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Outlook

Between Science and Big Business: Tapping Mary Jane's Uncharted Potential

Katharina Grotsch and Valery V. Fokin*

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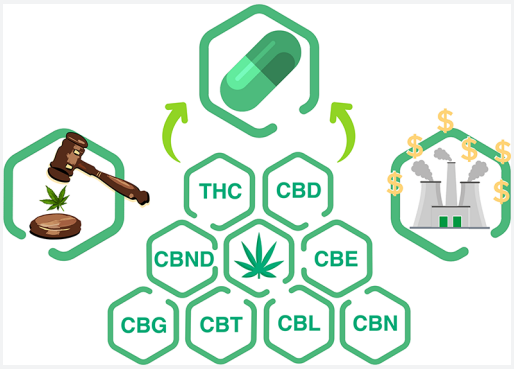
ABSTRACT: At the intersection of science and medicine, government policy, and pop culture, cannabis has prompted society since the beginning of recorded history. And yet, there is comparatively little replicable data on the plant, its constituents, and their capacity to modify human physiology. Over the past decades, several findings have pointed toward the importance of the endogenous cannabinoid system in maintaining homeostasis, making it an important target for various diseases. Here, we summarize the current state of knowledge on endogenous- and plant-based cannabinoids, address the issues related to cannabinoid-based drug discovery, and incite efforts to utilize their polypharmacological profile toward tackling diseases with a complex underlying pathophysiology. By fusing modern science and technology with the empirical data that has been gathered over centuries, we propose an outlook that could help us overcome the dearth of innovation for new drugs and synchronously redefine the future of drug discovery. Simultaneously, we call attention to the startling disconnect between the scientific, regulatory, and corporate entities that is becoming increasingly evident in this booming industry.



1. INTRODUCTION

New molecular entities (NME) are produced at the same rate today as they were 50 years ago, with the industry averaging about one NME every six years despite unprecedented pharmaceutical spending.[1,2] Over 96% of drug development efforts result in failure, with especially high rates of failure for diseases with a poorly understood pathophysiology.[3] The burden of this expensive and time-consuming R&D process often results in site closures, job loss, and inflated prices of the few drugs that surmount the demands of regulatory approval.[3] Perhaps even more troubling is the fact that it often discourages scientific innovation in favor of compounds with identical mechanisms of action to existing drugs (also known as "me too drugs") and deters efforts to develop therapies for treatment-resistant conditions. But what exactly are the current shortcomings of the drug development process? And how can we make an effort to minimize cost and maximize progress?

Our quest to address these questions takes us back thousands of years to the first reports of a plant that has adorned us throughout most of documented history—*Cannabis sativa*. As one of the oldest plants cultivated by man, cannabis has played an important role in many ancient civilizations ranging all the way from China, to India, and the Middle East.[4−7] The world's oldest pharmacopoeia, the *pen-ts'ao ching*, reported its use for rheumatic pain, constipation, and disorders of the female reproductive system in as early as 2,700 B.C.[8] The use of cannabis for mind-altering and medicinal purposes was explored by the Assyrians around the second millennium B.C., where it was referred to as *ganzi-gun-nu* ("the drug that takes away the mind") and illustrated a central theme in Arab poetry of the Middle Ages.[9,10] In Europe, Cannabis was introduced by Napoleonic soldiers returning from Egypt and British soldiers returning from India.[4] Famous intellectuals of the era described the "groundless gaiety" and "distortion of colors and sounds", as well as dissociation of ideas, errors of time and space, and fluctuation of emotions, associated with smoking cannabis.[11] However, the inception of a rampant political movement that originated at the beginning of the 20th century led to prohibition of cannabis throughout Western civilization.[12] Concurrently, regional medical practices became reliant on a heavily regulated system comprised mainly of single-molecule therapeutics, creating the highly competitive drug marketplace we know today.[12]

In the mid-20th century, a multitude of scientific discoveries shed light on the quintessential role of the endogenous

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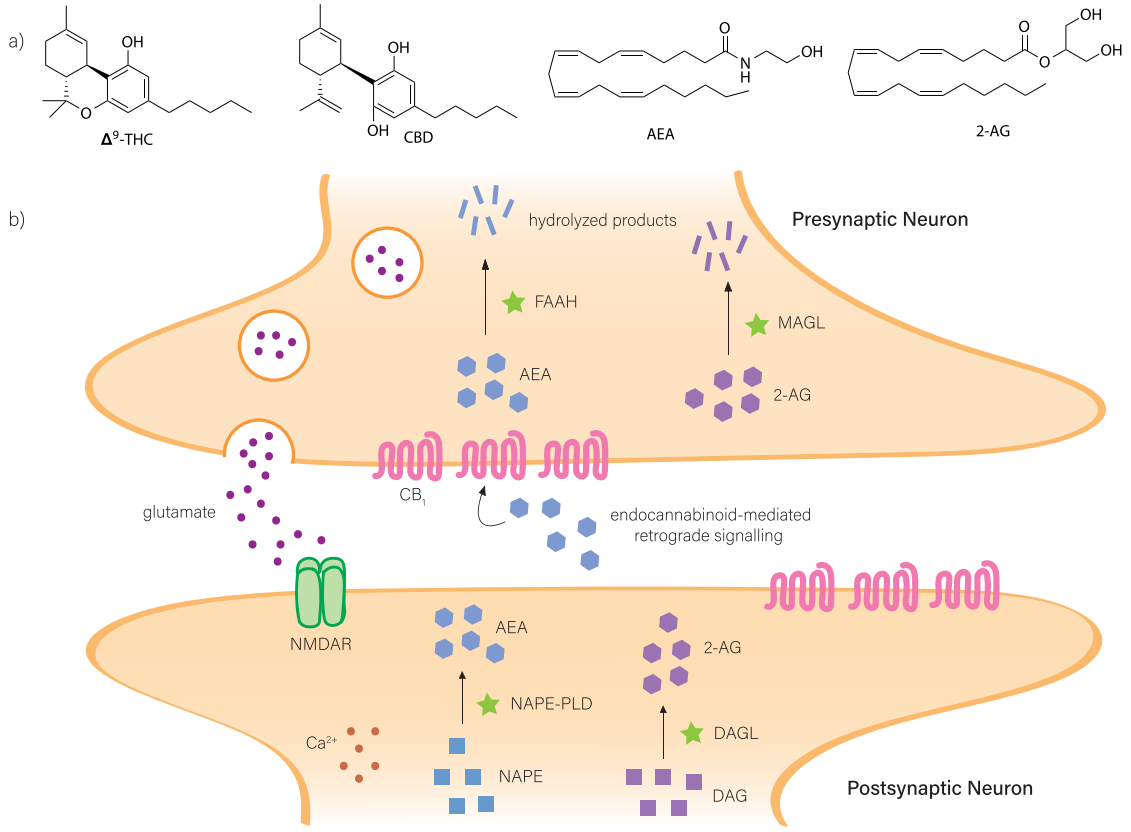

