) expression.[151] In addition to the role of the CB2 receptor in the immune response, cannabinoids are known to confer immunosuppression through other signaling pathways. CBN has been shown to modulate immunosuppression through disruption of the ERK signaling pathway.[152] CBN in low micromolar concentrations was associated with inhibition of ERK/MAP kinase phosphorylation of c-fos and c-jun. The modulation of phosphorylation levels results in blockage of activator protein complex 1 (AP-1) binding to these kinases. AP-1 is a heterodimeric complex of c-fos and c-jun proteins that is involved in the regulation of several genes including the gene for IL-2. CBN was shown to inhibit the binding of AP-1 to the promoter region of IL-2, resulting in a suppression of immune response.[152]

Cannabinoids have been investigated for neuroprotective properties and show potential as a treatment for Alzheimer's disease. A recent extensive study reported that a number of cannabinoids including CBN promote neuroprotection through the removal and degradation of preformed interneuronal amyloid β, through reduction of oxidative damage, and from the loss of energy or trophic support.[153] Interestingly, beneficial effects were observed for both neurons with and without cannabinoid receptors, suggesting a multimodal mechanism of neuroprotection. Pure CBN was shown to reduce cell death due to oxytosis (EC_{50} = 610 nM) in mouse HT22 hippocampal cells on its own. However, a significant synergistic response in the same HT22 cell line was observed in combination with Δ^9-THC. While not entirely clear, it is thought that the synergistic effect is due to enhanced agonism of the CB1 receptor.[153]

C. sativa has been used as a traditional treatment for metabolic diseases such as diabetes in South African indigenous populations.[154] CBN has been shown to have anticoagulant properties in a lean and obese rat model for diabetes.[154] Similar to Δ^9-THC, CBN has been shown to promote hyperphagia and increase food consumption in male rats. Although CBN is not as potent a CB1 agonist relative to Δ^9-THC, it has the advantage of not inducing Δ^9-THC-associated psychotropic effects. This feature allows for higher doses of CBN to be administered without the adverse side effects associated with a high dose of Δ^9-THC. A strong synergistic effect on hyperphagia was observed when CBN and Δ^9-THC were used in combination. Coadministration of CBN could be therapeutically advantageous for appetite stimulation, particularly if this allows for administration of a lower dose of Δ^9-THC to avoid undesired side effects.[155]

The antibacterial properties of CBN have been reported. CBN showed potent MICs of 1 μg/mL against a variety of methicillin-resistant *S. aureus* (MRSA) strains, although the mechanism of action is currently unknown.[72,156]

CBN has been shown to be an inhibitor of multidrug P-glycoprotein transporters such as ABCG2.[157] CBN is metabolized to the primary metabolites 11-hydroxy-CBN (**25**),[158] catalyzed by CYP2C9,[159] and 8-hydroxy-CBN (**26**),[160] catalyzed by CYP3A4. The major *in vivo* metabolite of CBN is **25**. This primary metabolite has been shown to have increased pharmacological activity relative to CBN.[161] In mouse models of catalepsy, hypothermia, locomotor activity, and pentobarbital-induced sleeping time showed that **25** was more active in each test compared to CBN, although not as potent as Δ^9-THC.[161]



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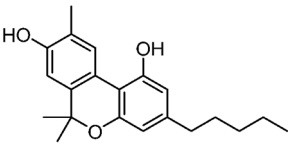

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CBN is an inhibitor of CYP2J2, the primary cytochrome P450 expressed in cardiac tissue. CYP2J2 is responsible for the oxidation of endogenous cannabinoids to cardioprotective epoxides of anandamide (EET-EAs). Inhibition of CYP2J2 is associated with lower levels of EET-EA.[162] The data for CYP inhibition by CBN is shown in Table 10.

Table 10. Metabolic Targets of CBN

CYP isoform	IC_{50} (μM)	K_i (μM)	type of inhibition
CYP2J2[162]	19	n/a	noncompetitive
CYP2A6[136]	30.4	1.0	noncompetitive
CPY2B6[136]	n/a	2.55[a]/39[b]	mechanism based/mixed inhibition

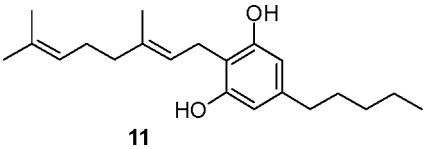
[a]Inhibition of 7-benzoxyresorufin *O*-debenzylase. [b]Inhibition of coumarin 7-hydroxylase.

