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Review Article

Small Molecules from Nature Targeting G-Protein Coupled Cannabinoid Receptors: Potential Leads for Drug Discovery and Development

Charu Sharma,[1] Bassem Sadek,[2] Sameer N. Goyal,[3] Satyesh Sinha,[4] Mohammad Amjad Kamal,[5,6] and Shreesh Ojha[2]

[1]*Department of Internal Medicine, College of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 17666, Al Ain, Abu Dhabi, UAE*
[2]*Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, United Arab Emirates University, P.O. Box 17666, Al Ain, Abu Dhabi, UAE*
[3]*Department of Pharmacology, R. C. Patel Institute of Pharmaceutical Education & Research, Shirpur, Mahrastra 425405, India*
[4]*Department of Internal Medicine, College of Medicine, Charles R. Drew University of Medicine and Science, Los Angeles, CA 90059, USA*
[5]*King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*
[6]*Enzymoics, 7 Peterlee Place, Hebersham, NSW 2770, Australia*

Correspondence should be addressed to Shreesh Ojha; shreeshojha@uaeu.ac.ae

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The cannabinoid molecules are derived from *Cannabis sativa* plant which acts on the cannabinoid receptors types 1 and 2 (CB_1 and CB_2) which have been explored as potential therapeutic targets for drug discovery and development. Currently, there are numerous cannabinoid based synthetic drugs used in clinical practice like the popular ones such as nabilone, dronabinol, and Δ^9-tetrahydrocannabinol mediates its action through CB_1/CB_2 receptors. However, these synthetic based *Cannabis* derived compounds are known to exert adverse psychiatric effect and have also been exploited for drug abuse. This encourages us to find out an alternative and safe drug with the least psychiatric adverse effects. In recent years, many phytocannabinoids have been isolated from plants other than *Cannabis*. Several studies have shown that these phytocannabinoids show affinity, potency, selectivity, and efficacy towards cannabinoid receptors and inhibit endocannabinoid metabolizing enzymes, thus reducing hyperactivity of endocannabinoid systems. Also, these naturally derived molecules possess the least adverse effects opposed to the synthetically derived cannabinoids. Therefore, the plant based cannabinoid molecules proved to be promising and emerging therapeutic alternative. The present review provides an overview of therapeutic potential of ligands and plants modulating cannabinoid receptors that may be of interest to pharmaceutical industry in search of new and safer drug discovery and development for future therapeutics.

1. Introduction

The endocannabinoid system (ECS), an important lipid signaling and immunomodulator system, has begun to reap attention as it is widely involved in modulating host of physiological responses ranging from appetite, respiration, metabolism, inflammation, pain, neurotransmission, and so forth. The ECS is comprised of the G-protein coupled receptors (GPCRs) such as cannabinoid receptors 1 and 2 (CB_1 and CB_2); cannabinoid receptor ligands also known as endocannabinoids are characterized by arachidonyl ethanolamide (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG) [1, 2] and the enzymes involved in synthesis and degradation of the endocannabinoids. The levels of the endocannabinoids in the tissues are maintained by the critical balance between their biosynthesis (involving phospholipase D and diacylglycerol

lipase-dependent and other pathways) and cellular uptake as well as degradation by the enzymes: fatty acid amide hydrolase (FAAH) and/or monoacylglycerol lipases (MAGL) [3]. Recently, some additional GPCRs such as GPR18, GPR55, and GPR119 have been recognized as members of the cannabinoid family; however the physiological significance is yet to be established [4].

The CB_1 and CB_2 receptors are well characterized members of the GPCR which couple to G-proteins in the $G_{i/o}$ family. The activation of the CB_1 and CB_2 receptors causes the numerous intracellular effects which may be cell type and ligand specific and involve the inhibition of various voltage gated Ca^{+2} channels and adenylate cyclase activity and the activation of K^+ channels, resulting in lower levels of cAMP along with activation of MAPK pathways [5]. The CB_1 receptors regulate the activities of adenylyl cyclase, ERK, glycogen synthase kinase 3, and calcium and potassium channels [5]. The CB_2 receptor couples to G_i to mediate their cellular effects via inhibition of adenylyl cyclase and regulation of transcription factors [5]. The inhibition of activation of cannabinoid receptors and inhibition of endocannabinoid degradative enzymes have been found to enhance endocannabinoid signaling and harness the therapeutic potential of the ECS as an important therapeutic target [6, 7].

In recent years, research is focusing on the unique neuromodulator system, ECS, which is named after the plant that led to its discovery [3]. The pervasive and varied regulatory actions of the ECS in maintenance of general health and diseases have supported the regulatory approval of several molecules of natural and synthetic origin as novel drugs that modulate the cannabinoid receptor signaling mediated by CB_1 or CB_2 receptors or alter the ECS activity by reducing the endocannabinoid tone by inhibiting FAAH and MAGL [6, 8]. The potential role for ECS-based therapies must be explored with a clear and complete picture of the potential beneficial and adverse effects that will occur from exogenous activation and/or inhibition of ECS using cannabinoid based medicines. The modulation of ECS by cannabinoid based medicines holds remarkable therapeutic promise in a variety of pathological conditions including neuropathic pain, diabetic complications, obesity, stroke, hypertension, cancer, psychosis, glaucoma, epilepsy, addiction, and neurodegenerative diseases including Alzheimer's disease, multiple sclerosis, and Parkinson's disease [7, 9].

The cannabinoids comprise compounds that produced endogenous (endocannabinoids), synthetic, and active components of *Cannabis sativa*, a traditional source of about 100 natural cannabinoids also known as phytocannabinoids [10]. The physiological effects of these phytocannabinoids derived from *Cannabis sativa* have been known since ancient times and used for both leisure and medicinal purposes and have generated immense interest for pharmaceutical development. Phytocannabinoids are defined as agents of plant origin that interacts with either of cannabinoid receptors or shares chemical similarity with cannabinoids or both. It is known that they arise from the interaction of Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive constituent of the plant; *Cannabis sativa* interact with cannabinoid receptors [11–13].

Several classes of synthetic cannabinoid agents have been developed for the therapeutic targeting of the several components of ECS. Among them, rimonabant (SR141716A; Acomplia), a CB_1 receptor antagonist/inverse agonist, makes a therapeutic success for the management of obesity but was withdrawn because of safety concerns about its psychiatric adverse effects, particularly increased incidence of depression, anxiety, and suicidal tendencies [10]. Numerous illicitly produced synthetic cannabinoid agonists typically acting as agonists at CB_1 receptors that mimic the effects of Δ^9-THC have been reported to drug monitoring agencies. Synthetic agents produce atypical pharmacological effects such as hypertension, seizures, and panic attacks. This is explained by atypical effect of CB_1 receptor agonist, which is apparently higher for synthetic cannabinoids: JWH-018 and JWH-073 compared with Δ^9-THC, the agent mainly accountable for the behavioral effects of cannabis [14].

In parallel to the development of synthetic analogues modulating ECS components, the pharmaceutical companies followed several approaches to target the cannabinoid receptors and modulate ECS activity including the development of phytocannabinoid compounds isolated from the plants. Currently, several drugs which modulate the CB_1 or CB_2 receptors are at present in the clinic such as Cesamet (nabilone), Marinol (dronabinol; Δ^9-THC), and Sativex (cannabidiol and Δ^9-THC). The agents, nabilone and dronabinol, are indicated to relieve chemotherapy-induced nausea and vomiting. Dronabinol is also used as appetizer, while the plant derived cannabis preparation. Sativex is frequently indicated for the symptomatic relief of neuropathic pain in adults with multiple sclerosis and spasticity and is also used as an adjunct to relieve pain in adult patients with advanced cancer.

The potential agents derived from plants targeting ECS have become a central focus of contemporary translational research for diverse indications with important unmet medical demands. The present review focuses on medicinal plants that have shown to modulate the ECS appearing as therapeutic possibility for diseases which involves ECS dysregulation. The present review focuses on natural small molecules, isolated and characterized as cannabinoid receptors modulator. These naturally derived molecules could offer the potential leads for future drug discovery and the targeting of endocannabinoid dysregulation or the diseases where endocannabinoid modulation represents an important therapeutic target. Additionally, the medicinal plants modulating ECS are also provided that can be subjected for the isolation of components possessing cannabinoid receptor agonist or antagonist activity. The actions of cannabinoid compounds partly involve several non-CB receptor dependent mechanisms and are regarded as an additive beneficial effect of phytocannabinoids molecules for multitargeting.

2. Phytochemicals as Lead Compounds Targeting ECS

Following the progress in chemical isolation and screening techniques, several novel lead molecules were isolated and characterized from the natural products for the development of new drugs. In current years, numerous molecules have

Figure 1: Cannabinoid receptor mediated medicinal and pharmacological activities of lead compounds isolated from medicinal plants.

been isolated and characterized which showed cannabinoid receptor affinity, efficacy, and therapeutic benefits in the *in vitro*, *in silico*, and *in vivo* studies [15–21]. The agents were also found to inhibit endocannabinoid metabolizing enzymes, FAAH, DAGL, and MAGL inhibitors, and exhibit their potential efficacy mediated by the cannabinoid mediated mechanism [7]. Figure 1 depicts the cannabinoid receptors and endocannabinoid metabolizing enzymes mediated pharmacological effects and therapeutic benefits of small molecules derived from nature.

Directly acting ligands are the compounds which exhibit high binding affinities (in low nanomolar to micromolar range) to the cannabinoid receptors and exert distinct functional effects behaving either as agonists, inverse agonists, partial agonists, or antagonist [22], whereas indirectly acting ligands target either the key proteins in the ECS which regulate endocannabinoid levels in tissues or the allosteric sites on the CB_1 receptors [6]. Recently, availability of different tools such as radioligand and [^{35}S]GTPγS binding assays facilitated the characterization of agonists, antagonists, and inverse agonists for cannabinoid receptors. Some practical guidelines and specific considerations in order to characterize the ligands using these assays are available for cannabinoid receptors. The agonists which bind to CB_1 and CB_2 receptors show little selectivity; however the CB_1 and CB_2 receptor antagonists are highly selective usually in nanomolar affinity at the respective receptor. This allows differentiating the CB_1 or CB_2 mediated mechanism and responses of *in vitro* and *in vivo* studies. In addition to the selective CB_1 and CB_2 antagonists that are used to block agonist effects, there are also genetic tools (CB_1/CB_2 receptor knockout mice) available to the research community. There are several nonselective agonists which are available which prefer either CB_1 or CB_2 receptors [4, 10].

In this review, the small molecules derived from natural products targeting ECS components are described in order to provide them as standard sources of templates for developing novel ligands for pharmaceutical development and clinical usage. The database searches using Medline/PubMed, EMBASE, Google Scholar, and Science Direct were conducted to include all the available published literature in the present review paper. The years of coverage for literature retrieval were from 1975 to May 25, 2015. The search was limited to English language publications; however if the abstract was available in English, then it is included in the present paper. For literature search, the standard MeSH such as natural products, cannabinoid receptor modulators, cannabinoid agents, medicinal plants, and cannabinoid ligands and articles all together on cannabinoid ligands were used in the database search engines. In almost all cases, the original articles were obtained and the relevant data was extracted.

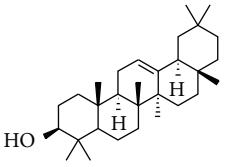
Table 1 depicts the physicochemical properties and drug likeness of phytochemicals and Figure 2 represents the chemical structure of phytochemicals modulating cannabinoid receptors and endocannabinoid metabolizing enzymes. Table 2 shows the therapeutic properties and underlying cannabinoid mediated mechanism of small natural molecules modulating cannabinoid receptors and endocannabinoid metabolizing enzymes. The cannabinoids are chemically defined as terpenoalcoholic compounds and chemical class of molecules identified till date is provided in Table 3. Recently, some selective full agonists and antagonists for specific CB_1 and CB_2 receptors have been recognized. Among the phytocannabinoids, β-caryophyllene is one which has been identified as a full agonist for CB_2 receptors and isolated from cannabis as well as noncannabis plant [18]. This generated interest in characterizing the cannabinoid-like compounds or CB receptor modulating ligands from plants other than cannabis, which is considered a traditional source of phytocannabinoids.

2.1. Alkylamides Derivatives

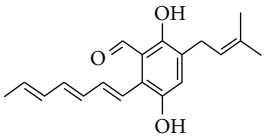
2.1.1. Alkylamides from Echinacea angustifolia. Various studies have demonstrated that the CB_2 receptors are primarily found in immune cells and participate in immune regulation [16, 17, 23, 24]. Thus, interactions of alkylamides with CB_2 receptors can be demonstrated by immunomodulatory effect of *Echinacea* preparations [21, 25, 26]. Two alkylamides, dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide and dodeca-2E,4E-dienoic acid isobutylamide, have been isolated from *Echinacea purpurea* and *Echinacea angustifolia* [21, 27]. Chemically, alkylamides show structural similarity with anandamide and bind with CB_2 receptors more potently than endogenous cannabinoids with the K_i values (CB_2 approximately 60 nM; $CB_1 > 1500$ nM) and act as full agonist on CB_2 receptors in nanomolar range. Also, the molecular modeling studies have shown that alkylamide compounds bind in the solvent-accessible cavity in CB_2 receptors which is directed by the H-bonding and pi-pi interactions [27]. Furthermore, these compounds raised total intracellular Ca^{2+} in CB_2-positive promyelocytic HL 60 cells as demonstrated by abrogation of the effects by SR144528 and also inhibit the enzyme, FAAH [27]. Though, the ketolactones found in *Echinacea pallida* (purple cornflower) did not show cannabinoid activity [28]. Another alkylamide, undeca-2-ene-8,10-diynoic acid isolated from *Echinacea* spp., stimulates 3T3-L1 differentiation mediated by PPAR-γ activity demonstrating that anti-inflammatory property of alkylamides is due to polyvalent activity [29, 30].

2.1.2. Alkylamides from Otanthus maritimus L. Several alkylamides have been isolated from dichloromethane root extract of *Otanthus maritimus* L. (family: Asteraceae), an aromatic herb growing on sandy beaches along the Mediterranean coasts. These compounds exhibit cannabinoid receptors binding affinity as demonstrated in the *in vitro*, *in silico*, and *in vivo* studies [15, 31]. The *in silico* studies were carried out by generating 3D models of hCB_2 receptors in homology modeling [31]. The root extract showed high binding affinity to CB_1 and CB_2 receptors with K_i values of 2.2 μg/mL and 1.3 μg/mL, respectively, and moderate affinity to μ- and δ-opioid receptors in radioligand assay. Among the several identified compounds from extract, a tertiary alkylamide, 1-[(2*E*,4*E*,8*Z*)-tetradecatrienoyl] piperidine, showed most potent binding affinity with both CB_1 and CB_2 receptors with a K_i value of 0.8 μM and 0.16 μM, respectively. It showed CB_2 selectivity with a $K_iCB_1/K_iCB_2 = 5$, with significant potency (K_i = 160 nM) [31]. Other isolated alkylamides as dodeca-2*E*,4*E*-dienoic acid isobutylamide, tetradeca-2*E*,4*E*-dienoic acid isobutylamide, tetradeca-2*E*,4*E*,8*Z*-trienoic acid isobutylamide, and 1-[(2*E*,4*E*,8*Z*)-tetradecatrienoyl] piperidine showed highest affinity for CB_2 receptors and show less affinity to opioid receptors. In regard to CB_2 receptor affinity, the structure activity relationship (SAR) studies reveal the influence of double bonds geometry in dodecatetraenoic acid isobutylamides. The alkylamides, N-substituted with an isobutyl or dimethylbutyl group and represented by a secondary alkylamide as the amide part, appear to be involved in the CB_2 receptor interaction [32]. However, it is observed that the tertiary amide 1-[(2*E*,4*E*,8*Z*)-tetradecatrienoyl] piperidine which contains a piperidinyl moiety linked to a C14 acyl chain appears to have more affinity and potency on CB_2 than dodeca-2*E*,4*E*-dienoic acid isobutylamide, an active principle of *Echinacea* species [15]. Overall, alkylamides from *Echinacea* and *Otanthus* spp. appear to be a good source of CB_2 receptors ligands in drug discovery.

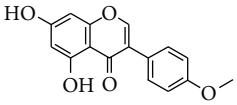
2.2. α,β-Amyrin. The pentacyclic triterpene and mixture (1 : 1) of two isomers, α,β-amyrin, are mainly constituent of the resin of *Protium kleinii* and *Protium heptaphyllum*. The CB receptor mediated anti-inflammatory and antinociceptive effect of α,β-amyrin has been shown in mice model of neuropathic pain [33]. It reduced mechanical and thermal hyperalgesia and inflammation induced by complete Freund's adjuvant and by partial sciatic nerve ligation in animal models. The antinociceptive responses were mediated by activation of the ECS and comparable to the synthetic molecules, ACEA and JWH-133. The reversals of antinociceptive effects by CB_1 or CB_2 receptor antagonists (AM251 and AM630, resp.) as well as knockdown of the CB_1/CB_2 gene demonstrate CB activity. It binds to CB_1 receptors with a high affinity (K_i = 0.133 nM) and to CB_2 receptors with a lower affinity (K_i = 1989 nM) along with absence of behavioral disturbances. The binding to CB_1 receptors was 200–300-fold more potent than Δ^9-THC. However, in contrast to Δ^9-THC and 2-AG, α,β-amyrin showed an unusual 15000-fold more binding selectivity for CB_1 receptors over CB_2. Furthermore, α,β-amyrin decreased proinflammatory cytokines and chemokines and prevented activation of the transcriptional factors: NF-κB and cyclic adenosine monophosphate response element binding (CREB) and the expression of cyclooxygenase-2 (COX-2) in footpads and spinal cords of mice. It also prevented upregulation of CB_2R mRNA but failed to affect CB_1 receptor mRNA upregulation as well as cortical levels of both CB_1 and CB_2 receptors. In another study, Chicca et al. [34] showed CB receptor binding interactions of α,β-amyrin using hCB_1/hCB_2 receptors transfected CHO-K1 cells and its effects on the endocannabinoid transport in U937 cells.