Figure 1. (a) Structures of the known phytocannabinoids Δ9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD), as well as the endogenous cannabinoids anandamide (AEA) and 2-arachidonyl glycerol (2-AG). (b) Schematic representation of the main components of the endocannabinoid system within the central nervous system (CNS). Here, glutamate release activates the NMDA receptor, leading to increased cytoplasmic calcium levels. Subsequently, the enzyme NAPE-PLD catalyzes the synthesis of AEA from NAPE, and DAGL catalyzes the synthesis of 2-AG from DAG. Release of AEA and 2-AG into the synaptic cleft triggers the activation of CB receptors at the presynaptic site and inhibits further neurotransmitter release. Once homeostasis is achieved, the endogenous cannabinoid molecules are degraded by their respective enzymes.

cannabinoid system (ECS) in maintaining homeostasis in the human body.[13−19] It is now known that the ECS is responsible for regulating sleep, appetite, stress, and memory among other things.[5] Unsurprisingly, it is an attractive target for the cure of various diseases, especially of the central nervous system (CNS).[18,20−22] As with opium poppies before, the study of an active component in cannabis has shed light on an endogenous system that controls various neurobiological functions, indicating significant promise for the development of novel pharmaceuticals.[23,24] Yet, relatively little progress has been made on exploring cannabinoids as therapeutic agents—despite their well-established safety profile. Is this a result of lacking scientific promise? Or is it simply a result of the multitude of social, political, economic, and technological developments that have shaped the world as it is today?

Here, we cursorily outline the role of the endocannabinoid system in regulating physiological functions to underline its importance and summarize the biological activity of known phytocannabinoids. With this background in mind, we attempt to understand to what extent the convoluted interplay of government regulations, economic developments, and shifts in the sociopolitical climate have influenced scientific progress. In doing so, we aim to highlight this underdeveloped area of research and propose a new outlook that amalgamates modern science with the empirical knowledge gathered over centuries, challenging the field of drug discovery as a whole.

2. THE ENDOCANNABINOID SYSTEM

The endogenous cannabinoid system (ECS) in its most rudimentary form is comprised of (a) the cannabinoid type I (CB_1) and cannabinoid type II (CB_2) cannabinoid *receptors*, (b) arachidonoylethanolamide (anandamide or AEA) and 2-arachidonyl glycerol (2-AG) as endogenous *ligands*, and (c) the *enzymes* involved in cannabinoid synthesis and degradation.[25,26] Its nomenclature is derived from the finding that various endocannabinoids and constituents of *Cannabis sativa* act on the same receptor targets.[4] In essence, the ECS provides protection against inflammatory and neuropathic stress, making it an attractive target for the treatment of chronic stress of the brain and body as a whole.[27] Given the dearth of effective medications for both chronic inflammation and neurological stress, there is a clear need for the development of new therapeutics to treat these conditions. For the purpose of this outlook, we will be focusing mainly on the endogenous cannabinoid system in the CNS. Importantly, alterations in the ECS are found in patients with most neurological diseases, outlining the critical role it plays and endorsing it as an important target for the development of new therapeutic agents for various CNS diseases including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), multiple sclerosis (MS), epilepsy, generalized anxiety disorder (GAD), obsessive compulsive disorder (OCD), social anxiety disorder (SAD), and post-traumatic stress disorder (PTSD).[28−31]

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2.1. Cannabinoid Receptors. The endogenous cannabinoid system consists of so far two identified G-protein coupled receptors (GPCRs), CB_1 and CB_2 that were named after their affinity for the agonist Δ^9-THC.[32,33] Both CB_1 and CB_2 are coupled through the $G_{i/o}$ family of proteins and are expressed both in the CNS and the immune system.[33−35]

CB_1 was first cloned by Tom Bonner's lab in 1990.[16] Autoradiographic studies have shown that CB_1 can be found mainly in the cerebral cortex, hippocampus, basal ganglia, and cerebellum—regions that are consistent with the known effects of cannabinoids on motivation and cognition.[16,34,36] Indeed, the physiological responses generally associated with Δ^9-THC consumption such as reduced stress, increased appetite, and euphoria, are generally attributed to activation of CB_1 receptors.[37−39]

The CB_2 receptor was first cloned in 1993 at the MRC Laboratory of Molecular Biology in Cambridge, England, and has a 44% sequence identity with CB_1.[17,32] Immunocytochemical evidence has identified the presence of CB_2 in spleen, thymus, tonsils, bone marrow, pancreas, mast cells, peripheral blood leukocytes, and several cultured immune cell models.[33,41] Although CB_2 is expressed mainly in the immune system, it is also present in the CNS, where it has been shown to control synaptic function and regulate synaptic plasticity, making it highly relevant target for many neurological disorders.[42,43]

