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Molecular Mechanism and Cannabinoid Pharmacology

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Abstract

Since antiquity, *Cannabis* has provoked enormous intrigue for its potential medicinal properties as well as for its unique pharmacological effects. The elucidation of its major cannabinoid constituents, Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), led to the synthesis of new cannabinoids (termed synthetic cannabinoids) to understand the mechanisms underlying the pharmacology effects of *Cannabis*. These pharmacological tools were instrumental in the ultimate discovery of the endogenous cannabinoid system, which consists of CB_1 and CB_2 cannabinoid receptors and endogenously-produced ligands (endocannabinoids), which bind and activate both cannabinoid receptors. CB_1 receptors mediate the cannabimimetic effects of THC and are highly expressed on presynaptic neurons in the nervous system, where they modulate neurotransmitter release. In contrast, CB_2 receptors are primarily expressed on immune cells. The endocannabinoids are tightly regulated by biosynthetic and hydrolytic enzymes. Accordingly, the endocannabinoid system plays a modulatory role in many physiological processes, thereby generating many promising therapeutic targets. An unintended consequence of this research was the emergence of synthetic cannabinoids sold for human consumption to circumvent federal laws banning *Cannabis* use. Here, we describe research that led to the discovery of the endogenous cannabinoid system, and show how knowledge of this system benefitted as well as unintentionally harmed human health.

Keywords

cannabinoid; endocannabinoid; phytocannabinoid; synthetic cannabinoid; *Cannabis* Use Disorder; CB_1/CB_2 receptor; allosteric modulation; opioid sparing effects; THC; CBD

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1: A Historical Perspective of, and Introduction to, Cannabinoids

Paleobotanical studies indicate that the *Cannabis* plant was present as long as 11,400 years ago during the Holocene epoch around central Asia (Tarasov et al. 2007; Clarke and Merlin 2013). The earliest evidence of *Cannabis* use dates back 10,000 years to the end of the ice age in Japan (Okazaki et al. 2011), as well as 4,000 years BCE in China as recorded in the ancient Pharmacopoea text “Shen Nung Pen Ts’ao Ching” (Jiang et al. 2006). Originally grown for its use as a fiber, food, and medicinal plant by shamans, *Cannabis* spread across the world due to human domestication and its adaptability to a wide range of climates (for an extensive account of the archeobotanical evidence for *Cannabis* use see Pisanti and Bifulco, 2019). The use of *Cannabis* as a recreational drug eventually became widespread, with an early description found in an 1857 article by The Hasheesh Eater (Lee 2013). *Cannabis* now represents the most commonly used psychoactive drug in the world after alcohol and tobacco.

Discussions surrounding the legal, ethical, and societal implications of *Cannabis* use have been ongoing for at least a century. The last several decades have seen an increasing rise in the frequency of physician-prescribed *Cannabis* for the treatment of various medical conditions such as chronic pain and psychiatric problems across a growing number of states in the United States (Whiting et al. 2015). The current renaissance of the medical employment of *Cannabis* as well as the changing legal landscape in 21st Century United States has forced issues associated with the safe use of *Cannabis* to a now higher prominence. These include; routes of administration, content identification and labelling, drug interactions, dispensing, safety and untoward side effects, contraindications, and use or unintended exposure in specialty populations (the young and the elderly), to name but a few. The inclusion of *Cannabis* Use Disorder into the third edition of the Diagnostic and Statistical Manual of Mental Disorders (DSMIII) in 1980 makes discussion of its safe use pertinent given the reinforcing value of this ancient plant and as such the potential that exists for dependence. As of 2010, global reports of *Cannabis* Use Disorder prevalence estimated that 0.2% or 13.1 million people met diagnostic criteria (Degenhardt et al. 2013), compared to the United States general population where a prevalence of 1.5% was reported in a 2015 National Survey of Drug Use and Health (Hasin et al. 2016; SAMHSA 2017). While *Cannabis* use is a necessary condition to develop *Cannabis* Use Disorder, not all users develop this disorder; therefore, use alone is not a sufficient predictor. The etiology of *Cannabis* Use Disorder is thus clearly complex.

Studying the reinforcing and rewarding effects of cannabinoids in preclinical settings remains a challenge. The most widely used preclinical investigative tool, murine species, do not show reliable or robust intravenous or oral self-administration of Δ^9-tetrahydrocannabinol (THC), the primary psychoactive constituent of *Cannabis* (Lefever et al. 2014; Wakeford et al. 2017; Barrus et al. 2018). Rat intracerebroventricular self-administration of THC (Braida et al. 2001; Zangen et al. 2006), as well as intravenous self-administration of the synthetic cannabinoid WIN 55,212–2 (Fattore et al. 2001; Mendizabal et al. 2006) has been reported. Whereas squirrel monkeys readily self-administer THC (Justinova et al. 2003), this phytocannabinoid functioned as a reinforcer in only half of rhesus and cynomolgus monkeys (John et al. 2017). Other behavioral measures have also

been employed to assess the rewarding effects of THC, such as conditioned place preference and intracranial self-stimulation, with inconsistent results (Braida et al. 2004; Hempel et al. 2016; Tanda 2016). As such, the limited success of modeling the rewarding effects of *Cannabis* in research model organisms remains a considerable barrier to preclinical research investigating the neurobiology underlying the abuse liability of cannabinoids as well as assessing drugable targets to treat *Cannabis* Use Disorder.

The chemicals collectively termed cannabinoids can be organized into three broad classes or categories: phytocannabinoids (plant based; the individual molecular constituents of the *Cannabis* plant), synthetic cannabinoids (manmade cannabinoids), and endocannabinoids (cannabinoids produced by and within the body).

1a. Phytocannabinoids

The *Cannabis* plant contains hundreds of phytochemicals, which include phytocannabinoids, terpenes, and phenolic compounds. To date, more than 560 chemicals have been identified in *Cannabis*, with approximately 120 of these constituents described as terpenophenolic cannabinoids or phytocannabinoids, primarily produced in the glandular trichomes of the plant (ElSohly et al. 2017). Phytocannabinoids are a broad group of closely related chemicals but with diverse structure as well as pharmacological actions. The availability of novel spectrometric methods in the 1960s facilitated the isolation of the primary psychoactive constituent of *Cannabis* Δ^9-THC (Gaoni and Mechoulam 1964) as well cannabidiol (CBD) (Mechoulam and Shvo 1963). For a full review of the chemical elucidation of phytocannabinoids through the 1970s see Mechoulam *et al.* (1976). The elucidation of these structures sparked an enormous amount of basic research that revealed the effects of these drugs in the brain and body. Moreover, the FDA approved THC (referred to as dronabinol) to treat nausea and emesis associated with cancer chemotherapy, as well as AIDS-related cachexia as an appetite stimulant. The FDA recently approved CBD to treat severe forms of pediatric epilepsy. Although THC and CBD represent the best known phytocannabinoids, other predominant constituents include cannabigerol (CBG), cannabichromene (CBC), and tetrahydrocannabivarin (THCV). The phytocannabinoids exist as acids (e.g., THCA-A, CBDA), which are non-enzymatically decarboxylated to their corresponding neutral forms (e.g., THC, CBD). This decarboxylation begins to occur after the plant is harvested during the drying process over time and/or the application of heat (Flores-Sanchez and Verpoorte 2008). Pharmacokinetic studies of cannabinoids have most often focused on THC. This phytocannabinoid is hydroxylated to the psychoactive metabolite 11-hydroxy-Δ^9-THC and then oxidized to the non-psychoactive Δ^9-THC-11-oic acid. THC and its metabolites remain sequestered in cell membranes and adipose tissues and are slowly released, which is why *Cannabis* use can be detected in urine long after use.

