Analysis of natural product regulation of cannabinoid receptors in the treatment of human disease☆

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ABSTRACT

The organized, tightly regulated signaling relays engaged by the cannabinoid receptors (CBs) and their ligands, G proteins and other effectors, together constitute the endocannabinoid system (ECS). This system governs many biological functions including cell proliferation, regulation of ion transport and neuronal messaging. This review will firstly examine the physiology of the ECS, briefly discussing some anomalies in the relay of the ECS signaling as these are consequently linked to maladies of global concern including neurological disorders, cardiovascular disease and cancer. While endogenous ligands are crucial for dispatching messages through the ECS, there are also commonalities in binding affinities with copious exogenous ligands, both natural and synthetic. Therefore, this review provides a comparative analysis of both types of exogenous ligands with emphasis on natural products given their putative safer efficacy and the role of Δ9-tetrahydrocannabinol (Δ9-THC) in uncovering the ECS. Efficacy is congruent to both types of compounds but noteworthy is the effect of a combination therapy to achieve efficacy without unideal side-effects. An example is Sativex that displayed promise in treating Huntington's disease (HD) in preclinical models allowing for its transition to current clinical investigation. Despite the *in vitro* and preclinical efficacy of Δ9-THC to treat neurodegenerative ailments, its psychotropic effects limit its clinical applicability to treating feeding disorders. We therefore propose further investigation of other compounds and their combinations such as the triterpene, α,β-amyrin that exhibited greater binding affinity to CB_1 than CB_2 and was more potent than Δ9-THC and the *N*-alkylamides that exhibited CB_2 selective affinity; the latter can be explored towards peripherally exclusive ECS modulation. The synthetic CB_1 antagonist, Rimonabant was pulled from commercial markets for the treatment of diabetes, however its analogue SR144528 maybe an ideal lead molecule towards this end and HU-210 and Org27569 are also promising synthetic small molecules.

Contents

Abbreviations: AC, adenyl cyclase; AD, Alzheimer's disease; 2-AG, 2-arachidonoylglycerol; ABHD6, α,β hydrolase 6; AEA, arachidonylethanolamide/anandamide; ALS, amyotrophic lateral sclerosis; BBB, blood brain barrier; cAMP, cyclic adenosine monophosphate; CBD, cannabidiol; CBN, cannabinol; CB, cannabinoid receptors; CNS, central nervous system; COX, cyclooxygenase; CYP, cytochrome P450; eCBs, endocannabinoids; ECS, endocannabinoid system; ERK, extracellular regulated kinases; FAAH, fatty acid amide hydrolase; GPCR, G protein coupled receptor; GTPγS, guanosine 5′-O-3-thiotriphosphate; HD, Huntington's disease; MAGL, monoacylglycerol lipase; mTOR, mammalian target of rapamycin; NO, nitric oxide; NOS, nitric oxide synthase; PD, Parkinson's disease; phyCBs, phytocannabinoids; PI3K, phosphatidylinositol-3-kinase pathway; PLC, phospholipase C; synCBs, synthetic cannabinoids; THCV, tetrahydrocannabivarin; Δ9-THC, Δ9-tetrahydrocannabinol.

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1. Introduction

The cannabinoid receptors (CBs), their ligands, machinery for ligand synthesis, metabolism, clearance and transport, along with G proteins and other effectors, participate in numerous signal relays. Altogether, these participants are canopied by the endocannabinoid system (ECS), which is uniquely poised to maintain cellular homeostasis, providing an avenue for disease preclusion. Endocannobinoids (eCBs), ligands of the CBs are synthesized upon demand from membrane phospholipids (*e.g.* arachidonylethanolamide/anandamide (AEA) and 2-arachidonoylglycerol (2-AG); Cadas, di Tomaso, & Piomelli, 1997; Di Marzo et al., 1994; Marrs et al., 2010; Muccioli, 2010; Schmid, Reddy, Natarajan, & Schmid, 1983) in response to increases in intracellular Ca^{+} followed by cellular uptake and rapid clearance. The two established CBs are $CB_{1,}$ which is highly expressed throughout the body and $CB_{2,}$ which is mainly localized in immune cells. Activation of presynaptic CB_1 blocks neurotransmitter release through a mixed function response in which potassium channels are stimulated, while calcium channels are inhibited (Howlett et al., 2002). Stimulation of CB_1 in extra-neuronal cells like the liver where immeasurable levels of CB_1 exist, results in an elevated expression of acetyl-CoA carboxylase and fatty acid synthase (Osei-Hyiaman et al., 2005). Meanwhile, CB_2 expression in immune cells participates in many immunosuppressive responses, such as inhibition of proinflammatory cytokine production (Maresz et al., 2007). The CBs, together with their associated targets within the ECS, function in copious signaling relays to modulate physiological and pathophysiological outcomes (appetite, respiration, metabolism, inflammation, pain, neurotransmission *etc.*) in different tissues. Therefore, dysregulation of these processes can effectuate acute or chronic diseases such as neurological and cardiovascular disorders, diabetes and cancers (Insel, Tang, Hahntow, & Michel, 2007).

As such, modulators of this unique system have garnered significant attention (Novack, 2016; Reuter & Martin, 2016; Sherman & McRae-Clark, 2016; Zlebnik & Cheer, 2016) in recent times. Furthermore, research on this system over the years have generated clinically applicable drugs (Cannabinor, Dexanabinol, Rimonabant, Sativex, Nabilone, and Dronabinol/Marinol), some of which have been withdrawn from commercial markets due to adverse effects. Despite these withdrawals, the promised efficacy of the ECS modulators continues to drive research towards regulatory approval of lead molecules, both natural and synthetic in origin.

Natural product research towards the development of drugs, also termed Pharmacognosy, dates back to the time of Hippocrates (470–350 BCE). It was the common practice then to use natural products, which at the time were primarily from plants, to achieve healing and also to protect against diseases (American Herbal Pharmacopoeia, 2013). As a result, the field of drug discovery developed from natural isolates. Moreover, Δ9-THC from the *Cannabis sativa* plant alluded to the eCBs, CBs and subsequently the ECS. Natural products continue to be ideal screening agents in the arena of drug development; indeed more than 50% of traditional drugs are either directly of natural origin or are templates of natural sources (Newman & Cragg, 2012). Furthermore, 30–50% of traditional medicines are believed to act through G protein coupled receptor proteins, of which CBs are members (Bjenning, Al-Shamma, Thomsen, Leonard, & Behan, 2004; Dahl & Sylte, 2005; Doggrell, 2004; Kroeze, Sheffler, & Roth, 2003). It is the belief of many that natural products do provide safer outcomes with less associated side-effects, however, research has shown that such notion tends to be more anecdotal than evidence based (Meier & Lappas, 2015). The father of toxicology, Roman physician Paracelsus believed that all things are poisonous, with the distinction between safety and efficacy residing in the dose (Borzelleca, 2000). It is unclear whether or not there are safer effects attributable to natural products' use, however it is evident that their chemistry continue to provide clues towards the treatment of diseases especially through novel targets.

This review presents an overview of the ECS, factors that cause its dysregulation, the use of natural and synthetic products towards ECS-associated morbidities and future perspectives. The emphasis will be on natural products' modulation of the ECS and whether based on a comprehensive overview, they provide better alternatives.

2. The physiology of the ECS

2.1. ECS signaling

CBs belong to the rhodopsin type family within the GPCRs and while structural elucidations for the CBs are still ongoing, a review by Kenakin and Miller (2010) outlines the conformational changes experienced by this family of GPCRs upon ligand binding and the modulation of allosteric and orthosteric ligands to functionally identify novel drug leads. Within the rhodopsin family, three regions were postulated to participate in their activation, i) Trp6.50, at the bottom of the major intra-helical ligand-binding pocket, ii) Tyr7.53, postulated to unite the bottom of TM7 and helix 8 at the cytosolic surface of the lipid bilayer, and iii) Arg3.50 at the cytosolic locale of TM3, where it possibly couples with the G protein. Together, these regions are believed to manipulate the rhodopsin type GPCRs' response to ligand binding and their conformational change to the cytosolic interface that aid or thwart coupling to specific effectors in order to transduce intracellular signaling.

A common feature of the GPCR signaling pathway is initiation by a ligand (hormones, neurotransmitters, inflammatory mediators and other bioactive molecules) that binds to a GPCR protein (Cabrera-Vera et al., 2003). Ligand-mediated activation of the GPCR in turn stimulates G proteins that are comprised of three subunits: α, β and γ and such stimulation prompts the replacement of GDP with GTP in the Gα subunit as shown in Fig. 1. The G_{α}-GTP complex is then dissociated from the $G_{\beta\gamma}$ subunits, an action that enables both molecular entities to transduce numerous signals through their various effectors (Cabrera-Vera et al., 2003) as shown in Fig. 2. In addition, CB_1 or CB_2 ligand signal transductions are also achieved through β-arrestin couplings that have been shown to participate in receptor desensitization internalization, resensitization, and down-regulation (Daigle, Kearn, & Mackie, 2008; Jin et al., 1999; Nguyen et al., 2012; Raehal & Bohn, 2014; Sim, Hampson, Deadwyler, & Childers, 1996).

GPCRs are usually named after their ligands and so this section will examine the signaling cascade involved in message relay initiated by the cannabinoid/CB pockets. The CB itself is coined after the plant, *Cannabis sativa* that led to its discovery (Di Marzo & Maccarrone, 2008). The structural identification of the plant's principal ingredient Δ9-THC (Gaoni & Mechoulam, 1964) and subsequently the bioactivity of its *l*-stereoisomer (Mechoulam, Braun, & Gaoni, 1967) together acted as a springboard for identifying to date 113 cannabinoids in the *Cannabis sativa* plant (Aizpurua-Olaizola et al., 2016), including eCBs and endogenous arachidonic acid derivatives, in addition to the synthesis of approximately 180 Δ9-THC analogues (Banister et al., 2015; Howlett et al., 2002; Rosati et al., 2014). Therefore, the rhodopsin CBs are regulated by three categories of ligands, eCBs, phytocannabinoids (phyCBs) and synthetic cannabinoids (synCBs). PhyCBs are referred to here as any natural product from the *Cannabis sativa* or other plants that either directly (modulate CBs, G proteins, eCBs or β-arrestins) or indirectly

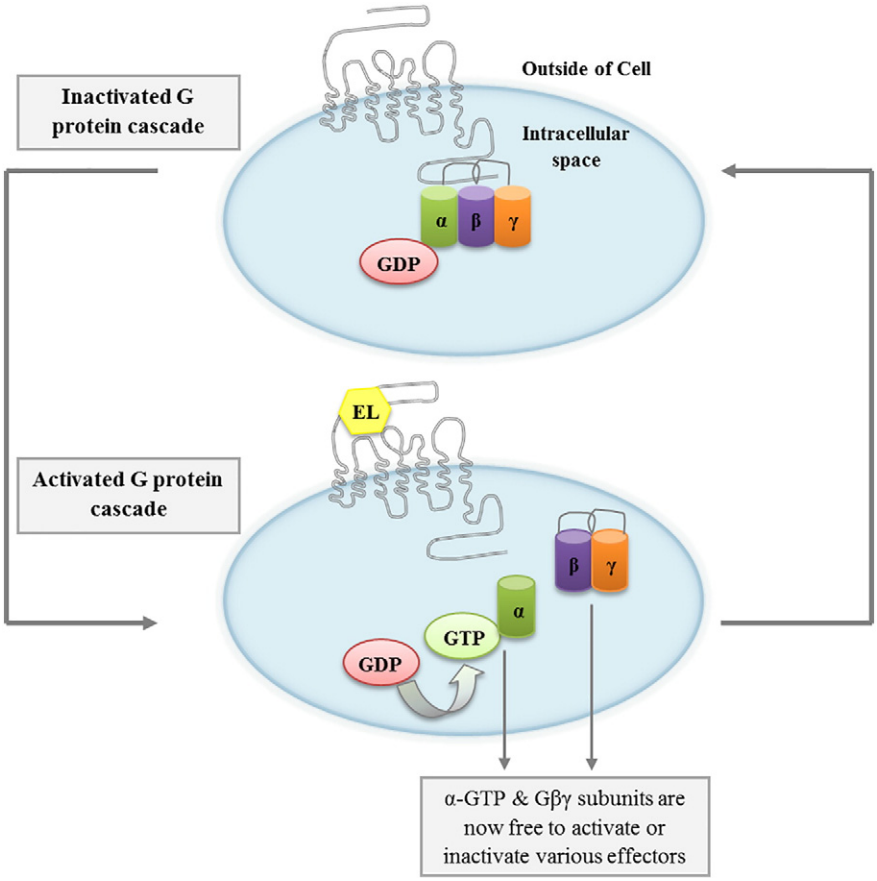


Fig. 1. GPCR mediated signal transductions. This figure offers a basic pictorial outline of the differences between an inactivated G protein (Gαβγ where Gα binds GDP and the entire unit binds to GPCR) and the activated G protein (Gα-GDP is replaced with GTP and Gα dissociates from Gβγ) which then is able to activate various effectors. The figure is not meant to depict appropriate ratios of the proteins to the cell itself. Key: EL—endogenous ligand.