CBN is a potent inhibitor of the hepatic esterase CES1 (carboxylesterase 1), K_i = 263 nM. CES1 is responsible for >80% of hydrolase-associated metabolism of many prescription drugs in the human liver.[163] To date, there is minimal data on the human pharmacokinetics for CBN. The strong CES1 inhibition should be taken into consideration when evaluating the therapeutic potential of CBN at physiologically relevant plasma levels.

In summary, CBN has a good safety profile in preclinical studies. As well, CBN has demonstrated promising results in animal models for sleep disorders, for appetite stimulation, and as a potential immunosuppressive agent, warranting further investigation. Very little is known about the phytocannabinoid CBV. Given the structural similarity to CBN and Δ^9-THCV, investigation of the *in vivo* pharmacology of CBV would be of interest.

CANNABIGEROL

Cannabigerol (**11**, CBG) is a monocyclic cannabinoid containing a five-carbon aliphatic side chain and a 10-carbon



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linear geranyl chain. CBG was first isolated by Gaoni and Mechoulam in 1964.[164]

CBG is a regulator of endocannabinoid signaling, binding to both CB1 and CB2 with low micromolar affinity as shown in Table 11. Radioligand binding assays with human CB1 and CB2

Table 11. Cellular Receptor Activity of CBG

receptor	EC_{50} (μM)	IC_{50} (μM)	K_i (μM)
CB1	>10[a]	1.1[b]	0.9[b]/3.1[c]/1.04[d]/0.38[f]/0.44[h]
CB2	1.1[k]	0.85[b]	0.15[b]/2.9[c]/1.22[d]/2.7[e]/2.6[g]/0.33[i]
α_2-adreno receptor	0.0002[j]/0.072[k]	n/a	n/a

[a][^{35}S]-GTPγS binding assay.[121] [b][^{3}H]-**20** binding assay in HEK293 cells.[121] [c][^{3}H]-**20** binding assay in Sf9 cells.[118] [d][^{3}H]-**20** binding assay in human receptor transfected to CHO cells.[165] [e][^{3}H]-**21** binding assay in human receptor transfected to CHO cells.[165] [f][^{3}H]-**20** binding assay in MF1 cells.[166] [g][^{3}H]-**20** binding assay in human receptor transfected into CHO cells.[166] [h][^{3}H]-**20** binding assay in mouse brain cells.[90] [i][^{3}H]-**20** binding assay in mouse spleen cells.[90] [j]CBG (10 nM) on [^{35}S]-GTPγS binding to MF1 mouse membrane.[166] [k]CBG on electrically invoked contractions in MF1 mouse vas deferens.[166]

receptors expressed in CHO cells using the synthetic ligand [^{3}H]-**20** or [^{3}H]-**21** revealed that CBG binds to CB2 in the low micromolar range. In the CB1 assay, low micromolar binding affinity was reported using the [^{3}H]-**20** binding assay. However, CBG did not displace [^{3}H]-**21** in the competitive CB1 binding assay.[165] CBG binds to the orthosteric site of CB2 and is a partial agonist. Similar results of low micromolar affinity were observed with binding assays of CB1 and CB2 receptors expressed in HEK293 cells using [^{3}H]-**20**. In a [^{35}S]GTPγS functional assay, CBG did not induce a response with the CB1 receptor; however, low micromolar efficacy was reported for CB2.[121]

The effect of CBG on cell signaling in HEK-293T expressing both CB1 and CB2 receptors has been reported. CB1−CB2 heteromeric cell models show negative cross-talk in cell signaling events. In this model, CBG was shown to influence cAMP levels as measured by dynamic cell mass redistribution; however, ERK1/2 phosphorylation or β-arrestin recruitment was not observed.[165] The minimal response on ERK1/2 phosphorylation was also observed in a LPI-mediated pERK stimulation assay as a measure of GPR55 activity with an observed EC_{50} of 2.16 μM.[97] CBG may also indirectly modulate CB1 and CB2 activity as the cannabinoid inhibits the uptake anandamide into RBL-2H3 cells at low micromolar levels.[49]