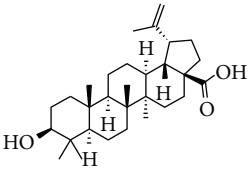
Amyrin



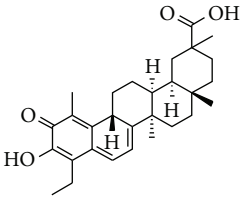
Auroglaucin



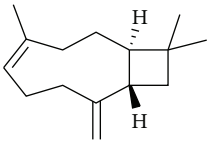
Biochanin A



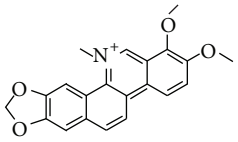
Betulinic acid



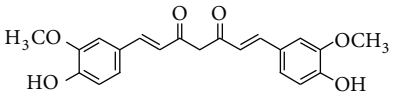
Celastrol



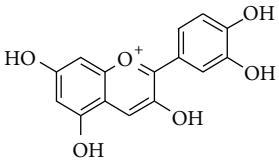
Beta-caryophyllene



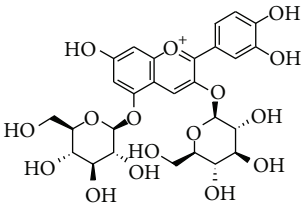
Chelerythrine



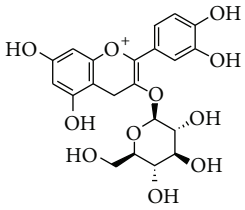
Curcumin



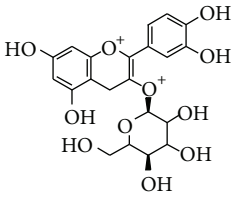
Cyanidin



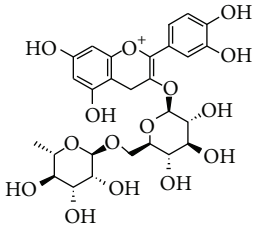
Cyanidin-3,5-di-O-glucoside



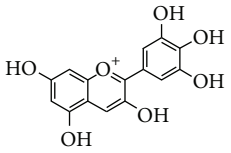
Cyanidin-3-O-glucoside



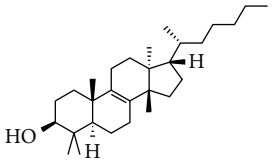
Cyanidin-3-galactoside



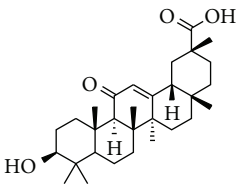
Cyanidin-3-O-rutinoside



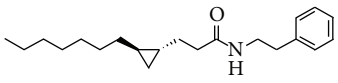
Delphinidin



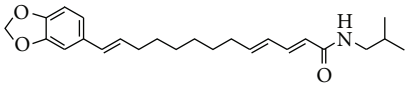
Euphol



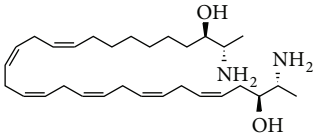
18β-Glycyrrhetinic acid



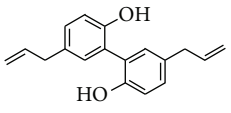
Grenadamide



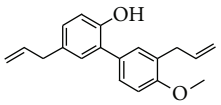
Guineensine



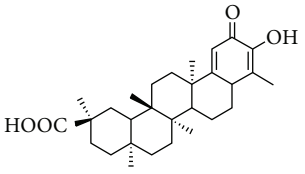
Leucettamol A



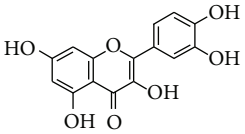
Magnolol



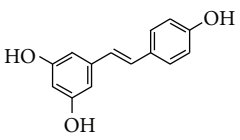
Methylhonokiol



Pristimerin



Quercetin



Resveratrol

Figure 2: Continued.

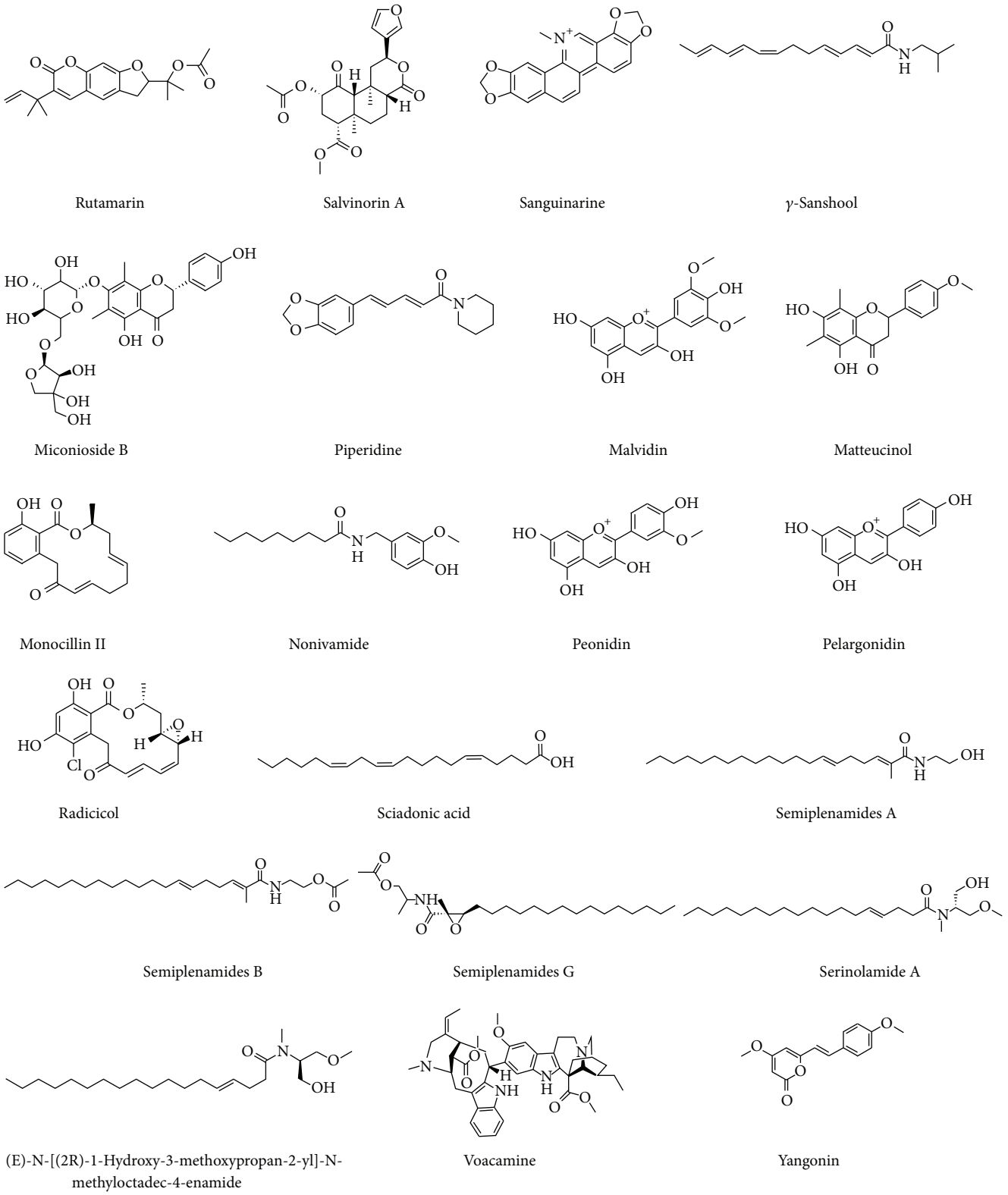


Figure 2: Chemical structure of isolated phytochemicals targeting endocannabinoid system.

Table 1: The physicochemical properties and common and IUPAC name of lead compounds modulating cannabinoid receptors.

Molecule & IUPAC name	Chemical properties	Common name(s)
Amyrin (3S,4aR,6aR,6bS,8aR,12aR,14aR,14bR)-4,4,6a,6b,8a,11,11,14b-Octamethyl-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-ol	M. Wt.: 426.71 [g/mol] M. formula: $C_{30}H_{50}O$ XLogP3-AA: 9.2 H-bond donor/acceptor: 1/1	Olean-12-en-3-beta-ol
Auroglaucin 2-[(1E,3E,5E)-Hepta-1,3,5-trienyl]-3,6-dihydroxy-5-(3-methylbut-2-enyl)benzaldehyde	M. Wt.: 298.37618 [g/mol] M. formula: $C_{19}H_{22}O_3$ XLogP3-AA: 5.4 H-bond donor/acceptor: 2/3	Auroglaucine
Biochanin A 5,7-Dihydroxy-3-(4-methoxyphenyl)chromen-4-one	M. Wt.: 284.26 [g/mol] M. formula: $C_{16}H_{12}O_5$ XLogP3-AA: 3 H-bond donor/acceptor: 2/5	5,7-Dihydroxy-4′-methoxyisoflavone
Betulinic acid (1R,3aS,5aR,5bR,7aR,9S,11aR,11bR,13aR,13bR)-9-Hydroxy-5a,5b,8,8,11a-pentamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysene-3a-carboxylic acid	M. Wt.: 456.70 [g/mol] M. formula: $C_{30}H_{48}O_3$ XLogP3-AA: 8.2 H-bond donor/acceptor: 2/3	3β-Hydroxy-20(29)-lupaene-28-oic acid
Celastrol (2R,4aS,6aR,6aS,14aS,14bR)-10-Hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydropicene-2-carboxylic acid	M. Wt.: 450.60962 [g/mol] M. formula: C29H38O4 XLogP3: 5.9 H-bond donor/acceptor: 2/4	Celastrol; tripterine; tripterin; celastrol, *Celastrus scandens*
Beta-caryophyllene (1R,4E,9S)-4,11,11-Trimethyl-8-methylidenebicyclo[7.2.0]undec-4-ene	M. Wt.: 204.35 [g/mol] M. formula: $C_{15}H_{24}$ XLogP3-AA: 4.4 H-bond donor/acceptor: 0/0	(−)-trans-Caryophyllene
Chelerythrine 1,2-Dimethoxy-12-methyl-[1,3]benzodioxolo[5,6-c]phenanthridin-12-ium	M. Wt.: 348.37 [g/mol] M. formula: $C_{21}H_{18}NO_4^+$ XLogP3-AA: 4.6 H-bond donor/acceptor: 0/4	1,2-Dimethoxy-12-methyl(1,3)benzodioxolo(5,6-c)phenanthridinium
Curcumin (1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)hepta-1,6-diene-3,5-dione	M. Wt.: 368.37 [g/mol] M. formula: $C_{21}H_{20}O_6$ XLogP3-AA: 3.2 H-bond donor/acceptor: 2/6	Diferuloylmethane
Cyanidin 2-(3,4-Dihydroxyphenyl)chromenylium-3,5,7-triol	M. Wt.: 287.24 [g/mol] M. formula: $C_{15}H_{11}O_6^+$ H-bond donor/acceptor: 5/5	Cyanidol, 3,5,7,3′,4′-pentahydroxyflavylium
Cyanidin-3,5-di-O-glucoside (2S,3R,4S,5S,6R)-2-[2-(3,4-Dihydroxyphenyl)-7-hydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromenylium-5-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	M. Wt.: 611.52 [g/mol] M. formula: $C_{27}H_{31}O_{16}^+$ H-bond donor/acceptor: 11/15	Cyanin, cyanidin 3,5-O-diglucoside
Cyanidin-3-O-glucoside 2-(3,4-Dihydroxyphenyl)-5-hydroxy-3-[(3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-7-one	M. Wt.: 448.37 [g/mol] M. formula: $C_{21}H_{20}O_{11}$ XLogP3-AA: −1.2 H-bond donor/acceptor: 7/11	—
Cyanidin 3-galactoside (2S,5R)-2-[2-(3,4-Dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-6-(hydroxymethyl)oxane-3,4,5-triol	M. Wt.: 449.38 [g/mol] M. formula: $C_{21}H_{21}O_{11}^+$ H-bond donor/acceptor: 8/10	Idaein, cyanidin 3-O-galactoside
Cyanidin 3-O-rutinoside 2-[[6-[2-(3,4-Dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methoxy]-6-methyloxane-3,4,5-triol chloride	M. Wt.: 630.97 [g/mol] M. formula: $C_{27}H_{31}ClO_{15}$ H-bond donor/acceptor: 10/15	Meralop, 3-O-rutinosylcyanidin,7,4′-dihydroxyflavilium chloride

Table 1: Continued.

Molecule & IUPAC name	Chemical properties	Common name(s)
Delphinidin 2-(3,4,5-Trihydroxyphenyl)chromenylium-3,5,7-triol	M. Wt.: 303.24 [g/mol] M. formula: $C_{15}H_{11}O_7^+$ H-bond donor/acceptor: 6/6	3,3′,4′,5,5′,7-Hexahydroxyflavylium
Euphol (3S,5R,10S,13S,14S,17S)-4,4,10,13,14-Pentamethyl-17-[(2R)-6-methylhept-5-en-2-yl]-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3-ol	M. Wt.: 426.71 [g/mol] M. formula: $C_{30}H_{50}O$ XLogP3-AA: 8.9 H-bond donor/acceptor: 1/1	Eupha-8,24-dienol
18β-Glycyrrhetinic acid (2S,4aS,6aR,6aS,6bR,10S,12aS,14bR)-10-Hydroxy-2,4a,6a,6b,9,9,12a-heptamethyl-13-oxo-3,4,5,6,6a,7,8,8a,10,11,12,14b-dodecahydro-1H-picene-2-carboxylic acid	M. Wt.: 470.68 [g/mol] M. formula: $C_{30}H_{46}O_4$ XLogP3-AA: 6.4 H-bond donor/acceptor: 2/4	18β-Glycyrrhetic acid, glycyrrhetinic acid
Grenadamide (5E)-N-[(E)-10-Chloro-4,6-dimethyl-5-oxodec-9-en-2-yl]-5-(chloromethylidene)octanamide	M. Wt.: 404.4141 [g/mol] M. formula: $C_{21}H_{35}Cl_2NO_2$ XLogP3-AA: 6.1 H-bond donor/acceptor: 1/2	—
Guineensine (2E,4E,12E)-13-(1,3-Benzodioxol-5-yl)-N-(2-methylpropyl)trideca-2,4,12-trienamide	M. Wt.: 383.52 [g/mol] M. formula: $C_{24}H_{33}NO_3$ XLogP3-AA: 6.8 H-bond donor/acceptor: 1/3	Pipyahyine
Leucettamol A (2S,3R,5Z,8Z,11Z,14Z,17Z,20Z,28R,29S)-2,29-Diaminotriaconta-5,8,11,14,17,20-hexaene-3,28-diol	M. Wt.: 472.74 [g/mol] M. formula: $C_{30}H_{52}N_2O_2$ XLogP3-AA: 6.2 H-bond donor/acceptor: 4/4	—
Magnolol 2-(2-Hydroxy-5-prop-2-enylphenyl)-4-prop-2-enylphenol	M. Wt.: 266.33 [g/mol] M. formula: $C_{18}H_{18}O_2$ XLogP3-AA: 5 H-bond donor/acceptor: 2/2	5,5′-Diallyl-2,2′-dihydroxybiphenyl
Methylhonokiol 2-(4-Methoxy-3-prop-2-enylphenyl)-4-prop-2-enylphenol	M. Wt.: 280.36 [g/mol] M. formula: $C_{19}H_{20}O_2$ XLogP3-AA: 5.3 H-bond donor/acceptor: 1/2	4′-Methoxy-3′,5-di-2-propenyl-(1,1′-biphenyl)-2-ol,4-methoxyhonokiol
Pristimerin Methyl (2R,4aS,6aR,6aS,14aS,14bR)-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14,14b-octahydropicene-2-carboxylate	M. Wt.: 464.63 [g/mol] M. formula: $C_{30}H_{40}O_4$ XLogP3-AA: 6.3 H-bond donor/acceptor: 1/4	24-Nor-D:A-friedooleana-1(10),3,5,7-tetraen-29-oic acid
Quercetin 2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	M. Wt.: 302.23 [g/mol] M. formula: $C_{15}H_{10}O_7$ XLogP3: 1.5 H-bond donor/acceptor: 5/7	—
Resveratrol 5-[(E)-2-(4-Hydroxyphenyl)ethenyl]benzene-1,3-diol	M. Wt.: 228.24 [g/mol] M. formula: $C_{14}H_{12}O_3$ XLogP3-AA: 3.1 H-bond donor/acceptor: 3/3	3,4′,5-Trihydroxystilbene
Rutamarin 2-[6-(2-Methylbut-3-en-2-yl)-7-oxo-2,3-dihydrofuro[3,2-g]chromen-2-yl]propan-2-yl acetate	M. Wt.: 356.41 [g/mol] M. formula: $C_{21}H_{24}O_5$ XLogP3-AA: 4.4 H-bond donor/acceptor: 0/5	—
Salvinorin A Methyl (2S,4aR,6aR,7R,9S,10aS,10bR)-9-acetyloxy-2-(furan-3-yl)-6a,10b-dimethyl-4,10-dioxo-2,4a,5,6,7,8,9,10a-octahydro-1H-benzo[f]isochromene-7-carboxylate	M. Wt.: 432.46 [g/mol] M. formula: $C_{23}H_{28}O_8$ XLogP3-AA: 2.5 H-bond donor/acceptor: 0/8	Divinorin A
Sanguinarine	M. Wt.: 332.32 [g/mol] M. formula: $C_{20}H_{14}NO_4^+$ XLogP3-AA: 4.4 H-bond donor/acceptor: 0/4	Dimethylene dioxybenzphenanthridine

Table 1: Continued.

Molecule & IUPAC name	Chemical properties	Common name(s)
γ-Sanshool (2E,4E,8Z,10E,12E)-N-Propan-2-yltetradeca-2,4,8,10,12-pentaenamide	M. Wt.: 259.38 [g/mol] M. formula: $C_{17}H_{25}NO$ XLogP3-AA: 4.3 H-bond donor/acceptor: 1/1	—
Miconioside B (2S)-7-[(2S,4S,5S)-6-[[(2R,3S)-3,4-Dihydroxy-4-(hydroxylmethyl)oxolan-2-yl]oxymethyl]-3,4,5-tri-hydroxyoxan-2-yl]oxy-5-hydroxy-2-(4-hydroxy-phenyl)-6,8-dimethyl-2,3-dihydrochromen-4-one	M. Wt.: 594.56 [g/mol] M. formula: $C_{28}H_{34}O_{14}$ XLogP3-AA: −0.5 H-bond donor/acceptor: 8/14	Farrerol 7-O-beta-D-apiofuranosyl(1->6)-beta-D-glucopyranoside
Piperine (2E,4E)-5-(1,3-Benzodioxol-5-yl)-1-piperidin-1-ylpenta-2,4-dien-1-one	M. Wt.: 285.33 [g/mol] M. formula: $C_{17}H_{19}NO_3$ XLogP3: 3.5 H-bond donor/acceptor: 0/3	1-Piperoylpiperidine
Malvidin 2-(4-Hydroxy-3,5-dimethoxyphenyl)chromenylium-3,5,7-triol	M. Wt.: 331.29 [g/mol] M. formula: $C_{17}H_{15}O_7^+$ H-bond donor/acceptor: 4/6	3′,5′-Dimethoxy-3,4′,5,7-tetrahydroxy flavylium acid anion
Matteucinol 5,7-Dihydroxy-2-(4-methoxyphenyl)-6,8-dimethyl-2,3-dihydrochromen-4-one	M. Wt.: 314.33 [g/mol] M. formula: $C_{18}H_{18}O_5$ XLogP3-AA: 3.4 H-bond donor/acceptor: 2/5	(2S)-5,7-Dihydroxy-2-(4-methoxyphenyl)-6,8-dimethyl-2,3-dihydro-4H-chromen-4-one
Monocillin II (4E,8E,11S)-15-Hydroxy-11-methyl-12-oxabicyclo[12.4.0]octadeca-1(14),4,8,15,17-pentaene-3,13-dione	M. Wt.: 300.349 [g/mol] M. formula: $C_{18}H_{20}O_4$ XLogP3-AA: 4.1 H-bond donor/acceptor: 1/4	—
Nonivamide N-[(4-Hydroxy-3-methoxyphenyl)methyl]nonanamide	M. Wt.: 293.40 [g/mol] M. formula: $C_{17}H_{27}NO_3$ XLogP3-AA: 4.2 H-bond donor/acceptor: 2/3	N-Vanillyl pelargonamide, pelargonic acid vanillylamide
Peonidin 2-(4-Hydroxy-3-methoxyphenyl)chromenylium-3,5,7-triol	M. Wt.: 301.27 [g/mol] M. formula: $C_{16}H_{13}O_6^+$ H-bond donor/acceptor: 4/5	3,4′,5,7-Tetrahydroxy-3′-methoxyflavylium
Pelargonidin 2-(4-Hydroxyphenyl)chromenylium-3,5,7-triol	M. Wt.: 271.24 [g/mol] M. formula: $C_{15}H_{11}O_5^+$ H-bond donor/acceptor: 4/4	3,4′,5,7-Tetrahydroxy flavylium chloride
Radicicol	M. Wt.: 364.77698 [g/mol] M. formula: $C_{18}H_{17}ClO_6$ XLogP3-AA: 3.4 H-bond donor/acceptor: 2/6	Monorderne, radisico, melanotetan II, monorden A
Sciadonic acid (5E,11E,14E)-Icosa-5,11,14-trienoic acid	M. Wt: 306.48 [g/mol] M. formula: $C_{20}H_{34}O_2$ XLogP3-AA: 6.7 H-bond donor: 1/2	Icosa-5,11,14-trienoic acid, 5c,11c,14c-eicosatrienoic acid
Semiplenamides A (2E,6E)-N-(2-Hydroxyethyl)-2-methylicosa-2,6-dienamide	M. Wt.: 365.59 [g/mol] M. formula: $C_{23}H_{43}NO_2$ XLogP3-AA: 7.7 H-bond donor/acceptor: 2/2	—
Semiplenamides B 2-[[(2E,6E)-2-Methylicosa-2,6-dienoyl]amino]ethyl acetate	M. Wt.: 407.62 [g/mol] M. formula: $C_{25}H_{45}NO_3$ XLogP3-AA: 8.3 H-bond donor/acceptor: 1/3	—
Semiplenamides G 2-[[(2S,3R)-2-Methyl-3-pentadecyloxirane-2-carbonyl]amino] propyl acetate	M. Wt.: 411.61 [g/mol] M. formula: $C_{24}H_{45}NO_4$ XLogP3-AA: 7.6 H-bond donor/acceptor: 1/4	—

Table 1: Continued.