2.2. Endocannabinoids. By inference, the presence of cannabinoid receptors indicates the existence of endogenous molecules that have the ability to modulate those receptors. These effects are mainly attributed to the two eicosanoids, AEA and 2-AG (Figure 1a).[44−47] The endocannabinoids (eCBs) are lipophilic, and, unlike most neurotransmitters, they are not stored in vesicles but rather synthesized "on demand" from membrane phospholipids as a result of increased intracellular Ca^{2+} levels at the postsynaptic site.[42,48] Their action is generally presynaptic rather than postsynaptic, meaning that once at their target site, eCBs bind to CB_1 receptors located at the presynaptic site in a retrograde manner, suppressing neurotransmitter release (Figure 1b).[33,48] Although they are inherently quite similar, the two ligands exhibit distinct functions in the ECS. While both AEA and 2-AG regulate presynaptic neurotransmitter release, the molecules mediate short-term and long-term synaptic plasticity in the brain by operating in phasic and tonic modes.[49] The available evidence suggests that AEA acts as the tonic signaling molecule, adapting slowly to stimulus and firing a sustained response, whereas 2-AG represents the phasic signal, adapting rapidly to stimulus and producing a more transient response during neuronal depolarization.[49] After the desired homeostatic response has been achieved, both AEA and 2-AG are removed from the synapse and degraded by their respective hydrolytic enzymes.[49]

2.3. Enzymes. Synthesis of 2-AG and other monoacylglycerols is catalyzed by diacylglycerol lipase α (DAGLα), and synthesis of anandamide and other N-acylethanolamines is catalyzed by N-acylphosphatidylethanolamine (NAPE)-specific phospholipase D-like hydrolase (NAPE-PLD).[50,51] The most notable and well-understood degradation enzymes in the endocannabinoid system are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), which hydrolyze AEA and 2-AG, respectively.[52,53] Experimental evidence indicates that FAAH is located primarily on the intracellular membrane of postsynaptic cells, whereas MAGL is generally located in presynaptic terminals in the vicinity of CB_1 receptors.[54,55]

2.4. Role of the Endocannabinoid System in the CNS. Entering the neurochemical, psychological, and philosophical realm of discussion, we are faced with three important questions about the endocannabinoid system: *how* do these components interact with each other to produce a physiological response?, *why*, from an evolutionary standpoint, do they work in this manner?, and *to what effect* do they influence our behavior?

To answer these questions, we must closely examine the known mechanisms of endocannabinoid signaling (Figure 1b). As previously mentioned, endogenous cannabinoids act as retrograde messengers to suppress neurotransmitter release. In other words, endocannabinoids are synthesized "on-demand" in response to neuronal stimulation and suppress the release of chemicals such as glutamate and GABA.[56,57] In essence, this molecular mechanism outlines the process of endocannabinoid-mediated synaptic plasticity.[58] The evidence for this is overwhelming, as three independent research groups in the early 2000s reported that postsynaptic depolarization-induced Ca^{2+} elevation in the hippocampus and cerebellar cortex triggers the postsynaptic synthesis of endogenous cannabinoids, which proceed to inhibit CB_1-mediated neurotransmitter release at the presynaptic site.[59−61] Since the early 2000s, eCBs have been shown to activate both short-term (depolarization-induced suppression of inhibition/excitation, or DSI/DSE) and long-term plasticity (long-term depression, or LTD) at synapses throughout the brain.[58,62] The most important and well-explored of these phenomena is LTD, which is defined by the reduction in neurotransmitter release upon binding of eCBs to CB_1 and has been reported in the dorsal striatum, nucleus accumbens, amygdala, and hippocampus among others.[63−68] The exact mechanisms underlying these changes are highly complex and still not fully understood. However, it is known that endocannabinoid-mediated LTD is a fundamental mechanism for inducing long-term changes to neural circuits and behavior.[62] Simply put, the endocannabinoid system exists to provide on-demand protection against excitotoxicity in CNS neurons.[69]

This brings us to the second question regarding the evolutionary purpose of the endocannabinoid system as a protective mechanism against fear, anxiety, and stress. Fear and anxiety are natural phenomena that occur as a result of a real or perceived threat, or the possibility of such a threat arising in the future.[18,40] Similarly, the stress response is a bodily reaction to this challenge in order to prepare it for upcoming danger, functioning as a protective mechanism that is essential to an organism's survival.[40] The body's response to stress consists of an autonomic and a neuroendocrine responses that are activated in parallel.[18] The autonomic nervous system consists of the sympathetic and parasympathetic nervous system, and functions mainly by using catecholamines like norepinephrine and acetylcholine as neurotransmitters.[70] In contrast, the neuroendocrine system is mediated by activation of the hypothalamic pituitary adrenal (HPA) axis, releasing cortisol, corticotropin, and other corticosteroids.[70] Although these mechanisms are integral in delegating the basic survival instinct, superfluous response to external stressors, especially when chronic, can prove detrimental to cognitive health and incite a shift in several neurobehavioral responses including anxiety, memory, pain sensitivity, and coping behaviors.[18,71,72] Therefore, it is vital that the domains of fear, anxiety, and stress

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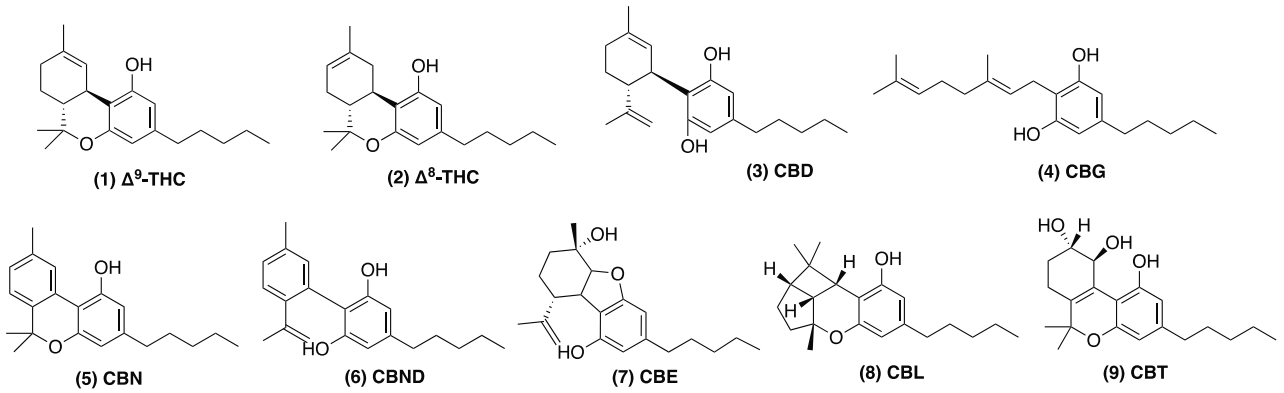