CBG binds to other receptors such as 5-HT_{1A} and α2-adrenoceptor.[166] The activity was determined through the observation that CBG significantly enhances [^{35}S]GTPγS binding to mouse MF1 membranes and acts as an inhibitor of electronically induced contractions of mouse vas deferens. CBG exhibits agonism of α_2-adrenoceptor and antagonism of 5-HT_{1A}.[166] *In vivo* activation of α_2-adrenoceptor by CBG was reported to reduce inflammatory pain in mice. Antinoception was achieved by treatment with CBG (10 mg/kg, i.p.) of mice injected with either formalin or λ-carrageenan in hind paw to induce pain. Modulation of the α_2-adrenoceptor was determined to be a contributing factor, as the nociceptive effect was reversed by dosing the mice with the α_2-adrenoceptor antagonist yohimbine (1 mg/kg, i.p.).[167] *In vivo* antagonism of 5-HT_{1A} was determined by the ability of CBG to reverse the antinausea effect of CBD.[168] Both CBD and the 5-HT_{1A} agonist 8-OH-DPAT were shown to suppress LiCl-induced nausea in rats. However, if the rats were pretreated with CBG (5−10 mg/kg, i.p.), the antinausea effect of both CBD and 8-OH-DPAT was inhibited.[168] This study suggests that the use of cannabis, particularly products containing CBG, may be less effective as a treatment for nausea than an individual cannabinoid with antinausea properties.

CBG has been reported to modulate the activity of a number of TRP ion channels as shown in Table 12. CBG was shown to

Table 12. Ion-Channel Activity of CBG

ion channel	EC_{50} (μM)	efficacy (%)	desensitization IC_{50} (μM)
TRPV1[a]	1.3	34	2.6
TRPV2[b]	1.7	74	1.5
TRPV3[c]	1.0	18	66
TRPV4[d]	5.1	24	1.3
TRPA1[e]	0.7	100	13
TRPM8[f]	n/a	n/a	0.16

[a]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 0.1 μM capsaicin. [b]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 3 μM lysophosphatidylcholine. [c]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 1 mM carvacrol. [d]Efficacy as a percentage effect of 4α μM ionomycin and desensitization in the presence of 1 μM α-PDD. [e]Efficacy as a percentage effect and desensitization in the presence of 100 μM isothiocyanate. [f]Desensitization in the presence of 0.25 μM icilin.

be an agonist of TRPA1, TRPV1, TRPV2, TRPV3, and TRPV4 expressed in HEK-293 cells and an antagonist to icilin activation of TRPM8. EC_{50}s and IC_{50}s in the submicromolar and low single digit micromolar range were reported for purified CBG.[49,102,169] A significant additive agonistic effect for TRPM8A was observed for a CBG-dominant botanical extract however. The IC_{50} for CBG alone was 0.16 μM. The extract elicited a 5-fold decrease in the IC_{50} to 0.034 μM, indicating that other unknown phytochemicals in the extract are contributing to the pharmacological activity. No significant difference between purified CBG and CBG dominant extracts was reported for any of the other ionotropic channels tested.[49]

CBG displays antimicrobial properties against a number of bacterial and fungal strains.[170] However, CBG was only reported to be effective against Gram-positive bacteria. In particular, CBG was effective against MRSA with reported MICs of 1−2 μg/mL.[72] This level of potency is in the range of approved antibiotics such as fluoroquinolones[171] and linazolide.[172]

CBG is reported to be an inhibitor of one of the enzymes involved in the bacterial fatty acid biosynthesis, enoyl-ACP-(acyl-carrier-protein) reductase (InhA). InhA is a validated therapeutic target for *M. tuberculosis* and its homologue FabI is a known target of the antibacterial agents triclosan[173] and AFN-1252.[174] Induced-fit molecular docking studies in a model based on the crystal structure of InhA showed CBG binds in a similar manner to the phenolic-based InhA inhibitor 5-pentyl-2-phenoxyphenol (5-PP). A similar hydrogen-bonding network to one of the phenolic moieties of CBG was observed, and the saturated aliphatic chain of CBG occupied the same hydrophobic pocket as the five-carbon chain of 5-PP. CBG inhibited InhA *in vitro* at an IC_{50} of 5.2 μM.[175] While inhibition of fatty acid synthases may contribute to the antimicrobial activity of CBG, a recent report revealed plant cannabinoids disrupt biofilm formation by targeting the bacterial outer cell