1b. Synthetic cannabinoids

Upon elucidation of the primary phytocannabinoids, medicinal chemists modified the structure of THC to understand the mechanisms underlying its pharmacological actions. Structurally diverse compounds that included bicyclic cannabinoids (Compton et al. 1992b) and aminoalkylindoles (Compton et al. 1992a; Wiley et al. 1998; Huffman et al. 2005) served as important tools that contributed to the eventual discoveries of cannabinoid

receptors (Devane et al. 1988; Munro et al. 1993; see Section 2) and the endocannabinoids (Devane et al. 1992; Mechoulam et al. 1995; Sugiura et al. 1995; see section 3c). Pharmaceutical companies also developed synthetic cannabinoids as potential medications. For example, the FDA approved nabilone, which is structurally similar to THC, for the treatment of nausea and vomiting associated with cancer chemotherapy.

An unforeseen consequence in studies publishing the synthesis and characterization of the synthetic cannabinoids in scientific journals was their diversion to recreational use and abuse (for full reviews see Ford et al. 2017; Wiley et al. 2017). As these compounds elicit even greater intoxicating effects as THC but would not be detected in common drug screening tests, their use circumvents the law and drug testing of *Cannabis*. The first generation of synthetic cannabinoids, such as JWH018 (Huffman et al. 2005), were added to plant material and sold over the internet and in convenience stores under various names such as “Spice” and “K-2” as marketing ploys. Administration of JWH018 and other synthetic cannabinoids has been linked to physiological toxicity (Freeman et al. 2013) and psychological complications (Celofiga et al. 2014). The first wave of synthetic cannabinoids were designated Schedule I drugs in 2011, and the United States also made them illegal. Since then, other synthetic cannabinoids emerged in a “cat-and-mouse” game between clandestine laboratories and law enforcement. One such example is the highly potent fubinaca, a Pfizer synthetic cannabinoid made Schedule I in 2014. Illicit synthetic cannabinoids frequently pose a greater public safety threat than *Cannabis*/THC as they have sparked a large increase in emergency room visits and often life threatening consequences (Gerostamoulos et al. 2015). The adverse effects of recreationally used synthetic cannabinoids are likely a result of their higher efficacy and potency at cannabinoid receptors as well as other non-cannabinoid receptor sites of action (Grim et al. 2016).

1c. Endocannabinoids

The endocannabinoid system refers collectively to cannabinoid receptors (CB_1 and CB_2) that are acted upon by endogenously produced cannabinoid ligands: the endocannabinoids (as well as by THC, other phytocannabinoids, and synthetic cannabinoids), and their biosynthetic and degradative enzymes (Blankman et al. 2007). The two most extensively studied endogenous ligands of cannabinoid receptors are arachidonylethanolamine (anandamide or AEA) and 2-arachidonoylglycerol (2-AG) (Devane et al. 1992; Mechoulam et al. 1995; Sugiura et al. 1995). The synthesis and degradation of these endocannabinoids are enzymatically regulated (Blankman and Cravatt 2013). The enzymes that regulate AEA and 2-AG are described below in Sections 3a and 3b. Other endogenous cannabinoid ligands have also been described (for a full review see Pertwee 2015). The majority of these ligands are lipids and include 2-arachidonylglyceryl ether (noladin ether) (Hanus et al. 2001), N-arachidonoyl dopamine (NADA) (Bisogno et al. 2000), and virodhamine (Porter et al. 2002).

2: Cannabinoid Receptor Discovery and Function

A major impetus for research geared towards understanding the molecular targets of cannabinoids included the identification of THC as the chief psychoactive constituent of

Cannabis. Additionally, extensive efforts in medicinal chemistry provided useful tools to bind and activate specific cannabinoid receptor binding sites in biological tissues. The development of highly selective antagonists for each of these receptors (Rinaldi-Carmona et al. 1994, 1998) has greatly aided the investigation of cannabinoid receptor pharmacology as well as provided insight into the function of the endogenous cannabinoid system. The creation of mutant mice in which the CB_1 receptor (Ledent et al. 1999; Zimmer et al. 1999) or CB_2 receptor (Buckley et al. 2000) was genetically deleted provided a powerful complementary tool to distinguish receptor targets of cannabinoid agonists and reveal potential functions of the endogenous cannabinoid system. Below we describe research leading to the discovery of the CB_1 and CB_2 receptors.

2a. CB_1 Receptor In Vitro Evidence

i) G proteins and adenylyl cyclase.—The history of cannabinoid receptors, and their phytocannabinoid, endocannabinoid ligands and analogs, has recently been reviewed by authors who have made major discoveries in cannabinoid pharmacology (Mechoulam et al. 2014; Ligresti et al. 2016). A highly comprehensive review of the CB_1 and CB_2 cannabinoid receptors was submitted by the Cannabinoid Receptor Subcommittee of the International Union of Basic and Clinical Pharmacology (IUPHAR) (Howlett et al. 2002), followed by an evaluation of other targets for the endocannabinoids anandamide and 2-AG (Pertwee et al. 2010). Cellular signaling evoked by the CB_1 receptor has been comprehensively reviewed (McAllister and Glass 2002; Turu and Hunyady 2010; Console-Bram et al. 2012; Howlett and Abood 2017).