Molecule & IUPAC name	Chemical properties	Common name(s)
Serinolamide A (E)-N-[(2R)-1-Hydroxy-3-methoxypropan-2-yl]-N-methyloctadec-4-enamide	M. Wt.: 383.6083 [g/mol] M. formula: $C_{23}H_{45}NO_3$ XLogP3-AA: 6.6 H-bond donor/acceptor: 1/3	(4E)-N-[(2R)-1-Hydroxy-3-methoxy-2-propanyl]-N-methyl-4-octadecenamide
Voacamine	M. Wt.: 704.89 [g/mol] M. formula: $C_{43}H_{52}N_4O_5$ XLogP3-AA: 6.1 H-bond donor/acceptor: 2/7	Voacanginine, voacamine
Yangonin 4-Methoxy-6-[(E)-2-(4-methoxyphenyl)ethenyl]pyran-2-one	M. Wt.: 258.26 [g/mol] M. formula: $C_{15}H_{14}O_4$ XLogP3-AA: 2.7 H-bond donor/acceptor: 0/4	4-Methoxy-6-(β-(p-anisyl)vinyl)-α-pyrone

The XLogP3-AA data, molecular weight, molecular formula, and H-bond donor/H-bond were collected from NCBI, http://www.ncbi.nlm.nih.gov/pccompound/?term.

Table 2: The cannabinoid receptor affinity, potency, and activity of lead molecules.

Compound	CB receptor mediated effect	CB receptor affinity/potency	References
γ-Sanshool	Diabetes	CB_2 agonist	Dossou et al. 2013 [106]
4′-O-Methylhonokiol	Alzheimer's diseases	CB_2 agonist	Gertsch and Anavi-Goffer 2012 [87] Schuehly et al. 2011 [85]
Yangonin	Anxiety	CB_1 receptor antagonist	Ligresti et al. 2012 [112]
Amyrin	Neuropathic pain	CB_1/CB_2 agonist, MAGL inhibitor	Simão da Silva et al. 2011 [33]
Betulinic acid	Cancer	CB_1 antagonist/CB_2 agonist	Liu et al. 2012 [38]
β-Caryophyllene	Ulcerative colitis Alzheimer's diseases Insulin resistance Alcohol addiction Anxiety Depression Nephrotoxicity Cerebral ischemia	CB_2 agonist	Bento et al. 2011 [40] Horváth et al. 2012 [47] Al Mansouri et al. 2014 [41] Bahi et al. 2014 [49] Choi et al. 2013 [43] Klauke et al. 2014 [50] Suijun et al. 2014 [44] Guo et al. 2014 [42] Gertsch et al. 2008 [18]
Celastrol	Neuropathic pain	CB_2 agonist	Yang et al. 2014 [55]
Chelerythrine	Neuropathic pain Neuroblastoma	CB_1 antagonist	Lim et al. 2003 [57]
Curcumin	Neuroprotective liver fibrosis	CB_1 antagonist/CB_2 agonist	Hassanzadeh and Hassanzadeh, 2012 [64]
Euphol	Neuropathic pain	CB_1/CB_2 agonist, MAGL inhibitor	Dutra et al. 2012 [71]
18β-Glycyrrhetinic acid	Obesity	CB_1 antagonist	Park et al. 2014 [73]
Pristimerin	Pain & inflammation	MAGL inhibitor	Chicca et al. 2012 [34]
Salvinorin A	Anxiety Depression Neuropathic pain Ulcerative colitis	CB_1 agonist, FAAH inhibitor	Fichna et al. 2012 [102] Aviello et al. 2011 [100] Capasso et al. 2008 [98] Braida et al. 2009 [95] Braida et al. 2007 [99]
Malyngamide B	Inflammation	CB_1/CB_2 agonist	Montaser et al. 2012 [82]
Rutin	Depression	CB_1 agonist	Su et al. 2014 [83]
Serinolamide B	Inflammation Cancer	CB_1 and CB_2 receptors action	Montaser et al. 2012 [82]

Table 3: The chemical class of compounds showing nature derived cannabinoid ligands.

Alkaloids	Terpenes and terpenoid	Polyphenols	Fatty acid derivatives
(i) Auroglaucin (ii) Chelerythrine (iii) Guineensine (iv) Bibenzyls (v) Isoperrottetin A (vi) Sanguinarine (vii) γ-Sanshool (viii) Voacamine (ix) 3,6-Oxidovoacangine (x) 5-Hydroxy-3,6-oxidovoacangine (xi) Haplosamates (xii) Desulfohaplosamates (xiii) Piperine (xiv) Neocosmosins (xv) Monocillins (xvi) Radicicol (xvii) Yangonin	(i) Amyrin (ii) Betulinic acid (iii) β-Caryophyllene (iv) Celastrol (v) Euphol (vi) Falcarinol (vii) 18β-Glycyrrhetinic acid (viii) Isoperrottetin A (ix) Pristimerin (x) Salvinorin A (xi) Thujone (xii) Yangonin (xiii) Thujone	(i) Biochanin A (ii) Curcumin and derivatives (iii) Cyanidin derivatives (iv) Desmodianones (v) Delphinidin (vi) (+)-Catechin derivatives (vii) Honokiol derivatives (viii) Peonidin (ix) Pelargonidin (x) Magnolol (xi) Malvidin (xii) Rutin (xiii) 6-Methyltetrapterol A (xiv) Magnolol (xv) Miconioside (xvi) Resveratrol	(i) Dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide (ii) Dodeca-2E,4E-dienoic acid isobutylamide (iii) 1-[(2*E*,4*E*,8*Z*)-Tetradecatrienoyl] piperidine (iv) Dodeca-2*E*,4*E*-dienoic acid isobutylamide (v) Tetradeca-2*E*,4*E*-dienoic acid isobutylamide (vi) Tetradeca-2*E*,4*E*,8*Z*-trienoic acid isobutylamide (vii) 1-[(2*E*,4*E*,8*Z*)-Tetradecatrienoyl] piperidine (viii) Malyngamides (ix) Serinolamides (x) Sciadonic acid (xi) Semiplenamides

The study showed that it did not bind to cannabinoid receptors ($K_i > 10\,\mu$M) whereas it inhibited 2-AG hydrolysis in pig brain homogenates and failed to inhibit AEA. Additionally, β-amyrin is found to weakly inhibit human MAGL in a rapid, reversible, and noncompetitive manner, similar to structurally related but more potent triterpene, pristimerin. Subsequently, Matos et al. [35] also showed the cannabimimetic activity of α,β-amyrin in dextran sulfate sodium-induced colitis in mice by diminishing disease activity, colonic damage, and activity of myeloperoxidase, N-acetylglucosaminidase, and attenuating induction of proinflammatory mediators: cytokines, chemokines, and adhesion molecules in the colon. The abrogation of the beneficial effects of α,β-amyrin by CB_1 receptor blocker, but not by CB_2 receptor blocker, demonstrates the CB_1 receptor mediated mechanism. Additionally, α,β-amyrin treatment reduced the MAGL and FAAH enzymes. Integrating the ECS modulatory properties α,β-amyrin seem to be a promising candidate for future therapeutics.

2.3. Anthocyanins. Anthocyanins are water-soluble polyphenol compounds abundantly found in colored fruits and vegetables particularly in red and blue fruits such as blueberry, cranberry, and red cabbage. These have been shown to regulate several intracellular functions. Numerous studies have shown that anthocyanins and anthocyanidins exhibit antioxidant, redox-inflammatory signaling which contributes to its analgesic, cardioprotective, neuroprotective, anticancer, atherogenic, antihyperlipidemic, and antihypertensive effects. The cannabinoid receptor activity has been demonstrated by competitive radioligand assays of cyanidin ($K_i = 16.2\,\mu$M) and delphinidin ($K_i = 21.3\,\mu$M) for hCB_1 receptors whereas similar affinities for CB_2 receptors have been shown by cyanidin ($K_i = 33.5\,\mu$M), delphinidin ($K_i = 34.3\,\mu$M), and peonidin ($K_i = 46.4\,\mu$M) [36]. However, the cyanidin derivatives such as cyanidin-3,5-di-O-glucoside, cyanidin-3-O-glucoside, cyanidin-3-O-galactoside, cyanidin-3-O-rutinoside, malvidin, and pelargonidin showed inhibition of both CB_1 and CB_2 receptors. Additionally, cyanidin-3-O-β-glucoside also reported to activate all forms of PPARs and reduces hepatic lipids by altering the expression of genes involved in lipid metabolic pathways. Taking altogether the multiple pharmacological properties, anthocyanins appear as polypharmacological agent for diseases involving dysregulation of ECS and PPARs [36].

2.4. Auroglaucin. Auroglaucin, a benzaldehyde compound, is obtained from ethyl acetate extract of fungus *Eurotium repens* collected from Tifton, GA. The extract as well as auroglaucin showed binding affinity for CB_1 (62.6%) and CB_2 receptors (43.1%) using CP55,940 assay in CHO-K1 cells [37]. The extract also showed affinity with opioid receptors with binding affinity more than 40%. The IC_{50} for CB_1 and CB_2 receptor was 15.2 and 19.9 μM, respectively [37].

2.5. Betulinic Acid. Betulinic acid is a widely distributed pentacyclic triterpenoid with a lupan skeleton in the plant kingdom. Betulinic acid isolated from the extract of several plants and its synthetic analogues exhibit a broad spectrum of activities including antioxidant, anti-inflammatory, antiangiogenic, immunomodulatory, and anticancer. Liu et al. [38] investigated the effects of CB_1 and CB_2 receptor antagonists AM251 and AM630, respectively, on betulinic acid-dependent repression of Sp1, Sp3, and Sp4 and survivin. Betulinic acid and either AM251 or AM630 attenuated the effects of betulinic acid persuaded downregulation of Sp1, Sp3, and Sp4 and survivin and AM251 and AM630 inhibited betulinic acid-mediated downregulation of ErbB2, p-ErbB2, p-MAPK, p-Akt, and YY1 in BT474 and MDA-MB-453 cells. Further, betulinic acid competitively bound to both cannabinoid receptors with K_i values of 36.7 ± 4.1 and $41.2 \pm 12.1\,\mu$mol/L for mCB_1 and hCB_2 receptors, respectively, in

radioligand binding assay. The role of CB receptor mediated activity was further confirmed in CB_1 and CB_2 knockdown mice partially reversed betulinic acid-induced downregulation of Sp1, Sp3, and Sp4. Betulinic acid-mediated repression of Sp1, Sp3, Sp4, and Sp-regulated genes found because of induction of the Sp repressor ZBTB10 and downregulation of microRNA-27a, which constitutively inhibits ZBTB10 expression, showed that the effects of betulinic acid were CB_1 and CB_2 receptor dependent. Further, it has also been shown to activate PPAR-γ, which encourages it as a multitargeted agent for future therapeutics.

2.6. Biochanin A. Biochanin A is an O-methylated isoflavone compound predominantly found in vegetable plants, red clover, soy, alfalfa sprouts, peanuts, and chickpea, and possesses potent antioxidant, anti-inflammatory, phytoestrogenic, and antineoplastic activities. It showed modest effects on CB_1 and CB_2 receptors in [^{3}H]CP55,940 assay and inhibited brain CB_1 receptors (27%) and recombinant CB_2 receptors (33%) [39]. No studies are available to demonstrate its other activities such as PPAR-γ modulation. It has been reported to inhibit FAAH (IC_{50} = 0.62 μM) at micromolar potencies in RBL2H3 cells [39].

2.7. β-Caryophyllene. β-Caryophyllene, a volatile sesquiterpene, is abundantly found in essential oil of many plants such as cloves, oregano, cinnamon, black pepper, hemp, rosemary, and hops [18]. It is popularly used in food, cosmetics, and fragrances as a preservative, additive, and flavoring agent. It is approved by several food and flavor regulatory agencies including United States Food and Drug Administration (FDA) for its use as a food additive and classified as a "generally regarded as safe" compound. Gertsch et al. [18] first time reported that the fractionation of cannabis essential oil yields β-caryophyllene which possesses an affinity for CB_2 receptors. In radioligand assays, (*E*)-β-caryophyllene and its isomer (*Z*)-β-caryophyllene dose-dependently displaced CP55,940 from hCB_2 receptors significantly expressed in HEK293 cells (K_i = 155 ± 4 nM) in the nanomolar range and exhibit selective full agonism on CB_2 receptors. (E)-β-caryophyllene exerts potent cannabimimetic anti-inflammatory effects in mice. Several studies have shown the CB_2 receptor dependent therapeutic effects in ulcerative colitis [40], alcohol addiction [41], cerebral ischemia [42, 43], insulin resistance [44], glutamate neurotoxicity [45], hypertriglyceridemia [46], renal injury [47], liver fibrosis [48], anxiety and depression [49], neuropathic pain [50], Alzheimer's disease [51], and CB_2 receptor knockout mice [47]. Taking together the cannabimimetic [18], opioidergic [52], and PPARs mediated activity [53], β-caryophyllene appears as most promising molecule of pharmaceutical interest with multifunctional and polypharmacological properties.

2.8. Catechins. Catechins are the group of polyphenol compounds abundantly found in the leaves of tea, the most popular beverage consumed worldwide and in many fruits and legumes. Catechins are known to maintain heath and general well-being and pharmacotherapeutic effects. The catechin compounds include (−)-epigallocatechin-3-O-gallate (EGCG), (−)-epicatechin-3-O-gallate (ECG), (−)-epigallocatechin (EGC), (−)-epicatechin, and (+)-catechin. These compounds have been comprehensively studied and shown to possess antioxidant, anti-inflammatory, GABAergic, glutamatergic, monoaminergic, opioidergic, and nitrergic modulatory activities and contribute to the several therapeutic benefits. For the first time, Korte et al. [54] evaluated the affinities of EGCG, ECG, EGC, (−)-epicatechin, and (+)-catechin for human CB_1 and CB_2 receptors in competitive radioligand binding assays in Chem-1 and CHO cells. All the compounds, namely, EGCG (K_i = 33.6 mM), EGC (K_i = 35.7 mM), and ECG (K_i = 47.3 mM) exhibited binding with CB_1 and CB_2 receptors in a dose-dependent manner. However, the weaker binding to CB_2 receptor was found with inhibition constants more than 50 mM for ECC and EGC. The epimers such as (+)-catechin and (−)-epicatechin in radioligand assays showed slight affinities for both CB_1 and CB_2 receptors. The study demonstrates that catechins possess a moderate affinity for CB_1 receptors whereas binding to CB_2 receptor was not very prominent. In SAR studies, the ungallated catechins were found to have negligible bioactivities for CB_1 and CB_2 and the 3^1,4^1,5^1-trihydroxyl substitution in the catechin B-ring partially contributing to antioxidant, apoptosis-inducer, and β-secretase inhibiting activity of catechins did not appear responsible for binding with cannabinoid receptors. Thus, the multifunctional effects of catechins could be further exploited for cannabinoid activities that with additional pharmacological properties may synergize the actions.

2.9. Celastrol. Celastrol, a quinone methide triterpenoid, is a pharmacologically active constituent from the root of *Tripterygium wilfordii* and *Celastrus regelii* (family: Celastraceae) also known as Thunder of God Vine in the Asian continent. It is used as a remedy of inflammatory and autoimmune diseases along with its antioxidant, anti-inflammatory, anticancer, and insecticidal activities. Celastrol showed cannabinoid mediated therapeutic activity in inflammatory and neuropathic pain induced by carrageenan and spared nerve injury in animal models [55]. It produces a dose-dependent inhibition of edema and allodynia evidenced by inhibition of inflammatory cytokines and hypersensitivity of nociceptive response. Further, the reversal of antihyperalgesic effects of celastrol by SR144528, a specific CB_2 receptor antagonist, but not by SR141716, a specific CB_1 receptor antagonist, demonstrates the analgesia effects of celastrol through CB_2 signaling. Although celastrol shows an effect on CB_2 receptors in neuropathic pain and inflammation, further studies would explore its potential as a novel candidate for pain relief.

2.10. Chelerythrine and Sanguinarine. Chelerythrine and sanguinarine are the alkaloids of quaternary benzophenanthridine class in several medicinal plants and reported as a potent protein kinase C (PKC) inhibitor. These compounds showed to modify behavior mediated by CB_1 receptors [56]. The CB_1 receptor modulatory property of chelerythrine was first reported in a chronic constriction sciatic nerve injury

model of neuropathic pain [57]. The application of chelerythrine was found to inhibit CB_1 receptors mainly within the ipsilateral superficial spinal cord dorsal horn mediating tyrosine kinase receptors. Chelerythrine also inhibits desacetyl levonantradol-dependent activation of CB_1 receptor in the neuroblastoma cells (N18TG2) and this was supported with modulation of a downstream PKC by CB_1 receptor [58]. The pseudobase forms of chelerythrine and sanguinarine inhibit CB_1 receptors similar to Δ^9-THC at low micromolar concentrations in mouse brain membrane [59]. In [^{3}H]CP55,940 binding assay, the IC_{50} of sanguinarine and chelerythrine appears in the 1-2 μM range, which has similar potency like cannabidiol, virodhamine, various Δ^8-THC derivatives, and certain bicyclic resorcinols [60]. However, these were found weaker than Δ^9-THC and Δ^9-tetrahydrocannabivarin, which inhibit the binding of [^{3}H]CP55,940 at low nanomolar concentrations [61]. Chelerythrine and sanguinarine showed lesser potency in comparison with several conventional CB_1 receptor blockers but act differently to AM251 by the reverse modulation of CB_1 receptors [56]. A recent study showed that chelerythrine produces the sequential activation of muscarinic (M_3) receptors and CB_1 receptors which synergistically induce contractile effects of the bovine ciliary muscle by involving the activation of Rho-kinase and PKC [62]. Considering the CB selectivity these molecules may serve as a template for potent CB_1 receptor blocking drugs of natural origin negatively regulating the ECS.