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membrane.[156] CBG was shown to inhibit biofilm formation in MRSA with reported MICs of 0.5 μg/mL as well as eradicate slow-growing persister cells. Of interest was the low incidence of acquired resistance observed with CBG when MRSA cells were challenged repeatedly at 2−16× MIC. As well, a MIC_{90} of 4 μg/mL was reported for a range of 96 clinical isolates of MRSA. CBG was also effective in a mouse systemic infection model. CBG (100 mg/kg, i.p.) was administered immediately following *S. aureus* USA300 infection in a single dose. The treatment was well-tolerated and resulted in a 2.8-$\log_{10}$ reduction in bacterial load compared to untreated control. The *in vivo* efficacy was also comparable to treatment with vancomycin (100 mg/kg, i.p.) in the same systemic infection model. Similar to previous reports, CBG did not show any antimicrobial efficacy toward Gram-negative strains, likely due to reduced membrane permeability. However, CBG did show moderate efficacy (MIC 4 μg/mL) in *E. coli* when administered in combination with sublethal levels of the cell permeability enhancing agent polymyxin B.[156]

CBG has reported anti-inflammatory properties. It has been investigated as a treatment for IBD[176] and colitis[73] in preclinical models. In a DNBS-induced mouse model of colitis, CBG reduced both nitrite production in macrophages and oxidative stress in intestinal epithelial cells. CBG was shown to be both preventive (1 mg/kg, i.p.) and curative (30 mg/kg, i.p.) in the DNBS model.[176] The anti-inflammatory effect was assessed by measurement of colon weight/length ratio and neutrophil infiltration by myeloperoxidase activity. In a meta-analysis of cannabinoid studies on IBD, CBG was determined to have the strongest effect of any phytocannabinoid studied on MPO activity and intestinal inflammation.[73]

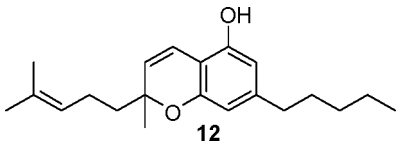
Two hallmarks of neurodegenerative disease are inflammation and oxidative stress, leading to neuronal cell death. As CBG was previously shown to attenuate both of these processes, it has been investigated for neuroprotective properties.[177] When NSC-34 motor-neuron-like cells were exposed to LPS-stimulated macrophages, pretreatment of the cells with CBG was shown to both increase cell viability and decrease apoptosis compared to untreated cells. Also observed was a significant decrease in levels of the proinflammatory cytokines IL-1β, IFN-γ, and TNF-α as well as protein levels of PPARγ. The attenuation of inflammation was coupled with a reduction in oxidative stress in this assay. Expression levels of both nitric oxide synthase (iNOS) and superoxide dismutase 1 (SOD1) were reduced upon pretreatment with CBG.[177] The neuroprotective properties of CBG were also investigated in a mouse model for Huntington's disease.[178] Mice were treated with 3-nitropropionate (3-NP) to induce Huntington's-like symptoms such as dystonia. As well, 3-NP treatment induces elevated expression levels of the proinflammatory cytokines TNF-α and IL-6 and the pro-inflammatory enzymes iNOS and COX-2. Treatment with CBG was shown to attenuate the levels of these inflammation markers. Unfortunately, in a transgenic mouse model of HD, the effects of CBG were not significant, suggesting CBG alone may not be an effective therapeutic for HD. However, CBG may be beneficial in combination with other phytocannabinoids or anti-inflammatory agents that show promising results in models for HD.[178]

In addition to being reported as a potential antitumor agent,[179] CBG has also been investigated as a treatment from chemotherapy-induced cachexia.[180−182] Purified CBG and a CBG-dominant extract were shown to induce hyperphagia in Lister rats treated with CBG (120−240 mg/kg, p.o.). The treated rats were observed to feed more frequently with an increase in feeding duration or individual meal consumption.[180,181] The CBG extract, which contained 72% CBG, displayed similar efficacy to pure CBG. However, the extract showed increased potency in the model (maximal effect 120 mg/kg for the extract vs 240 mg/kg for pure CBG). The hyperphagia may be related to CBG agonism of TRPV1 and/or α_2-adrenoceptor.[180] In a cisplatin-induced cachexia model in Lister rats, administration of CBG (120 mg/kg, p.o.) reduced weight loss and increased food intake over 72 h, suggesting CBG as a treatment option for chemotherapy-induced muscle atrophy and weight loss.[182]