Cannabinoid receptors were initially identified and pharmacologically characterized based upon the ability of THC and antinociceptive analogs developed by Pfizer Central Research to attenuate cAMP accumulation in neuronal cells and brain (Howlett et al. 1988). The N18TG2 hybrid cell line, derived from rat neonatal dorsal root ganglia and mouse neuroblastoma, played an essential role in the discovery and function of CB_1 cannabinoid receptors. Based on the observation that pertussis toxin, which eliminates $G_{i/o}$ coupling, abrogated the inhibitory effect on cAMP accumulation, the CB_1 receptor was determined to be a G protein-coupled receptor (GPCR) (Howlett et al. 1986; Houston and Howlett 1993). Immunoprecipitation studies indicated that CB_1 receptors are pre-coupled to $G_{i/o}$ proteins in membrane preparations without the addition of exogenous agonists (Mukhopadhyay et al. 2000; Mukhopadhyay and Howlett 2001, 2005). These studies demonstrated that agonists promote dissociation of the G protein from the CB_1 receptor whereas antagonist/inverse agonists maintain the CB_1 receptor-G_i protein interaction in a more stable form. We now know from antibody-capture scintillation proximity assays for [^{35}S]GTPγS binding, that members of the $G_{i/o}$ family constitute about 75 % of the G protein activation by the high-efficacy receptor agonist CP55,940 (Eldeeb et al. 2016, 2017). G_s, $G_{q/11}$, G_{12} and G_{13} each contributed 5–10% of the total activation under those assay conditions (Eldeeb et al. 2017). G_z, G_s and $G_{q/11}$ were not found to be pre-associated with the CB_1 receptor in 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)-solubilized immunoprecipitation protocols (Mukhopadhyay et al. 2000), which are used to isolate and purify the proteins of interest. Specifically, pertussis toxin pre-treatment did not lead to increased G_s activation (Eldeeb et al. 2016). Evidence for agonist-selective regulation

of various G proteins has come from G-protein activation studies (Glass and Northup 1999; Prather et al. 2000). Immunoprecipitation studies indicated that full agonists could activate all $G_{i/o}$ subtypes, whereas "partial agonists" only activate certain G_i subtypes and act as inverse agonists at other subtypes (Mukhopadhyay and Howlett 2005). These studies at the level of G-protein activation suggest that agonists may select differing signal pathways depending upon the G-protein availability in the environment of the CB_1 receptor.

Numerous reviews have identified the role of neuronal CB_1 receptor signal transduction based upon $G\beta\gamma$ protein release and $G_{\alpha i}$-mediated reduction of cAMP in the regulation of neurotransmission (Kano 2014; Lu and Mackie 2016), neurodevelopment (Diaz-Alonso et al. 2012; Gaffuri et al. 2012; Maccarrone et al. 2014) and synaptic plasticity (Garcia et al. 2016; Araque et al. 2017). Following $G_{i/o}$ activation and dissociation, CB_1 receptors can be phosphorylated by G-protein receptor kinases (GRKs), leading to interactions with β-arrestins 1 or 2 (Breivogel et al. 2013; Chen et al. 2014). β-arrestins are scaffolding proteins that internalize the CB_1 receptors from the plasma membrane, and/or regulate CB_1 receptor-mediated signal transduction that is not related to G proteins (Nogueras-Ortiz and Yudowski 2016). For example, extracellular signal regulated kinase (ERK)1 and 2 phosphorylation and activation can be regulated by both CB_1-mediated $G\beta\gamma$ release with diminished cAMP and PKA signaling, followed by an extended phase mediated by β-arrestins (Rubino et al. 2006; Daigle et al. 2008; Dalton and Howlett 2012; Franklin et al. 2013; Mahavadi et al. 2014).

ii) Radioligand binding and cloning of cannabinoid receptors in the CNS.— The biological activity of Δ^9-THC and analogs being attributed to cannabinoid receptors was first identified using radioligand binding to a Pfizer analog [^{3}H]CP55,940 (Devane et al. 1988). Using this assay, brain cannabinoid receptors were demonstrated to be among the most abundant GPCRs, being highly expressed in the cortex, hippocampus, basal ganglia, and cerebellum consistent with cannabinoid effects on cognition, memory, hypoactivity, and sedation (Herkenham et al. 1990; Glass et al. 1997; Tsou et al. 1998).

An orphan 7-transmemebrane receptor from rat appeared to exhibit neuroanatomical localization similar to that identified as the brain cannabinoid receptor, and using the [^{3}H]CP55,940 radioligand binding assay, was subsequently identified to be what we now refer to as the CB_1 cannabinoid receptor (Matsuda et al. 1990). Based upon this rat clone, the human CB_1 receptor was shown to have 97% amino acid sequence identity, but was shorter by one residue (Gerard et al. 1991). The mouse and rat exhibit identical amino acid sequences (Chakrabarti et al. 1995; Ho and Zhao 1996; Abood et al. 1997). Although there appears to be very similar pharmacological properties between human and rodent CB_1 receptors, some variation in ligand binding has been noted (McPartland et al. 2007).

The first reported splice variant of the human hCB_1 receptor, referred to as hCB_{1a}, is reduced by 167 base pairs in the coding region, thereby reducing the N-terminal extracellular domain by 61 residues, and changing 28 residues in the remaining sequence (Shire et al. 1995). The second reported splice variant, hCB_{1b}, was the result of removal of 99 base pairs from the human mRNA, eliminating 33 residues in the N-terminal domain (Ryberg et al. 2005). It should be noted that these variants are not possible in rodents due to a sequence difference (Bonner 1996). An investigation of the pharmacological profile for

the hCB_{1a} variant compared with hCB_1 expressed in CHO cells found that agonist ligands and the antagonist SR141716 bound to the receptor with three-fold lower affinity; however, the cellular signaling via cAMP and ERK phosphorylation was not appreciably different (Rinaldi-Carmona et al. 1996; Xiao et al. 2008). CB_{1a} and CB_{1b} variants expressed in HEK293 cells exhibited no significant difference compared with CB_1 receptors in receptor binding affinity for Δ^9-THC, CP55,940, WIN55,212–2, HU210 or SR141716, and two to three-fold lower affinity for 2-AG, but 200-fold lower affinity for anandamide (Ryberg et al. 2005). Both variants exhibited [^{35}S]GTPγS activation EC_{50} values and efficacies similar to CB_1 receptors; however, 2-AG acted as an inverse agonist in the [^{35}S]GTPγS activation assay (Ryberg et al. 2005). When expressed in $CB_1{-/-}$ mouse hippocampal neurons, hCB_{1a} and hCB_{1b} were less efficacious than CB_1 in producing depolarization-induced suppression of excitation (Straiker et al. 2012). These hCB_{1a} and hCB_{1b} mRNAs are expressed in low-abundance, <5% of that of CB_1 (Shire et al. 1995; Ryberg et al. 2005; Xiao et al. 2008). However, the protein levels of splice variants were immuno-detectable in human and macaque brains (Bagher et al. 2013). Thus, the relevance of these splice variants to human cannabinoid receptor function is not readily apparent.