2.11. Curcumin. Curcumin, chemically known as diferuloylmethane, is a well-known polyphenol molecule and an active constituent of the dietary spice turmeric (*Curcuma longa*) used for dietary and medicinal purposes since centuries. Numerous studies demonstrate that curcumin regulates various signaling molecules including inflammatory molecules, cytokines and chemokines, adhesion molecules, transcription factors, enzymes, protein kinases, protein reductases, carrier proteins, cell survival proteins, cell-cycle regulatory proteins, drug resistance proteins, growth factors, receptors, DNA, RNA, and metal ions. Seely et al. [63] first showed that curcumin binds to CB_1 receptors with nanomolar affinities and in micromolar affinities with CB_2 receptors. Structurally, curcumin also shares structural motifs with some cannabinoid receptor ligands. Further, curcumin has been showed to cause sustained elevation of brain derived nerve growth factor and endocannabinoids in brain region-specific and dose-dependent manner similar to the conventional antidepressant amitriptyline [64]. However, pretreatment with AM4113, a CB_1 receptor neutral antagonist, but not with SR144528, a CB_2 receptor antagonist, prevents induction of brain derived nerve growth factors and suggests CB_1 receptor mediated ECS as novel targets for curcumin. Recently, Witkin et al. [65] reported that curcumin did not potently alter GTP-γ-35S binding, which suggests its functional CB_1 antagonist (K_i = 2080 nM). Further, curcumin did not prevent the hypothermic effects of the CP55,940 and the anti-immobility effects of curcumin did not occur in CB_1 knockout ($CB_1^{-/-}$) mice. In a recent study, Zhang et al. [66] demonstrated the cannabinoid mediated antifibrotic activity of curcumin in liver fibrosis induced by carbon tetrachloride. Curcumin treatment upregulated CB_2 receptors and downregulated CB_1 receptors in hepatic stellate cells and modulated the expression of extracellular matrix (ECM) proteins. The abrogation of inhibition of curcumin effects on ECM expression revealed that inverse agonism/antagonism of CB_1 receptors contributed to curcumin inhibition of ECM expression. Further, *in silico* studies showed its binding to CB_1 receptors with two hydrogen bonds. In a very recent study, bisdemothoxycurcumin, a derivative of curcumin, has been showed to induce apoptosis in activated hepatic stellate stem cells by impairing cellular energetics and downregulating cytoprotective proteins, likely through a mechanism that involves CB_2 receptors as evidenced by reversal of the BDMC-induced apoptosis with cotreatment of SR144528, a CB_2 antagonist, and confirmed with genetic downregulation of the receptor using siCB_2 receptors [67]. The studies conclude that the effects of curcumin in chronic liver disease are mediated by cannabinoid receptors and may offer therapeutic benefits in hepatic fibrosis. Integrated all together, cannabinoid mediated effects of curcumin and well established manifold properties of curcumin; it holds a strong propensity in diseases where ECS is dysregulated.

2.12. Haplosamate. Haplosamate derivatives are first naturally derived cannabinomimetic compound belonging to steroid family representing a new chemical class of cannabinoid receptor ligands. It is a group of steroids including haplosamate A and haplosamate B [68, 69]. Haplosamate A is a C28 sterol containing seven oxygenated carbons and a rare six-member ether ring connecting C-16 and C-23 with a sulfate group at C-3 as well as a methyl phosphate at C-15. For the first time, it was isolated from a sponge, *Xestospongia* sp., and later on from other sponges such as Haplosclerida spp. and *Cribrochalina* spp. [68] and Indonesian marine sponge, *Dasychalina* spp. (family: Niphatidae). The isolated haplosamate compounds, haplosamate A and desulfohaplosamate, as well as semisynthetic derivatives were screened for the interaction and affinity to cannabinoid receptor. Haplosamate A and desulfohaplosamate exert opposite effects as haplosamate A showed significant affinity for CB_1 receptor, whereas desulfohaplosamate showed higher affinity for CB_2 receptor. The 7-monoacetylated derivative of haplosamate A exhibits affinity to both cannabinoid receptors in comparison with its parent compound. However, acetylation at C-4 or dialdehyde derivative showed the loss of affinity on both CB_1 and CB_2 receptors.

2.13. Euphol. *Euphol*, a tetracyclic triterpene alcohol, is the key constituent in the sap of *Euphorbia tirucalli* L. (family: Euphorbiaceae), a plant grown in Africa and South America, Brazil, and Amazonas. King et al. [70] first reported that euphol inhibits MAGL in a reversible and noncompetitive manner. The SAR studies reveal that euphol is a bioisoester of pristimerin and lacks the quinone methide group and is found devoid of CNS side effects in the tetrad tests, such as deficit locomotor, catalepsy, analgesia, and hypothermia, typical features of cannabinoids. Euphol showed potent immunomodulator and anti-inflammatory effects in animal

models of ulcerative colitis and autoimmune encephalomyelitis where CB_2 receptors play a vital role in pathogenesis [71]. The antihyperalgesic effect of euphol appears similar to the effects caused by ACEA, a CB_1 receptor agonist, and JWH-133, a CB_2 receptor agonist. The reversal of the antinociceptive effects of euphol on pretreatment with CB_1 antagonist AM251 or with CB_2 selective antagonist AM630 showed CB_1 and CB_2 receptor dependent mechanisms. Euphol was found efficacious in preventing the neuropathic behavior mediated through the modulation of both CB_1 and CB_2 receptors. These findings suggest that euphol has excellent potential for use in neuropathic pain and persistent inflammation owing its ability to interact with ECS and is devoid of the CNS adverse effects even at high doses.

2.14. Falcarinol. Falcarinol is a C17-polyacetylene compound with two carbon-carbon triple bonds and two double bonds and possesses a reactive polyyne structure and is found predominantly in carrot, celery, fennel, parsnip, and Gamisans, members of Araliaceae and Apiaceae family. It is a phytoalexin also known as panaxynol and isolated for the first time from *Panax ginseng*. It showed to bind with both cannabinoid receptors nonselectively but selectively alkylates the CB_1 receptors and induces CB_1 receptor mediated functional signals by covalent and irreversible interaction with the CB_1 receptors (K_i = 0.59 μM) [72]. Though, falcarinol is not a functional ligand at CB_2 receptor as it did not interfere with constitutive or forskolin-stimulated cAMP but appears as a weak partial agonist on CB_2 receptor and acting through G_o signaling [72]. Falcarinol is unstable and upon exposure to sunlight causes the formation of secondary alcohol with the loss of binding affinity to the cannabinoid receptors. Thus, only freshly obtained falcarinol exerts significant cannabinoid receptor binding affinity. Recently, falcarinol showed inverse agonist/antagonism for the CB_1 receptors in keratinocytes and causes expression of proallergic chemokines in keratinocytes, the effects similar to rimonabant. Furthermore, a structural analog of falcarinol, pontica epoxide, was found devoid of affinity either for cannabinoid or for opioid receptors [15].

2.15. 18β-Glycyrrhetinic Acid. 18β-Glycyrrhetinic acid and its diastereomer 18α-GA are the triterpenoid saponins obtained from the roots of *Glycyrrhiza glabra* L., popularly known as licorice. It is generally used as a natural sweetener and flavoring additive in food and as traditional medicines owing to its antimicrobial, anticancer, and anti-inflammatory properties. The inhibitory activities of licorice extract in hCB_1 receptor-expressing Chem-1 cells showed a dose-dependent decrease in intracellular Ca^{2+} levels (IC_{50} = 1.96 ± 0.05 μM) [73]. Other active constituents of licorice like liquirtin, glabridin, and 18α-glycyrrhetinic acid also exhibited inhibitory activity against Ca^{2+} flux induced by AEA, whereas 18β-glycyrrhetinic acid showed stronger potency evidenced by more than 90% inhibition in responses to CB_1 receptor agonist. The 18β-glycyrrhetinic acid was also found to regulate CB_1 receptors implicated in antiadipogenesis responses in 3T3-L1 cells and exerts antiobesity effects by correcting lipid dysregulation, body weight gain in diet-induced obese animals [73]. Further, it also alleviated effects of AEA, a CB_1 receptor agonist, and suppressed adipocyte differentiation in 3T3-L1 cells by downregulating the AEA-induced MAPK activation and expression of adipogenic genes including C/EBP-α and PPAR-γ. The 18β-glycyrrhetinic acid in licorice extract appears to be an active constituent possessing CB_1 receptor downregulatory effect and confers therapeutic effects against obesity.

2.16. Guineensine. Guineensine possesses potent cytotoxic, insect repellents, anti-inflammatory, insecticidal, and antifeedant activities from black pepper, *Piper nigrum* (family: Piperaceae). It appears as a potent novel inhibitor (EC_{50} = 290 nM) of cellular uptake of the AEA and 2-AG [74, 75] in nanomolar range. Though, guineensine did not inhibit the enzyme FAAH or enzyme MAGL or interact with cannabinoid receptors or fatty acid binding protein 5 (FABP5), a major cytoplasmic AEA carrier, or serine hydrolases. The SAR studies suggest the significance of alkyl chain length interconnecting the pharmacophoric isobutylamide and benzodioxol moieties for AEA cellular uptake inhibition. Studies have shown cannabimimetic effects such as catalepsy, hypothermia, reduced locomotion, analgesia, and blockade of the effects by CB_1 receptor antagonist, rimonabant (SR141716A) in animals. Other common constituents of black pepper, piperine, dose-dependently reduce intestinal fluid accumulation induced by castor oil and pretreatment with SR141716A; a CB_1 receptor antagonist showed that the effects were not dependent on cannabinoid receptors [76]. Similarly, Izzo et al. [77] studied the effect of capsaicin, piperine, and anandamide on upper gastrointestinal motility in mice and showed the inhibitory effect of anandamide but not piperine using a noneffective dose of SR141716A, a CB_1 receptor antagonist. Piperine appears to reduce upper gastrointestinal motility independent of CB_1 receptors. Guineensine appears as a novel plant derived compound which inhibits endocannabinoid uptake independent of FAAH [74, 75]. Thus, the scaffold of guineensine could be useful in finding future tools for ECS transport and modulatory mechanism in therapeutics.

2.17. Hydroxyeicosatetraenoic Acid (HETE) and Hydroxyl-Anandamide (HAEA). The oxylipin, 3-hydroxyarachidonic acid (3(*R*)-HETE), is an intermediate of the β-oxidation of arachidonic acid and plays an important biological role in the life cycle of fungi. The fungal pathogen *Candida albicans* transforms arachidonic acid into 3(*R*)-HETE. It has been showed that *Diposascopsis uninucleata* converts AEA into 3-HAEA and established an enantiodivergent synthesis to study its pharmacological activity [78]. The affinity of AEA, 3(*R*)-HAEA, and 3(*S*)-HAEA for CB_1 receptors was 0.02 ± 0.015 μM, 1.85 ± 0.275 μM, and 1.46 ± 0.33 and for CB_2 receptors was 0.11 ± 0.025 μM, 6.43 ± 0.7710 μM, and 4.85 ± 0.38 μM, respectively. Thus, yeasts producing 3(*R*)-HETE convert AEA released by the host cells at the site of infection into 3(*R*)-HAEA which leads to the inflammatory and algogenic responses associated with fungal diseases. Both the enantiomers of 3-HAEA exhibited similar affinity for hCB_1

and hCB_2 receptors but significantly (approximately 70–90-fold and approximately 40–60-fold) lower affinity than the parent compound AEA. Further, studies are needed in order to utilize these compounds in drug discovery through biotransformation.

2.18. Magnolol. Magnolol, a biphenyl neolignan from *Magnolia officinalis*, was used popularly in traditional Chinese medicine for insomnia, anxiety, and allergic diseases. Rempel et al. [79] examined the extract and biphenyls honokiol, magnolol, 8,9-dihydromagnolol, tetrahydromagnolol, and trans-isomagnolol for its cannabinoid affinity and activity. The study showed that magnolol behaved as partial agonist for CB_2 receptor, while honokiol was less potent but showed full agonistic activity at CB_1 and antagonistic properties at CB_2 receptor. However, further studies showed no inhibition activity for FAAH and MAGL in rat brain preparations. Thus magnolol showed partial agonist affinity at both CB receptor subtypes, while tetrahydromagnolol showed higher affinity for CB_2 receptor and antagonist at GPR55, a CB-related orphan receptor in β-arrestin translocation assays.

Fuchs et al. [80] synthesized analogs of magnolol and investigated affinity at hCB_1/CB_2 receptors using CP55,940 radioligand studies and also examined SAR of these analogs with variations of alkyl chains and phenolic groups which may improve the potency. The study showed that methylation of phenolic hydroxyl group abolishes the preference of magnolol analogs for CB_2 receptors; however depending on which of the two phenolic groups was methylated the resulting compounds exhibited an enhanced affinity to CB_1 receptors. Full agonism on CB_1 and CB_2 receptors was observed following methylation of the hydroxyl group in the *para*-position to the propyl residue for derivatives. But methylation of the hydroxyl group in the *para*-position of the hexyl residues results in CB_1 antagonist and partial CB_2 receptors agonist activity, emphasizing the importance of the free phenolic hydroxyl group for high intrinsic activity. Further, activity of new analogs at G_i-coupled CB_1 and CB_2 receptor subtypes on forskolin-stimulated adenylate cyclase activity in cAMP accumulation assays confirmed that potency and efficacy of magnolol can be easily altered by methylation of one of the phenolic hydroxyl groups and depending on the position of the methoxy group, full agonism on both receptors with antagonist activity at CB_1 and partial agonist activity at CB_2 receptors can be achieved. Magnolol also exhibited dual agonism of RXRα and PPARβ/γ and appears as an important agent to target this heterodimer [81]. The manipulation of the biphenyl scaffold appears as a putative pharmacophore for the further development of novel CB receptor ligands.

2.19. Malyngamides. Malyngamides are the fatty acid amide compounds abundantly found in marine cyanobacterial metabolites from *Lyngbya* spp. Till date, more than 30 malyngamide analogues have been isolated and screened for their cannabinoid affinity and activity. Among numerous analogues, malyngamide B appeared to bind to both CB_1 and CB_2 receptors, with moderate potencies as agonist. Further tests reveal its anti-inflammatory properties like cannabimimetic compounds and it was found to inhibit NO production with an IC_{50} of 6.2 μM without affecting cellular viability up to 25 μM. It appears devoid of inhibitory activity on FAAH, which catalyzes anandamide hydrolysis and terminates anandamide signaling [82].

2.20. Rutin. Rutin is a flavonoid from *Saussurea involucrata* also known as snow lotus, in different regions of China. The cannabinoid mediated antidepressant activity of rutin shown in mice models employing weight-loaded forced swim test. Rutin treatment showed upregulation of CB_1 receptors in mouse brain tissue demonstrating antifatigue activity and CB_1 receptor-interacting proteins. Further, in brain tissues, an increase in expression of peroxisome proliferator-activated receptor-α coactivator (PGC-1α) and sirtuin 1 (SIRT1) was also demonstrated [83]. Integrating together the cannabinoid, PPAR-γ, and opioid receptor activities, rutin may be a potential multitargeted polypharmacological agent in prevention and treatment of diseases involving dysregulation of PPAR and ECS.

2.21. Serinolamides. Serinolamides are fatty acid amides found in a marine cyanobacterium, *Lyngbya* spp., collected from Piti Bomb Holes from Guam. Among the isolated compounds, the analogue serinolamide A isolated from the marine cyanobacterium, *Lyngbya majuscula*, *Oscillatoria* spp., showed structural similarity to the endocannabinoids anandamide and 2-AG. Serinolamide A showed binding affinity to the human cannabinoid receptors and found 5-fold more selective agonist activity for the CB_1 receptors with moderate binding affinity [84], whereas serinolamide B appeared to inhibit forskolin-stimulated cAMP accumulation mediating both CB_1 and CB_2 receptors with moderate potencies along with more CB_2 receptor selectivity in binding as well as functional assays. However, serinolamide B showed an opposite trend in binding affinities compared to serinolamide A, where it exhibited a moderate affinity and higher selectivity for CB_2 (K_i = 5.2 μM) over CB_1 receptor (K_i = 16.4 μM) [82]. Serinolamide B like other cannabimimetic compounds exerted anti-inflammatory effects in lipopolysaccharide-(LPS-) induced murine macrophages RAW 264.7 with an IC_{50} > 25 μM. The observations indicate that presence of a secondary amide versus a tertiary amide is not a major element for specific receptor selectivity. Though, the compounds represent a novel scaffold from a marine organism for the development of cannabinoid modulators.

2.22. Methylhonokiol. 4′-O-Methylhonokiol is a polyphenolic compound isolated from *Magnolia grandiflora* L., a tree growing in Northern Mexico and the USA. Schuehly et al. [85] first reported methylhonokiol as a potent agonist on CB_2 receptors, triggering a novel type of heteroactive signaling in the radioligand displacement assays in HEK293 cells. In an *in vitro* study, methylhonokiol only showed ligand binding interactions with CB_2 receptors but no effects on GPR55 and CB_1 receptors. It also acts both as inverse agonist and as agonist dependent on the specific signal pathways. A prominent effect of methylhonokiol observed is inhibition of macrophage migration induced by 2-AG, even though

it shows anti-inflammatory properties similar to 2-AG and other endocannabinoids [86].

Based on the reports that orally administered 4′-O-methylhonokiol prevents amyloidogenesis and progression of Alzheimer's disease by inhibiting neuroinflammation in mouse model of Alzheimer's disease [87], authors also suggested that 4′-O-methylhonokiol exerts its beneficial effects by modulation of CB_2 receptors significantly expressed in astrocytes and microglia. Its structural similarity with HU308, a synthetic CB_2 receptor-selective agonist, has been shown to inhibit osteoclastogenesis and be useful as bone resorption inhibitors support its cannabinoid property [88]. Overall, with activities such as GABAergic, PPAR-γ, and AChE modulatory, methylhonokiol seems to be a novel agent to target CB_2 receptors in treatment of osteoarthritis, Alzheimer's disease, neuroinflammation, neuropathic pain, and chronic bowel disease.

2.23. Miconioside. Miconioside compounds are flavanone glycoside isolated from the methanolic extract of the stems of *Miconia prasina* growing in tropical and subtropical regions of the Americas. These compounds include miconiosides B and C which showed their affinity to bind with CB_1 and CB_2 receptors. They showed weak inhibition for CB_2 receptors, but no activity on CB_1 receptors in radioligand binding studies [89].

2.24. Pristimerin. Pristimerin is a natural quinone methide triterpenoid isolated from the *Celastrus* and *Maytenus* spp. exhibiting anti-inflammatory, antioxidant, chemoprotective, and antimalarial activity. Pristimerin exhibits reversible inhibition of MAGL [70] as the quinone methide group to react with cysteine residues of proteins to form covalent adducts [90] and this was confirmed by using a rapid dilution assay. The molecular docking studies showed that lipophilic portion of the molecule lies on a pocket located within the lid domain of MAGL and its 3-hydroxyl group [70]. The binding of pristimerin to MAGL strengthens by the formation of a polar interaction with a regulatory cysteine, possibly Cys^{208}. Chicca et al. [34] also showed that pristimerin and JZL184 both produce potent inhibition of MAGL activity. Pristimerin produced inhibition of [^{3}H]-glycerol formation and accumulation of intracellular [^{3}H]2-AG and was found less potent than β-amyrin, another MAGL inhibitor. Based on the *in vitro* and *in vivo* studies, it has been concluded that pristimerin inhibits MAGL in a rapid, reversible, and noncompetitive manner.