In summary, CBG displays interesting anti-inflammatory properties in preclinical rodent models for both IDB and colitis. CBG also shows potential for the treatment of chemotherapy-induced cachexia. Hyperphagia was observed for both pure CBG and a CBG-dominant botanical extract and should be investigated in additional preclinical models. The CBG extract displayed an apparent increase in potency over CBG itself, suggesting a potential synergistic benefit with other components of the extract. Of note however is the observation of the beneficial effect of a CBG extract may be indication specific. It was observed that CBG can potentially attenuate the positive antinausea effect of other cannabinoids such as CBD. While this effect is shown in a rodent model, it is worth noting that some chemotherapy patients opt for cannabis-based medicine products to treat chemotherapy-induced nausea and vomiting. Of significant interest is the potent antimicrobial properties of CBG against drug-resistant strains of *S. aureus*. Further investigation of CBG against a wider panel of clinically relevant bacterial infections should be conducted. In addition, CBG is present in many commercially available cannabis-based products, necessitating the need for studies of how CBG interacts with cytochrome P450 enzymes.

CANNABICHROMENE

Cannabichromene (**12**, CBC) is a cannabinoid that was first isolated from *C. sativa* in 1966.[183,184] CBC belongs to a unique



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class of cannabinoids in which the core structure consists of a benzopyran (chromene) moiety.[185] CBC carries a stereocenter on the benzopyran ring. However, CBC isolated from cannabis is not enantiopure, but scalemic. The enantiomeric excess as determined by chiral-phase HPLC has been reported to range from 25% to 66%; however, the absolute configuration of the major enantiomer remains unknown.[186,187]

CBC is reported to bind to both the CB1 receptor and CB2 receptor with K_i = 0.71 and 0.26 μM.[118] Although CBC binds with high affinity, no activity was observed in a CB1 functional assay. With respect to CB2, CBC has been reported as an agonist.[188] CBC was shown to promote cellular hyperpolarization in mouse AtT20 cells transfected with human CB2, albeit at a therapeutically irrelevant concentration of 30 μM.[188]

CBC has been shown to activate the ionotropic channels TRPV3, TRPV4, and TRPA1, and the channels are desensitized to subsequent treatment as shown in Table 13. Agonism of TRP channels expressed in HEK-293 cells was observed for TRPV3, TRPV4, and TRPA1. Low single digit micromolar (TRPV3) and submicromolar EC_{50}s and IC_{50}s (TRPV4 and TRPA1) were

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Table 13. Ion-Channel Activity of CBC

ion channel	EC_{50} (μM)	efficacy (%)	desensitization IC_{50} (μM)
TRPV1[a]	24.2	<10	>50
TRPV2[b]	n/a	<10	6.5
TRPV3[c]	1.9	20	201
TRPV4[d]	0.6	23	9.9
TRPA1[e]	0.09	119	0.37
TRPM8[f]	n/a	n/a	40.7

[a]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 0.1 μM capsaicin. [b]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 3 μM lysophosphatidylcholine. [c]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 1 mM carvacrol.[102] [d]Efficacy as a percentage effect of 4 μM ionomycin and desensitization in the presence of 1 μM 4αPDD. [e]Efficacy as a percentage effect and desensitization in the presence of 100 μM isothiocyanate. [f]Desensitization in the presence of 0.25 μM icilin.

reported for purified CBC. There was no significant change in activity observed in the activity of an equivalent experiment with a CBC-dominant botanical extract.[49,102]