Figure 2. Chemical structures of the common phytocannabinoids (1) Δ^9-tetrahydrocannabinol, (2) Δ^8-tetrahydrocannabinol, (3) cannabidiol, (4) cannabigerol, (5) cannabinol, (6) cannabinodiol, (7) cannabielsoin, (8) cannabicyclol, (9) cannabitriol.

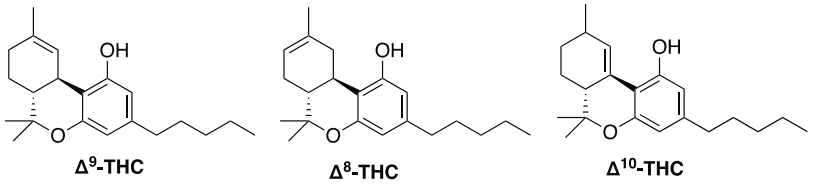

Figure 3. Some known isomers of THC.

are regulated by the endocannabinoid system in an effort to maintain homeostasis in a healthy brain.[40]

Lastly, it is important to touch upon the effects of endocannabinoid-mediated synaptic plasticity on human behavior. Several clinical and preclinical studies have been conducted in an effort to explore how the ECS acts as a buffer against the effects of stress. As previously discussed, the ECS controls several brain regions related to fear and anxiety, generally regulating overactivation. Acute exposure to stress results in an increase of FAAH activity and thus a reduction of AEA levels in the amygdala and prefrontal cortex. This leads to activation of the HPA axis and an increase in the concentration of 2-AG, which in turn inhibits the release of glutamate and GABA in the hypothalamus and prefrontal cortex, respectively.[40,73,74] However, the repeated exposure of the brain to nonhabituating, chronic stress results in desensitization of CB_1 receptor signaling.[40,75] This becomes important as chronic stress can trigger or exacerbate a variety of psychiatric disorders including schizophrenia and major depressive disorder (MDD).[76,77]

2.5. The Endocannabidiome. The endocannabinoid system, as currently defined, is an oversimplification of the complex action of mediators and alternative metabolic processes. The modulation of its components is part of a larger network known as the endocannabidiome.[78] This system spans from GPCRs (GPR55, GPR119), to ion channel receptors (TRPV1) and nuclear receptors (PPAR-γ), and includes mediators such as *N*-acyl amino acids and *N*-acyl neurotransmitters.[78−82] Notably, the existence of the endocannabidiome exposes the flaws of reducing a physiological response to confined ligand-target interactions. Despite the ever-evolving progress in science that has allowed us to "zoom in" on explicit mechanisms of interest, we must not forget that the human body is not composed of a combination of isolated systems but should instead be thought of as a complex web of highly intertwined molecular entities.

Particularly interesting is the interplay between the eicosanoid and endocannabinoid signaling systems. Although the two have traditionally been investigated separately, there are a multitude of factors pointing toward a potential biological dialogue.[83] Both the endogenous cannabinoids 2-AG and AEA, as well as other eicosanoids such as prostaglandins, thromboxanes, and leukotrienes are synthesized from arachidonic acid (AA).[84] In addition, the lipases that initiate both pathways respond to some of the same secondary messengers, meaning that they will be activated together, and some of the enzymes involved in eicosanoid biosynthesis can metabolize both AA and endogenous cannabinoids.[83] Interestingly, endocannabinoids can also be converted to a number of prostanoids—both prostaglandin (PG)-glyceryl esters as well as PG-ethanolamides (prostamides) can be formed from 2-AG and AEA, respectively.[85,86] Despite the mounting evidence that these two systems are deeply entangled, not much research has been done on the role of these pathways in human health and wellbeing.

3. PHYTOCANNABINOIDS

Having elucidated the function of the endocannabinoid system and explored the role of endogenous cannabinoids, it is more than fitting to take a closer look at their illustrious namesakes. The *Cannabis sativa* plant is distributed as hashish (resin from upper leaves and flower buds) and marijuana (dried leaves and flowering heads), which both contain a variety of cannabinoids and noncannabinoids.[87] There are over 500 known compounds and at least 120 unique phytocannabinoids that have been identified as of today. These can be divided into 10 subclasses; Δ^9- and Δ^8-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG), cannabinol (CBN), cannabinodiol (CBND), cannabielsoin (CBE), cannabicyclol (CBL), cannabitriol (CBT), and miscellaneous type (Figure 2).[12] Additionally, there are several other constituents in the plant that may or may not contribute to the overall pharmacological effect, including terpenes, nitrogenous com-

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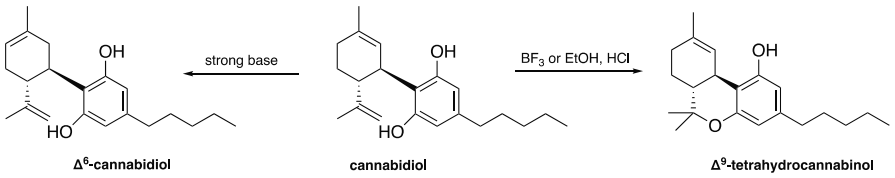

Figure 4. Some possible transformations for CBD and related compounds.

pounds, amino acids, proteins, enzymes and glycoproteins, sugars, hydrocarbons, simple alcohols and aldehydes, and steroids, among others.[12] Although many of the natural products in cannabis have been synthesized, isolated, and characterized, several questions remain open about the activity of these molecules and their possible synergistic interactions.