CB_1 receptors in the CNS function at the pre-synaptic terminals of neurons to curtail release of neurotransmitters, particularly in GABAergic more so than glutamatergic neurons (Katona et al. 1999; Szabo and Schlicker 2005; Puighermanal et al. 2009). However, CB_1 receptors are indeed present across all plasma membrane components including lipid rafts (Bari et al. 2005; Barnett-Norris et al. 2005), and intracellularly in endosomes and mitochondria (Benard et al. 2012). In addition to neurons, CB_1 receptors are expressed by astrocytes (Han et al. 2012; Oliveira da Cruz et al. 2016), oligodendrocytes and their precursors (Ilyasov et al. 2018), and perhaps other glial subtypes (Stella 2010). It should also be noted that the CB_1 receptor can be expressed in tissues outside the nervous systems, including heart, lung, prostate, liver, uterus, ovary, testis, vas deferens, and bone (Galiegue et al. 1995). As such, peripheral CB_1 receptors mediate physiological processes such as; gastrointestinal motility and energy balance, reproduction and fertility, pain, and skeletal muscle energy metabolism.

2b. CB_1 Receptor In Vivo Evidence

The elucidation of the structures of the many phytocannabinoids present in *Cannabis* (e.g., THC, CBD, CBG, CBC, THCV) led to a great deal of studies investigating their pharmacological actions. The tremendous breadth of pharmacological actions of these compounds was initially hypothesized to reflect nonspecific or specific interactions (as reviewed in (Martin 1986). The well-described effects of cannabinoids in inhibiting cAMP accumulation through GPCR-dependent mechanisms (see section 2a above) provided strong evidence supporting specific mechanisms. However, given the extremely hydrophobic nature of THC and other cannabinoids, their much higher affinity for cell membranes than for aqueous media is not surprising. Accordingly, early studies hypothesized that cell membrane perturbation mediated the pharmacological effects of THC (Hillard et al. 1985, 1990). This perturbation of neuronal cell membranes was also proposed for the intoxicating effects of ethanol (Lyon et al. 1981) and volatile anesthetics (Seeman 1972). In assays using cholesterol liposomes, THC and other psychoactive cannabinoids elicited perturbation,

while CBD elicited stabilizing effects in this artificial membrane system (Lawrence and Gill 1975). However, structure activity relationship (SAR) studies did not bear out a correlation between membrane fluidization and intoxicating effects of cannabinoids (for a full review, see Martin 1986). It should be noted that the high concentration of THC necessary to disrupt membrane fluidity far exceeds typical physiological relevant concentrations. Finally, the n-octanol/water partition coefficients of a series of naturally occurring and synthetic cannabinoids did not correlate with their behavioral activity in measures of spontaneous activity, rectal temperature, tail-flick response, and ring-immobility, suggesting that lipophilicity may represent a component, but not a primary determinant in driving the pharmacological activity of the cannabinoids (Thomas et al. 1990).

SAR studies investigating common *in vivo* pharmacological effects of cannabinoids demonstrated stereoselectivity in rodents, dogs and nonhuman primates (Martin 1986), which strongly supported a receptor mechanism of action. Early studies reported that dogs displayed particular sensitivity to the ataxic effects of *Cannabis* extracts (Walton et al. 1938). Accordingly, the dog static ataxia test offered utility to investigate the SAR of synthetic cannabinoids (Adams et al. 1948a, b; Martin et al. 1975, 1984; Pars et al. 1976; Beardsley et al. 1987; Little et al. 1989; Compton and Martin 1990), and was also used to examine *in vivo* cannabimimetic effects of anandamide (Lichtman et al. 1998) prior to the knowledge of its rapid metabolism by FAAH. Over time, employment of the dog static ataxia assay gave way to rodent high throughput screening and drug discrimination assays.

A high throughput screening, developed by the late Professor Billy Martin for SAR studies and eventually coined the “tetrad test”, evaluates the occurrence of decreased spontaneous activity, hypothermia, catalepsy, and thermal antinociception (Little et al. 1988). Whereas noncannabinoid drugs produce one or a subset of pharmacological actions in this series of tests (Wiley and Martin 2003), THC (Little et al. 1988), potent synthetic THC analogs (Little et al. 1989), synthetic bicyclic cannabinoid analogs (Little et al. 1988; Compton et al. 1992b), synthetic aminoalkylindole analogs (Compton et fal. 1992a), and synthetic anandamide analogs (Thomas et al. 1996) produce the entire constellation of tetrad effects in a stereoselective manor. Indeed, the pharmacological effects of synthetic cannabinoids in the tetrad assay highly correlate with binding affinity to the CB_1 receptor (Compton et al. 1993). Additionally, this assay can be used to estimate pA_2 and pK_B values of cannabinoids (Grim et al. 2017), as well as be modified to determine efficacy, which yields values of efficacy that highly correlate with agonist stimulated [^{35}S]GTPγS binding (Grim et al. 2016).

In contrast to the tetrad assay, the drug discrimination paradigm offers a high degree of specificity in capturing the subjective effects of CB_1 receptor agonists (for a full review see Wiley et al. 2018). In this assay, laboratory animals are trained in an operant food motivated task to discriminate between the subjective effects of a psychoactive drug and vehicle (Solinas et al. 2006). A large body of drug discrimination studies examining drugs from a multitude of classes demonstrate its tremendous utility and its exquisite sensitivity and specificity. Specifically, drugs that fully substitute for the training drug act through a similar mechanism of action. In a career spanning over 40 years beginning in the 1970s, Järbe and colleagues pioneered the drug discrimination paradigm to investigate cannabinoids (Järbe and Henriksson 1973, 1974; Henriksson et al. 1975; Jarbe et al. 1977).

This work was particularly useful in identifying synthetic cannabinoids with cannabimimetic activity (Järbe and Gifford 2014; Järbe et al. 2016a, b). Studies employing CB_1 receptor antagonists confirm that this receptor mediates the discriminative stimulus of THC, synthetic cannabinoids, and MAGL inhibitors (Wiley et al. 1995; Owens et al. 2017). Moreover, other pharmacological agents leading to CB_1 receptor activation substitute for these training drugs. Finally, Jarbe and colleagues demonstrated that drug discrimination can determine efficacy of CB_1 receptors agonists (Järbe et al. 2014).