(modulate the enzymatic systems that synthesize, metabolize, transport and clear the eCBs including fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL), diacylglycerol lipase (DAGL) α,β hydrolase 6 and 12 (ABHD6 and ABHD12)) interact with the ECS and/or share chemical similarity with the eCBs.

There are to date, two main identified types of CBs, CB_1 that is primarily localized in the brain (although it is also found in lungs, liver and kidneys) and CB_2 that is mostly expressed in peripheral tissues, in particular, the immune and skeletal systems (Ameri, 1999). Very recent X-ray crystal structures (Fig. 3 shows identified amino acid residues that function in CB_1's regulation) have become available for CB_1 (Hua et al., 2016; Shao et al., 2016) after considerable attempts at preparing CB homology models (Feng et al., 2014; Liu, Patel, & Doerksen, 2014; Shim, Welsh, & Howlett, 2003). Both CBs couple to the G protein effector, G_{io} and their signal transductions are complex and only summarized here where the following observations are not necessarily similar in all tissues expressing CBs. CB_1 couples to Gs G proteins although G_i coupling is the canonical coupling for CB_1. More comprehensive reviews on the ECS signaling cascade (Dalton, Bass, Van Horn, & Howlett, 2009; Howlett and Shim, 2000-2013; Howlett et al., 2004; Maccarrone et al., 2015) exist for consultation. Ligand induced CBs/G_{io} associations typically result in inhibition of the cyclase activity of AC usually the AC 1, 3, 5, 6 and 8 isoforms, while CBs/Gβγ associations activate AC 2, 4 and 7 isoforms (Rhee, Bayewitch, Avidor-Reiss, Levy, & Vogel, 1998). Receptor dimerization has also been observed to participate in AC activation when GPCR dopamine D_2 and CB_1 are simultaneously activated (Glass & Felder, 1997; Kearn, Blake-Palmer, Daniel, Mackie, & Glass, 2005). This dimerization has also been linked to CB_1/Gα coupling while CB_1/$G_{q/11}$ coupling leads to an increase in intracellular calcium ions (Lauckner, Hille, & Mackie, 2005) and Phospholipase C (PLC) activation (Piiper, Stryjek-Kaminska, Klengel, & Zeuzem, 1997). Meanwhile, CB_1/$G_{\beta\gamma}$ coupling modulates phosphorylation of different ion channels, as calcium channels are inhibited (Gebremedhin, Lange, Campbell, Hillard, & Harder, 1999; Mackie & Hille, 1992) whereas potassium channels are activated (Mackie, Lai, Westenbroek, & Mitchell, 1995). CB_1/$G_{i\alpha}$ coupling also participates in the phosphorylation of ion channels, however, this is mediated through inhibiting AC (1, 3, 5, 6, and 8 isoforms), an outcome that stimulates protein kinase A. In addition, CB_1/G_{io} coupling i) induces all three families of multifunctional mitogen-activated protein kinases, including p44/42 (Davis, Ronesi, & Lovinger, 2003; Wartmann, Campbell, Subramanian, Burstein, & Davis, 1995), p38 kinase (Derkinderen, Ledent, Parmentier, & Girault, 2001; Liu et al., 2000), and JUN-terminal kinase (Liu et al., 2000; Rueda, Galve-Roperh, Haro, & Guzman, 2000); ii) activates the phosphatidylinositol-3-kinase (PI3K) and MAPK pathways directly (Gomez Del Pulgar, De Ceballos, Guzman, & Velasco, 2002) and independently (Asimaki & Mangoura, 2011) and iii) activates calcineurin (Stefano et al., 1997). The CB_1/G protein coupling specificities remain to be fully elucidated with regards to NOS stimulation as information is steered to CB_1/ligand coupling (Stefano et al., 1997). More recently, CB_1 was shown to activate extracellular regulated kinases also known as ERK1/2 and neuronal induction in primary neurons from chick embryo telencephalon through lipid rafts (Asimaki & Mangoura, 2011).

Unlike CB_1, the elucidation of many signal transduction pathways for CB_2 are yet to be discovered. Current CB_2 mediated effects include CB_2/$G_{i/o}$ trafficking that participates in the inhibition of cAMP (Rhee et al., 1998) and the activation of MAPK, p42/44 family (Bouaboula et al., 1996). However, CB_2 agonists failed to activate PKB/Akt in HL60 cells, suggesting that a PI3K mechanism may not be engaged by CB_2 receptors (Gomez Del Pulgar et al., 2002). In contrast, the expression of the

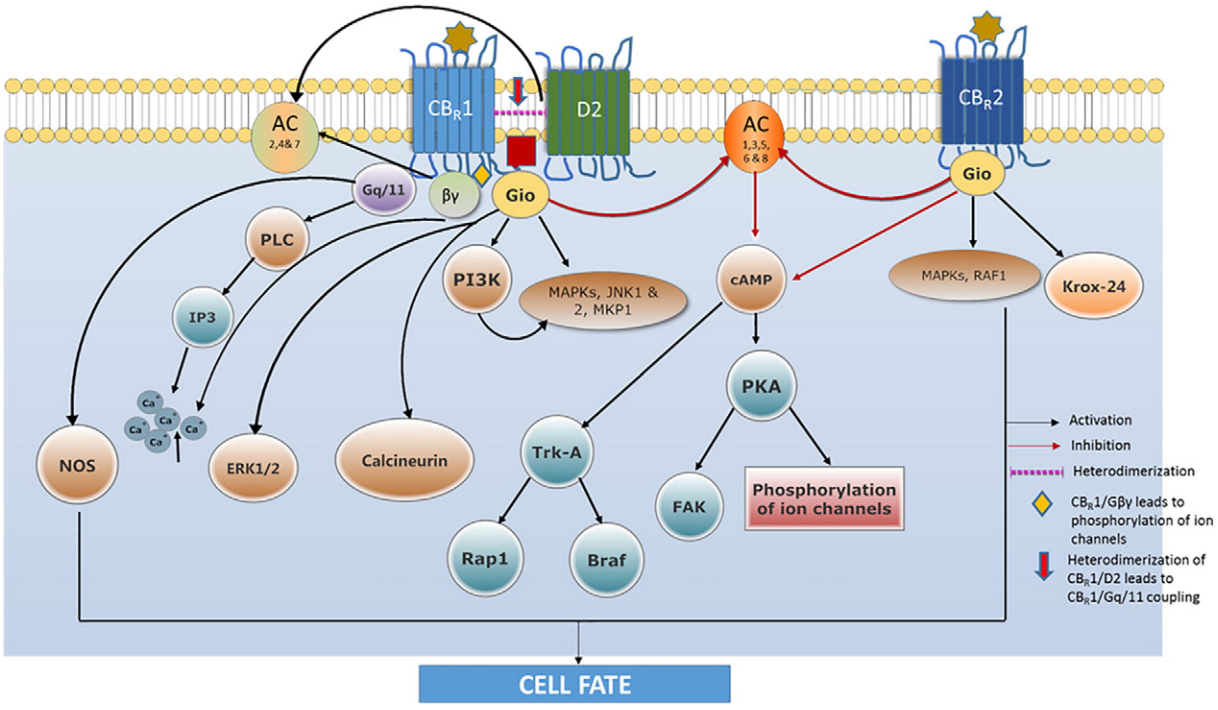


Fig. 2. Ligand induced intracellular signal transductions by cannabinoid receptors associated with G proteins. When a ligand, such as an endocannabinoid, synthetic derivative or natural product binds to either CB_1 or CB_2 exhibiting agonistic effects, various signaling cascades can ensue. These pathways to date are largely dependent on the isoform of G protein that couples to the CB. In the event, $G_{i\alpha}$ binds to CB_1 and one of many effectors can become activated, the outcome of which is largely due to tissue specificity and ligand/CB pocket association not described in this figure. Currently, the cumulative targets modulated by this association are i) inactivation of AC (1, 3, 5, 6 & 8 isoforms) and activation of ii) PI3K, iii) MAPK (p38, p42/44), iv) ERK1/2, v) calcineurin and vi) various ion channels *via* protein kinase A induction. In addition, under this CB/$G_{i\alpha}$, coupling cAMP can induce other targets and these include, Trk-A, Rap1 and Braf and FAK. Ligand association with $CB_1/G_{\beta\gamma}$ complex can result in the activation of the AC 2, 4 and 7 isoforms and Ca^+ increases concentration and the phosphorylation of various ion channels. Activation of these AC (2, 4 & 7) isoforms can also ensue from CB_1/D2 dimerization. The $CB_1/G_{\beta\gamma}$ coupling has also been shown to activate PLC and NOS. The dimerization between CB_1 and D2 have also been linked to the $CB_1/G_{q/11}$ coupling which can result in an increase in PLC activation and an increase in Ca^+ ions. Together, the careful regulation of these target molecules determines the fate of the cell. Not shown in this figure are the effects of β-arrestins which can also couple with the CBs initiating G protein independent signaling such as the activation of p42/44MAPK cascade or Src kinases in addition to CB desensitization and internalization.

nuclear protein that functions as a transcriptional regulator, Krox-24 also known as EGR-1 (early growth response protein 1) was stimulated by CB_2 receptors in HL60 promyelocytes (Bouaboula et al., 1996).

2.2. Endocannabinoids, cues for cannabinoid modulation

Documentation of Δ9-THC as a ligand of the CBs paved the way for identifying eCBs and elucidating their mechanisms of action. The varied binding modalities by these eCBs as either orthosteric (primary unmodulated) agonists, inverse agonists or antagonists contribute to ECS mediated physiological outcomes. The first eCB, anandamide, also referred to as *N*-arachidonoylethanolamine or AEA was discovered in 1992 by Devane et al. (1992). This discovery was made only a year after the elucidation of CB_1 (Matsuda, Lolait, Brownstein, Young, & Bonner, 1990) and before the elucidation of CB_2 (Munro, Thomas, & Abu-Shaar, 1993). The second eCB, 2-AG, was discovered three years later (Mechoulam et al., 1995; Sugiura et al., 1995). Since then, other putative eCB targets have been identified (Johns et al., 2007; Overton et al., 2006) but the biosynthesis, metabolism, transport and function of eCBs have been primarily focused on AEA and 2-AG.

The production of these eCBs occurs when there is an elevation in intracellular Ca^{2+} subsequent to either neuron depolarization or activation of metabotropic $G_{q/11}$-coupled receptors. AEA is a fatty acid derivative neurotransmitter that is synthesized from *N*-arachidonoyl phosphatidylethanolamine by many pathways, derived from the non-oxidative metabolism of arachidonic acid (Cadas et al., 1997; Di Marzo et al., 1994; Schmid et al., 1983) and discussed in more detail in Bosier, Muccioli, Hermans, & Lambert (2010), Pacher, Batkai, & Kunos (2006), and Pertwee (2009). In contrast, 2-AG is produced from activities of diacylglycerol lipase (DAGL) and PLC and different pathways are engaged in its *in vitro versus in vivo* synthesis (Savinainen, Saario, & Laitinen, 2012). Once taken up into the cell, AEA's metabolism is achieved by FAAH while MAGL is primarily observed to catalyze 2-AG although ABHD6 (Marrs et al., 2010), ABHD12 and FAAH (Blankman, Simon, & Cravatt, 2007) also participate in the hydrolysis of 2-AG. The inducible form of COX, COX2 also plays a role in oxidizing AEA and 2-AG (Kozak et al., 2002).

Both eCBs are agonists of CB_1 and CB_2 receptors, however, AEA exhibits low CB_1 efficacy and an even lower efficacy towards CB_2. Meanwhile, 2-AG binds to the CBs with reduced affinity but is fully effective at both CBs (Gonsiorek et al., 2000). The eCBs are released upon demand, bind to the coupled CB/G protein complex inducing signaling cascades as observed in Fig. 2, prior to being rapidly cleared from the extracellular matrix. Their transport mechanism however remains to be fully unraveled although a putative eCB transporter and heat shock proteins (Hsp70s) are implicated in AEA transport (Gerdeman, Ronesi, & Lovinger, 2002; Maccarrone et al., 2000), while fatty acid binding proteins are implicated in the transport of both eCBs (Kaczocha, Glaser, & Deutsch, 2009; Oddi et al., 2009).