3.1. Δ^9-Tetrahydrocannabinol (Δ^9-THC). In 1964, Gaoni and Mechoulam reported the isolation of Δ^9-THC as the first structurally elucidated active component of *Cannabis sativa*.[13] There are several constitutional and stereoisomers of THC, but (−)-trans-Δ^9-tetrahydrocannabinol or (6aR, 10aR)-delta-9-tetrahydrocannabinol is the main plant-derived isomer and, by extension, also the most well explored. Interestingly, it is far less stable than its Δ^8 and Δ^{10} analogues, with Δ^{10} being the most stable as a result of the double bond in conjugation with the aromatic ring (Figure 3).

Δ^9-THC acts as a partial agonist on both CB_1 and CB_2, with K_i values in the low nanomolar range.[88] The psychoactive effects of Δ^9-THC are mediated by CB_1, and its potential immunological or anti-inflammatory effects are thought to be a result of CB_2 receptor agonism.[89] The effects of this molecule are fairly well studied, but the complexity of the interactions leaves several questions open. Effectively, it is known that Δ^9-THC perturbs GABA and glutamatergic neurotransmission in a similar fashion to endogenous cannabinoids, producing many of the common effects associated with consumption of cannabis.[88,89] Notably, however, neuronal CB_1 receptors are targeted in a less selective manner by phytocannabinoids than the respective endogenous cannabinoids.[89] Emerging evidence over the last two decades has shown that *in vivo* administration of Δ^9-THC can actually *increase* the release of certain neurotransmitters, i.e., acetylcholine in rat hippocampus, acetylcholine, glutamate, and dopamine in rat prefrontal cortex, and dopamine in mouse and rat nucleus accumbens.[89−91] These combined stimulatory−inhibitory influences could be responsible for the excitant and depressant effects of Δ^9-THC.[92,93]

The implications of Δ^9-THC administration on psychosis, addiction, and memory and cognition remain controversial. Generally, cognitive deficits observed from acute exposure to cannabis are transient.[88,94] In contrast, prolonged use is associated with more pronounced chronic deficits in learning and memory.[95] It is worth noting that more recent studies have not replicated this conclusion.[96,97]

3.2. Cannabidiol (CBD). (−)-Cannabidiol (CBD) is the second major constituent of *Cannabis sativa*. It was first isolated in 1940 by Adams and co-workers, but its structure was not fully elucidated until almost 30 years later when Mechoulam's group was able to isolate CBD from Lebanese hashish and establish its structure and stereochemistry.[14,15,98] It differs from the THC in that it has a pyran ring and can easily undergo acid- and base-catalyzed transformations to produce Δ^9-THC and Δ^6-CBD, respectively (Figure 4).[98]

Although structurally similar to Δ^9-THC, CBD exhibits none of the addictive or psychoactive properties associated with its infamous relative and is known to have very low affinity to both known cannabinoid receptors. This lack of affinity seems to be a result of the two rings in CBD being oriented in a perpendicular fashion, as compared to the planar conformation of Δ^9-THC.[99] Unlike the endogenous cannabinoids and Δ^9-THC, CBD possesses a highly complex and diverse pharmacological profile, relying on interactions with a myriad of receptors. Here, we will highlight only the most important interactions. One known mechanism of cannabidiol action is its function as an antagonist of cannabinoid receptor agonists.[100] It was able to block the effects of CB_1 agonists WIN55212 and CP55940 at a far lower dose than is required for receptor activation by CBD. Studies have also shown that it enhances endogenous adenosine signaling through inhibition of uptake, providing an explanation for its anti-inflammatory properties.[101,102] In addition, CBD is a modest agonist of the serotonin ($5\text{-}HT_{2A}$) receptor, which may be responsible for its analgesic and anxiolytic effects.[103] It is also a potent antioxidant, as studies by Hampson et al. have shown that CBD prevents hydrogen peroxide-induced oxidative damage as well as or better than vitamin C and vitamin E.[104] Furthermore, there is evidence for activity at the δ- and μ-opioid receptors and TRPV1 cation channels.[89]

CBD has a well-established safety profile and generally is well tolerated in doses up to 1500 mg/day orally, without any reported negative effects on mood or motor skills.[105] Evidence from human studies has highlighted the potential of CBD for treatment against anxiety at 300−600 mg PO daily.[20] With this in mind, interest in the therapeutic potential of cannabidiol has skyrocketed over the past decades. Increasing amounts of preclinical and clinical data have been gathered to support the application of CBD as an antipsychotic, analgesic, antiemetic, antioxidant, antiepileptic, anti-inflammatory, and anticonvulsant.[20,88]

3.3. Approved Cannabinoids. The only two pharmaceutical forms of Δ^9-THC on the U.S. market are nabilone (a synthetic derivative of Δ^9-THC) and dronabinol (synthetic Δ^9-THC).[106] Both medications are used in the treatment of chemotherapy-related nausea and AIDS-associated weight loss and anorexia.[88] On June 25, 2018, the FDA approved Epidiolex, a highly purified botanical CBD extract, for the treatment of Dravet syndrome and Lenox Gastaut syndrome, two forms of childhood-onset epilepsy.[12,107] Almost a decade after a study conducted by the lab of Ben Whalley highlighted the antiseizure properties of cannabidiol, Epidiolex is the first cannabis-derived medicine approved for clinical use. The only currently approved combined formulation, Sativex, contains a 1:1 ratio of CBD/Δ^9-THC.[12] Interestingly, users have oftentimes described vastly different sensations based on whether the administered drug was synthetic or plant-derived, although the two were chemically identical.[12]