2c. CB_1 Receptor Allosteric Modulation

The CB_1 cannabinoid receptor has been suggested as a therapeutic target for a number of disorders including chemotherapy-induced nausea, wasting syndrome associated with cancer and AIDS, pain, obesity, neurodegenerative disorders, and substance use disorders (Mackie 2006a; Pacher et al. 2006). Traditionally, therapeutic manipulation of the function of the CB_1 cannabinoid receptor is mainly achieved through the application of exogenous compounds that bind to the CB_1 receptor orthosteric site where the endogenous cannabinoids such as anandamide and 2-AG bind. Most endogenous compounds bind the orthosteric site, which is the main active site of the receptor. However, agonists or antagonists targeting the orthosteric sites of CB_1 receptors have been found with either psychotropic (Grotenhermen and Muller-Vahl 2012) or psychiatric adverse effects (Cridge and Rosengren 2013). These untoward side effects have made orthosteric CB_1 ligands challenging to develop into therapeutic agents. To overcome the on-target side effects of CB_1 orthosteric ligands, novel ligands interacting with CB_1 receptors via a new mechanism of action have been vigorously pursued. To this end, several classes of allosteric modulators, which bind to CB_1 receptor sites different from the orthosteric sites, have been discovered (Figure 1). These new CB_1 ligands include Org27569 (**1**) (Price et al. 2005), PSNCBAM-1 (**2**) (Horswill et al. 2007), RTI-371 (**3**) (Navarro et al. 2009), ZCZ011 (**4**) (Ignatowska-Jankowska et al. 2015), and others have been suggested (Laprairie et al. 2015; Priestly et al. 2015) including endogenous (Bauer et al. 2012; Pamplona et al. 2012; Vallee et al. 2014) molecules. Theoretically, a receptor can form a multitude of active and inactive conformations through selective stabilization by various ligands. Allosteric modulators can induce receptor conformations distinct from those stabilized by orthosteric agonists and antagonists but are generally substrates of active or inactive receptor conformations. Thus, novel mechanisms of action from ligand binding to cytosolic signal transduction can be achieved. Preliminary studies of these allosteric modulators have revealed new mechanisms of action in regulating CB_1 receptor function. For instance, Org27569 enhances binding of CB_1 orthosteric agonists and promotes β-arrestin-1 mediated phosphorylation of ERK1/2, and is a positive allosteric modulator (PAM). This allosteric modulator also inhibits G-protein binding and CB_1 agonist-induced G-protein mediated phosphorylation of c-Jun *N*-terminal kinase (JNK) (Ahn et al. 2013; Baillie et al. 2013). An active form of the receptor may produce signal transduction (e.g. and phosphorylation of some kinases) that differs depending on the nature of the coupling partner (e.g. isoform of G protein and/or β-arrestin). Unlike Org27569, the CB_1 allosteric modulator ZCZ011 enhanced the CB_1 stimulated G-protein binding and augmented G-protein mediated ERK1/2 phosphorylation induced by CB_1 agonists anandamide and CP55,940 (Ignatowska-Jankowska et al. 2015). This evidence suggested functional selectivity in signal transduction and provided for

the possibility of separating therapeutic effects from untoward adverse effects when the physiologically important CB_1 receptors are manipulated with allosteric modulators.

Translational research using preclinical models of several disorders have shown that the CB_1 PAM ZCZ011 and its analogs exhibit exciting promise for potentiating CB_1 receptor activity without eliciting adverse effects typically found in the CB_1 orthosteric agonists (Ignatowska-Jankowska et al. 2015; Cairns et al. 2017; Slivicki et al. 2018b). On the other hand, the well-characterized CB_1 PAM Org27569 failed to show CB_1 dependent anorectic activity in a mouse model (Gamage et al. 2014) whereas it attenuated both cue- and drug-induced reinstatement of cocaine- and methamphetamine-seeking behavior in experimental rats (Jing et al. 2014).

To date, growing evidence from *in vitro* and *in vivo* characterization indicate that allosteric modulators of the CB_1 receptor can regulate the function of the CB_1 receptor with novel mechanisms of action. To translate the exciting *in vitro* pharmacological activities of this class of CB_1 ligands into clinical relevant therapies demand further investigations.

2d. CB_1 Receptor Functional Selectivity (Biased Agonism)

GPCR signaling is multifaceted and different ligands can induce multiple receptor micro-conformations that generate diverse pharmacological responses (Luttrell 2014). Multiple micro-conformations give rise to a variety of activated receptor sub-states that best couple to different G proteins (e.g. $G_{i/o\ subtypes}$, G_s, G_q, etc) or β-arrestins (1 or 2). Both orthosteric (Lauckner et al. 2005; Mukhopadhyay and Howlett 2005) and allosteric (Khurana et al. 2017) ligands can induce a preference for coupling and downstream signaling. Allosteric modulators that evoke β-arrestin-1 binding to signal include ORG27569 (Ahn et al. 2013) and PSNCBAM-1 (Jagla et al. 2019).

Fay and Farrens (2015) used a fluorescence probe to examine changes in the orientation of helices with ORG27659 binding. They found that ORG27569 binding precludes outward movements of helix 6 that are key to G-protein activation. Further, this and movement of helix 7 (and helix 8 on the carboxy-terminus) may help explain why β-arrestin binds preferentially to G-proteins thereby mediating alternative signal transduction. It is also possible that allosteric modulator binding to the CB_1 receptor is in the same region as G-protein or β-arrestin binding, physically precluding some coupling agents from a receptor interaction. Functional selectivity via an allosteric modulator, with or without probe dependence of orthosteric ligand binding, gives one strategy for providing specificity of a therapeutic response.

2e. CB_2 Receptors

A major impetus for the development of new synthetic cannabinoids was to create molecules that retained therapeutic actions without the occurrence of cannabimimetic side effects. To this end, the CB_2 receptor (Munro et al. 1993) offers great promise. This receptor shares approximately 44% amino acid homology with the CB_1 receptor. Similar to the CB_1 receptor, it is coupled predominantly to $G_{i/o}$ proteins, and linked to signaling cascades that involve adenylyl cyclase and cAMP, mitogen-activated protein kinase (MAPK), and the regulation of intracellular calcium. In addition, an extensive characterization of a panel of

ligands binding CB_2 receptors revealed compelling evidence of biased agonism with respect to GTPγS, cAMP, β-arrestin, pMAPKs and G protein-gated inwardly rectifying potassium channel (GIRKs) (Soethoudt et al. 2017). Several agonists emerged as highly selective for CB_2 receptors, including HU910, HU308 and JWH133. Unlike the CB_1 receptor, the CB_2 receptor is sparsely expressed in the CNS, but it is highly expressed in cells of the immune system. A great deal of effort dedicated to developing selective CB_2 receptor agonists as research tools and candidate medications has revealed that these drugs produce antinociceptive actions without the occurrence of cannabimimetic side effects (for reviews see Anand et al. 2009; Donvito et al. 2018). Although a variety of CB_2 receptor agonists lacked efficacy in clinical trials, trials are underway with other compounds for a variety of chronic pain conditions (Aghazadeh Tabrizi et al. 2016).