The anti-inflammatory properties of CBC have been reported dating back to the 1980s at a high dose of 120 mg/kg p.o. in rats.[189,190] CBC was reported to induce an anti-inflammatory response through downregulation of nitric oxide synthesis in LPS-stimulated macrophages in mouse models of colitis.[191] The positive effect was observed for dosing as low as 1 mg/kg (i.p.). Similarly, CBC was shown to reduce intestinal hypermotility in a croton oil-induced mouse model. The mode of action for the positive response induced by CBC in these models is not fully understood; however, it did not appear to result from modulation of either CB1, CB2, or TRPA1.[192] The lack of CBC mediation of CB1 and CB2 receptors was also reported in results of the murine tetrad test, as CBC activity was not blocked by either the CB1 inverse agonist **5** or the CB2 inverse agonist **24**.[193] CBC alone only exhibited cataleptic, hypothermic, antinociceptic, and locomotor suppression effects at a high dose of 100 mg/kg, although the analgesic response in the tail-flick model was minimal.[193,194] However, in combination with Δ^9-THC there was a significant additive effect in the LPS-induced paw edema model at 40 mg/kg CBC and 4 mg/kg Δ^9-THC compared to control.[193]

CBC was reported to show increased latency in a rat tail-flick model for pain through intraventrolateral−periaqueductal gray microinjection at a concentration of 6 nM. The antinociception activity was accompanied by an increase in intracellular levels of anandamide and 2-AG, indicating the compound is likely inducing analgesic effects via multiple modalities.[195]

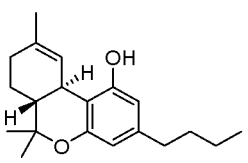
The CNS depressant properties of CBC were reported in a mouse sedation study. Acute dosing of CBC (50 mg/kg, i.p.) was shown to prolong the sleep time of hexobarbital-treated mice. The effect was not observed for prolonged dosing (10 mg/kg, i.p.) over 7 days.[196]

Neuroprotective properties of CBC were examined with respect to the viability of neuronal stem/progenitor cells (NSPCs). CBC was reported to affect intracellular signaling by upregulating ERK1/2 phosphorylation and nestin and downregulation of glial fibrillary acidic protein and increased expression levels of adenosine A1 receptor. Increased levels of ATP and adenosine were observed in CBC-treated NSPCs during differentiation. This effect stimulated purinergic signaling and enhanced cell viability. In contrast, CBC was shown to inhibit differentiation into astrocytes, which is potentially detrimental to neurogenesis. Further studies of CBC on neuronal stem cells *in vivo* to determine the effect on neurogenesis should be conducted.[197]

In summary, there is preclinical evidence in rodent models to support further investigation of CBC in the treatment of inflammatory pain and as a potential neuroprotective agent. Isolation and purification of each CBC enantiomer is highly important to determine the specific activity of each stereoisomer.

Δ^9-TETRAHYDROCANNABUTOL AND CANNABIDIBUTOL

Analogues of both CBD and Δ^9-THC that carry a four-carbon butyl side chain have been reported as minor components of *C. sativa*.[198,199] The structures of each were confirmed by comparison of purified isolates with samples prepared by stereoselective synthesis using high-resolution mass spectrometry, NMR, IR, UV, and circular dichroism.[74,200] Initially described as THC-C4 and CBD-C4, they have recently adopted the International Nonproprietary Name of Δ^9-tetrahydrocannabutol (**13**, Δ^9-THCB) and cannabidibutol (**27**, CBDB).[74,200]



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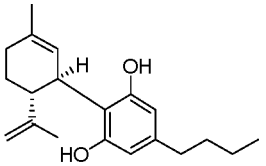

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No pharmacological data for CBDB have been reported to date; however, *in vitro* and *in vivo* activity for Δ^9-THCB has been reported. As the structure of Δ^9-THCB differs only in the reduced length of the aliphatic side chain by one methylene unit, it is not surprising that the biological activity mimics closely Δ^9-THC. In the human CB1 and CB2 radioligand receptor binding assay using [^{3}H]-**20** and [^{3}H]-**21**, Δ^9-THCB was found to bind with high affinity, exhibiting K_i values of 15 and 51 nM, respectively. Comparison of the binding affinity with both Δ^9-THC and Δ^9-THCV shows that the CB1 value is similar to Δ^9-THC (5−40 nM) and five times more active than Δ^9-THCV (75 nM). With respect to CB2 binding affinity, there is no significant difference in binding affinity among Δ^9-THCB, Δ^9-THC, and Δ^9-THCV.[74]

The *in vivo* pharmacological effect of Δ^9-THCB was evaluated by a tetrad behavioral test in mice. These four tests evaluate physiological effects related to cannabinoids or cannabimimetic activity that include catalepsy, analgesia, changes in rectal temperature, and changes in behavioral activity. Mice were dosed with Δ^9-THCB (10 mg/kg and 20 mg/kg, i.p.). The tetrad results were consistent with partial agonism of CB1 and disruption of endocannabinoid signaling.[74]