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3.4. Entourage Effect. Here lies the pressing question: how can two chemically identical compounds produce different effects based purely on the method of their isolation? Given that chemistry is an exact science and spectroscopic methods can confirm the identity of the molecules in question, there are only two scenarios that could explain this phenomenon: (a) one of the substances was mistakenly identified, or (b) one of the substances contains an impurity that contributes to the overall pharmacological profile.[12] The so-called "entourage effect" provides a strong case for the latter and was first described by Ben-Shabat in 1998 with reference to the enhanced activity of the endogenous cannabinoid 2-AG by inactive fatty acid glycerol esters.[108] Since then, the term has been extended to incorporate other cannabinoids and noncannabinoids that enhance the activity of cannabis preparations.[109] As stated by Mechoulam, "this type of synergism may play a role in the widely held view that in some cases, plants are better drugs than the natural products isolated from them".[110]

At this stage, it is only logical to ask: what therapeutic advantage, if any, does utilizing the entire cannabis plant provide as opposed to the government-approved synthetic formulations like dronabinol? One indication that the non-psychoactive components of the cannabis plant alter the physiological response is demonstrated by the markedly different effects of the *Cannabis sativa* and *Cannabis indica* chemovars.[111] Although both contain Δ^9-THC, the former tends to enhance creativity and productivity, while the latter is known to induce relaxation. As a matter of fact, the disparities between the different chemovars are so significant that the species assignation of cannabis itself is subject to heavy debate.[111] The question remains: why do cannabis users experience such divergent strain-dependent sensations if the main active ingredient is the same?

Since the original discovery of the entourage effect, it has been shown on several occasions that THC monotherapy is not as effective as the dual administration of THC in combination with CBD or terpenoids.[109] In 2010, Johnson and co-workers conducted a multicenter, double-blind, randomized placebo-controlled study of cannabis-based extracts in patients with cancer-related pain.[112] In their findings, the THC-predominant extract produced results similar to the placebo, whereas a plant extract containing a mixture of CBD and THC was statistically significantly better than both.[111,112] In another study, researchers found that small doses of pure CBD reduce pain until a peak is reached, after which further increases are ineffective.[113] This bell-shaped dose−response curve was, however, not observed for a full-spectrum cannabis extract with equivalent doses of CBD, which resulted in a linear dose−response curve with no observed ceiling effect.[113] Thus, counterintuitively, higher purity formulations of the active ingredients in cannabis did not guarantee higher therapeutic efficacy. Further evidence for the entourage effect was provided by a study conducted in 2018, which employed five distinct cannabis extracts with a uniform concentration of CBD on mice with induced seizures.[114] The results of this study showed that all five extracts were beneficial when compared to the control, but there were pronounced differences between the number of mice developing tonic-clonic seizures (21.5−66.7%) as a result of the varying amounts of the "minor" components in each extract.[111,114] In summary, these findings show that isolating or synthesizing only the active components of marijuana may significantly limit the plant's therapeutic potential and, by extension, limit the variability of interbreeding and hybridization within the highly versatile cannabis genome.

Currently, the lack of hard scientific evidence to back these empirical findings limits the utility of the entourage effect in therapeutic applications.[115] Neither the effects of cannabinoid−cannabinoid interactions nor the effects of cannabinoid−terpenoid interactions have been clearly elucidated. If there are to be significant advances in cannabinoid-based drug discovery, it is essential that more comprehensive studies are designed and performed to gather conclusive scientific evidence.

3.5. Cannabis as a Medical Armory of Weapons. For most of modern history, efforts in the field of drug discovery have centered around the idea of creating highly potent and highly specific molecules to treat diseases, with the aim of avoiding unwanted side-effects. As we move into the third decade of the 21st century, it becomes clear that this reductionist approach has not even begun to unravel the multifarious mysteries of medicine. In cases where the underlying disease pathophysiology is more complex than the dysfunction or dysregulation of a single target, enzyme, or receptor, there is no hope of developing a single drug with a single target to treat that condition. Specifically, psychiatric and neurodegenerative diseases seem to be polygenic in origin, given that the most effective medications on the market have complex pharmacology and ill-defined mechanisms of action.[116] This empirical observation, in combination with the repeated failure of using highly potent and target-specific drugs in clinical development, allows us to infer retrospectively that treatment of CNS diseases is highly convoluted and requires the modulation of multiple biological targets.[117] It seems that the development of antipsychotic and antidepressant medications should be approached with the prospect of restoring physiological balance by administering drugs with pleiotypic actions, rather than by aggressively pursuing a specific target.

In cases where the underlying disease pathophysiology is more complex than the dysfunction or dysregulation of a single target, enzyme, or receptor, there is no hope of developing a single drug with a single target to treat that condition.

The term combination therapy, or polypharmacy, refers to the combined administration of two or more single-target molecules to yield a more favorable outcome. As such, it is the simplest approach to circumventing the limitations of single-molecule-defined target drug discovery. Nonetheless, the efficacy of combining two or more single-target drugs is limited by pharmacokinetic properties such as half-life and distribution, as well as unwanted drug−drug interactions.[117] In contrast, the development of one multitarget drug that address several biological targets as "magic shotguns" instead of "magic bullets", is known as polypharmacology.[116,117] This approach provides the added promise of reducing treatment complexity and lowering drug dosage to produce adequate pharmacological effects due to synergistic multitarget modulation,

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without the aforementioned complications. Since the introduction of the term by Bryan Roth in 2004, several developments in machine learning, statistical analysis, network analysis, and *in silico/in vitro* approaches have facilitated the inception of *de novo* methods to evaluate and rationally design multitarget compounds. Notably, in 2012 Besnard et al. described an automated approach for the rational design of polypharmacological ligands by designing focused libraries of analogues of an initial compound through machine learning and built Bayesian models to prioritize these compounds according to a multidimensional set of objectives.[118] Other approaches include Keiser's similarity ensemble approach (SEA) and Reker's self-organizing map-based prediction of drug equivalence relationships (SPiDER).[119,120] In addition, an increasing number of chemical probes and empirical models are being developed to facilitate the experimental validation of target synergies. For example, the "Therapeutic Handshake" has been successfully applied to explain the efficacy of the combination of CBD and THC in Sativex.[121] Albeit that these developments have facilitated the rational design of new polypharmacological ligands, safety issues surrounding multitarget interactions remain the biggest limitation of this approach.