In the CNS, signaling of the eCB/CB_1/G protein complex is to date largely achieved using retrograde synaptic relay (Chevaleyre, Takahashi, & Castillo, 2006; Wilson & Nicoll, 2002). As retrograde messengers, eCBs are released from depolarized postsynaptic neurons in a calcium-dependent manner as they reversely inhibit periglomerular cells by way of glutamatergic neurons that are presynaptic to these cells. Therefore, retrograde signaling induced inhibition of the periglomerular cells controls the cell's GABA release, which in turn controls primary cell activity (Thomas, Ze-Jun, Edward, & Paul, 2016). Findings show that retrograde signaling by the eCBs may be important for treating feeding disorders (Pertwee, 2006b) as elucidations indicate

that some mechanisms are involved in CB_1's participation in increasing food intake in mice via elevating odor detection. These include a reduction in the excitatory drive of the olfactory cortex areas in the main olfactory bulb (Soria-Gomez, Bellocchio, & Marsicano, 2014). Further, it is believed that the eCBs' involvement in the inhibition of periglomerular cells provides novel insights into their potential role to mitigate substance abuse (Thomas et al., 2016). Endocannabinoids also participate in regulating cell migration and the production of cytokines and chemokines which play a role in maintaining homeostatic immune function (Cabral, Ferreira, & Jamerson, 2015). Conversely, elevated levels of the eCB, AEA, have been associated with stunted embryonic development, foetal loss and pregnancy failure (Maccarrone, 2009; Maccarrone et al., 2001). Altogether, supporting evidence exists which underlies eCBs as neuromodulators and immunomodulators and their inhibition towards enhanced fertility. These translate to antinociceptive action (Kaczocha et al., 2014), anxiolytic-like properties (Marco et al., 2015), antidepressant activity (Trezza & Campolongo, 2013), anti-tumour efficacy (Picardi, Ciaglia, Proto, & Pisanti, 2014), and the lowering of blood pressure in hypertensive experimental models (Batkai, Pacher, Jarai, Wagner, & Kunos, 2004). The continuous elucidation of the pharmacological properties of the eCBs underscores their value in restoring cellular homeostasis and treating certain diseases while taking into account their adverse effects.

3. ECS signaling gone awry and its restoration

3.1. Factors that affect ECS and its signaling relay

Di Marzo, Stella, and Zimmer (2015) discuss at length in a review, factors that are associated with a dysregulated ECS highlighting age, neurological diseases and cancer and so will only be briefly mentioned here in addition to mutations within the CBs caused by genetic or epigenetic factors (Gyombolai, Toth, Timar, Turu, & Hunyady, 2015).

Clinical and pre-clinical evidence exist that demonstrate the impact ageing has on varied aspects of the ECS signal relay. Firstly, a reduction in CB_1 gene expression levels was observed in the extrapyramidal structures of aged rats compared to their younger counterparts (Berrendero et al., 1998; Rodriguez de Fonseca, Ramos, Bonnin, & Fernandez-Ruiz, 1993). Furthermore, the CB_1 binding affinity was also impacted in these preclinical models and other research confirms this effect in a clinical setting (Rodriguez de Fonseca et al., 1993). In humans, the expression of key genes in the ECS that regulate CB's orthosteric ligands and their inactivating enzymes were impacted with maturation of brain function (Long, Lind, Webster, & Weickert, 2012). CB/G protein coupling was also affected by ageing in the limbic forebrain of mouse models (Wang, Liu, Harvey-White, Zimmer, & Kunos, 2003).

Classical (*e.g.* Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophiclateral sclerosis (ALS)) or inflammatory associated (*e.g.* multiple sclerosis (MS) and HIV-associated dementia) degenerative disorders of the CNS which may occur concomitantly with or independently of ageing (Stella, 2010) exhibit similar etiologies, neuroinflammation, excitotoxicity, deregulation of intercellular communication, mitochondrial dysfunction and disruption of brain tissue homeostasis. Induced signaling by CB_1 and CB_2 are important to Ca^{2+} homeostasis, trophic support, mitochondrial activity (Ranieri, Laezza, Bifulco, Marasco, & Malfitano, 2016), the generation, specification and maturation of neurons in addition to brain maturation (Skaper & Walsh, 1998). Preclinical evidence supports an association between inflammation and ECS derived neurological disease (Hegde, Nagarkatti, & Nagarkatti, 2010) in mice that underwent experimental allergic encephalomyelitis (EAE) or multiple sclerosis. Other findings (Maresz et al., 2007) demonstrated the role of low levels of CB_2 in non-CNS cells to prevent and control neuroinflammation and its accompanied disorders. Consequently, disrupted $CB_{1/2}$ signaling may play a role in degenerative disorders of the CNS.

The mammalian target of Rapamycin (mTOR) is a target molecule associated with cancer and it is one of the downstream signaling effectors of CB_1 and leptin through PI3K activation. mTOR enables both cellular nutrient sensing and energy homeostasis through the ERK/MAPK–Akt pathway, in addition to the expression of c-myc and cyclin D1 that contributes to cell growth and survival and the transcription of a number of hypoxia-inducible genes including vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) (Rini & Atkins, 2009). These genes have implications for mitogenesis, permeability, vascular tone and the production of vasoactive molecules (Zachary, 1998). Therefore, the mTOR complex participates in a wide array of biological functions including angiogenesis, synaptic plasticity and cognitive function, and usage of the mTOR inhibitor, Rapamycin, blocked mTOR mediated angiogenesis (Sekiguchi et al., 2012) and conferred cellular cytoprotectivity (Busquets-Garcia et al., 2013; Rubinsztein, Marino, & Kroemer, 2011). Interestingly, antagonism of CB_1 was shown to inhibit autophagy in a mTOR mediated manner (Hiebel, Kromm, Stark, & Behl, 2014), a phenomenon possibly related to ageing and certain neurodegenerative disorders such as ALS and PD (Centonze, Finazzi-Agro, Bernardi, & Maccarrone, 2007).

Finally, CB mutations have been associated with anomalies in the ECS signal relay that are concomitant to certain diseases like certain neurodegenerative disorders, discussed more by Maccarrone, Bernardi, Agrò, & Centonze (2011). The review highlights the general role cholesterol plays with respect to the CBs which can either be through a direct impact of the CB conformation states *via* CRAC, cholesterol recognition amino acid sequence consensus in helix 7 on the CBs or indirectly altering the physiochemical properties of the bilayer as shown in Fig. 3. More definitive mutations such as single-, double- and/or triple-alanine mutations in the highly conserved DRY motif within the CB_1 have been recently found to display either bias towards G proteins or β-arrestins (Gyombolai et al., 2015). Single alanine mutations decreased G_o protein activation and enhanced basal β-arrestin 2 recruitment while a double mutant (CB_1-D3.49A/R3.50A) augmented β-arrestin 1 and 2 recruitment but decreased G-protein activation. CB_2 mutations have been linked to oncogenic effects as *CB_2* gene transforming mechanisms using ligands or retroviral insertions resulted in altered cellular migration and a reduction in neutrophilic development in addition to aberrant expression of CB_2 in human myeloid cell lines respectively (Alberich Jorda et al., 2004).

3.2. Synthetic CB ligands

The identification of the natural isolate, Δ9-THC from the *Cannabis sativa* plant not only led to the identification and isolation of other naturally derived phyCBs (natural products from the *Cannabis sativa* or other plants that modulate the ECS) but to synCBs, synthetic products that also modulate the ECS. These synCBs are structurally classified as either classical, non-classical, aminoalkylindoles, eicosanoids or others and are represented in Table 1. Classical ligands are analogues of the Δ9-THC isolate and these tend to behave as agonists of the CBs showing minimal selectivity but in some cases showing increased affinity. One such example is HU-210 whose increased affinity to the CB is believed to be a result of replacing the pentyl side chain on Δ9-THC with a dimethylheptyl group (Griffin et al., 1997; Ross et al., 1999; Sanchez et al., 2001). HU-210 was shown to participate in maximal CB_1 coupling to the G proteins, G_i and G_o compared to the eCB, AEA and the phyCB, Δ9-THC in addition to CB_1 receptor-catalyzed activation of G_i (Glass & Felder, 1997). Moreover, activation of the $G_{i/o}$ complex achieved by a synthetic peptide fragment from the juxtamembrane C-terminal region of the CB_1 receptor independently activated both [35S] guanosine 5′-O-(3-thio)triphosphate (GTPγS) binding to G proteins and inhibition of adenylate cyclase (Howlett, Song, Berglund, Wilken, & Pigg, 1998; Mukhopadhyay, Cowsik, Lynn, Welsh, & Howlett, 1999), therefore indicating the role this domain plays in $G_{i/o}$ activation.

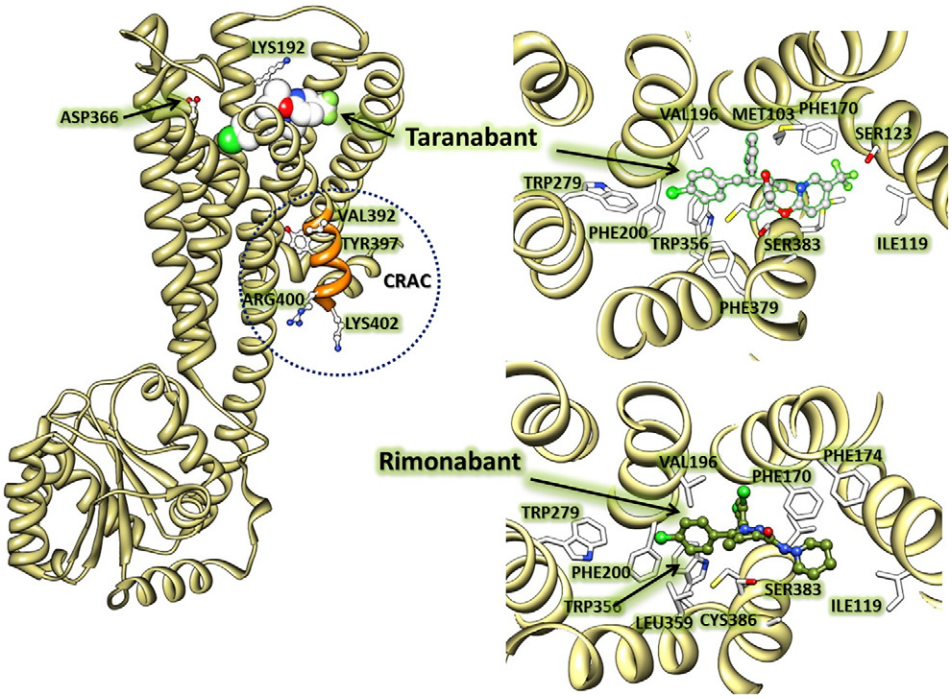


Fig. 3. X-ray crystal structure of human CB1 cannabinoid receptor (rendered in gold; PDB ID: 5U09) bound to inverse agonist ligand taranabant (spheres). Cholesterol interaction motif "CRAC" is highlighted in orange. Ligand binding has disrupted the LYS192-ASP366 salt bridge interactions. A closer look at the taranabant (B; rendered in grey) binding site and the top docked pose of the inverse agonist ligand Rimonabant (C; rendered in olive green) reveal similar ring stacking interactions with PHE200/TRP356 and PHE170.