3: The endocannabinoids and their enzymatic regulation

The cannabinoid receptors discussed in Section 2 mediate most of the psychomimetic effects of *Cannabis*. Yet the evolutionary significance of CB_1 and CB_2 receptors is greater than their activation by *Cannabis* in that they are primarily acted upon by endogenous cannabinoid ligands (endocannabinoids), which together form a neuromodulatory network.

Several unique properties of the endocannabinoid system set it apart from the functional profile of a classical neurotransmitter system. These differences include the direction of endocannabinoid cell-to-cell communication, the unique biosynthesis of endocannabinoid lipids in location and temporal regulation, and the manner of achieving endocannabinoid signalling selectivity. The first property, retrograde neurotransmission, distinguishes endocannabinoids from classical neurotransmitters by their release and action sites and as such their direction of cell-to-cell communication. In contrast to classical neurotransmitters that are released from presynaptic terminals to act at postsynaptic neurons, endocannabinoids are released from the postsynaptic neuron and travel retrogradely across the synaptic cleft to act at pre-synaptic CB_1 receptors. The activation of presynaptic cannabinoid receptors ultimately dampens presynaptic neurotransmitter release (Mackie 2006b), which is how endocannabinoids modulate synaptic strength. This functional consequence gives the endocannabinoid system its label as a neuromodulatory system.

A second property, biosynthesis of endocannabinoids, is also dramatically different from classical neurotransmitters which are synthesized in the cell body and packaged into secretory vesicles, transported to axon terminals, and stored for release upon propagation of an action potential. Endocannabinoid biosynthesis occurs “on-demand” in response to increased intracellular Ca^{2+} (Kondo et al. 1998) or activation of the phospholipase C pathway (Prescott and Majerus 1983; Sugiura et al. 1995) at the level of the plasma membrane from phospholipids present within the cell membrane. The manner by which these highly bioactive yet hydrophobic lipids traverse the aqueous environment of the synaptic space as well as following reuptake in the aqueous intracellular environment remains to be fully understood, though carrier proteins such as fatty acid binding proteins (FABPs) are likely candidates (Haj-Dahmane et al. 2018). The location of the endocannabinoid biosynthetic machinery at the cellular membrane and their hydrophobic nature all contribute to their localized sites of action (20 μm area (Wilson and Nicoll

2001)) and short half-life (less than 5 minutes (Willoughby et al. 1997)), all of which makes endocannabinoid signalling directed, short lived, and occurring in response to discrete stimuli.

A third property, signalling specificity, also distinguishes the endocannabinoid system from that of classical neurotransmission. In traditional neurotransmission, differential activation of signalling pathways are achieved through binding of distinct receptor subtypes by one single neurotransmitter (Siegel 1999). However, endogenous cannabinoids produce functional selectivity at CB_1 and CB_2 receptors. The endocannabinoid ligands and their abundance and action at cannabinoid receptors are a key component of the endocannabinoid system. However, the anatomical and cellular distribution of their biosynthetic and degradative enzymes exerts precise regulatory control of the actions of these endogenous cannabinoid ligands.

3a. N-arachidonylethanolamine (Anandamide)

Anandamide acts as a partial agonist at CB_1 and CB_2 receptors (Hillard 2000), as well as binding to TRPV1 receptors (Melck et al. 1999; Zygmunt et al. 1999) and GPR55 (Baker et al. 2006). The best-characterized biosynthetic pathway for anandamide is the conversion of *N*-acylphosphatidylethanolamines (NAPEs) by NAPE phospholipase D-type (NAPE-PLD) (Okamoto et al. 2004). NAPE-PLD is highly expressed in brain as well as kidney, spleen, lung, heart and liver (Degenhardt et al. 2013). However studies using NAPE-PLD knock-out mice show no changes in brain anandamide levels suggesting the existence of alternative biosynthetic pathways (Leung et al. 2006). A unique feature of anandamide biosynthesis is the existence of several further redundant pathways: the conversion of *N*-acyl-lysophosphatidylethanolamine by a lysophospholipase-D (lyso-PLD) (Sun et al. 2004); the conversion of NAPE or lyso-NAPE by α/β-hydrolase 4 (Simon and Cravatt 2006); and finally the hydrolysis of NAPE by phospholipase C to phosphoanandamide which is then dephosphorylated to anandamide (Liu et al. 2006). The multiple redundant pathways of anandamide biosynthesis perhaps suggest an evolutionarily conserved mechanism for the importance of preserving endocannabinoid tone. The primary deactivation enzyme of anandamide is fatty acid amide hydrolase (FAAH) (Cravatt et al. 1996, 2001), the degradative product of which is arachidonic acid. FAAH is found in soma and dendrites of the postsynaptic neuron and is associated with membranes of cytoplasmic organelles (Gulyas et al. 2004) in areas such as the neocortex, cerebellar cortex, and hippocampus (Egertova et al. 1998). Other enzymes are also responsible for anandamide degradation, specifically through oxidation. Cyclooxygenase-2 (Kozak et al. 2001), lipoxygenases (Hampson et al. 1995), and cytochrome P450 monooxygenases (Snider et al. 2010), all of which convert anandamide to oxygenated derivatives that have biological activity of their own in eicosanoid inflammatory pathways.

3b. 2-Arachidonoylglycerol (2-AG)

2-AG acts as a high efficacy agonist at both CB_1 and CB_2 receptors (Hillard 2000), as well as binds $GABA_A$ receptors (Sigel et al. 2011). The biosynthesis of 2-AG occurs through the conversion of diacylglycerols (DAG) by the diacylglycerol lipases (DAGL) (Bisogno et al. 2003), in which DAGL-α is predominantly expressed on neurons and DAGL-β is expressed

on immune cells (Yoshida et al. 2006; Hsu et al. 2012). The distribution of DAGLs markedly differ between development and adulthood. In the developing mouse forebrain projection neuron, DAGLs are located on elongating axons (co-expressed with CB_1 receptors) and implicated in growth cone guidance (Bisogno et al. 2003). Post-development, DAGLs accumulate on post-synaptic dendrites and participate in endocannabinoid mediated modulation of synaptic strength (Keimpema et al. 2011). Additional, but less well studied, 2-AG biosynthetic pathways include PLA1 activation of lyso phospholipase C (lyso-PLC) (Higgs and Glomset 1994) and dephosphorylation of arachidonoyl-lyso-phosphatidic acid (Nakane et al. 2002).