With the outlook of building on the well-established safety profile of plants like *Cannabis sativa* with modern scientific discoveries, we propose an extension of the "multi compound-single target" and "single compound-multi target" approaches in the form of a "multi compound-multi target" approach. Rather than attempting to find a "magic bullet" or a "magic shotgun" to treat complex diseases, we suggest gathering an "armory of weapons" that consists of multiple compounds with multiple targets and can be combined and administered as necessary. Not only does this significantly reduce the time spent on rational design of novel ligands for each specific condition, it also has the potential to reduce the rate of failure in clinical trials because of unwanted side-effects, making the process both faster and more cost-efficient. Here, we find ourselves at the intersection of modern drug discovery and ancient herbal medicine, with the prospect of building on our empirical knowledge of plant material with modern scientific methods. By doing so, we hope to gather concrete data to support and evaluate these complex natural compounds, the multitude of targets they interact with, and the physiological responses they produce. Not only will this enable us to fine-tune formulations of multiple compounds to elicit a specific desired effect, it also has the potential to enhance our understanding of the nature of CNS diseases as a whole.

Cannabis sativa is one of the oldest plants known to man, and yet there is a shocking lack of conclusive knowledge on its individual constituents, their mechanisms of action, the physiological responses they evoke, and their possible synergistic interactions.

4. THE WAR ON DRUGS

Cannabis sativa is one of the oldest plants known to man, and yet there is a shocking lack of conclusive knowledge on its individual constituents, their mechanisms of action, the physiological responses they evoke, and their possible synergistic interactions. While multiple studies demonstrated that marijuana smokers have impaired cognitive performance, just as many failed to observe such effects.[122−126] While there is evidence that combined administration of cannabinoids can result in an "entourage effect", the few reported small-scale studies that were conducted did not confirm such interactions.[111,127,128] In summary, the lack of decisive and replicable evidence leaves many open-ended questions making it difficult to build on the vast empirical knowledge that has been gathered over centuries.

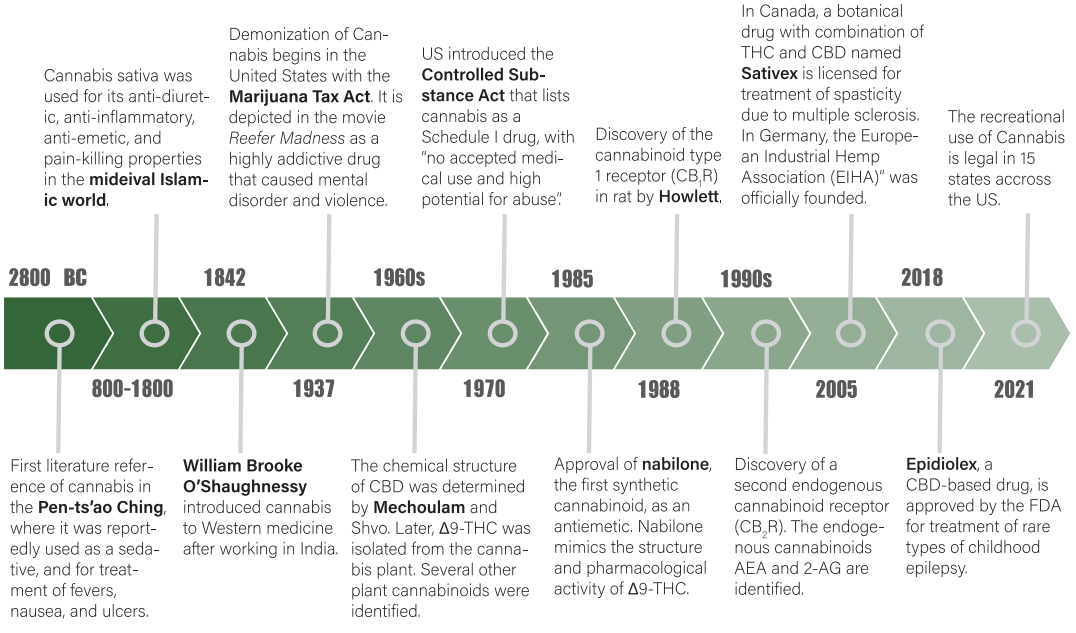

Figure 5. A select timeline of the history of cannabis as medicine.

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4.1. The Tangled History of Cannabis. What is the reason for this lack of progress? And why has cannabinoid-based drug development been so stagnant in comparison to opioids? With the introduction of cannabis, opium, and coca into Western culture at a time of rapid technological and scientific developments, the blurred lines between religious, social, and medicinal uses of these plants became ever more defined.[129] However, early efforts to identify and isolate the active components of cannabis for medicinal purposes proved to be too big of a challenge for the state of knowledge at the time.[130] Availability of other therapeutics discouraged physicians from prescribing such preparations, and cannabis was swept under the rug as a useless remedy. Henceforth, cannabis became looped into the efforts to eliminate illegitimate use of drugs under a series of international drug conventions in the early 20th century. The results of this have shaped the portrayal of cannabis in popular culture, as well as efforts in science up to this day (Figure 5).

4.2. Regulatory Status and Academic Research. In the United States, federal law prohibits the possession, production, and distribution of cannabis. The Controlled Substance Act (CSA) of 1970 lists cannabis (in the form of resin, extracts, tincture, pure THC, and pure CBD) as a Schedule I drug with no medical use, in the same category as heroin and worse than methamphetamine and cocaine.[129,131,132] As a consequence, obtaining permission to conduct clinical research on cannabis is a lengthy process and requires approval from both the FDA and the DEA.[133] In addition, all cannabis used for research purposes must be obtained exclusively from the University of Mississippi, which inherently limits the quality and diversity of samples.[107,134] This is troubling on several accounts. First off, it is widely accepted that samples obtained from this source have more resemblance with marijuana from the 1980s than the wide variety and increased potency of cannabis products available on the commercial market today. In addition, restricting research to samples from just one source neglects to acknowledge both the biggest advantage and the greatest challenge associated with plant medicine: the idea that different strains produce different effects. Without access to the wide variety of cannabis products that are available to the consumer, the research becomes tenuous.