2.25. Resveratrol. Resveratrol is a stilbenoid compound isolated from fruits and plants and widely studied for its pharmacological properties. Recently, the uncharacterized trans-resveratrol receptor has shown to share many characteristics with cannabinoid receptors. The affinity of trans-arachidins, trans-resveratrol, and trans-piceatannol for CB_1 and CB_2 receptors was investigated in CHO cells expressing cannabinoid receptors and it was found that trans-resveratrol and all analogs bind to CB_1 receptors, whereas isoprenylated trans-resveratrol derivatives tA1 and tA3 bind to CB_2 receptors [63]. The study showed affinity of trans-resveratrol and trans-piceatannol for CB_2 receptors is 5- to 10-fold lower than that observed for CB_1 receptors. All compounds except for tA3 exhibit approximately 2- to 10-fold selectively for binding to CB_1 receptors relative to CB_2 receptors. In molecular docking, trans-arachidins, trans-resveratrol, trans-piceatannol, and their glucuronidated metabolites bind with CB_2 receptors while isoprenylated analogs tA1 and tA3 bind with both CB_1 and CB_2 receptors. Trans-resveratrol and Trans-picetamol also bind to mCB_1 receptors; however they lack affinity for hCB_2 receptors. The docking studies showed that prenylated stilbenoids trans-arachidins 1 and 3, the more lipophilic isoprenylated analogs of trans-resveratrol and trans-piceatannol, may be preferable alternatives to trans-resveratrol due to increased bioavailability via slowed metabolism. Both parent and isoprenylated compounds bind to CB_1 receptors and were confirmed by the antagonistic actions produced by CB_1 receptor agonists. However, the analogs possess an isoprenyl group, trans-arachidin 1 and trans-arachidin 3, showed affinity for CB_2 receptors, and were further confirmed by molecular docking [91]. Though, resveratrol has been well investigated in numerous experimental and clinical studies; however the cannabinoid mediated pharmacological effects need to be ascertained.

2.26. Resorcylic Acid Lactones. Resorcylic acid lactones neocosmosin A, neocosmosin B, neocosmosin C, monocillin IV, monocillin II, and radicicol are obtained from ethyl acetate extracts of *Neocosmospora* spp. The extracts as well as the compounds were found to exhibit moderate affinity with opioid receptor and cannabinoid receptors in a high throughput screen employing a receptor binding assay. Among these compounds, neocosmosin B, monocillin II, and radicicol showed a binding affinity for CB_1 receptors using CP55,940 as standard. However, compounds, neocosmosin A, neocosmosin B, neocosmosin C, monocillin II, and radicicol, exhibited binding affinity to CB_2 receptors with respect to CP55,940 as standard. Neocosmosin C, monocillin II, and radicicol also showed good affinity for binding with the human opioid receptors [92, 93]. These findings are implicated in neuropathic pain and neuroinflammatory disorders where opioid and cannabinoid systems are dysregulated.

2.27. Salvinorin A. Salvinorin A, a trans-neoclerodane diterpenoid, is the principal constituent of *Salvia divinorum*, a plant used in Mexico for spiritual and medical purposes. It possesses psychotropic activity that resembles with the structure and mode of action of typical hallucinogens. The radioligand displacement studies show salvinorin A as a potent, selective, and full agonist on κ-opioid receptors [94–96], but not μ- or δ-opioid receptors. Other studies have shown that salvinorin A possesses ECS mediated activity and interaction with κ-opioid in rats and Zebra fish models [94, 96–101]. It provides a new lead compound for developing antiallodynic agents via opioid and CB_1 receptors activation. Fichna et al. [102] demonstrated that salvinorin A impedes gastrointestinal motility and ion transport, mediated by κ-opioid receptors in mice. Further, it significantly attenuated chemical-induced colitis in mice and the antinociceptive

action was blocked by opioid and CB_1 receptor antagonists. Salvinorin A also slows colonic motility *in vitro* and *in vivo* and alters neurogenic ion transport [103, 104]. Further, Fichna et al. [105] reported the inhibitory effects of salvinorin A on endotoxin-induced ileal hypercontractility in mouse stomach mediated by opioid receptors and cannabinoid receptors. The inhibitory effect of salvinorin A on motility demonstrates functional interaction between CB_1 and κ-opioid receptors in the inflamed gut but in normal control animals [98].

Further, Aviello et al. [100] reported that salvinorin A reduced inflammation and pain in animal models of LPS- and carrageenan-induced paw edema as well as formalin-induced inflammatory pain. The actions were found mediated by the κ-opioid receptors and CB_1 receptors-dependent anti-inflammatory actions on macrophages and in experimental animals. A study evaluated salvinorin A in a set of *in vitro* and *in vivo* tests and demonstrated that salvinorin A did not bind or activate CB_1 receptors but effects are mediated by its activation of κ-opioid receptors [96]. Braida et al. [95] reported the anxiolytic- and antidepressant-like effects of salvinorin A which are mediated by both κ-opioid and CB_1 receptors. In addition to a weak affinity for CB_1 receptors, it also reduced FAAH activity in amygdale. Based on the cannabinoid and opioid modulatory activity, salvinorin A or its synthetic or semisynthetic derivatives could be useful in the treatment of lower gastrointestinal disorders because inflammation in the intestine upregulates cannabinoid receptors and endogenous cannabinoids.

2.28. γ-Sanshool. γ-Sanshool is an alkylamide compound isolated from *Zanthoxylum clava-herculis* L. (family: Rutaceae) also known as pepperwood, native to the southeastern United States. Dossou et al. [106] have shown its CB_2 receptor activity. Subsequently, a novel plate-based assay was developed in order to determine both CB_1 and CB_2 receptors antagonist and agonist activity and the ligand effect on internalization of the CB_1/CB_2 receptors in different extracts of the plant genus *Zanthoxylum* [106].

Later, it was found that γ-Sanshool isolated from *Zanthoxylum bungeanum* shows potent agonism on the CB_2 receptor and antagonism on CB_1 receptors. In addition to its interactions with CB_1 and CB_2 receptors, it showed antagonist activity at the follicle stimulating hormone receptor (68%) and at the prolactin-releasing hormone receptor (52%). These findings reveal that, given the role of cannabinoid receptors in diabetes pathophysiology, γ-Sanshool with a dual function on CB_1 receptors inhibition in combination with CB_2 activation may be useful in the treatment of diabetes.

2.29. Sciadonic Acid. Sciadonic acid is obtained from the seeds of a coniferous plant, *Sciadopitys verticillata* (umbrella pine) in Japan. Sciadonic acid structurally resembles with 2-AG, the endogenous natural ligand for the cannabinoid receptor. Nakane et al. [107] showed that sciadonic acid exhibits cannabimimetic activity by inducing rise of intracellular Ca^{2+} levels in neuroblastomaxglioma hybrid cells (NG108-15) expressing CB_1 receptors. This was the first study showing the occurrence of a cannabimimetic monoacylglycerol in higher plants exhibiting CB_1 receptor dependent mechanism.

2.30. Semiplenamides. Semiplenamides (semiplenamide A to G) belong to a series of novel fatty acid amides similar to endocannabinoid, anandamide. These were isolated from marine blue green algae, *Lyngbya semiplena* collected from Papua New Guinea. Semiplenamides A, B, and G derivatives exhibited weak affinity for the CB_1 receptors [108]. Additionally, semiplenamide A was found to be a moderate inhibitor of the anandamide membrane transporter thereby inhibiting anandamide breakdown. The results indicate that these compounds may appear as future cannabinoid specific drugs of natural origin.

2.31. Thujone. Thujone, a monoterpene ketone, is found in variable amounts in several food and medicinal plants such as *Juniperus* spp., *Cedrus* spp. It has been regarded as a severe neurotoxicant causing exciting and convulsive effects in the CNS by inhibiting $GABA_A$ receptors in a dose-dependent manner. It is known for its notoriety being an important component of the once-popular drink absinthe. Thujone possesses psychoactivity similar to cannabinoids but does not mimic cannabinoids in inhibiting the synaptosomal enzyme [109].

Meschler and Howlett [110] investigated the affinity of thujone for the brain CB_1 receptor in radioligand assay and found that thujone affinity with the CB_2 receptor is approximately similar to the CB_1 receptor. In bioassays and forskolin-stimulated adenylate cyclase assays, thujone did not show any activity on CB_1 receptor. Thujone treatment in rats exhibited different behavioral characteristics, the open-field test for locomotor activity, the ring-stand test for immobility (catalepsy), and hot-plate test for antinociception comparable with a potent cannabinoid agonist, levonantradol. Though, thujone was found devoid of stimulatory activity on brain cannabinoid receptors and does not elicit cannabimimetic behavioral effects in animals at physiologically relevant doses.

2.32. Voacamine and Analogues. Voacamine, 3,6-oxidovoacangine, and 5-hydroxy-3,6-oxidovoacangine are the indole alkaloids isolated from methanolic extract of root bark of *Voacanga africana*, a tropical African tree. Several compounds have been isolated and screened for the cannabinoid activity in Aequorin/GPCR cell-based Ca^{2+} functional assay using CP55,940 or rimonabant as a positive control for cannabinoid receptors ligands [111]. These compounds exhibited potent CB_1 receptor antagonist activity in a concentration-dependent manner compared to rimonabant, whereas the other coexisting alkaloids, such as voacangine, vobasine, and tabersonine, fail to exhibit any CB receptor mediated activity. This was the first study showing that naturally occurring alkaloids are also source of CB_1 receptor antagonists and this could be further evaluated for cannabimimetic activity and potential therapeutic benefits.

2.33. Yangonin. Yangonin is a kavalactone extracted from *Piper methysticum* Forster, popularly known as Kava, and cultivated in the South Pacific Island Countries. Several

compounds, known as kavalactones, are isolated and the most common are kavain, 7,8-dihydrokavain, methysticin, 7,8-dihydromethysticin, yangonin, and desmethoxyyangonin. Ligresti et al. [112] examined their CB receptor binding affinity and inhibitory activity on endocannabinoid metabolizing enzymes, FAAH and MAGL involved in endocannabinoid degradation. Only yangonin emerged as the most interesting compound as evidenced by the binding affinity to the CB_1 receptor ($K_i = 0.72\,\mu$M). However, all other compounds were found inactive in inhibiting activities of FAAH and MAGL enzymes.

The study also reported that 250–1250 mg yangonin, which is 10% of the total kavalactone-content taken orally, may provide sufficient serum concentrations of yangonin to affect CB_1 receptors in the CNS. The authors suggested that yangonin which possesses an extensive conjugated double bond system bears a little structural resemblance to the phytocannabinoids. The kavalactones may also be a target for GABA and BZDs, voltage gated Na^+/Ca^{2+} channels, monoamine uptake, and arachidonate cascade which may synergize and contribute to the psychopharmacological profile of the Kava.

Miscellaneous Compounds Isolated from Nature. Desmodianone derivatives, desmodianones D and E and 6-methyltetrapterol A, are isoflavonoids isolated from *Desmodium canum*. It is known for soil preserving property and used as forage with some application in traditional medicine. These isoflavonoids possess cannabinoid-like moieties; however no further reports on their cannabimimetic or cannabinoid modulatory activity are available in the literature [113]. Isoperrottetin A, a bibenzyl compound along with several bisbenzyls, prenyl bibenzyls, and sesquiterpenoids, has been isolated from the ether extract of the liverwort, *Radula perrottetii*. All these compounds are known to structurally consist of cannabinoid moiety; however there is no report available on their ECS modulating property [114].

Leucettamols are the bifunctionalized sphingoid-like compounds obtained from a marine sponge, *Leucetta* sp. In preliminary studies, they appear inactive on CB_1, CB_2, and TRPV1 receptors. Soderstrom et al. [115] also extracted numerous endocannabinoid-like purified unsaturated fatty acids from green algae (Chlorophyta), the brown alga *Laminaria angustata*, and the sponge *Mycale micracanthoxea*. The authors did not find endocannabinoid compound from *L. majuscula*. Also, AEA has been detected in dietary chocolate and cocoa obtained from *Theobroma cacao*, a popular plant [116]. Recently, in a study, several compounds such as sinostrobin, naringenin 7,4′-dimethyl ether, 2′,6′-dihydroxy-4′-methoxychalcone, 4-methoxy-6-(2-phenylethenyl)-2H-pyran-2-one, naringenin 7-methyl ether, and 3,5-heptanediol, 1,7-diphenyl are isolated from the dichloromethane extract of *Renealmia alpinia* subjected to either opioid or cannabinoid receptors *in vitro* binding affinity assays. Though, the plants show antinociceptive and analgesic effect in the *in vivo* model but the constituents and plant failed to show affinity to cannabinoid receptors [117]. The compound isolated from the soil microfungus, *Eupenicillium parvum*, showed selective μ-opioid receptor and CB_1 receptor binding affinities, *in vitro* binding assays [118]. These findings provide insight into the potential therapeutic utility of this class of compounds.

3. Medicinal Plants Modulating Cannabinoid Receptors and Metabolizing Enzymes

In the last few years, several medicinal plants have been reported to modulate the ECS activity by inhibiting or activating the cannabinoid receptors and the endocannabinoid metabolizing enzymes [22]. The plants have been reported to interact with cannabinoid receptors directly or indirectly in experimental studies designed to evaluate the pharmacological properties and therapeutic benefits using pharmacological challenge of CB receptor agonists and antagonists or utilizing the CB_1/CB_2 receptor knockout mice [6, 19, 22]. Several medicinal plants other than cannabis have been shown to alter the ECS signaling pathways and exhibit cannabimimetic effects and put forward their potential therapeutic and dietary application [6, 16–19]. The therapeutic and pharmacological activities presented by these plants involving cannabinoid mediated activity are present in Table 4.

In the modern era of medicine medicinal plants and phytochemicals derived from plants continue to play an important role in drug discovery and development [6, 16, 17, 41]. The plants have become the key resource for bioactive agents and played a vital role in the search of lead compounds for novel drug discovery and development. The isolated bioactive agents and their synthetic or semisynthetic analogs can be developed into promising drug candidates by the processes of highly efficient bioactivity-directed fractionation and isolation, following analog synthesis using modern medicinal chemistry-based molecular modifications. The next paragraphs focus on medicinal plants other than cannabis which have been reported to interact with the molecular components of ECS and are detailed below. The bioactive constituents of such plants display a rich source for the discovery of novel cannabinoid compounds with potential for pharmacological applications and drug development. Besides the small molecules, secondary metabolites also play an important role in search of novel compounds.

3.1. Corydalis yanhusuo. *Corydalis yanhusuo* (family: Papaveraceae) is one of the traditional Chinese medicines used as sedative, hypnotic, and pain killer possessing a number of potent alkaloids. The CB_1 receptor mediated effect of *Corydalis yanhusuo* was tested in an animal model of trigeminal neuralgia pain induced in rats by chronic constriction injury of the infraorbital branch of the trigeminal nerve [119]. *Corydalis* binds to CB_1 receptors and exerts antinociceptive effect in animal models of inflammation and pain. In addition, tetrahydropalmatine [127] an active component isolated from *Corydalis* has shown to improve anxiolysis and decreased motor movements, independent of the $GABA_A$ receptors [127]. The analgesic and anti-inflammatory effect mediated by CB_1 receptors along with anxiolytic activity is an advantage over synthetic CB_1 receptor modulators [127].

3.2. Echinacea purpurea. *Echinacea purpurea* is commonly used worldwide for the prevention and treatment of

Table 4: The cannabinoid receptor affinity, potency, and activity of medicinal plants.

Medicinal plants	CB mediated effect	CB affinity/potency	References
Corydalis yanhusuo	Neuropathic pain	CB_1 antagonist	Huang et al. 2010 [119]
Echinacea purpurea	Immunomodulation	CB_2 agonist	Chicca et al. 2009 [25]
Linum usitatissimum	Inflammation	CB_2 agonist	Styrczewska et al. 2012 [120]
Melilotus suaveolens	Lung injury	CB_2 agonist	Liu et al. 2014 [121]
Morinda citrifolia	Immunomodulation	CB_1 antagonist/CB_2 agonist	Palu et al. 2008 [122]
Nelumbo nucifera	Obesity	CB_2 agonist	Velusami et al. 2013 [123]
Olea europaea	Colon cancer	CB_1 agonist	Cotrim et al. 2012 [124]
Rubus coreanus	Osteoporosis	CB_1 antagonist/CB_2 agonist	Lim et al. 2015 [125]
Ruta graveolens	Diabetes	CB_2 agonist	Rollinger et al. 2009 [126]

common cold, cough, bronchitis, influenza, and allergic respiratory diseases. It has been shown to exert antioxidant, anti-inflammatory, and immunostimulatory properties owing to the chemical constituents, alkamides, and ketoalkenes/alkynes. The alkamides were the first compounds identified in plants besides cannabis to possess cannabimimetic properties on both the cannabinoid CB_1 and CB_2 receptors, revealing their structural similarity to the endogenous cannabinoid ligand anandamide [20, 128]. The extract of *Echinacea* roots was studied in $^{[35S]}$GTPcS-binding experiments on rat brain membrane preparations using arachidonyl-20-chloroethylamide (ACEA), a full agonist ligand at the CB_1 receptor [128]. Among the isolated compounds, some displayed partial agonist property while others exhibit inverse agonist effects to CB_1 receptor. Despite their relatively low efficacy at the cannabinoid receptors, the compounds behave as inverse agonist was capable of inhibiting the full agonist effect of ACEA. The compounds showed partial agonistic property that also significantly increased the G-protein-stimulatory action of ACEA. The SAR studies showed an exchange of isobutylamide moiety (inverse agonist activity) of the molecule for 2-methylbutylamide (partial agonist activity).

The CB_2 receptor activity of alkamides, demonstrated by binding assays, is believed to be the most probable mechanism of action of alkamides as immunomodulator agents isolated from *Echinacea* [21, 25, 27, 129]. The interaction between anxiety and cannabinoids is known to be complex and activation of the CB_1 receptors by endogenous ligands was believed to play a role in the control of anxiety [128]. The dry and fresh herb of *Echinacea* provides a different yield of alkamides [29, 30]. All together the studies convincingly suggest that *Echinacea* could provide scaffolds for future CB_2 ligands in drug discovery and development.

3.3. Linum usitatissimum. Linum usitatissimum (family: Linaceae), also known as flax, is considered a distinct source of fibers and oil for industrial and medicinal application. The transgenic plants are generated in order to enhance the production of phenylpropanoids; a class of new terpenoid has shown to possess health-beneficial properties. The plant has shown to alter the expression of genes involved in inflammatory processes in mouse and human fibroblasts and activates the gene expression of CB_2 receptor [120]. The findings reveal that flax can be a source of cannabinoid-like compounds which may influence the immunological responses and aid in designing the fabric for wound dressing with putative anti-inflammatory properties [120].

3.4. Melilotus suaveolens. Melilotus suaveolens Ledeb. (family: Leguminosae), a traditional Tibetan medicine, is also known as wild alfalfa or "cold-tasting" annual or biennial herb. It has been reported to contain compounds such as coumarin, flavonoids, phenolic acids, steroids, and triterpenes. It has been found effective in inflammation, pain, and antimicrobial activity. The cannabinoid mediated anti-inflammatory activity of *M. suaveolens* has been demonstrated in a rat cecal ligation and puncture- (CLP-) induced animal model of acute lung injury representing sepsis in human [121]. It has shown to upregulate the CB_2 expression in peripheral blood mononuclear cells, reduce the number of neutrophils, lymphocytes, and total cells, and inhibit the induction of proinflammatory cytokines and transcription factors, NF-$\kappa\beta$65. The CB_2 expression was shown to be correlated negatively with NF-$\kappa\beta$ mRNA and supported by a significant reduction in CLP-induced lung inflammation. These findings suggest that *M. suaveolens* may have therapeutic potential in the treatment of CLP-induced acute lung injury.

3.5. Morinda citrifolia. Morinda citrifolia L. (family: Rubiaceae), also known as Noni, has been used by Polynesians for over 2000 years for numerous diseases. The advent of Tahitian Noni Juice generated interest in medicine for its possible beneficial effects on human health and well-being. Almost all parts of the plants are used medicinally in treating a variety of ailments. Palu et al. [122] showed the binding affinities of Noni samples (Tahitian Noni Juice and Noni fruit juice concentrates) for CB_1 and CB_2 receptors in CHO-K1 cells expressing hCB receptors using WIN-55,212-2, a nonspecific ligand, and *in vivo* in mice. Both juices were found to activate CB_2 receptor but inhibit CB_1 receptors.