A characteristic of non-classical CB ligands is an A-ring accompanied by an aryl C-3 side chain bicyclic structure also termed, AC bicyclic and ACD-tricyclic structures that have an opening in the D rings. The CB bicyclic analog, CP55940, is a CB full agonist that is 45 times more potent than Δ9-THC, has parallel affinity for both CB_1 and CB_2 receptors and is highly potent *in vivo* (Rinaldi-Carmona et al., 1996). In 2005, the first CB_1 allosteric site was identified (Price et al., 2005) and a synthetic compound, Org27569 was shown to enhance CB agonist binding (specifically CP55940), inhibit G protein-dependent agonist signaling using *in vitro* models expressing CB_1 receptor (Ahn, Mahmoud, & Kendall, 2012) and participate in β-arrestin CB_1 biased signaling (Ahn, Mahmoud, Shim, & Kendall, 2013). Org27569 demonstrated preclinical hypophagic activity that has implications for weight loss (Ding et al., 2014) and propelled the synthesis of other allosteric compounds with parallel binding affinities (Qiao et al., 2016). Allosteric modulation welcomes a novel approach to the manipulation of the ECS for therapeutic benefit. Recent research identified R-(+)-WIN55212, an aminoalkylindole that has a high affinity for both CBs with a slightly enhanced affinity for CB_2, a property also displayed by the other aminoalkylindoles, JWH-015 and L-768242 (Showalter, Compton, Martin, & Abood, 1996). R-(+)-WIN55212 also maximally activates CB_1 receptor-catalyzed activation of G_i, while sub-maximally (at around 70%) engages in CB_1 stimulation of G_o (Glass & Northup, 1999). Methylation of AEA results in methanandamide being 9-fold more specific than AEA for CB_1 (Showalter et al., 1996). Overall, agonists of the CBs are involved in cognition, memory, anxiety, control of appetite, emesis, motor behavior, sensory, autonomic and neuroendocrine responses, immune responses and inflammatory effects (Svíženská, Dubový, & Šulcová, 2008), liver injury and hepatocellular carcinoma (Sathyapalan et al., 2016) and so the reverse conditions are anticipated in the presence of their antagonists and these are highlighted in Fig. 4. CB_1's primary locale in the hypothalamus underscores its potential value in treating feeding disorders since the hypothalamus plays a role in feeding regulation and is connected to the mesolimbic dopamine pathway, the so called 'reward' system.

Antagonists of the CB_1 receptors are therefore believed to be important to the weight loss paradigm (Black, 2004) and drug (nicotine and Δ9-THC) cessation is important to weight gain (Filozof, Fernandez Pinilla, & Fernandez-Cruz, 2004; Schindler et al., 2016). After a decade of attempts to synthesize the first CB antagonist, first through isoforms of Δ9-THC, Rinaldi-Carmona et al. (1994) reported the successful synthesis of SR141716A also called Rimonabant, a potent CB_1 antagonist, that propelled the synthesis of other CB antagonists. Analogues of Rimonabant, AM251 and AM281 also block CB_1 receptor-mediated effects while SR144528 is a CB_2 antagonist (Rinaldi-Carmona et al., 1994, 1998). CBs active states can be induced by their respective ligands or by spontaneous shifting between inactive to active states (Ortega-Gutierrez & Lopez-Rodriguez, 2005). Rimonabant, the inverse agonist, binds to the CB_1 and interaction is thought to exist through hydrogen bonding between the carbonyl group of Rimonabant and the Lys192 residue of the CB_1 receptor, shown in Fig. 3. This bond stabilizes the Lys192-Asp366 salt bridge in CB_1 helices 3 and 6, believed to be specific to the inactive CB_1 state (Lange & Kruse, 2005; McAllister et al., 2003). Rimonabant, through direct stacking of its 2,4-dichlorophenyl ring to the Trp279/Phe200/Trp356 residues (on CB_1) on one end and the para-chlorophenyl ring (on Rimonabant) to the Tyr275/Trp255/Phe278 (on CB_1) on the other end, binds within the transmembrane-3-4-5-6 aromatic microdomain of the CB_1 (Fan et al., 2009; Lange & Kruse, 2005). These binding interactions with CB_1 seem to be important to Rimonabant's clinical efficacy to treat diabetes (Muccioli & Lambert, 2005) and a proposed mechanism is through increased insulin sensitivity in an age-dependent manner (Lipina et al., 2016) and oxidation of fatty acids in muscles and the liver (Patel & Pathak, 2007). Current preclinical and *in vitro* findings suggest that Rimonabant through its CB_1 antagonism treats non-alcoholic fatty liver disease (NAFLD) by blocking fatty liver metabolism (Sathyapalan et al., 2016). More recently, the effects of Rimonabant to minimize drug dependence of nicotine and Δ9-THC in squirrel monkeys were mimicked by a neutral antagonist, AM4113 (Schindler et al., 2016). Rimonabant as an antagonist/inverse agonist of the CB_1 is much concretized and its implications in weight loss, anti-diabetes and reduced drug dependency are established. Also

Table 1
Synthetic CB ligands, their structures, binding affinities and bioactivities.

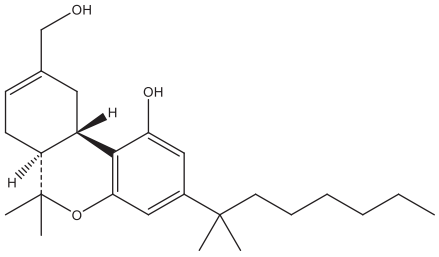
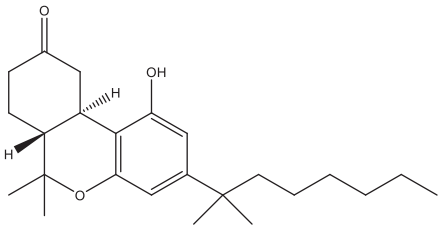
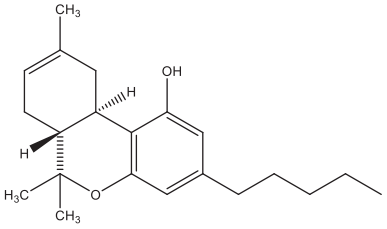
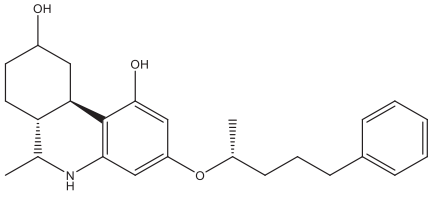
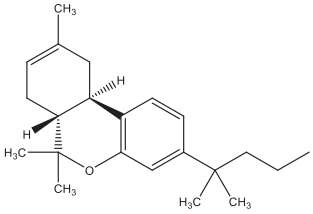
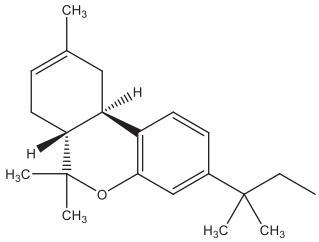
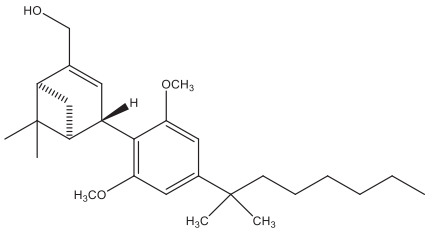
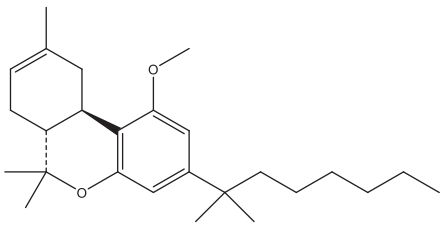
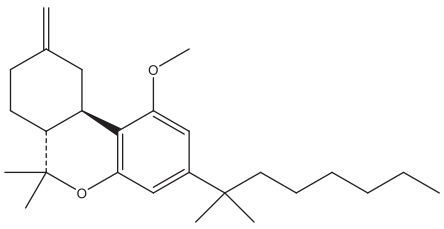
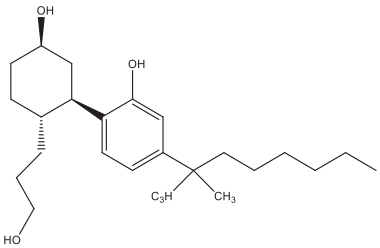
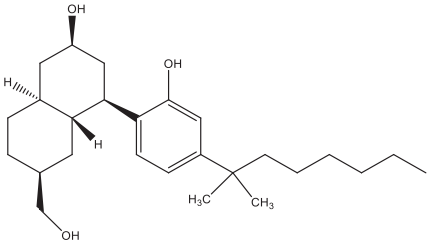
Synthetic cannabinoids	Binding type/CB	Bioactivity	Reference
Classical			
 HU-210	Agonist/CB_1, CB_2	Analgesic, neuroprotective	Guhring et al. (2001), Lax, Esquiva, Altavilla, and Cuenca (2014)
 Nabilone	Agonist/CB_1, CB_2	Analgesic, antiemetic, anti-inflammatory	Conti, Costa, Colleoni, Parolaro, and Giagnoni (2002), Darmani, Janoyan, Crim, and Ramirez (2007)
 Δ8-THC	Agonist/CB_1,CB_2	Antiemetic, orexigenic	Avraham et al. (2004), Webster and Sarna (2007)
 Desacetyl-L-nantradol	Agonist/CB_1,CB_2	Analgesic	Pertwee (2005)
 JWH-133	Agonist/CB_2	Anti-cancer	Chakravarti et al. (2014)
	Agonist/CB_2	Unknown	Chakravarti et al. (2014)

Table 1 (*continued*)

Synthetic cannabinoids	Binding type/CB	Bioactivity	Reference
 JWH-139	Agonist/CB_2	Analgesic, anti-inflammatory, lowers blood pressure	Hanuš et al. (1999)
 HU-308	Agonist/CB_2	Analgesic	Ross et al. (1999)
 L-759633	Agonist/CB_2	Analgesic	Howlett et al. (2002)
 L-759656			
Non-classical			
 CP55940	Agonist/non-selective	Anti-nociceptive, antiemetic,	Darmani et al. (2003), Pugh, Mason, Combs, and Welch (1997)
 CP55244	Agonist/CB_1	Anti-nociceptive	Little, Compton, Johnson, Melvin, and Martin (1988)

(continued on next page)

Table 1 (*continued*)

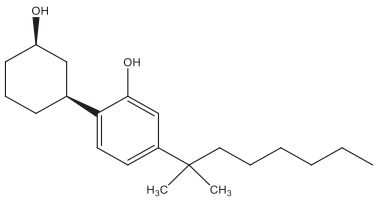
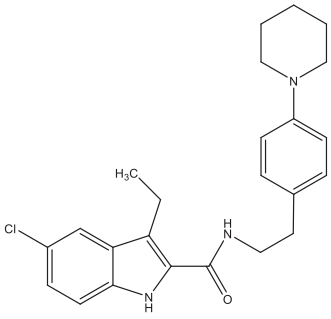
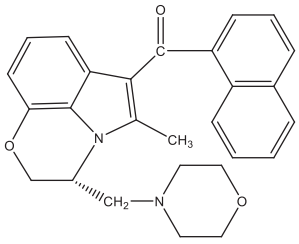
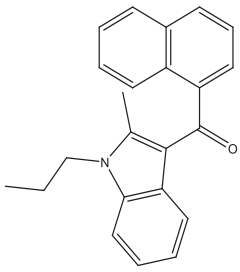
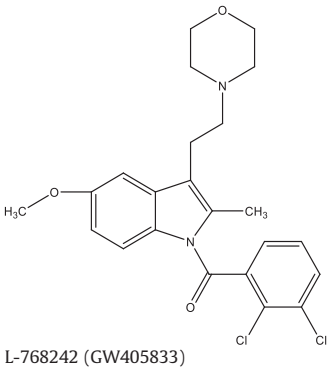
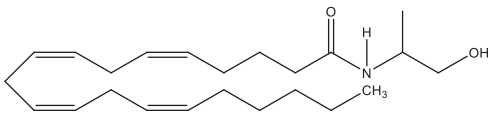
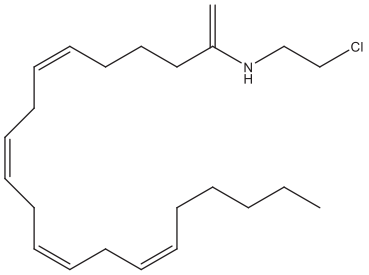
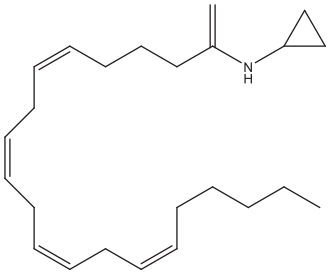
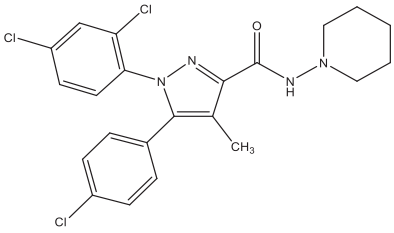
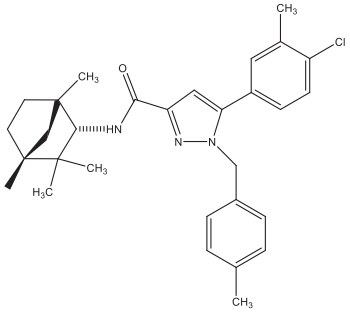
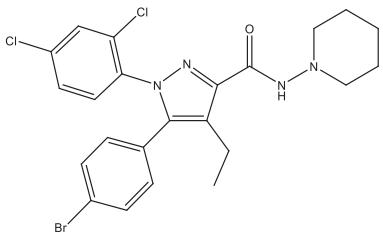
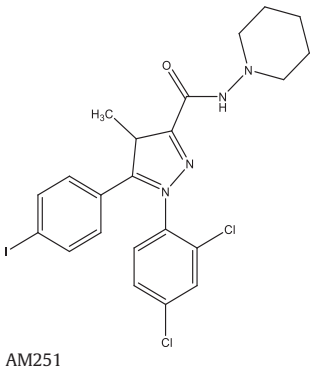
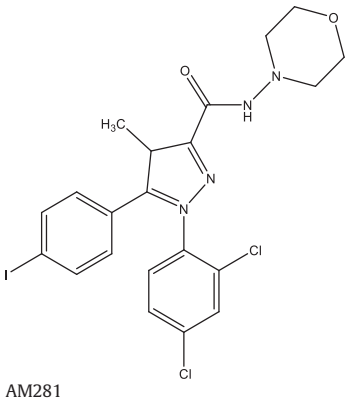
Synthetic cannabinoids	Binding type/CB	Bioactivity	Reference
 CP47497	Agonist/CB_1	Analgesic, anticonvulsive, motor depressive	Melvin et al. (1993), Weissman, Milne, and Melvin (1982)
 Org27569	Allosteric modulator/CB_1	Hypophagic	Ding et al. (2014)
Aminoalkylindoles			
 R-(+)-WIN55212	Agonist/CB_1	Analgesic, anti-inflammatory	Herzberg, Eliav, Bennett, and Kopin (1997), Marchalant, Rosi, and Wenk (2007)
 JWH-015	Agonist/CB_2	Immunomodulatory, anti-inflammatory	Lombard, Nagarkatti, and Nagarkatti (2007)
 L-768242 (GW405833)	Agonist/CB_2	Analgesic, anti-inflammatory	Clayton, Marshall, Bountra, and O'Shaughnessy (2002)