2-AG inactivation occurs by a variety of enzymes which either hydrolase 2-AG into its component parts (arachidonic acid and glycerol) or transform it by acylation or phosphorylation. The hydrolysis of 2-AG occurs primarily through monoacylglycerol lipase (MAGL) (Dinh et al. 2002; Blankman et al. 2007), which is highly expressed at presynaptic terminals (Gulyas et al. 2004) in brain areas including the cortex, hippocampus, cerebellum, and thalamus (Dinh et al. 2002) and functions in the bulk clearance of 2-AG. To a lesser extent (< 10%), 2-AG is also hydrolyzed by ABHD6 and ABHD12 (Blankman et al. 2007) as well as FAAH (Di Marzo et al. 1998). Both ABHD6 and ABHD12 are post-synaptic integral membrane proteins but ABHD6 has an intracellular facing active site and the active site of ABHD12 is extracellular. The location of ABHD6/12 and their modest contributions to 2-AG metabolism contribute to the hypothesis that they might act as a form of regulatory break for 2-AG production. Enzymes that participate in the deactivation of 2-AG through transformation include COX-2 (Kozak et al. 2000), cytochrome P450 (Chen et al. 2008), lipoxygenases (Maccarrone et al. 2000), as well as MAG kinases (Kanoh et al. 1986) and MAG acyl transferases (Coleman and Haynes 1986).

2-AG levels are 1,000 times more abundant in brain than those of AEA. This high level of production is particularly pertinent given that the metabolism of 2-AG contributes to the availability of free arachidonic acid, the major precursor for the production of pro-inflammatory eicosanoids. Specifically, MAGL is the rate limiting enzyme for free arachidonic acid production in brain, liver, and lung (Nomura et al. 2011). As such, MAGL not only serves as the major enzyme terminates 2-AG signalling but also plays an important role in the production of free arachidonic acid production in a tissue specific manner. Importantly, MAGL does not mediate the production of arachidonic acid in the gastrointestinal tract. Ultimately 2-AG production and metabolism serves to facilitate both neuromodulation and immunoregulation respectively (an extensive review of 2-AG biosynthesis and degradation can be found in Murataeva *et al*, 2014).

3c. Other endocannabinoids, hemopressins, and related lipids

Endocannabinoids are not restricted to AEA or 2-AG. They are members of an ever-growing family of bioactive lipids (Di Marzo 2018). Other described endocannabinoids with cannabimimetic properties include; noladin ether (Hanus et al. 2001), NADA (Bisogno et al. 2000), virodhamine (Porter et al. 2002), and LPI (Pineiro and Falasca 2012). Fatty acid amides such as palmitoylethanolamine and oleoylethanolamine while lacking affinity for CB_1 or CB_2 receptors (O'Sullivan and Kendall 2010), activate GPR55 and

GPR119 receptors (Godlewski et al. 2009), as well as enhance AEA and 2-AG activity by competition for FAAH (Ben-Shabat et al. 1998; Jonsson et al. 2001). Hemopressin is a nonapeptide produced from the cleavage of hemoglobin which acts as an inverse agonist at CB_1 receptors (Heimann et al. 2007). Hemopressin shows several physiological effects such as antinociception, hypophagy, and hypotension (Heimann et al. 2007; Monti et al. 2016). Indeed, docking studies have shown that hemopressin binds to the same CB_1 receptor pocket as SR141716, a CB_1 receptor competitive antagonist/inverse agonist used for metabolic syndrome, but withdrawn from the European market in 2009 due to psychiatric side effects (Motaghedi et al. 2011).

4: Drug Interactions

The endocannabinoid system can interact with a wide range of other neurotransmitter systems including opioids, GABA, glutamate, dopamine etc., and modulate the effects of ethanol, NSAIDs, and substrates for various enzymes. Here we will focus on two such interactions: the potential of drugs targeting the endocannabinoid system to increase opioid potency (termed opioid-sparing effects) which minimizes side effects, and cannabinoid competition for cytochrome P450 (CYP) enzymes making them a potential contraindication for diseases with CYP dysregulation or patients taking drugs metabolized through these enzymes.

4a. Opioid-sparing effects

One consequence of the opioid epidemic crisis is the need to identify alternative drug classes of analgesics that can replace opioids or can reduce the dose of opioids necessary to ameliorate pain. Modulating the endocannabinoid system represents a promising strategy to reduce the effective analgesic doses of opioids, while concomitantly decreasing opioid abuse liability as well as unwanted dose-dependent side effects such as constipation and respiratory depression. Substantial pre-clinical evidence suggests that cannabinoid agonists might produce opioid-sparing effects (for reviews see Nielsen et al. 2017; Donvito et al. 2018). THC represents the most widely selected cannabinoid evaluated in combination with opioids in rodent models of pain. The Welch group pioneered this area of research by employing an isobolographic approach revealing that THC synergistically enhances the antinociceptive effects of various opioids in rodent models of acute pain (Welch and Stevens 1992; Smith et al. 1998; Cichewicz et al. 1999, 2001, 2005; Cox et al. 2007). Likewise, the synthetic cannabinoids CP55,940 and WIN55,212–2 augmented the antinociceptive effects of morphine, but did not affect the discriminative stimulus effects of morphine or heroin self-administration in rhesus monkeys (Maguire et al. 2013). The periaqueductal gray (Wilson-Poe et al. 2012, 2013) has been implicated as a potential brain site contributing to the augmented antinociceptive effects resulting from combined administration of opioids and cannabinoids. Inhibitors of endocannabinoid catabolic enzymes also augment the antinociceptive effects of opioids. Combination of the brain penetrant FAAH inhibitor URB597 or peripherally restricted FAAH inhibitor URB937 plus morphine produced synergistic antinociceptive effects in the mouse paclitaxel model of neuropathic pain (Slivicki et al. 2018a). Similarly, combined injections of the MAGL inhibitor MJN110 and morphine produced synergistic antinociceptive effects in the mouse chronic constrictive

injury model of neuropathic pain (Wilkerson et al. 2016). Curiously, co-administration of the dual FAAH-MAGL inhibitor and morphine produced an additive antinociceptive effect in this assay (Wilkerson et al. 2017).

In contrast to the well-established findings from pre-clinical studies showing that cannabinoids augment the antinociceptive effects of opioids, translation to clinical settings remains to be established, as discussed in a recent meta-analysis (Nielsen et al. 2017). Based on preclinical studies, clinical case reports, and a highly cited population study (Bachhuber et al. 2014), the idea of opioid-sparing effects of cannabinoid agonists has been touted as rationale for the legalization of "medical" *Cannabis*. While large controlled clinical studies provide some evidence of analgesic benefits of THC, opioid dose changes have rarely been reported (Seeling et al. 2006; Johnson et al. 2010). However, three recent phase 3 clinical trials failed to achieve statistical significance of the primary endpoint (average pain Numerical Rating Scale) of nabiximols (an oral-mucosal spray consisting of approximately equal parts of THC and CBD) in advanced cancer patients with chronic pain not alleviated by optimized opioid treatment (Fallon et al. 2017; Lichtman et al. 2018). However, nabiximols showed efficacy on secondary endpoints including sleep disruption as well as patient (Subject Global Impression of Change and Patient Satisfaction Questionnaire) and physician (Physician Global Impression of Change) questionnaires. Substantial differences (e.g., endpoints, species differences, type of pain, etc.) exist between preclinical studies of pain and treatment of clinical pain (Negus 2018, 2019). Moreover, preclinical studies typically use opioid-naïve or non-tolerant laboratory animal subjects, whereas patients in clinical trials have generally been on large dose regimens for prolonged periods of time. Thus, it would be advantageous in future clinical investigations to initiate cannabinoid treatment in cancer pain patients prior to establishing an aggressive opioid treatment regimen. In addition, it is possible that cannabinoid drugs produce opioid-sparing effects for only specific types of cancer pain or in certain patients.