Tahitian Noni Juice produced inhibition of CP55,940 for CB_1 receptors and enhancement for CB_2 receptors. Noni fruit juice concentrate caused stimulation of [^{3}H]WIN-55,212-2 binding. At different concentrations the CB_1 receptor was inhibited whereas CB_2 receptor showed stimulations at the same concentrations.

The binding activity of Tahitian Noni Juice for CB_1 receptors was similar at each concentration, even at fivefold increased concentration. However, at both concentrations a remarkable selectivity for CB_2 binding/activation was observed for CB_2 receptor [122]. In mice orally administered Tahitian Noni Juice decreased IL-4 and increased IFN-γ suggesting that Noni juice favorably alters the immune system and exhibits immunomodulatory effects by activating the CB_2 receptors which are involved in the immune regulation. The dual activity of Noni juice as CB_1 receptor inhibitor and CB_2 receptor activator has potential benefits in inflammation and immunomodulation.

3.6. Nelumbo nucifera. Nelumbo nucifera Gaertn. (family: Nymphaeaceae), also known as a sacred lotus, is widely distributed across the world and used as food and medicine. The seeds, rhizomes, leaves, flowers, and roots of the plant have been reported to contain megastigmanes including eudesmane sesquiterpenes, nelumnucifosides A and B, alkaloids such as roemerine, nuciferine, nornuciferine, nelumboside, anonaine, 5-methoxy-6-hydroxyaporphine, liensinine, asimilobine, and flavonoids. The cannabinoid activity of both methanol and aqueous extracts of *N. nucifera* was studied in measuring inhibition of CP55,940 elicited CB_2 activity in the G_i/G_o-coupled CHO-K1 cell line [123]. The methanolic extract showed antagonism against CP55,940 activity towards CB_2 receptor, whereas water extract was found inactive. A potent antagonist activity towards CP55,940 activated CB_2 receptor with an IC_{50} value of ~62.3 nM was demonstrated by AM630. The study indicated that *N. nucifera* petal extract possesses potential benefits in metabolic disorders mediated by antagonistic effect on CB_2 receptors.

3.7. Olea purpurea. Olea europaea (family: Oleaceae), a traditional tree of the Mediterranean basin, is the source of olive oil. The effects of olive oil and its phenolic constituents on gene expression in ECS have been studied in human colon cancer cells (Caco-2). A selective and transient upregulation of CNR1 gene-encoding for CB_1 receptor was induced by exposure of Caco-2 cells to the oil. However, the other ECS components such as CB_2, GPR55, and TRPV1 receptors and endocannabinoid metabolizing enzymes, NAPE-PLD, DAGL, FAAH, and MAGL, remained unaffected [130].

Further, dietary oil supplementation was found to increase the expression of CB_1 in the colon of rats. Following oil supplementation, the methylation of Cnr1 promoter, miR23a, and miR-301a, previously shown to be involved in the pathogenesis of colorectal cancer, was predicted to target CB_1 mRNA and appears reduced. In another study, the phenolic compounds of olive oil were developed to allow the preparation of unsaturated derivatives altered food intake in rats owing to their molecular similarity with CB_1 endogenous ligands and PPAR-α as potential targets [124]. Taken together, the findings demonstrate modulation of CB_1 by olive oil or its phenolic compounds and may provide a new therapeutic avenue for prevention and treatment of cancer and obesity.

3.8. Rubus coreanus Miquel. Rubus coreanus Miquel (family: Rosaceae), also known as Korean black blackberry, is known for its benefits in liver and kidney diseases, spermatorrhoea, prostate, and urinary diseases. It is known to contain tannins such as sanguiin H-4 and sanguiin H-6, flavonoids such as 3,4-dihydroxybenzoic acid, nigaichigoside F1, nigaichigoside F2, and coreanoside F1, a dimeric triterpene glycosyl ester, and anthocyanins. Its supplementation has shown to enhance antioxidant capacity in men [131]. The cannabinoid receptors mediated activity of *Rubus coreanus* has been shown in osteoporosis and occurs with N-methyl-N-nitrosourea-(MNU-) induced prostatic hyperplasia in aged rats as well as diabetic osteoporosis rats [125] following streptozotocin or ovariectomization [132]. The upregulation of CB_1 and CB_2 receptors were increased in rats that were ovariectomized and treated with streptozotocin and *Rubus coreanus* but decreased in those treated with streptozotocin and *Rubus coreanus* alone. The study revealed that in postmenopausal diabetic and aged rats the antiosteoporotic effect is attributable to the CB receptor-related upregulation of osteoblastogenesis and inhibition of prostatic hyperplasia. *Rubus coreanus* rescued bone loss in diabetic and aged osteoporosis by simultaneous alteration of activation in osteoblasts and osteoclasts dependent on upregulation of the ECS. Though, the active component responsible for an effect is yet to be determined.

3.9. Ruta graveolens. Ruta graveolens L. (family: Rutaceae) is a plant of medicinal and culinary importance native to Mediterranean region of southern Europe and northern Africa and Balkans. The plant and phytochemicals isolated have shown to be effective in different types of skin diseases including psoriasis, vitiligo, and cutaneous lymphoma. The dichloromethane and methanol extracts of *Ruta graveolens* yielded several constituents and were subjected to *in silico* studies using hitting model for CB_2 ligands consisting of the five selective agonists AM1241, GW405833, HU-308, JWH-133, and JWH-267 [126]. Of all the molecules subjected to parallel screening, rutamarin showed selective affinity to the CB_2 receptor with a K_i of 2.64 ± 0.2 μg/mL or 7.4 ± 0.6 μM in radioligand displacement assay. The findings reveal that rutamarin may provide a novel scaffold for the discovery of CB_2 specific ligands.

Miscellaneous Medicinal Plants. Recently, *Withania somnifera* Dunal, a popular medicinal plant, possesses immunomodulator activity shown to prevent tolerance to the analgesic effect of morphine and suppress rebound hyperalgesia found devoid of affinity for cannabinoid receptors [133]. *Hypericum perforatum* also known as St. John's wort is a popular plant remedy for depression that did not show cannabinoid property studied using the pharmacological challenge with several agonists and antagonists including SR141716A, CB_1 receptor antagonist. However, naloxone significantly reduced the inhibitory effect of *Hypericum perforatum* on contractions induced by electrical field stimulation mediated

by opioid receptors [134]. Yuliana et al. [135] evaluated the effects of several dietary spices in the antiobesity related bioactivity screening assays and found that nutmeg, mace, black pepper, and turmeric are capable of modulating the CB_1 receptors. El-Alfy et al. [136] also showed that nutmeg extract showed a concentration-dependent inhibition for both FAAH and MAGL. Inhibition of endocannabinoid metabolizing enzymes by nutmeg extracts explains the cannabis-like effect exerted by nutmeg.

4. Concluding Remarks and Future Prospects

Compared to synthetic compounds, natural products are known to offer huge structural diversity and the availability of modern techniques for separation, structure elucidation, and screening and combinatorial synthesis will lead to revitalization of plant products as sources of novel drugs. In recent years, several new selective CB_1 and CB_2 receptor agents from natural products have been described. Though, several have been identified by these ligands *in vitro* and *in silico* studies. However, these molecules are used at micromolar concentrations in the *in vitro* studies and therefore may show affinity at both receptors. Therefore, additional controls are needed to be performed in order to ensure the selectivity, affinity, potency, and site of action of these molecules.

The *in vivo* characterization, pharmacokinetic considerations, and the cannabinoid mediated mechanism should be demonstrated for the pharmacological benefit and pharmaceutical development. Moreover, many of these ligands exert prominent CB receptor-independent pharmacological effects, such as activation of the opioid receptors, nicotinic acetylcholine receptors, G-protein-coupled receptor GPR55, peroxisome proliferator-activated receptor gamma, and the transient receptor potential vanilloid channels. The characterization of CB-dependent and CB-independent mechanisms could be further beneficial in developing the multitargeted polypharmacological compound for diseases which involve multiple mechanisms particularly the neurodegenerative and neuropsychiatric diseases where endocannabinoid system dysregulation plays a critical role. Based on current knowledge, the components of ECS may be a system that, under the appropriate conditions, produces synergy with established therapeutic agents in different diseases particularly autoimmune inflammatory diseases.

Currently, there are no clinical data indicating that the use of these ligands as adjuvant or cotreatment could improve the efficacy of the available agents or reduce the dosage thereby reducing the adverse effects and maximizing efficacy. Thus, such clinical comparisons would be very interesting and more research should be directed towards the potential synergism and antagonism of cannabinoid ligands in pharmacotherapeutics. The potential of the ECS in a wide range of disorders has been demonstrated; therefore, it is tempting and reasonable to speculate that the nature derived small molecules modulating cannabinoid receptors will have to demonstrate therapeutic efficacy and elucidate underlying potential mechanism of therapeutic benefits by cannabinoids. Additionally, lack of toxicity along with additional anxiolytic activity which appears with synthetic CB_1 receptor antagonists, the phytocannabinoids, can potentially be promising for future armamentarium of the cannabinoid based therapeutics. The data on acute and chronic toxicity and safety is also desired in order to undergo the translation of the observed experimental benefits into humans.

The medicinal plants are part of diet since civilization and therefore based on the evidences of cannabimimetic activity of many more plants could be promoted for inclusion in the diet as these could indirectly exert immunomodulatory, nonpsychoactive, and anti-inflammatory action. This could potentially modulate inflammatory and other pathophysiological processes. The wide availability, easy accessibility, high lipophilicity, and wide therapeutic window make them an excellent candidate for therapeutic intervention. Further, the isolation and characterization of pharmacophores from these plants may provide a model for drug leads using combinatorial chemistry and *in silico* approaches for future drug discovery. These plants may also offer dietary means of treatment for targeting of endocannabinoid dysregulation or the diseases where endocannabinoid modulation represents an important therapeutic target.

The development of new drugs remains an important task for the pharmaceutical industry. The natural compounds from these herbs could provide a rich source in the search for new candidates targeting GPCRs in particular cannabinoid receptors and ECS. Developing phytocannabinoids possess cannabimimetic activity and being devoid of psychotropic activity will enhance their therapeutic spectrum. To explore this possibility, several herb-based natural compound library and cell-based cannabinoid receptor assays were developed to perform high throughput screening. We believe that the process of assay development for cannabinoid receptors, compound screening using these assays, and hit compounds identification will lead to a successful compound for future therapeutic use.

Conflict of Interests

The authors declare no conflict of interests.

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Terpenes and Lipids of the Endocannabinoid and Transient-Receptor-Potential-Channel Biosignaling Systems

David R. Janero[†] and **Alexandros Makriyannis**[*,†,‡]

[†]Center for Drug Discovery and Departments of Chemistry and Chemical Biology and Pharmaceutical Sciences, Northeastern University, Boston, Massachusetts 02115-5000, United States

[‡]King Abdulaziz University, Jeddah, 22254, Saudi Arabia

Abstract

Endocananbnoid-system G-protein coupled receptors (GPCRs) and transient receptor potential (TRP) cation channels are critical components of cellular biosignaling networks. These plasma-membrane proteins are pleiotropic in their ability to interact with and engage structurally diverse ligands. The endocannabinoid and TRP signaling systems overlap in their recognition properties with respect to select naturally occurring plant-derived ligands that belong to the terpene and lipid chemical classes, the overlap establishing a physiological connectivity between these two ubiquitous cell-signaling systems. Identification and pharmacological profiling of phytochemicals engaged by cannabinoid GPCRs and/or TRP channels has inspired the synthesis of novel designer ligands that interact with cannabinoid receptors and/or TRP channel as xenobiotics. Functional interplay between the endocannabinoid and TRP-channel signaling systems is responsible for the antinocifensive action of some synthetic cananbinoids (WIN55,212-2 and AM1241), vasorelaxation by the endocannabinoid *N*-arachidonylethanolamide (anandamide), and the pain-relief afforded by the synthetic anandamide analogue *N*-arachidonoylaminophenol (AM404), the active metabolite of the widely used nonprescription analgesic and antipyretic acetaminophen (paracetamol). The biological actions of some plant-derived cannabinoid-receptor (e.g., Δ^9-tetrahydrocannabinol) or TRP-channel (e.g,, menthol) ligands either carry abuse potential themselves or promote the use of other addictive substances, suggesting the therapeutic potential for modulating these signaling systems for abuse-related disorders. The pleiotropic nature of and therapeutically relevant interactions between cananbinergic and TRP-channel signaling suggest the possibility of dual-acting ligands as drugs.

Graphical abstract

[*]**Corresponding Author**: Mailing address: Northeastern University Center for Drug Discovery, 360 Huntington Avenue, 116 Mugar Hall, Boston, MA 02115-5000. Phone: 617-373-4200. Fax: 617-373-7493. a.makriyannis@neu.edu.

Author Contributions

Both authors contributed to the writing of the manuscript.

Notes

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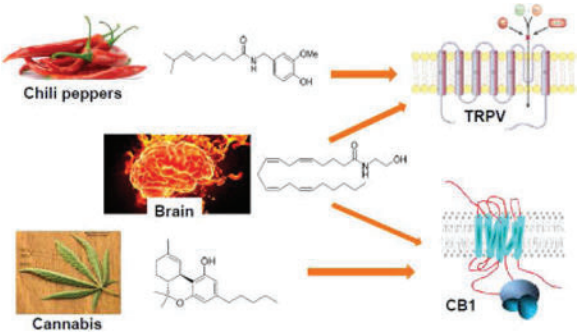

Keywords

Drug discovery; endocananbinoid; G-protein coupled receptors; ion channels; ligands; phytochemicals; phytocannabinoid; signal transduction

At the cellular level of biological organization, a fundamental paradigm for communication utilizes plasma-membrane proteins (receptors, ion channels, transporters, etc.) as components of organized signaling circuits that interact with molecules (ligands) for the purpose of transmitting information from the external milieu to the intracellular compartment, thus enabling the target-cell to respond to its environment.[1] This core principle of signal transduction is well exemplified by two ubiquitous mammalian signaling systems, one of which employs cannabinoid (CB) receptors; the other, transient receptor potential (TRP) cation channels.

Discovery and molecular characterization of the first CB receptor to be cloned (CB1) was spurred by identification and synthesis of the major psychotrophic component of marijuana, (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC), a plant-derived CB ("phytocannabinoid") (Figure 1A). A second CB receptor, designated CB2, was subsequently identified through homology cloning, and other putative CB receptors have been suggested. Both CB1 and CB2 are class-A G-protein coupled receptors (GPCRs) featuring characteristic 7-transmembrane helical domains, significant homology with one another in their transmembrane domains, and distinct distributions: CB2 is found mainly in peripheral tissues (principally immuneassociated), whereas CB1 is a major GPCR in the central nervous system at presynaptic neurons and is also expressed in the periphery.[2] CB1 and CB2, along with a growing family of their endogenous activators ("endocannabinoids") and the enzymes that synthesize and inactivate those agonists, are constituents of the endocannabinoid system, a biosignaling network ubiquitous in mammals.[3] The best-studied endocan-nabinoids, 2-arachidonoylglycerol (2-AG) and N-arachidonylethanolamide (or anandamide) (AEA), are lipid mediators derived from diacylglycerol and *N*-acylphophatidycholine, respectively (Figure 2A). They originate from membrane phospholipids by distinct enzymatic pathways and possess specific functional and pharmacological properties.[4] In the central nervous system (CNS), the presynaptic serine hydrolase, monoacylglyceorol lipase (MGL), is primarily responsible for 2-AG inactivation in vivo along with the α,β-hydrolase domain-containing proteins 6 and 12 (ABHD6 and ABHD12), whereas AEA is inactivated by fatty acid amide hydrolase (FAAH) postsynaptically.[5] Aside from its canonical role in the CNS as

a key retrograde modulator of neurotransmitter release,[6] the endocananibinoid system, either alone or in concert with other neuromodulatory signaling systems, is involved in a number of fundamental (patho)physiological processes, including energy balance, emotional processing, reward and motivation, immune function, and pain sensing.[7–10]

Across animal phyla, TRP channels constitute a superfamily of over 50 nonselective cation-channel membrane proteins that function as cellular sensors whose activity in response to external stimuli ultimately elicits a change in cell-membrane potential.[11] All known TRP channels evidence six transmembrane segments (TMS1-TMS6) and a short, hydrophobic, cation-permeable pore domain between TMS5 and TMS6. TRP channels are polymodal: their ion permeability can be modulated by diverse mechanisms including G-protein coupled signaling, membrane depolarization, and direct ligand binding. The 28 distinct mammalian TRP channels identified have been classified on the basis of sequence homology into six subfamilies, each subfamily characterized by distinct gating mechanisms and cation selectivities.[12] Of these subfamilies, the six vanilloid or "thermo" TRP channels (TRPV1–6) have garnered the most attention, with TRPV1 being the best-characterized and first-cloned TRPV due to its physiological role in nervous tissue as a molecular integrator of diverse noxious chemical and thermal stimuli, notably its extreme responsiveness to thermal (heat) activation from the pungent vanilloid capsaicin (8-methyl-*N*-vanillyl-6-nonenamide) (Figure 3A), an irritant found in hot chili peppers, as well as to other phytochemical toxins and acid.[13] Reminiscent of the endocananbinoid system, TRP-channel signaling is involved in many (patho)physiological processes, for example, cell proliferation/differentiation, neurotransmitter release, chemical sensing, cell death, and inflammation.[11,14,15] Indeed, both endocanabinoid-system proteins[8–10] and TRP channels[15–18] are aggressively being pursued as drug targets for indications including diabetes, pain, cancer, neurodegeneration, and substance-abuse disorders.

The goal of this Review is to summarize and discuss select concepts related to the most important ligands associated with the (in)activation of the CB and TRP-channel signaling systems. A particular focus will be on ligands belonging to the terpene or lipid chemical classes that interact with both systems.