The cannabinoid was also evaluated in a mouse formalin-induced nociception test. At subcutaneous doses of 3 mg/kg, both the first and second phases of the formalin test were reduced. When mice were pretreated with the CB1 inverse agonist **21**, the analgesic effect of Δ^9-THCB was negated. This again suggests Δ^9-THCB-mediated disruption of endocannabinoid signaling.[74] The potential interaction of Δ^9-THCB with other nociceptive pharmacological targets such as TRP receptors should be investigated.

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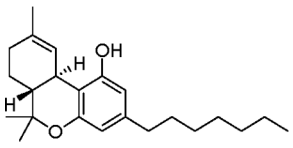
Δ⁹-TETRAHYDROCANNABIPHOROL AND CANNABIDIPHOROL

The length of the lipophilic side chain of natural and synthetic cannabinoids plays a critical role as a major pharmacophore



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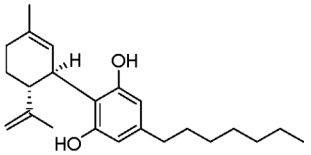

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required for CB1 and CB2 activity.[201] Structure−activity relationship (SAR) studies on synthetic Δ⁸-THC analogues varying both the length and substitution pattern of the side chain have been reported.[202] Side chains of five to eight carbon units were found to be optimal for CB1 activity. C7 and C8 analogues generally showed the strongest binding affinity to CB1 in the single digit to subnanomolar range.[203] Side chain lengths greater than eight carbon units resulted in a decline in CB1 activity.

Most naturally occurring phytocannabinoids present in *C. sativa* contain side chain units of five or few carbons. Recently, naturally occurring phytocannabinoids carrying a seven-carbon side chain were identified.[75] The two new phytocannabinoids were isolated and the structures confirmed by stereoselective synthesis. They were found to be direct structural analogues of Δ⁹-THC and CBD and subsequently named Δ⁹-tetrahydrocannabiphorol (**14**, Δ⁹-THCP) and cannabidiphorol (**28**, CBDP).

The CB1 and CB2 binding affinity of Δ⁹-THCP was determined using [³H]-**20** and [³H]-**21** binding assays. Δ⁹-THCP was found to bind with high affinity to both CB1 and CB2 with measured K_i values of 1.2 and 6.9 nM, respectively. Similar to observations from the CB1 SAR study, the longer side chain resulted in a greater than 30× and 60× increase in affinity to CB1 relative to Δ⁹-THC and Δ⁹-THCV. With respect to CB2, a 5× and 10× increase in affinity relative to Δ⁹-THC and Δ⁹-THCV was observed.[75]

The cannabimimetic activity of Δ⁹-THCP was evaluated *in vivo* by a tetrad test in mice. The results showed induction of catalepsy and analgesia as well as decreased locomotor activity and rectal temperature. These results were consistent with outcomes expected for a full agonism of the CB1 receptor and endocannabinoid signal modification. The *in vivo* activity of Δ⁹-THCP at 5 mg/kg in three of the four tetrad tests is similar to the effect of a higher dose of Δ⁹-THC at 10 mg/kg.[75]

The level of Δ⁹-THCP in other varieties of *C. sativa* is currently unknown. However, the increased potency of Δ⁹-THCP relative to Δ⁹-THC suggests the possibility of synergistic or enhanced psychotropic effects of some varieties of cannabis may derive from Δ⁹-THCP or from potentially other highly active phytocannabinoids. The bioavailability and PK/PD profile of Δ⁹-THCP are also unknown. The structural and lipophilic character of Δ⁹-THCP suggests the pharmacodynamic profile would be similar to that of Δ⁹-THC. Currently, there is increased focus on cannabis formulations that are delivered orally. As a BCS class II molecule of high permeability and low solubility, Δ⁹-THC is known to have low bioavailability (<25%). Δ⁹-THCP falls into the same class. The advantage of developing Δ⁹-THCP compared to Δ⁹-THC for oral delivery is the potential for lower drug load to deliver the same pharmacological response. To date, no pharmacological data have been reported for CDBP.