These are issues that researchers have faced for decades, but they become ever more relevant as both the medical and recreational use of cannabis are skyrocketing. At its core, the CSA provides a legal foundation for the government's fight against drugs with a high potential for abuse. But what defines a "drug of abuse", and why are some drugs viewed differently than others? To what extent does the policy on drugs like alcohol, tobacco, marijuana, and several prescription drug families reflect their true dangers? And how have these policies been modified and skewed in order to facilitate the regulating body's political agenda? Here, the lines between government policy and scientific progress become blurred, especially as most academic research institutes rely heavily on funding provided by government agencies.[135] Really, it is a Catch-22 situation—as long as academic research on cannabinoids remains so heavily restricted, efforts to enforce the appropriate regulations on their consumption will remain futile.

4.3. Cannabis in Big Business. While federal regulations have not changed much since the 1970s, several states across the United States have loosened their restrictions on marijuana, creating a new legal cannabis market. In 1996, California passed Prop 215, the country's first medical marijuana law, in an effort to provide relief to patients suffering from chronic illnesses. Since then, the movement has spread across the US in what has been called "medicine by popular vote".[12,136] As of November 2021, medical marijuana is legal in 36 states across the United States, and 18 states as well as the district of Columbia have enacted legislation to regulate the nonmedical use of cannabis.[137] Consequently, the legal medical and recreational cannabis market has become a multibillion dollar industry and is expected to continue growing at a compound annual rate of 26% per year.[138]

Really, it is a Catch-22 situation—as long as academic research on cannabinoids remains so heavily restricted, efforts to enforce the appropriate regulations on their consumption will remain futile.

In fact, Big Marijuana has become so powerful that indirect competitors in Big Tobacco, Big Alcohol, and Big Pharma have recently announced deals with cannabis companies in response to the plethora of social and political campaigns against opioid, alcohol, and tobacco use.[139] In past years, the pharma giant Novartis, the alcohol firm Molson Coors Brewing, and several tobacco companies have joined forces with marijuana businesses in an effort to capitalize on this new movement.[139]

4.4. Dangers of Cannabis in a Free Market. This in and of itself should ring alarm bells, as each of the aforementioned industries have a history of actively campaigning to change legislation, influence public opinion, and distort research in their favor, demonstrating the dangers of leaving public health in the hands of Big Business. In addition, large corporations have a monetary incentive to breed a steady population of heavy users for their personal benefit—a concept known as the 80:20 rule where 20% of users account for 80% of consumption. In a marketplace where profit is the driving factor, consumer welfare is secondary.

At this stage, Big Marijuana has an enormous amount of regulatory freedom, especially in comparison to researchers at academic institutions. From prohibition to becoming one of the fastest-growing industries in North America in less than a decade, the cannabis industry has expanded at a rate with which the scientific community is unable to keep up.

5. OUTLOOK

With all this in mind, we revisit the inaugral question: how can we efficiently overcome the stagnating progress in drug discovery to develop new therapies for complex diseases? In his highly cited 1964 article on "Strong Inference", John R. Platt raises the question why some fields of science are moving forward faster than others.[140] Platt reduces this down to the manner in which the scientific method is approached, arguing that the following steps of inductive reasoning should be applied to every problem that is encountered: (1) identify an interesting observation, (2) enumerate the hypotheses, (3) carry out the experiment, and (1′) reject each hypothesis until a single hypothesis remains.[140] In addition, Don L. Jewett points out the importance of "seed observations" upon which these alternative observations can be based.[141]

As one of the oldest plant remedies known to man, the potential of *Cannabis sativa* to heal various ailments is no

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Unless there is an active effort to fund and facilitate unbiased academic research on cannabinoids and the endocannabinoid system, the cannabis industry could be setting itself up for its own downfall.

secret.[142,143] The cannabis plant has a well-established safety profile and a multitude of active and nonactive natural compounds that could contribute to its overall pharmacological effect. The "seed observations", in this case, have been gathered in an exploratory phase over centuries. As such, the plant, its individual components, and their combinations have the potential to elucidate the relevant mechanisms associated with complex diseases, making it an ideal starting point to explore the entire endocannabidiome and modify it according to a desired therapeutic outcome.

Despite its enormous potential, the rules and regulations surrounding cannabis in research have presented a major roadblock in this endeavor. Simultaneously, a unique patient-centric movement propagating the legalization of cannabis across North America has created a new multibillion dollar industry that is continuing to grow exponentially. In this extraordinary situation, individual commercial entities in several states across the United States have the liberty to grow and distribute marijuana and marijuana-based products without being subjected to the lengthy FDA-approval process. As a result, the fate of millions of consumers is left in the hands of profit-oriented corporations. This is not to discredit the use of marijuana on an individual level to relieve stress, pain, or inflammation. However, if we have learned anything from the opioid crisis, it is to be weary of simple solutions for complex problems. Unless there is an active effort to fund and facilitate unbiased academic research on cannabinoids and the endocannabinoid system, the cannabis industry could be setting itself up for its own downfall.

AUTHOR INFORMATION

Corresponding Author

Valery V. Fokin − *Bridge Institute and Loker Hydrocarbon Research Institute, University of Southern California, Los Angeles, California 90089-3502, United States;* orcid.org/0000-0001-7323-2177; Email: fokin@usc.edu

Author

Katharina Grotsch − *Bridge Institute and Loker Hydrocarbon Research Institute, University of Southern California, Los Angeles, California 90089-3502, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acscentsci.1c01100

Notes

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