Table 1 (*continued*)

Synthetic cannabinoids	Binding type/CB	Bioactivity	Reference
Eicosanoids			
	Agonist/CB_1	Analgesic, antiemetic, orexigenic, anti-proliferation, anti-migration	Chakravarti et al. (2014), Guindon and Hohmann (2011)
Methanandamide			
	Agonist/CB_1	Anti-depressant, anti-nociceptive	Guindon and Hohmann (2011), Rutkowska and Fereniec-Goltbiewska (2006)
Arachidonyl-2-chloroethylamide (ACEA)			
	Agonist/CB_1	Anti-nociceptive	Jafari, Ghiasvand, Golmohammadi, Zarrindast, and Djahanguiri (2008)
Arachidonylcyclopropylamide (ACPA)			
Others			
	Antagonist/CB_1	Anti-obesity, smoking cessation, neuropsychiatric effects	Boekholdt and Peters (2010), Boyd and Fremming (2005), Carai, Colombo, and Gessa (2005)
SR141716A (Rimonabant)			
	Inverse agonist/CB_2	Anti-nociceptive	Clayton et al. (2002), Portier et al. (1999)
SR144528			

(*continued on next page*)

Table 1 (*continued*)

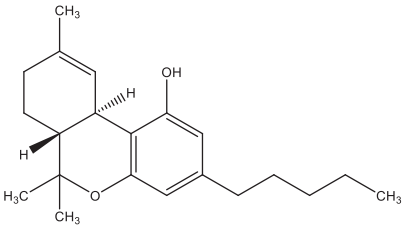
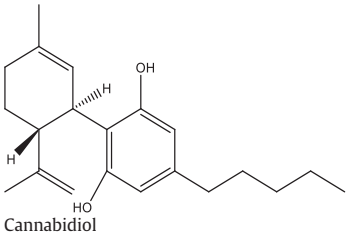
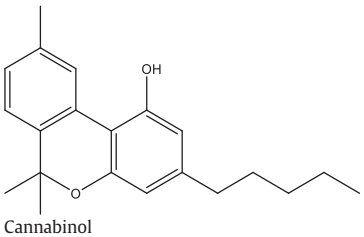
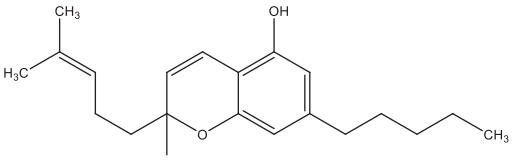
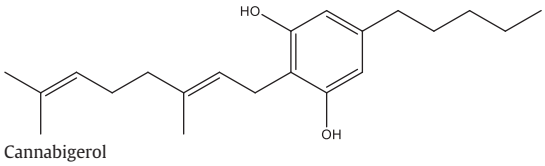
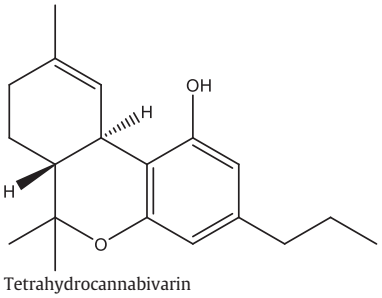
Synthetic cannabinoids	Binding type/CB	Bioactivity	Reference
 SR147778 (Surinabant)	Antagonist/CB_1	Anorectic, smoking cessation; suppression of alcohol preference	Lallemand and De Witte (2006), Lamota et al. (2008), Rinaldi-Carmona et al. (1998)
 AM251	Inverse agonist/CB_1	Antidepressant, anorectic	McLaughlin et al. (2003), Shearman et al. (2003)
 AM281	Antagonist/CB_1	Improves cognitive deficits	Vaseghi, Rabbani, and Hajhashemi (2012)

recently, CB_1's modulation has been implicated in olfaction, a process involved in food intake, visual perception and social interaction and a proposed putative marker for schizophrenia and autism (Hu, 2016). Rimonabant was also shown to participate in pharmacological and behavioral effects independent of CB_1 receptor activation as it is an agonist/antagonist of the transient receptor potential vanilloid receptor 1 (TRPV1) (Hu, 2016). Therefore, Rimonabant exerted olfactory discrimination deficit by modulating both CB_1 and TRPV1 receptors.

Direct interactions with the CBs are not the only pathways to participate in disease amelioration. Research on the CB_2 antagonist, Rimonabant analogue, SR144528 is believed to exhibit anti-obesity and anti-diabetic properties achieved in part through its inhibition of CB_1/CB_2 induction of orexin A/orexin 1 receptor (OXR1). OXR1 is a GPCR protein that regulates feeding disorder and like CB_1 is expressed in the lateral hypothalamus. In a heterologous model that co-expressed both GPCRs, CB_1 was shown to induce orexin-mediated mitogen-activated protein kinase activation more than 100 fold, an effect that was attenuated by the CB_1 antagonist/inverse agonist, SR141716/Rimonabant (Hilairet, Bouaboula, Carriere, Le Fur, & Casellas, 2003). Furthermore, cannabinoid-opioid cross-modulation has been implicated in antinociception, hypothermia, sedation and reward (Wang, Zhang, et al., 2016). Recent studies have shown that a CB_1 antagonist delayed long-term hyperexcitability after brain injury by i) inhibiting long-term up-regulation of CB_1 receptors in the hippocampus, ii) exhibiting long-term potentiation of dynorphin, iii) no impact on the up-regulation of κOR (another rhodopsin GPCR) in hippocampus and iv) reverse the overexpression of mGluR5 in the late stage of brain injury (Wang, Zhang, et al., 2016). SR141716 also participated in the enhancement of ischemia-induced glutamate release after prolonged alcohol withdrawal (Zheng, Wu, Dong, Ding, & Song, 2015).

The research on the synthetic CB antagonists although promising are associated with unideal convoys and so many have been pulled from commercial markets and clinical trials. Rimonabant was approved by the European Union in 2006 to treat diabetes but was discontinued from commercial markets and clinical trials two years later because of serious risk of psychiatric disorders (Sanofi-aventis, 2008). Taranabant (MK-0364) and otenabant (CP-945,598) were both discontinued in phase III clinical trials for treating obesity due to the risk/reward ratio (Aronne et al., 2010; Pfizer, 2008; Pharmacodia, 2008) and Surinabant (SR147778) was discontinued from clinical trials for smoking cessation (R & D Focus Drug News, 2008). Some therefore believe that the current thrust towards tackling these adverse effects is to restrict binding associations to peripheral CB_1 and so limit the crossing of the blood brain barrier (BBB) by small molecules (Chorvat, 2013).

Table 2
Natural CB ligands, their structures, binding affinities and bioactivities.

Phytocannabinoids	ECS modulation	Bioactivity	Reference
Classical			
 Δ9-THC,dronabinol/Δ9-THC	Agonist/CB_1, CB_2	Analgesic, anorexia, antiemetic, orexigenic	Abrams (2016), Beal et al. (1995), Gonzalez-Rosales and Walsh (1997)
 Cannabidiol	Antagonist, weak affinity for CB_1/CB_2	Anti-inflammatory, antioxidant, anti-proliferative, apoptosis inducer, neuroprotective	Ligresti et al. (2006), Pertwee (2008)
 Cannabinol	Agonist/CB_1,CB_2	Analgesic	Sofia, Vassar, and Knobloch (1975)
 Cannabichromene	Agonist, antagonist/CB_1, CB_2, TRPA1, TRPV1	Analgesic, anti-inflammatory. Antimicrobial, anti-proliferative in tumour models < increase neural stem cell viability	Appendino et al. (2008), Izzo et al. (2012), Ligresti et al. (2006), Maione et al. (2011)
 Cannabigerol	Agonist/CB_1, CB_2, TRPA1, TRPV1, TRPV2	Anti-inflammatory, anti-cancer, neuroprotective, orexigenic	Borrelli et al. (2013), Granja et al. (2012), Valdeolivas et al. (2015)
 Tetrahydrocannabivarin	Antagonist, partial agonist/CB_1, CB_2	Anti-inflammatory	Bolognini et al. (2010)
	CB_1 independent	Anticonvulsant	Amada, Yamasaki, Williams, and Whalley (2013), Hill et

(continued on next page)

Table 2 (*continued*)