COMMONALITIES BETWEEN CANNABINERGIC AND TRP BIOSIGNALING SYSTEMS

Aside from their basic physiological role as routes of cellular communication and focuses of contemporary drug discovery, CB-receptor- and TRP-channel-dependent signaling share several fundamental properties. Cannabinergic and TRP-signaling systems are both pleiotropic. Their pleiotropic, or "opportunistic", nature is manifested in the ability of CB1, CB2, and TRPV1 to interact with and engage structurally diverse ligands belonging to a wide variety of chemical classes, some of which may also interact with other signaling systems. Most relevant to the present discussion, the endocannabinoid and TRP systems overlap in their ligand-recognition properties with respect to select naturally occurring ligands.[19] The shared endogenous ligands are found in plants and animals and generally belong to the lipid and terpene chemical classes. The seminal discovery in this regard,

demonstration in 1999 that the endocannabinoid AEA can activate TRPV1 channels,[20] also established physiological connectivity between endocananbinoid-system and TRP-channel-mediated cellular signaling, a relationship particularly relevant in the CNS where CB1 and TRPV1 activation provides a framework for Δ^9-THC's psychobehavioral manifestations and the transmission and modulation of thermal and pain effects. The spectrum of natural cannabinergic and TRP-channel ligands has been greatly expanded by medicinal chemistry efforts that have produced designer synthetic ligands that interact as xenobiotics with CB receptors and/or TRP channels.[9,21,22]

PHYTOCANNABINOIDS AND LIGANDS BASED UPON A TERPENOID CHEMOTYPE

Among its approximately 500 endogenous phytochemicals,[23] the cannabis plant contains some 70 unique cannabinoids ("phytocannabinoids"), of which the most well-studied are shown in Figure 1A. Δ^9-THC is the archtypical "classical CB", encompassing a fused-ring tricyclic terpenoid derivative incorporating a polar benzopyran ring with a terminal, hydrophobic alkyl (*n*-pentyl) side-chain, a characteristic lipophilic domain, and hydrogen-bonding phenolic group.[24] Δ^9 -THC can engage and activate both CB1 and CB2 receptors with low nanomolar affinity, although the action of Δ^9-THC as a partial agonist at presynaptic CB1 receptors in the CNS is thought to account for its psychotropic activity as the main psychoactive cannabis phytocannabinoid. Δ^9-THC and its much less prominent natural isomer, (−)-Δ^9-tetrahydrocannabinol (Δ^8-THC), are virtually equivalent as to CB-receptor affinity and pharmacological activity, although Δ^8-THC is the more chemically stable isomer.[25] As compared to Δ^9 -THC, the phytocannabinoid (−)-cannabidiol (CBD) has significantly less affinity for CB receptors, modest CB2 selectivity, and negligible psychotropic activity.[26,27] Another classical terpenoid phytocannabinoid, (−)-Δ^9 -tetrahydrocannabivarin (Δ^9 -THCV), is a shorter side-chain Δ^9 -THC homologue with a similar CB-receptor affinity and selectivity profile. As a "neutral" CB1 antagonist in some systems, Δ^9-THCV at low doses can antagonize the effects of Δ^9-THC in a manner distinct from that of typical CB1 antagonists/inverse agonists and may also have CB receptor-independent pharmacological activities.[27,28]

The classic terpenoid phytocannabinoids THC, CBD, and Δ^9-THCV have inspired the synthesis of several structurally related cannabinergic compounds that display varying degrees of selectivity as CB1/CB2 agonists and, generally, improved CB-receptor affinity versus Δ^9-THC. Many of the synthetic CB agonists based on classic phytocannabinoids have been designed with therapeutic application in mind and, consequently, have been profiled preclinically for both their molecular pharmacology in vitro and potential salutary effects in disease models in vivo. Most prominent of these xenocannabinoids include nabilone, the first CB drug to be synthesized and used to treat chemotherapy-associated nausea, and CP-55,940, the first tritiated CB that, as a radiolabeled ligand, played a key role in the discovery of CB1.[2,7,8] Other related xenocannabinoids include HU-210,[29] AM4054,[30] AM841,[31] and AM2389[32] (Figure 1B. Of note, the isothiocyanate AM841 has been identified as an exceptionally potent "megagonist" at CB2 whose molecular mechanism of action involves covalent modification of a critical cysteine residue in the receptor's

transmembrane helix 6.[33] Similarly, the analgesic potency of the novel, high-efficacy, CB1-selective agonist AM2389 is 1000-fold greater than that of morphine in the standard rat tail-flick model of pain.[34]

ENDOCANNABINOIDS AND RELATED LIPID LIGANDS

Endocannabinoid lipid mediators that activate CB1 and CB2 are exemplified by AEA and 2-AG (Figure 2A). AEA is a partial CB1 activator with modest affinity and a relatively weak CB2 ligand with low overall efficacy, whereas 2-AG is a full agonist at CB1 and CB2, albeit with lower affinity and greater efficacy relative to AEA.[3,4] As is the case for synthetic phytocannabinoid analogues, several lipid cananbinergic ligands structurally related to AEA/2-AG have been synthesized by medicinal chemists for potential therapeutic application. These include arachidonoylcyclopropylamide (AM860, ACPA), a potent, selective CB1 agonist with anxiolytic and vasorelaxant properties in vivo;[35] R-methanandamide (AM356), the first metabolically stable, chiral AEA analogue with partial CB1 efficacy and higher potency as compared to AEA itself that exerts therapeutic neuroprotective, antinociceptive, vasorelaxant, and anti-inflammatory effects;[36] AMG313, the first AEA analogue with a chiral methyl arachidonoyl side chain;[37] and AM9017, the first AEA analogue with high CB2 affinity (Whitten and Makriyannis, 2014, unpublished results) (Figure 2B).

PHYTO-TRPs AND RELATED TERPENOID LIGANDS

The prototypic, plant-derived TRPV1 agonist and homovanillic ester, capsaicin, is one of the five principal capsaicinoids present in Cayenne chili pepper (*Capsicum annuum* L.)[38,39] (Figure 3A). Several other structurally diverse phytochemicals have been identified as naturally occurring TRP-channel modulators of various TRP channel subfamilies. The diterpene capsaicin analogue resiniferatoxin is an extremely potent TRPV1 agonist,[40] and the phytocannabinoid cannabidiol is both a CB1/CB2 agonist as well as an activator of TRPV1, TRPV2, and TRPV3[41,42] (Figure 3A). Other phytochemical TRP-channel activators include the four transient potential receptor channel ankyrin 1 (TRPA1) agonists cinnamaldehyde (found in cinnamon),[43] eugenol (found in cloves),[44] gingerol (found in ginger),[45] and umbellulone (found in California bay laurel, the “headache tree”)[46] and the two marine sphingoids leucettamol-A and leucettamol-B, which activate TRPA1 and inhibit transient receptor potential channel melastatin 8 (TRPM8)[47] (Figure 3A). Some plant-derived TRP-channel agonists have served as templates for medicinal chemistry efforts aimed at producing TRP-channel modulators with improved pharmacological profiles as potential drugs, for example, gingerol analogues.[48]

ENDOGENOUS TRP-CHANNEL AND RELATED LIPID LIGANDS

Several naturally occurring lipids act on TRP channels to modify cation flux through them.[49–51] The endocannabinoid AEA modulates CB1/CB2 and also acts as a TRPV1 agonist[20] (Figure 2A), while oleoylethanolamide (OEA) is a TRPV1 agonist that also binds to peroxisome proliferator-activated receptor alpha (PPAR-α) and the GPR119 CB-like receptor[52] (Figure 4A). Other endogenous *N*-acyl-amide lipids structurally related to AEA

include the FAAH inhibitor and TRPV1 antagonist *N*-arachidonoylserotonin[53] and the CB1 and TRPV1 agonist *N*-arachidonoyldopamine (NADA).[54] NADA was the first endogenous compound ("endovanilloid") identified in mammalian nervous tissue with potency comparable to the phytochemical capsaicin at TRPV1 and is a long-chain fatty-acid amide, as are AEA and capsaicin.[55] *N*-Acyl-amide lipids that act at TRP channels have inspired synthetic analogues including *N*-vanillylarachidonamide ("arvanil"), a TRPV1 agonist and CB1 partial agonist.[56] Similarly, *N*-arachidonoylaminophenol (AM404), the first potent lipid-amide inhibitor of cellular AEA uptake, was subsequently shown to be a potent TRPV1 activator and cyclooxygenase-1/2 inhibitor as well[57–59] (Figure 4B).

Lipids produced by the lipoxygenase-mediated oxygenation of polyunsaturated 20-carbon fatty acids (especially arachidonic acid), including the eicosanoids leukotriene B4 (LTB_4) and 12-hydroperoxyeicosatetraenoic acid (12-HPETE), are potent, endogenous TRPV1 activators[60,61] (Figure 4A). Formed by macrophages in vivo, an endogenous lipid mediator involved in resolving inflammation, maresin-1 (4*Z*,7*R*,8*E*,10-*E*,12*Z*,14*S*,16*Z*,19*Z*)-7,14-dihydroxy-4,8,10,12,16,19-docosahexaenoic acid, is synthesized from docosahexaenoic acid lipoxygenation and acts as a TRPV1 antagonist[62] (Figure 4A). Other endogenous lipid-derived mediators have been identified as modulators of TRP channels from nonvanilloid subfamilies or TRP channels in the vanilloid subfamily other than TRPV1; for example, 4-hydroxynonenal produced from polyunsaturated fatty acid peroxidation activates TRPA1,[63] and epoxytrienoic acids (EETs), including 5′,6′-epoxyeicosatrienoic acid (5′,6′-EET) produced from epoxygenation of 20-carbon polyunsaturated fatty acids by cytochrome P450, activate TRPV1and TRPV4[64,65] (Figure 4A).

THERAPEUTICALLY RELEVANT FUNCTIONAL INTERPLAY BETWEEN CANNABINERGIC AND TRP-CHANNEL-MEDIATED SIGNALING

The ability of the synthetic aminoalkylindole cannabinoids *R*-(+)-WIN55,212-2 and AM1241 (Figure 5) to elicit peripherally mediated antinocieption and antihyperalgesia in acute pain models[66] and alleviate capsaicin-induced hyperalgesia/allodynia[67,68] prompted investigation of their mechanism of action. Particularly intriguing was the notion that both *R*-(+)-WIN55,212-2 and AM1241 can inhibit nociceptive sensory neurons while differing in their activation profiles at CB receptors: *R*-(+)-WIN55,212-2 is a full CB1 agonist,[69] whereas AM1241 is a CB2-selective agonist.[70] Cellular and in vivo animal data demonstrate that these CB agonists exert peripheral antinocifensive actions against the phytochemicals capsaicin and mustard oil by desensitizing TRPA1 and TRPV1 channels on sensory neurons.[71] Thus, these cannabinergic compounds first act to activate TRPA1 and TRPV1, which is then followed by desensitization of these TRP channels.

Another level of interaction between endocananbinoid-system and TRP-channel signaling is illustrated by the metabolic cascade responsible for TRPV4 activation by the lipids AEA (Figure 2A) and 5′,6′-EET (Figure 4A). As elucidated by Watanabe et al.,[72] enzymatic hydrolysis of the endocananbinoid AEA by FAAH produces arachidonic acid. This FAAH-dependent AEA hydrolysis is a metabolic conversion that is obligatory for AEA to activate TRPV4, since the arachidonic acid so produced serves as substrate for EET production

through the cytochrome-P450 epoxygenase pathway. A resulting lipid epoxide product, 5′, 6′-EET, is able to activate and open TRPV4, leading to calcium influx into the target cell, a phenomenon important to the physiological modulation of vascular tone and AEA's vasorelaxant property.

A further example of the functional crosstalk between the endocananibnoid system and TRP channel-mediated information transduction has emerged from a series of laboratory studies in rodents on the mechanism of action of acetaminophen (*N*-acetyl-*p*-aminophenol), a widely used over-the-counter analgesic and antipyretic drug.[59,73,74] Spanning a decade, these studies by Zygmunt and colleagues demonstrated that the antinociceptive effect of acetaminophen is dependent upon brain TRPV1 and that acetaminophen is biotransformed to the synthetic lipid AM404 through the action of the endocananbinoid-system enzyme, FAAH, in rat and mouse brain. The mechanism of acetaminophen's TRPV1-mediated antinociception was demonstrated to reflect acetaminophen hepatic metabolism to *p*-aminophenol, which is subsequently conjugated with arachidonic acid in FAAH-containing neurons expressing TRPV1, leading to the formation of the TRPV1 activator AM404, which directly interacts with this TRP channel to elicit a therapeutic effect (analgesia, reduce fever). Notably, neither acetaminophen nor *p*-aminophenol interacts with TRPV1. Prior to this work, AM404 was shown to inhibit cellular AEA uptake and cyclooxygenase-1/2 and activate TRPV1.[57,59] Thus, AM404's potent analgesic activity in vivo may reflect its pleiotropic activity profile and effects on multiple endocananbinoid-system and TRP-channel targets.

INVOLVEMENT OF CANNABIBERGIC AND TRP-CHANNEL SIGNALING IN DRUG ABUSE

Potentiation of cannabinergic and TRP-channel signaling by phytochemicals has been linked to substance-abuse-related disorders, with great implications for human health. Stimulation of CB1 in the CNS by the phytocannabinoid Δ^9-THC is generally accepted to be the basis for the negative cognitive effects of marijuana and its abuse liability,[75] inviting novel medicinal chemistry approaches (e.g., CB1 agonists with limited CNS penetration[76]) for modulating CB1 activity without inviting adverse psychobehavioral events. Observations that changes in cannabinergic activity and/or endocannabinoid tone have been associated with a variety of physiological challenges and disease states involving the nervous system and most every peripheral organ suggest that the endocananbinoid system contributes to normal physiological conditions by responding to injurious or disease-provoking insults in order to attenuate or delay their potentially damaging consequences and help maintain homeostatic balance.[77,78] Thus, modulation of endocannabinoid-system activity has been of great therapeutic interest with respect to two general modalities: (a) regulating endocananbinoid-system activity with an agent whose dosing regimen/molecular pharmacology does not itself invite adverse events; (b) enhancing cyto- and tissue-protective endocannabinoid-system activation in a time-, event-, and tissue/organ-specific manner so as to reduce the potential for adverse responses. Examples of the former modality are the successful introduction into the clinic in certain markets of Sativex, a mixture of Δ^9-THC and the nonpsychoactive phytocannabinoid CBD (Figure 1A), for relief of neuropathic pain

associated with multiple sclerosis, as an adjunctive pain reliever for advanced cancer, and for treating spasticity due to multiple sclerosis[79] and CB1 (periphero-)neutral antagonists for cardiometabolic disease.[80] The latter modality would include FAAH inhibitors that enhance the CNS endocannabinoid elevation observed after brain injury to therapeutic levels.[10]

The well-known cooling sensation of menthol (Figure 3A), a constituent of the wild mint plant (*Mentha arvensis*), reflects this phytochemical's ability to trigger chemically the cold-sensitive TRPM8 receptors in cold-sensitive sensory neurons. Menthol also has complex olefactory- and somatosensory-stimulating properties and interacts with TRPA1, an irritant-sensing TRP channel expressed by nociceptors in the lung and respiratory tract.[81] In humans, a common haplotype of the gene encoding for TRPA1 provides a functional TRPA1 channel associated with a preference for mentholated cigarettes among heavy smokers.[82] This genetic and biological profile along with the ubiquitous presence of menthol as an additive in most commercial cigarettes, the preference for menthol-containing cigarettes during smoking initiation, and the lower smoking-cessation rates for menthol smokers have prompted investigation as to menthol's potential role in promoting smoking behavior/nicotine addiction and its contribution to the incidence of tobacco-related diseases.[83] Data in mice demonstrate that menthol, through TRPM8 activation, acts as a potent respiratory counterirritant to suppress the respiratory irritation caused by a wide variety of irritants in tobacco smoke.[84] In this manner, menthol's biological activity at TRPM8 may facilitate smoke inhalation and promote tobacco smoking/nicotine addiction, an enormous health problem as the leading cause of preventable death and illness underserved by current pharmacotherapeutic strategies.[85] In the clinic, the CB1 antagonist/inverse agonist rimonabant has been shown to increase the likelihood that smokers will quit,[86] and inhaled CBD reduces cigarette consumption, perhaps by modulating the craving-related salience of smoking cues.[87] These aggregate in vivo and clinical data regarding menthol pharmacology support the proposition that TRP-channel and endocannabinoid-system signaling are involved in sustaining tobacco smoking and may be leveraged for therapeutic gain against nicotine addiction.

Perhaps instigated by menthol's biological properties as related to tobacco smoking, the very potent synthetic TRPM8 agonist, icilin (Figure 3B), has been studied by the tobacco industry as a flavor enhancer for cigarettes.[88] It is noteworthy, however, that icilin, but not menthol, requires calcium as a coagonist to attain maximal levels of TRPM8 activation, suggesting that discrete structural requirements must be fulfilled for ligand-induced TRP channel activation and degree of thermosensitivity.[89–91] This proposition was indeed supported by mutagenesis experiments demonstrating that specific residues in the cytoplasmic loop interconnecting TMS2 and TMS3 (i.e., N799, D802, and G805) are critical for TRPM8's icilin sensitivity, just as residues in analogous positions (i.e., Y511 and S512) are critical for activation of TRPV1 by capsaicin.[91] Further evidence for ligand-sensitive functional domains in TRP channels comes from demonstration that many noxious TRPA1-activating compounds are electrophiles whose covalent modification of select reactive cysteine residues in this TRP channel is critical for the rapid signaling of potential tissue damage through the neural pain pathway.[92] Despite their noxious and abuse-related properties, then, some phytochemicals have proven their value for interrogating the molecular mechanisms by which TRP channels are activated.

CONCLUSIONS

Both naturally occurring and synthetic terpenes and lipids stimulate cannabinergic or TRP channel-mediated signaling in mammals. Some of these signaling molecules are co-opted by both information systems and are able to act at both discrete CB and TRP-channel protein targets. Interaction of phytochemcials and synthetic ligands with both CB receptors and TRP channels and the significant degree to which CB1 and TRPV1 are coexpressed in several brain regions (including the hypothalamus, striatum, hippocampus and substantia nigra)[93] carry implications for human health and disease treatment. For instance, demonstration that the competitive TRPV1 antagonist AMG9810 (Figure 3B) further reduces the inflammatory activation of human endothelial cells elicited by the synthetic CB *R*-(+)-WIN55,212-2 or the naturally occurring CB1 and TRPV1 agonist NADA, whereas TRPV1 inhibition with AMG9810 alone potentiated the inflammation suggests that cannabinergic and TRP-mediated signaling work in concert to regulate endothelial inflammatory sensitivity/homeostasis.[94] The anticonvulsant effect of dual FAAH and TRPV1 blockade with *N*-arachidonoyl-serotonin depends upon potentiation of CB1 activity as a result of the increased AEA levels consequent to FAAH inhibition with a component of TRPV1 blockade against the neuroexcitatory effect of TRPV1 activation by AEA.[95] Such findings suggest that discrete functional and pharmacological interactions between TRP channels and endocannabinoid-system proteins offer opportunities to develop novel, dual-acting ligands both as both probes for interrogation of their independent and integrative functionality and as drugs that modulate these two biosignaling systems for therapeutic gain.