4b. Cytochrome P450 enzymes

Cytochrome P450 enzymes are highly expressed in liver and intestine among other tissues and are necessary for the metabolism of steroid hormones, cholesterol, vitamin D, bile acids, and eicosanoids (Hasler et al. 1999). Diseases of CYP dysregulation include hypertension, hepatotoxicity, infection and chronic inflammation among many others (Setchell et al. 1998; Hiratsuka et al. 2006; Capdevila et al. 2007) (for a full review of CYP roles in disease see Pikuleva and Waterman, 2013). CYP activity is also a major factor in the pharmacokinetics of drugs and thus drug responses (Zanger and Schwab 2013). Endocannabinoids, phytocannabinoids (specifically THC and CBD), as well as synthetic cannabinoids are all substrates of various CYP enzymes (for a full review of cannabinoid interactions with CYP enzymes see Zendulka *et al*, 2016). As such, binding of phytocannabinoids to CYP enzymes could potentially produce treatment failure if taken with clinically co-administered drugs. Competitive inhibition of CYP enzymes raises concerns of drug toxicity from clinical medications. Medications metabolized by CYP enzymes taken in combination with phytocannabinoids or synthetic cannabinoids may interfer with metabolism, thereby increasing drug blood levels and/or extending duration of action. While the perceived risk for clinically significant drug interactions between cannabinoid-based

drugs and the metabolism of CYP medications are generally not considered, limited human data quantifying these interactions exist that are necessary to make definitive conclusions. Given the increased use of “medical” and recreational cannabis, a great need exists to understand the metabolic and pharmacodynamic interactions between cannabinoid-based drugs and other pharmaceuticals.

5: Conclusions

Since antiquity, *Cannabis* has been recognized for its wide range of therapeutic actions as well as for its intoxicating effects. Studies identifying the active constituents of *Cannabis* provoked an enormous body of research that led to the creation of new research probes revealing mechanisms underlying its pharmacological effects, the discovery of the endocannabinoid system, and cannabinoid-based medications approved by the FDA. However, further research is needed to understand the pharmacological effects and the mechanisms of action underlying the minor phytocannabinoids and terpines, both alone and in combination. Further work geared towards exploiting promising therapeutic targets within the endocannabinoid system (e.g., allosteric sites on the CB_1 receptor, CB_2 receptors, FAAH, MAGL, FABPs) may yield new medicines. In particular, preclinical research demonstrates that phytocannabinoids, synthetic cannabinoids, and inhibitors of endocannabinoid regulating enzymes produce antinociception and augment the antinociceptive effects of opioids in a great variety of acute and chronic models of pain. An enormous amount of preclinical studies demonstrate potential efficacy of drugs acting upon the endocannabinoid system in various laboratory animal models of disease and injury. Thus, it remains to examine whether this basic knowledge translates into the clinic. Moreover, given the wide availability of *Cannabis*, *Cannabis* extracts, and phytocannabinoids in dispensaries throughout the US and legal availability of CBD derived from hemp, a tremendous need exists for evidenced-based practice for therapeutic needs (e.g. mental illness and *Cannabis* Use Disorder), which includes understanding potential harms and minimizing abuse of synthetic cannabinoids. A great need also exists for further research to understand the long-term consequences of *Cannabis* on the developing brain, not only in developing fetuses, but also in adolescent and young adults. In sum, great strides have been achieved in the understanding of cannabinoid pharmacology, including the tremendous complexity existing between the endogenous cannabinoid system and the numerous physiological systems it regulates.

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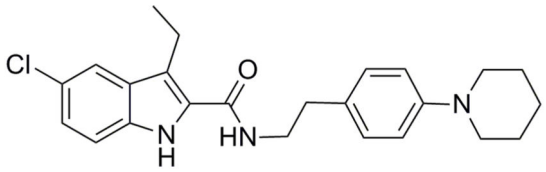

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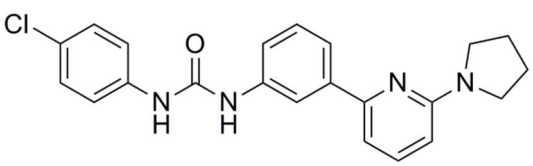

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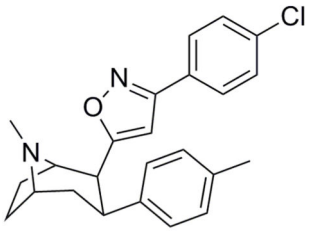

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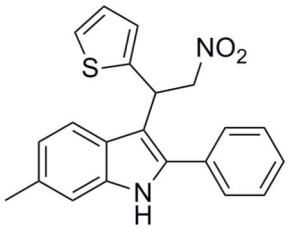

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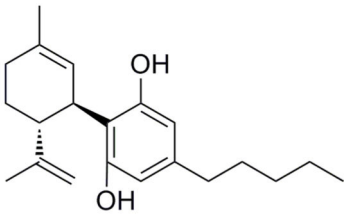

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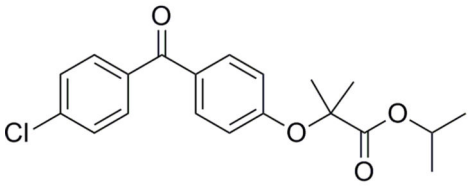

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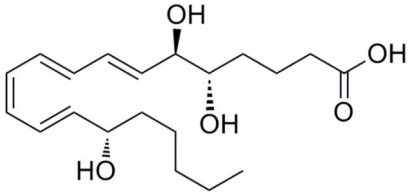
Fenofibrate



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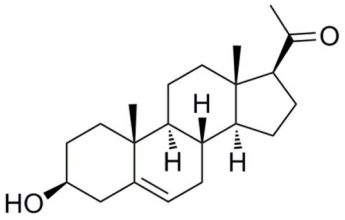

8
Pregnenolone

Arg-Val-Asp-Pro-Val-Asn-Phe-Lys-Leu-Leu-Ser-His

9
Pepcan-12

Figure 1.
Structures of proposed CB_1 receptor allosteric agonists.