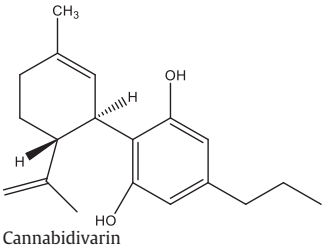
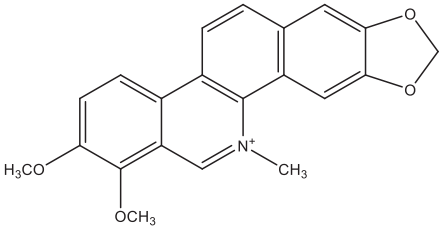
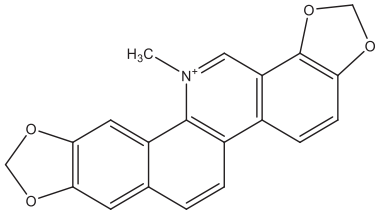
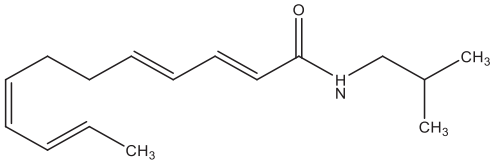
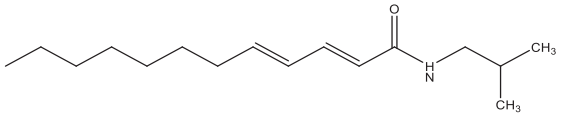
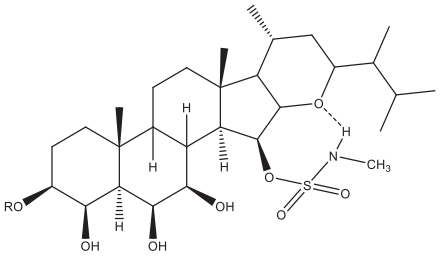
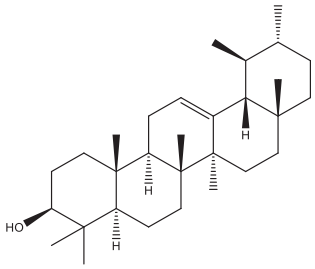
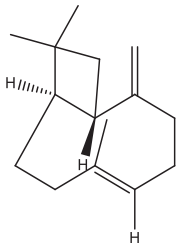
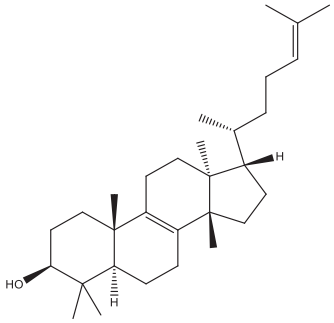
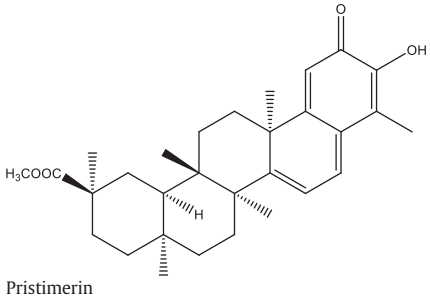
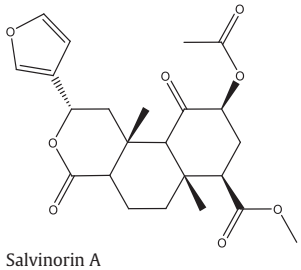
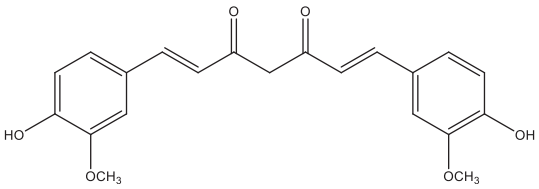
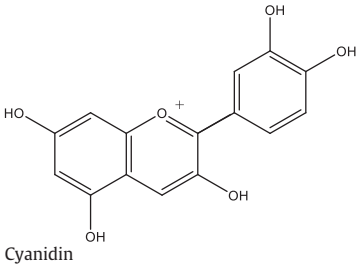
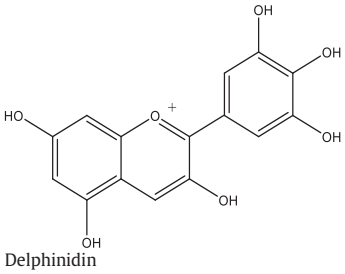
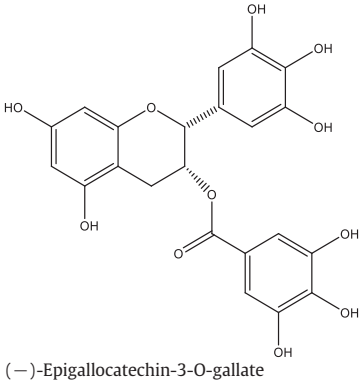
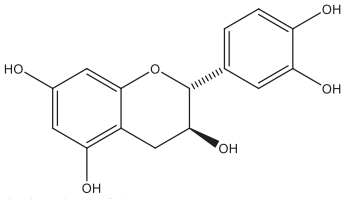
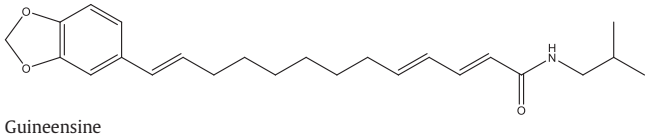
Phytocannabinoids	ECS modulation	Bioactivity	Reference
 Cannabidivarin	mechanism		al. (2013)
Nonclassical (alkaloid derivatives)			
 Chelerythrine	Antagonist, CB_1	Analgesic, antimicrobial	Dhopeshwarkar et al. (2011), Lim, Sung, Ji, and Mao (2003), Mitscher et al. (1978)
 Sanguinarine	Antagonist, CB_1	Anticancer, apoptosis inducer, improved gut peristalsis	Das and Khanna (1997), Dhopeshwarkar et al. (2011), Sun et al. (2010)
Nonclassical (alkylamide derivatives)			
 Dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide	CB_2 agonist, inhibit FAAH	Immunomodulatory	Guiotto et al. (2008), Raduner et al. (2006)
 Dodeca-2E,4E-dienoic acid isobutylamide	CB_2 agonist, inhibit FAAH	Anti-inflammatory, immunomodulatory	Guiotto et al. (2008), Raduner et al. (2006)
Nonclassical (cannabinomimetic derivative)			
 Haplosamates A (R = O, SO_3Na)	Agonist/CB_1/CB_2	Unknown	Pereira, Pfeifer, Grigliatti, and Andersen (2009)

Table 2 (*continued*)

Phytocannabinoids	ECS modulation	Bioactivity	Reference
Nonclassical (terpenoid derivatives)			
 α,β-Amyrin	Agonist/CB_1, CB_2	Anti-inflammatory, anti-nociceptive	da Silva et al. (2011)
 β-Caryophyllene	Agonist/CB_2	Anti-inflammatory	Jürg Gertsch et al. (2008)
 Euphol	CB_1, CB_2 dependent mechanisms	Analgesic, anti-inflammatory, anti-nociceptive	Dutra et al. (2012)
 Pristimerin	Inhibitor/MAGL	Anti-inflammatory, antioxidant, chemoprotective	King et al. (2009)
 Salvinorin A	Weak affinity/CB_1/CB_2	Anti-inflammatory, anti-nociceptive, neuromodulatory	Braida, Limonta, Pegorini, et al. (2007), Capasso, Borrelli, Cascio, et al. (2008)

(*continued on next page*)

Table 2 (*continued*)

Phytocannabinoids	ECS modulation	Bioactivity	Reference
Nonclassical (polyphenol derivatives)			
 Curcumin	Antagonist, inverse agonist/CB_1, CB_2	Anti-inflammatory, antioxidant, immunomodulatory, neuroprotective	Hassanzadeh and Hassanzadeh (2012), Seely, Levi, and Prather (2009)
 Cyanidin	Unknown/CB_1, CB_2	Anti-inflammatory, neuroprotective	Korte et al. (2009)
 Delphinidin	Unknown/CB_1, CB_2	Anti-cancer, anti-inflammatory, antioxidant, hepatoprotective, neuroprotective	Korte et al. (2009), Patel, Jain, and Patel (2013)
 (−)-Epigallocatechin-3-O-gallate	Moderate affinity/CB_1, weak affinity/CB_2	Anti-inflammatory, neuroprotective	Korte et al. (2010)
 (−)-Epicatechin	Negligible affinity for CB_1/CB_2	Anti-inflammatory, neuroprotective	Korte et al. (2010)
Others			
 Guineensine	Indirect agonist/CB_1	Cannabimimetic effects including analgesic and thermoregulation	Nicolussi et al. (2014)

3.3. Natural products as ligands of the ECS, better efficacy than synCBs or not?

It has been highlighted that a natural product, Δ9-THC paved the way for understanding the role of CBs and their eCBs. Of the over 100 phyCBs present in *Cannabis sativa*, the major constituents within the plant are Δ9-THC, cannabidiol (CBD) and cannabinol (CBN) (Russo, 2011). Unlike Δ9-THC (Cumella et al., 2012), CBD and CBN are non-psychotropic phytocannabinoids (Pertwee, 2006a) and exhibit poorer binding affinities to the CBs. However, findings thus far have uncovered enhanced efficacy (considering the reduced psychotropic effects) when using the combination therapy, CBD and Δ9-THC which will be the primary focus of this section. Δ9-THC binds with similar affinities to CB_1 and CB_2 receptors at nanomolar concentrations. It behaves like a CB_1 receptor partial agonist and CB_1/CB_2 receptor antagonist (Pertwee, 2008). Under the trademark Marinol, Δ9-THC is used to treat anorexic associated disorders in patients with AIDS and nausea related conditions in patients on certain chemotherapies (Hazekamp & Grotenhermen, 2010). The lack of euphoric accompaniment by Marinol could be due to its dose, while the other trademark, Nabilone is used to treat chemotherapy induced nausea and vomiting. Δ8-THC has similar affinities for CB_1 and CB_2 receptors akin to Δ9-THC (Paronis, Nikas, Shukla, & Makriyannis, 2012). Other classically derived phyCBs are Cannabigerol (CBG), Cannabichromene (CBC), Tetrahydrocannabivarin (THCV), Cannabidivarin (CBDV) and these and more along with modulatory effects on the ECS are outlined in Table 2.

PhyCBs have been shown to exhibit promising efficacy towards many conditions including, i) CNS disorders (Hill, Williams, Whalley, & Stephens, 2012); ii) convulsions (Hill et al., 2010); iii) neurodegeneration (Gilbert, Kim, Waataja, & Thayer, 2007; Zani, Braida, Capurro, & Sala, 2007), iv) epilepsy (Cunha et al., 1980; dos Santos, Hallak, Leite, Zuardi, & Crippa, 2015); v) sleep disorders (Murillo-Rodríguez (Murillo-Rodriguez, Millan-Aldaco, Palomero-Rivero, Mechoulam, & Drucker-Colin, 2006) and vi) inflammation (Costa et al., 2004).

The structural similarities shared between Δ9-THC and the eCBs, AEA and 2-AG explain the ability of this phyCB to activate CB_1 and CB_2. Δ9-THC binds to both receptors (CB_1 and CB_2) with higher affinity (nM range) than its corresponding (+)-*cis* (6aS, 10aS) enantiomer (+)-Δ9-THC) and with equal or greater affinity than other phyCBs, (−)-Δ8-THC, Δ9-THCV, CBD, CBG and CBN. However, Δ9-THC exhibits lower affinity and efficacy than synCBs, HU-210, CP55940 and R-(+)-WIN55212 (Pertwee, 2008) and the eCBs (Pertwee, 2008). Additional to its partial agonism of the CBs, Δ9-THC activated G proteins by 44% (specifically for the interaction of CB_2 receptors with G_i) and CB_1-catalyzed G_i by 56% (Glass & Northup, 1999). These observed effects were weaker than the synCB, HU-210 and the eCB, AEA. Akin to the eCBs, Δ9-THC can inhibit ongoing neurotransmitter release through neuronal presynaptic CB_1 receptors (Pertwee & Ross, 2002). This is believed to account for many of the Δ9-THC mediated CB_1 effects such as analgesia, muscle relaxation, anti-emesis or appetite stimulation as well as psychotropic properties (Pertwee, 2000). Furthermore, Δ9-THC antinociceptive effects were shown to be mediated through β-arrestin-2 as β-arrestin-2-KO mice failed to desensitize their CB_1 receptors after Δ9-THC stimulus and therefore were more sensitive to Δ9-THC (Breivogel, Lambert, Gerfin, Huffman, & Razdan, 2008; Nguyen et al., 2012). Δ9-THC is not as selective for CB_1 (Kreitzer, 2005; Vaughan & Christie, 2005), as its (CB_1) mediated activation has been linked to the activation of other neurotransmitters like dopamine and acetylcholine (Gardner, 2005; Nagai et al., 2006; Pertwee & Ross, 2002; Pisanu, Acquas, Fenu, & Di Chiara, 2006; Pistis et al., 2002), which is believed to restrict its clinical applicability to treating anorexia, nausea and vomiting. Although CB_1 commonly facilitates inhibition of continuous neuronal transmitter release (on which it is located), it's (CB_1) activation as shown prior, sometimes results in an opposite effect. These mixed effects that Δ9-THC participates in *in vivo* is a possible reason why this phyCB exhibits both excitant and depressant effects as it has been shown to demonstrate anticonvulsant properties in certain *in vivo* models but proconvulsant activities in others (Berrendero & Maldonado, 2002; Braida, Limonta, Malabarba, Zani, & Sala, 2007; Chiu, Olsen, Borys, Karler, & Turkanis, 1979; Colasanti, Lindamood, & Craig, 1982; Dewey, 1986; Fish, Consroe, & Fox, 1983; Patel & Hillard, 2006; Schramm-Sapyta et al., 2007; Turkanis & Karler, 1981; Wallace, Blair, Falenski, Martin, & DeLorenzo, 2003). Therefore, perhaps combination therapies of inhibitors of these other neurotransmitters (acetylcholine and dopamine), further discussed (Babitha, Sahila, Bandaru, Nayarisseri, & Sureshkumar, 2015; Čolović, Krstić, Lazarević-Pašti, Bondžić, & Vasić, 2013; Wang, Shen, et al., 2016) with Δ9-THC can be explored. One such example is rivastigmine (Gawel et al., 2016). It should be noted however, that a comprehensive overview of the function, production and associated targets of these neurotransmitters is critical when deciding ideal combinations. For example, the loss of dopaminergic neurons in the substantia nigra par compacta has been implicated in the onset of certain neurodegenerative diseases like PD, and so some studies demonstrate that the stimulation of dopamine