In summary, the animal and receptor data suggest Δ⁹-THCB and Δ⁹-THCP have potential for the treatment of pain. Further studies are required to isolate which specific subtypes of pain respond to these cannabinoids.

FUTURE DIRECTIONS

The upregulation and downregulation of endocannabinoid processing by individual cannabinoids and their promiscuity to act on numerous receptors, transporters, and enzymes make it challenging to elucidate the therapeutic effects of individual phytocannabinoids.[204] Finding an ideal phytocannabinoid composition that imparts the optimal therapeutic benefit is an even greater challenge. Standardization of cannabis extracts also presents a challenge because of a wide variety of cannabis cultivars, variability in growing conditions, method of plant extraction, and route of administration of the product, which may result in different pharmacological outcomes.[205,206] While the ECS is complex, details are emerging on the mechanism by which both endogenous and exogenous cannabinoids activate receptors and metabolic enzymes and how intracellular and extracellular transmembrane transporters conduct the flow of lipids through the ECS signaling network.[31]

The data supporting the therapeutic benefit of Δ⁹-THC and CBD is strong and continues to grow. However, focusing solely on only these two major phytocannabinoids would limit medical cannabis from reaching its full potential, particularly if patients want to avoid the psychoactive effects associated with Δ⁹-THC.

The next wave of cannabinoid therapeutics is also rich with data to support further research. Crystal structures of both CB1 and CB2 offer a tool to predict the binding mode of phytocannabinoids to these receptors and serve as a platform for structure-based drug design. Currently, Δ⁹-THCV and CBDV hold the most promise based on clinical data for safety and tolerability and in the treatment of diabetes and neurological disorders such as epilepsy and autism spectrum disorder. Preclinical studies support additional work to assess the potential use of phytocannabinoids for the treatment of inflammation (Δ⁹-THCV, CBDV, CBG, CBN, and CBC) and neurological disorders (Δ⁹-THCV, CBDV, and CBG) or as an antibiotic (CBG). Newly discovered cannabinoids such as Δ⁹-THCP and CBDP are promising Δ⁹-THC and CBD surrogates that may offer similar therapeutic benefit at lower doses.

The preponderance of data presented herein was collected using individual phytocannabinoids. This is a critical first step in determining the pharmacology and pharmacodynamics of these interesting natural products, particularly in light of multitarget modality. Sativex was a great initial demonstration of the benefit of a two-component formulation of CBD and Δ⁹-THC, yet does not go so far as to prove that these cannabinoids are acting in an entourage-like fashion similar to endogenous cannabinoids.[11] The potential benefits and risks of multicomponent cannabis-based formulations still remain largely an enigma, with most of the positive results coming from anecdotal reports. Empirically, there are data to support the potential benefits of Δ⁹-THCV attenuation of the negative physiological effects of Δ⁹-THC or CBD/CBDV combinations for epilepsy. Additionally, there is *in vitro* data to support the synergistic enhancement of potency of cannabis extracts in TRP assays over single compounds.[49,102] In contrast, not all multicomponent cannabinoid preparations are beneficial. As shown, CBG potentially counteracts the effectiveness of CBD as an antinausea agent, and Δ⁹-THCV-dominant extracts were less effective than Δ⁹-THCV alone in models of hyperphagia and inflammation. Clearly, the use of

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mono- or multicomponent cannabinoid therapies will be indication specific. Phytocannabinoid synergism is very challenging to test clinically as part of a randomized controlled trial, considering the vast number of potential cannabinoid combinations and formulations. Insight into the effectiveness of phytocannabinoid combinations is more likely to be determined by critically evaluating real world evidence studies that are conducted using standardized products.[207]

While the prescription components of cannabis present in Marinol and Epidiolex have an acceptable safety profile for some therapeutic indications, the same cannot be assumed for all components of cannabis. Safety studies must be conducted with each individual cannabinoid, particularly when the therapeutically relevant dose is far greater than the levels generally available in the cannabis plant. The challenge of obtaining sufficient quantities of compound to conduct clinical studies will likely be met through biosynthesis or total chemical synthesis rather than extraction. The future for cannabis-based medicine will be to strengthen the *in vivo* data of this next generation of phytocannabinoids, either individually or in combination, to support their evolution into therapeutics of value to patients.

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Notes

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