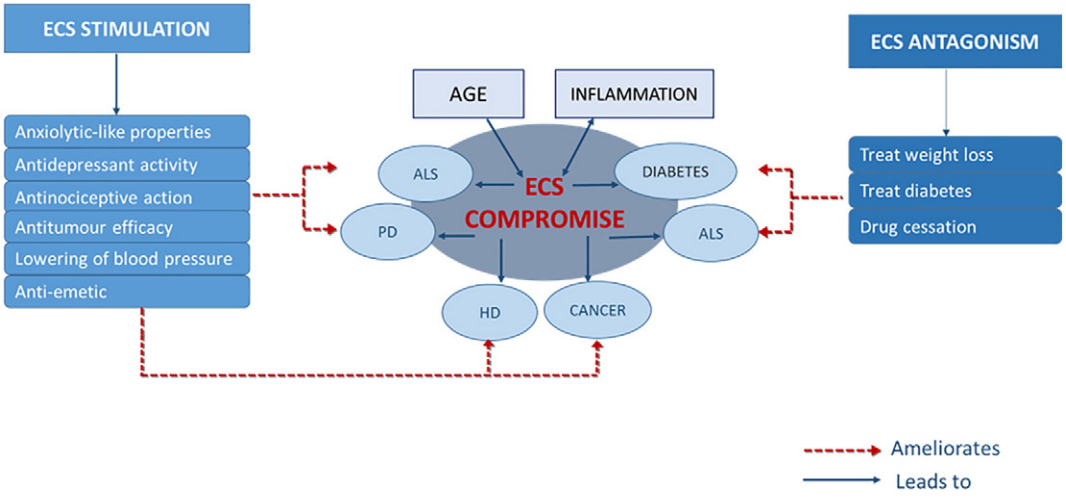


Fig. 4. Synopsis of research approach based on current research findings when treating various disease conditions that ensue from ECS compromise. Factors like age and inflammation participate in ECS compromise, while a cause and effect relationship with the latter requires further delineation. Research findings thus far have linked stimulation of the ECS system, occurring mainly through CB agonism, with various phenotypic properties. These include: anxiolytic, antidepressant, antinociceptive, antiemetic, anti-tumour and reduction in blood pressure effects. Stimulants of the ECS system have been associated with the treatment of HD, PD, ALS and cancer. Meanwhile, antagonists of the ECS, occurring mainly through CB, have been associated with treating weight loss (which has implications for treating cardiovascular morbidities), diabetes and drug cessation.

confers neuroprotective properties (Ablat et al., 2016). On the other hand, overstimulation of dopamine participates in drug dependence (Dackis & Gold, 1985). Hence, further delineation is needed to identify appropriate combinations with Δ9-THC that could generate optimum efficacy with minimal side-effects. In addition to the mixed stimulatory-inhibitory effects of Δ9-THC on CB_1 mediated neurotransmission release/inhibition, the phyCB can inhibit central neurotransmission. As Δ9-THC exhibits lower affinity and efficacy compared to some phyCBs and eCBs, its particular demonstrable bioactivity is believed to be governed by the CBs' density and their coupling efficiencies (Childers, 2006; Pertwee, 2006b). Hence, in certain cells/tissues, Δ9-THC may not exhibit agonistic traits but block the efficacy of other ligands that demonstrate higher binding affinities (Patel & Hillard, 2006; Sim et al., 1996). The partial agonistic traits of Δ9-THC can therefore be clinically applicable to disease conditions that result in an upregulation of the CBs in a cell/tissue specific manner. Such upregulation is thought to increase the selectivity and efficacy of partial CB agonists like Δ9-THC.

The other well researched phyCB is CBD, a key cannabinoid constituent in *Cannabis sativa* that represents up to 40% of cannabis extracts (Grlic, 1976). However, unlike Δ9-THC, it is well tolerated and does not present with psychoactive properties; CBD's bioactivities are thought to be *a priori* its innate chemical structure, specifically its hydroxyl groups (Mechoulam, Parker, & Gallily, 2002) rather than its ligand-mediated signal transduction effect. CBD's bioactivities are more defined around its neuroprotective effects although there is preclinical evidence of its anti-tumorigenic property (McAllister, Christian, Horowitz, Garcia, & Desprez, 2007). This is believed to occur because of one or more of the following; its ability to impede adenosine uptake (Liou et al., 2008), down-regulate the enzymes FAAH and 5-lipoxygenase (Capasso, Borrelli, Aviello, et al., 2008; Massi et al., 2008), or bind both transient receptor potential vanilloid 1 (TRPV1) (Iannotti et al., 2014) and 5-hydroxytryptamine (serotonin) receptor 1A (5-HT1A) receptors (Russo, Burnett, Hall, & Parker, 2005). The neuroprotective properties of CBD are thought to be independent of the ECS (De Filippis et al., 2010) except in select conditions (Bisogno et al., 2001; Castillo, Tolon, Fernandez-Ruiz, Romero, & Martinez-Orgado, 2010; De Filippis et al., 2008). Nonetheless, emerging research shows that despite the low affinity CBD displays for the CBs, this phyCB exhibits possible noncompetitive binding to CB_1 and CB_2, possibly as an inverse agonist at certain concentrations below which it binds to both (CB_1 and CB_2) orthosteric sites (Lunn et al., 2006; MacLennan, Reynen, Kwan, Bonhaus, & Martin, 1998; Thomas et al., 2007). Further, CBD also demonstrates antagonistic effects on CB_1 agonists, CP55940 (Petitet, Jeantaud, Reibaud, Imperato, & Dubroeucq, 1998) and *R*-(+)-WIN55212 (Pertwee & Ross, 2002) in various *in vitro* and preclinical models.

CBD's neuroprotective effects are thought to be because of i) its ability to restore the normal balance between oxidative events and antioxidant endogenous mechanisms (Fernandez-Ruiz, Garcia, Sagredo, Gomez-Ruiz, & de Lago, 2010), often dysregulated in neurodegenerative disorders; ii) the upregulation of endogenous antioxidant enzymes to control oxidative stress, specifically transcription factor nuclear factor-erythroid 2-related Factor 2 (Nrf-2) induced signaling; iii) its anti-inflammatory activity *via* the canonical pathway (Walter et al., 2003), limiting ATP induced-increases in intracellular Ca^+ levels and NO production in microglial cells (Martin-Moreno et al., 2011) and other mechanisms discussed further (Fernandez-Ruiz et al., 2013). While CBD has also shown evidence of neuroprotection *via* an ECS independent pathway (Abood, Rizvi, Sallapudi, & McAllister, 2001; Gilbert et al., 2007), the modulation of CB_1 also plays a role in this paradigm as observed in *in vitro* (Abood et al., 2001; Gilbert et al., 2007) and preclinical (Chen & Buck, 2000; El-Remessy et al., 2003; van der Stelt et al., 2001; Zani et al., 2007) models. Furthermore, other phyCBs, Δ9-THC and THCV's agonistic properties of the CB_2 have also been implicated in preclinical neuroprotection (Garcia et al., 2011; Tourino, Zimmer, & Valverde, 2010).

The promising neuroprotective potential of CBD has propelled its elevation of research from basic science to clinical, especially since it seems to attract less psychoactive side-effects than its Δ9-THC counterpart. There is compelling preclinical evidence supporting the efficacy of CBD's neuroprotection against neonatal ischemic insults (Alvarez et al., 2008; Castillo et al., 2010; Fernandez-Ruiz et al., 2010; Lafuente et al., 2011). Additionally, CBD was shown to minimize necrotic and apoptotic damage brought about by glucose-oxygen deprivation in newborn mice using the newborn hypoxic-ischemic brain damage (NHIBD) model (Castillo et al., 2010). The mechanism of action is believed to occur through CBD's normalization of glutamate and cytokines in addition to the inhibition of iNOS and COX-2. Co-incubation with a CB_2 antagonist abolished the observed effects which strongly suggests the role of CBs in CBD's observed neuroprotection cascade in newborns (Castillo et al., 2010).

CBD's application in combination with other phyCBs has revealed ideal synergism. CBD combined with Δ9-THC, akin to the drug Sativex was shown to be effective in treating Huntington's disease in preclinical models (rats lesioned with 3-nitropropionic acid) (Sagredo, Ramos, Decio, Mechoulam, & Fernandez-Ruiz, 2007; Sagredo et al., 2011) and believed to occur *via* CB_1 and CB_2 dependent pathways. Another model that utilized malonate induced lesions in rats seemed to engage a CB_2 pathway only as CBD alone was ineffective while other CB_2 agonists were effective (Sagredo et al., 2009). The combination therapy of CBD and Δ9-THC towards treating HD has thus far exhibited promising efficacy which has already transitioned them to clinical trials (Fernandez-Ruiz et al., 2013). PD is another neurodegenerative disorder that seems to be mitigated by the action of CBD by CB_1 independent mechanisms (Garcia et al., 2011; Garcia-Arencibia et al., 2007; Lastres-Becker, Molina-Holgado, Ramos, Mechoulam, & Fernandez-Ruiz, 2005) as CB_1 is associated with reduced motor activity as evidenced from clinical studies (Fernandez-Ruiz, 2009). Therefore, it is believed that the activation of CB_2 and not CB_1 might be more ideal for treating ECS associated diseases. However, such an angle could potentially preclude opportunities to treat CB_1 associated morbidities. Hence, like Sativex, perhaps other combinations with CBD could be explored *e.g.* CBD with CB_1 inhibitors like the phyCB, THCV that showed preclinical evidence of treating PD (Garcia et al., 2011) and CBD with α,β-amyrin, a potent CB_1 triterpenoid phyCB (da Silva et al., 2011) discussed more below.

Currently, Sativex, a 1:1 combination of Δ9-THC and CBD is used to treat neuropathic pain, spasticity, overactive bladder and other symptoms of multiple sclerosis (Rahn & Hohmann, 2009; Russo, 2008). Many phyCBs are undergoing clinical trials for various ailments as outlined in a review (Hazekamp & Grotenhermen, 2010), this includes tetrahydrocannabinol, Δ9-THC, CBD, dronabinol, marinol, nabilone,

The challenge that exists when utilizing exogenous CB_1 agonists is the onset of psychosomatic symptoms and recently, overactive CB_1 was shown to participate in type 2 diabetic nephropathy (Jourdan et al., 2014). So the question lies in how to strike a balance, maintaining the efficacy of the phyCBs while blocking the side-effects. So far we have explored a possible mechanism that accounts for the side-effects of Δ9-THC which could be the upregulation of certain neurotransmitters, acetylcholine and dopamine upon CB_1 activation. Indeed, the partial agonism that Δ9-THC seems to exert is thought to reduce its efficacy especially when there is normal to suboptimal levels of CB_1. On the other hand, this type of binding is believed to hold promise in instances where CB_1 and CB_2 activation are elevated and particularly localized to certain tissues as binding affinity to the phyCB would be more restricted to those tissues/cells with elevated CB expression. This phenomenon is not uncommon as the cytochrome P450 (CYP) CYP1B1 enzyme whose presence is usually induced by the activation of the aryl hydrocarbon signal transduction pathway is believed to hold promise in treating certain cancers. CYP1B1's presence is often times elevated in cancerous tissues like prostate relative to normal neighbouring tissue (Yang et al., 2008) and so chemotherapy prodrugs that are metabolized only by CYP1B1 are believed to hold promise as target-specific anticancer drugs (Roos & Bolt, 2005). Although

CYP1B1 is an enzyme and CB_1 is a receptor, the principle remains as the upregulation of CBs can be a positive angle for utilizing Δ9-THC, the potential side-effects of this phyCB can be mitigated using combination therapies as discussed prior. A polypharmacology approach is not uncommon when treating diseases especially since the lock and key approach towards disease amelioration seem to produce less than ideal outcomes (Medina-Franco, Giulianotti, Welmaker, & Houghten, 2013).

Currently, we know that ligand binding of CB_1 can participate in numerous outcomes as shown in Figs. 2 and 4 and even much more remains to be elucidated. Many reviews have been published on the effects of natural products on the CB_1 at the *in vitro* and preclinical levels (Di Marzo & Piscitelli, 2015; Gertsch, Pertwee, & Di Marzo, 2010; McAllister, Soroceanu, & Desprez, 2015; Sharma, Sadek, Goyal, Sinha, & Kamal, 2015) and so will not be reiterated here. These reviews have highlighted the modulatory effects of phyCBs that either exhibit direct or indirect impact on the ECS. Overall, many alkaloids, alkylamide derivatives, terpenes, polyphenols, polyacetylene and various fatty acids and fatty acid amides, from terrestrial and marine plant sources have shown *in vitro* and/or preclinical evidence of ECS modulation towards efficacy. Table 2 in this review highlights those phyCBs that have exhibited CB selectivity and/or potent direct or indirect ECS modulation, of these, noteworthy ones are discussed below.

We previously examined in detail the effects of 2 classical phyCBs that have and continue to be researched for efficacy. However, other phyCBs also hold promise. Non-classical CBs, fatty acid derivatives, *N*-alkylamides from the *Echinacea* spp. that show some structural similarity to the eCB, AEA, demonstrate preferred binding affinity to the CB_2 resulting in elevated levels of intracellular Ca^+ ions (Gertsch, Raduner, & Altmann, 2006), also observed with CB_1/ligand coupling in human neuroblastoma SH-SY5Y cells (Marini et al., 2009). The select binding of CB_2 is believed to be an ideal route and so compounds with restrictive capacity to peripheral tissues and so unable to cross the BBB (Pertwee, 2009) may minimize the psychoactive properties exhibited with CB_1 agonists. However, alkylamides (dodeca-2E,4E,8Z,10Z-tetraenoicacidisobutylamide) have been shown to cross the BBB; nonetheless their poor binding affinity to CB_1 might present a promising option for treating CB_2 associated disorders. Furthermore, the observed effects of the alkylamides hold additional implications, such as the treatment of congestive heart failure, as an elevation of intracellular Ca^+ ions is known to effectuate a positive ionotropic effect. This effect parallels the mode of action exhibited by the cardiac glycosides, digoxin and digitoxin, that inhibit the target molecule, Na/K^+ ATPase enzyme. Inhibition of this enzyme causes an imbalance in the ratio of Na^+ to K^+ ions that results in elevated intracellular Ca^+ ions. When this occurs, there is a subsequent increase in the strength of the heart muscles. This has implications for the treatment of congestive heart failure. The elevation in Ca^+ levels parallel the mode of action exhibited by the cardiac glycosides, digoxin and digitoxin, that inhibit the target molecule, Na/K^+ ATPase enzyme. Inhibition of this enzyme causes an imbalance in the ratio of Na^+ to K^+ ions that results in elevated intracellular Ca^+ ions. When this occurs, there is a subsequent increase in the strength of the heart muscles. Alkylamide-based modulation of the ECS also demonstrated implications for anti-inflammatory effects (Raduner et al., 2006) in addition to indirect influence on the ECS through interfering with the bioavailability of eCB precursors (Banni & Di Marzo, 2010; Di Marzo & Despres, 2009).

Another family of compounds with promising ECS mediated efficacy is terpenes. α,β-Amyrin, a pentacyclic triterpene exhibited CB mediated anti-inflammatory and antinociceptive effects and the latter were comparable to synthetic molecules, ACEA and JWH-133 (da Silva et al., 2011). While α,β-Amyrin modulated the activities of both CB_1 and CB_2, α,β-Amyrin was 15,000 fold more selective for CB_1 (da Silva et al., 2011), 200–300 fold more potent than Δ9-THC (Chicca, Marazzi, & Gertsch, 2012) and did not present with behavioral effects making this phyCB an ideal small molecule warranting further investigations especially in combination therapies. The terpene, β-caryophyllene of *Cannabis sativa* is a selective agonist of CB_2 receptors (Gertsch, 2008) that exerts anti-inflammatory and analgesic effects in addition to easing neuropathic pain (Zimmer, Treschan, Meier, & Nosch, 2009). A recent review highlights β-caryophyllene's multifaceted therapeutic effects including its observed synergy with other GPCR signaling pathways (μ-opioid receptor dependent pathways) strongly indicating the value of this phyCB to treat ECS associated morbidities (Sharma et al., 2016). The diterpene salvinorin has also confirmed effects from a polypharmacological angle as it is a selective κOR GPCR agonist and CB ligand (Fichna et al., 2009).

More recently, *in vitro* research shows the antiproliferative effects of the flavonoid, quercitin, mediated by CB_1 (Refolo et al., 2015). A review by Guzman (2003) highlights the potential anticancer effects of select cannabinoids, isolates from *Cannabis sativa* and a fairly recent review (Chakravarti, Ravi, & Ganju, 2014) provides a more detailed representation of the efficacy of these isolates to treat cancer. On another note, indirect modulation of the ECS was observed in the presence of naturally occurring quinoid terpenoids, pristimerin and euphol through reversibly inhibiting the activity of the enzyme, monoacylglycerol lipase (MAGL). For other phyCBs that exhibit efficacy through the CBs and the ECS, see Table 2.

4. Concluding remarks and future perspectives

CBs play an important role effecting many biological switches and so they are targets for treating autoimmune diseases (Cabral & Griffin-Thomas, 2009), inflammatory and/or neuropathic pain (Guindon & Hohmann, 2008), osteoporosis (Karsak et al., 2005), cancer (Velasco, Sanchez, & Guzman, 2016) and most recently, diabetes (Lu, Dopart, & Kendall, 2016; Vemuri, Janero, & Makriyannis, 2008). The latter is an interesting tangent to the well-researched insulin-mediated signal transduction pathway that has been explored for many decades but continues to present challenges of drug resistance and toxicity effects (Kooti, Farokhipour, Asadzadeh, Ashtary-Larky, & Asadi-Samani, 2016). The treatment of diabetes through either the ECS or the insulin-mediated signal transduction pathway holds promise to treating diabetes associated comorbidities, cardiovascular and metabolic disorders, collectively known as the metabolic syndrome.

The transduction of signals through the CBs by means of endogenous ligands has established a foundation for possible exogenous modulators that can mitigate disease initiation and progression. So far, agonists of the CBs are theoretically important for ameliorating neurodegenerative disorders, treating pain and many cancers and evidence of these exists at various stages of research as discussed earlier. On the other hand, antagonists have been shown to primarily play a role in weight loss, diabetes and treating feeding disorders. Yet, the theory of these expectations are not necessarily realised in clinical research and if they are, they are usually accompanied by unbearable side-effects and thus the many drug withdrawals. This is possibly due to the gross oversimplification of the ECS which encompasses a myriad of factors that surround bioactivity outcome. These factors include tissue locale, CB's shapeshifting myriads, CBs' density and their coupling efficiencies, CBs' dimerization with other GPCRs and protein receptors, and other CBs' direct and indirect targets.

Both synthetic and natural ligands of the CBs have demonstrated promising efficacy and indeed the lack of clinical applicability is congruent to both types of exogenous CB ligands. For example, despite the promising efficacy of Δ9-THC to treat various neurodegenerative disorders (Scotter, Abood, & Glass, 2010) and oncology (Walsh, Nelson, & Mahmoud, 2003), its psychoactive side-effects continue to be a deterrent to its treatment of AD, ALS, HD and cancer. So currently, Dronabinol, the international nonproprietary name of Δ9-THC is used to treat anorexia in people with HIV/AIDS in addition to nausea and vomiting in persons undergoing chemotherapy (Haney et al., 2007). Rimonabant, the synCB antagonist that showed promising efficacy to the treatment of diabetes and was therefore approved by the European

Union in 2006 was later discontinued from commercial markets and clinical research because of grave risk of psychiatric disorders.

Indeed, natural products paved the way for the current understanding of the ECS and their value in structural diversification, ECS signal transduction pathway elucidation and the synthesis of CB ligands continue to steer future directional ECS research. But the efficacy of both natural and synthetic compounds seems to lie in their specific association with the CBs and CB associated targets and their concentration, the latter confirming the theory of Paracelsus. This indeed requires more mechanistic investigations in a systematic way in order to delineate the mode of action of the phyCBs and synCBs. Understanding further, the mechanism used by synthetic *versus* natural drugs to elicit beneficial effects specifically through the various targets outlined in Fig. 2 is paramount. One such example is the effects of allosterism on the ECS' functionality as was delineated for the synthetic allosteric modulator, ORG27569. ORG27569 enhanced the current selective direct CB agonists, competitive antagonists and enzyme inhibitors. The concept of allosterism to provide an enriched landscape for novel therapeutics is shared by Changeux & Christopoulos (2016). Further, delineating the CB conformational states and their desensitization, internalization, resensitization, and downregulation (Raehal & Bohn, 2014) regulated through the β arrestins may be pivotal to advancing the development of CB exogenous ligands with enhanced efficacy. What is now needed is evidence of the clinical efficacy of CB induced β-arrestin modulation that are aligned with the observed efficacy of combination therapies of μOR analgesic, morphine and μOR antagonists, naloxone and naltrexone, usually given minutes apart. These antagonists diminish β-arrestin recruitment resulting in the improved analgesic properties of morphine while endowing significantly reduced side-effects caused by morphine, like nausea (Gan et al., 1997; Rebel, Sloan, & Andrykowski, 2009). The role of β-arrestin modulation warrants further research in ECS associated disorders and preclinical findings demonstrate the antinociceptive potential of Δ9-THC in β-arrestin2-KO mice (Raehal & Bohn, 2014). Nonetheless, alternatives to treat nausea and vomiting with reduced CNS related side-effects exist like the class of setron (*e.g.* Ondansetron).

Crystal structures of the CBs are necessary and could assist in identifying more neutral or peripherally exclusive drug leads especially in light of the many drug withdrawals. Given the recent advance in CB_1 crystal structure elucidation, advances in further delineating binding associations of the natural and synthetic leads to the CBs will be propelled further, paving the way for maximizing potential synergism within combination therapies. Understanding how the efficacy of one product whether natural or synthetic, impacts another, in that, if it enhances/diminishes bioactivities of other eCBs and exogenous CBs may only be achieved with a more comprehensive overview of the ECS. Even though significant strides have been made in this area, there remains much to be uncovered, for example, other putative CBs like orphan GPR55 and GPR18 receptors that are controlled by cannabinoid like molecules in addition to their participatory role in the ECS (Haugh, Penman, Irving, & Campbell, 2016). Additionally, there also remains much to be uncovered about the G proteins and the modulatory effects of phyCBs compared to synCBs with respect to the CB/G protein complexes. Furthermore, findings suggest that particular G protein mutations are more favorable to bind nonclassical cannabinoid ligands (Shim, Bertalovitz, & Kendall, 2011) compared to classical ones. G protein modulation seems to hold promise in cancer treatment and possibly prevention (Fan et al., 2013), yet the precise mechanism of action especially in relation to the ECS including which of the numerous subunits under the G protein family are impacted and how, remains unknown. Dated research demonstrates the effects of phyCBs and synCBs on various G protein activation dependent and independent of CB activation. Overall, the synCB, HU201 was found more potent than the eCB, AEA and the phyCB, Δ9-THC (Glass & Northup, 1999).

Finally, what is needed is a high-throughput systematic mechanism for screening the phyCBs and their analogues and is discussed further in the review article by Zhang and Xie (2012) that readily integrates not only therapeutic signal transduction pathways but also relevant adverse pathways in order to truly ascertain phyCBs that have novel therapeutic actions not mirrored by synthetic drugs. The seven helical scaffolds of the CBs warrant pharmacological assays that can compute biased rejoinders and measured expression in chemical scaffolds to enhance biased effects. Classical *in vitro* assays are limiting yet efficient while preclinical research findings contribute to clinical translation, although limitations on efficiency and costs continue to be a challenge. Hence, an *in vitro* method that enables rapid results emblematic of clinical efficacy taking into account age, inflammation, genetic and epigenetic alterations within the CBs and its targets is ideal though a momentous task. Therefore organ-on-chip technology (van der Helm, van der Meer, Eijkel, van den Berg, & Segerink, 2016) when fully streamlined maybe an ideal route that will propel the development of phyCBs in treating ECS associated morbidities.

5. Expert opinion by David Puett

This paper presents a comprehensive and critical timely overview of cannabinoid receptors and the endogenous and exogenous ligands, both synthetic and naturally occurring, that act *via* CB_1 and CB_2. These receptors are members of the G protein-coupled receptor (GPCR) superfamily that constitutes the largest gene family in the human genome, indeed with an estimated 800 members and accounting for approximately 1% of the total protein coding genes. The crystallographic structures of several GPCRs, and most recently the CB_1 as well as members of the four families of the associated G proteins, have been elucidated in recent years, thus facilitating detailed structure-function studies. The review of signaling mechanisms presented by the authors and the discussion of endocannabinoids set the stage nicely for the discussion that follows on exogenous synthetic and naturally occurring ligands that hold promise in treating a variety of disorders. The concluding section outlines the importance of research on this topic and offers cogent recommendations for advancement of the field. This paper will make a timely and valuable contribution to the literature.

Conflict of interest

The authors report no conflict of interest at this time.

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The authors report that the manuscript is not being considered for publication elsewhere and its publication is approved by all authors.

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