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ABBREVIATIONS

CB	cannabinoid
TRP	transient receptor potential
CB1	cannabinoid 1 receptor
(−)-Δ^9-THC	(−)-Δ^9-tetrahydrocannabinol
CB2	cannabinoid 2 receptor
GPCR	G-protein coupled receptor
2-AG	2-arachidonoylglycerol
AEA	anandamide
CNS	central nervous system
MGL	monoacylglycerol lipase
ABHD6	α,β-hydrolase domain-containing protein 6

ABHD12	α,β-hydrolase domain-containing protein 12
FAAH	fatty acid amide hydrolase
TMS	transmembrane segment
TRPV	transient receptor potential vanilloid
(−)-Δ^8-THC	(−)-Δ^8-tetrahydrocannabinol
CBD	cannabidiol
Δ^9-THCV	Δ^9-tetrahydrocannabivarin
ACPA	arachidonoylcyclopropylamide
TRPA1	transient potential receptor channel ankyrin 1
TRPM8	transient receptor potential channel melastatin 8
OEA	oleoylethanolamide
PPAR-α	peroxisome proliferator-activated receptor alpha
NADA	*N*-arachidonoyl dopamine
LTB_4	leukotriene B_4
12-HPETE	12-hydroperoxyeicosatrie-noic acid
EET	epoxytrienoic acid
5′,6′-EET	5′,6′- epoxytrienoic acid

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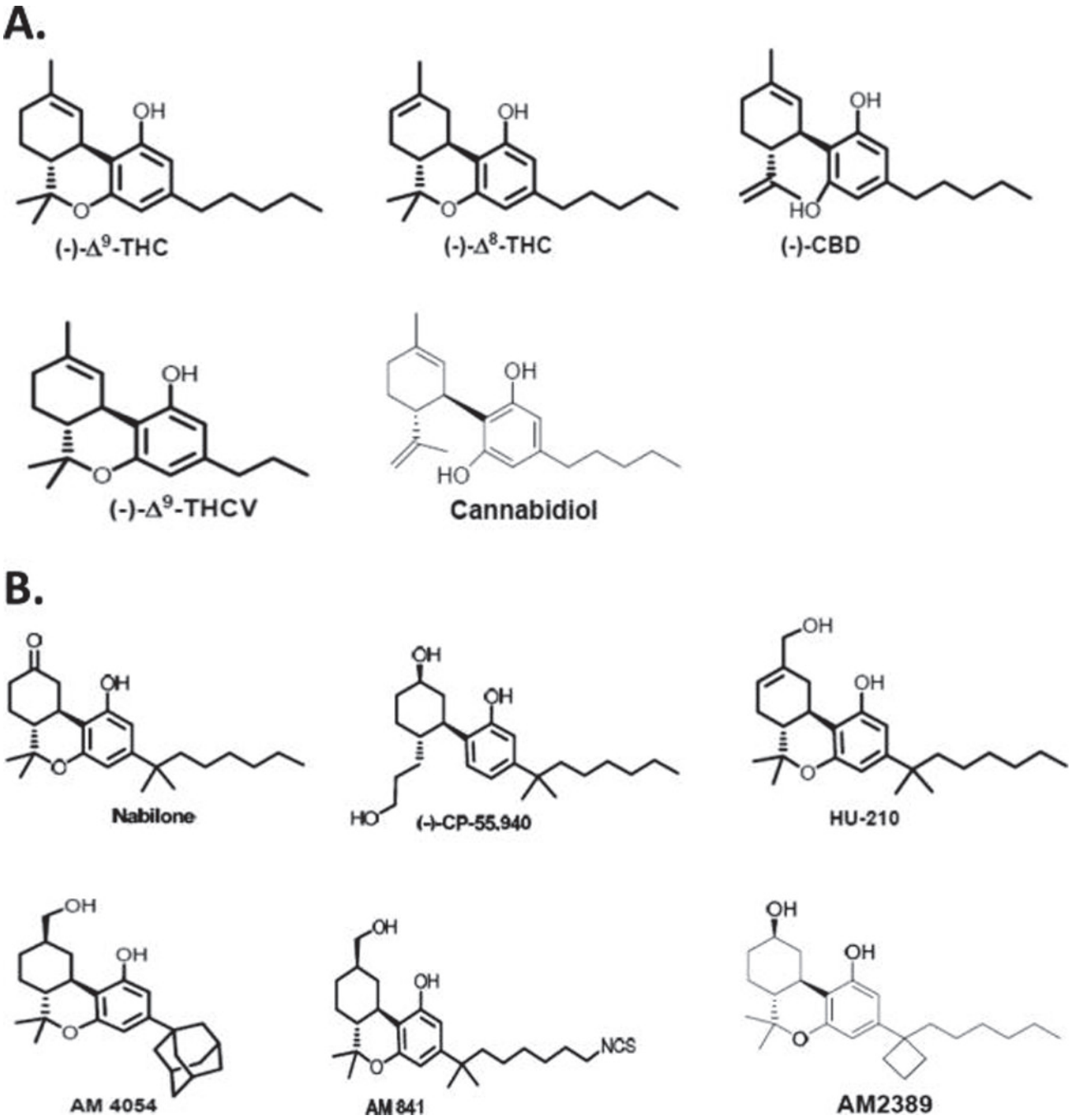

Figure 1.

Chemical structures of select plant-derived terpenoid cannabinoids (phytocannabinoids) (A) and select synthetic terpenoid cannabinoids (B) discussed in the text.

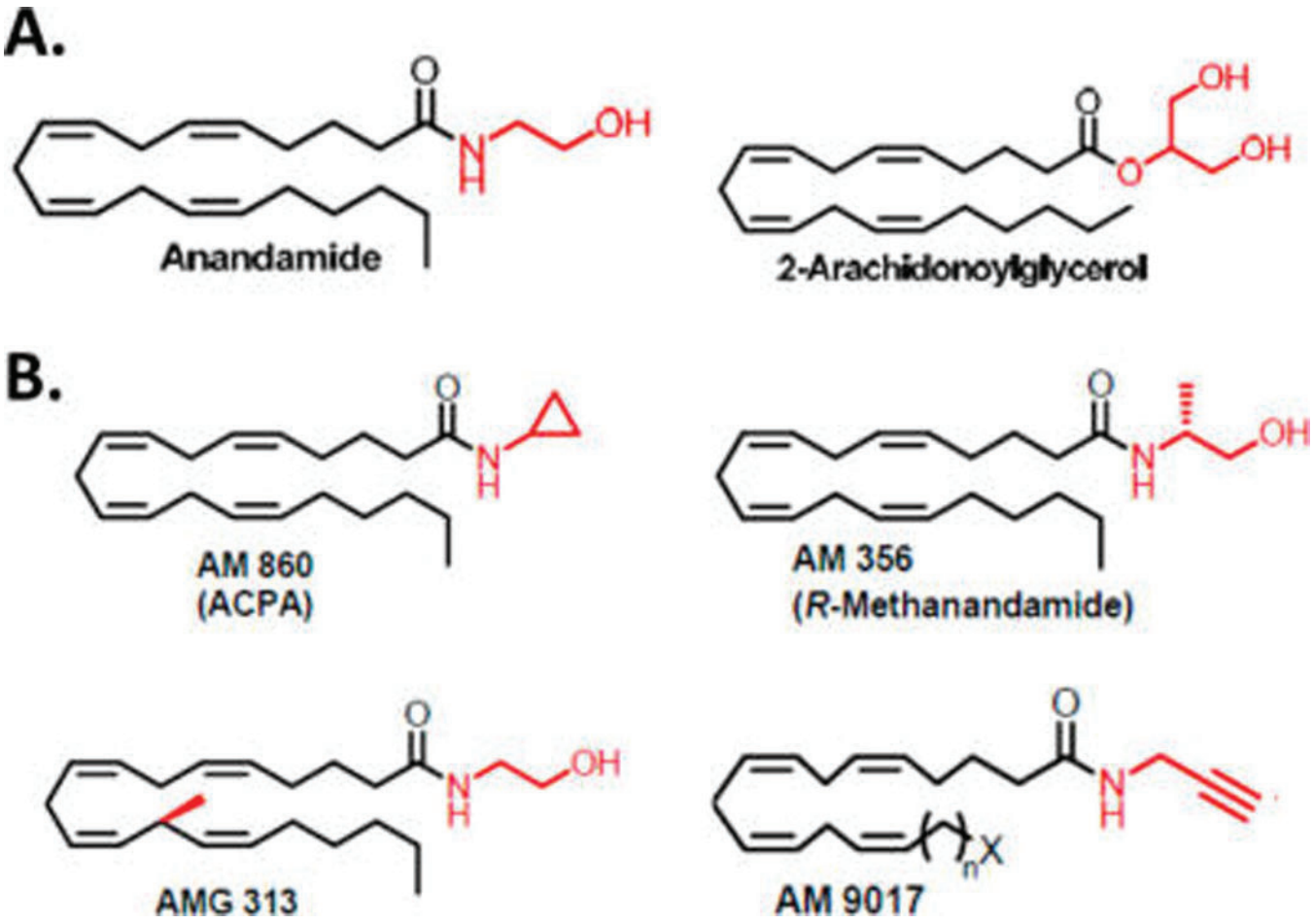

Figure 2.
Chemical structures of select lipid endocannabinoids (A) and structurally related synthetic cannabinergic agents (B) discussed in the text.

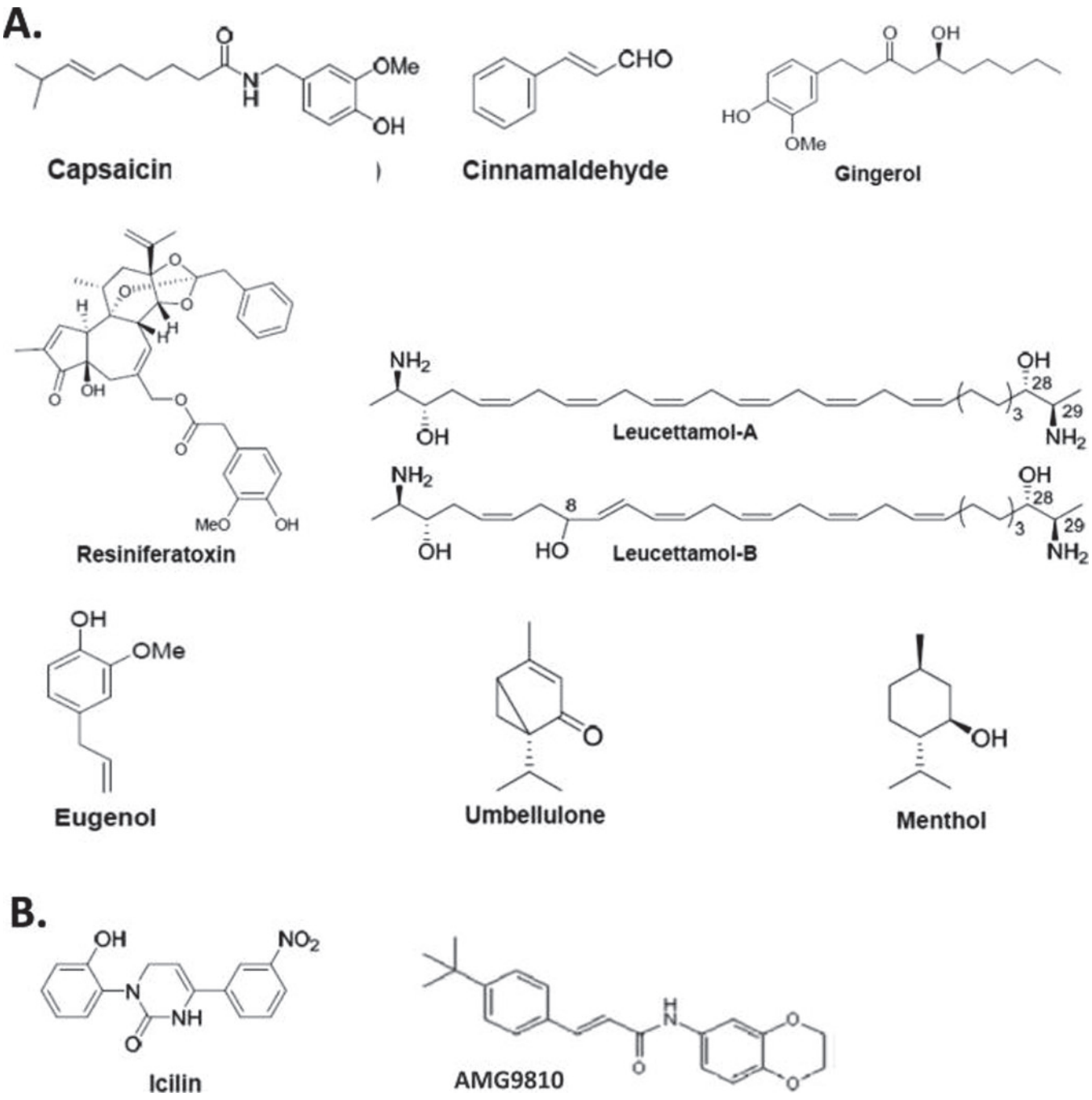

Figure 3.

Chemical structures of select plant-derived terpenoid TRP-channel ligands (A) and synthetic TRP-channel ligands (B) discussed in the text.

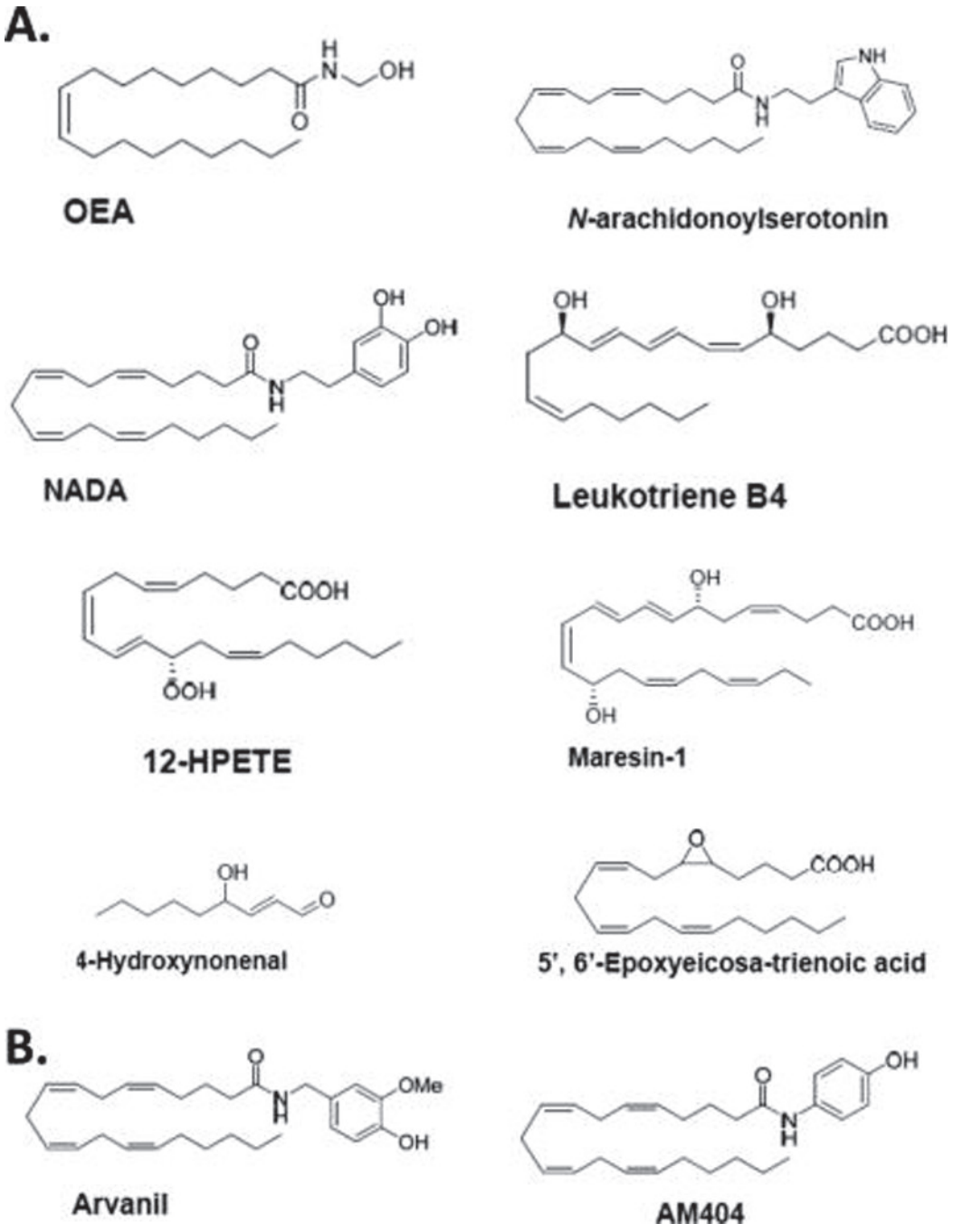

Figure 4.
Chemical structures of select endogenous (A) and synthetic (B) lipid TRP-channel ligands discussed in the text.

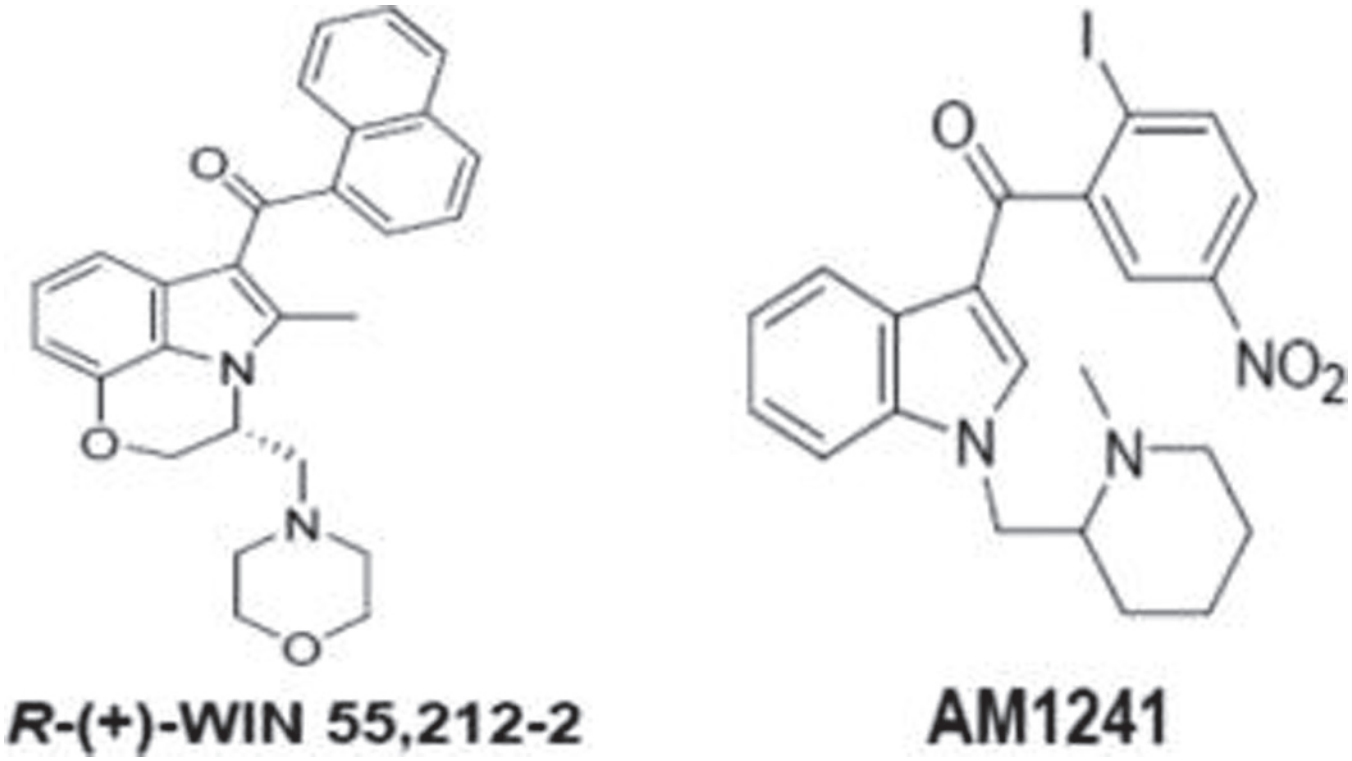

Figure 5.
Chemical structures of synthetic aminoalkylindole cannabinoids discussed in the text.

molecules

MDPI

Review

Terpenoids, Cannabimimetic Ligands, beyond the *Cannabis* Plant

Elaine C. D. Gonçalves [1,2], Gabriela M. Baldasso [1,†], Maíra A. Bicca [3,†], Rodrigo S. Paes [1], Raffaele Capasso [4,*] and Rafael C. Dutra [1,2,*]

1 Laboratory of Autoimmunity and Immunopharmacology (LAIF), Department of Health Sciences, Campus Araranguá, Universidade Federal de Santa Catarina, Araranguá 88906-072, Brazil; elainecdalazen@gmail.com (E.C.D.G.); baldasso.gabriela@gmail.com (G.M.B.); rodrigosebbenp@gmail.com (R.S.P.)

2 Graduate Program of Neuroscience, Center of Biological Sciences, Campus Florianópolis, Universidade Federal de Santa Catarina, Florianópolis 88040-900, Brazil

3 Neurosurgery Department, Neurosurgery Pain Research institute, Johns Hopkins School of Medicine, Baltimore, MD 21287, USA; bicca.ma@jhmi.edu

4 Department of Agricultural Sciences, University of Naples Federico II, 80,055 Portici, Italy

* Correspondence: rafcapas@unina.it (R.C.); rafaelcdutra@gmail.com or rafael.dutra@ufsc.br (R.C.D.); Tel.: +39-081-678664 (R.C.); +55-48-3721-21678 (R.C.D.); Fax: +55-48-3721-6448 (R.C. & R.C.D.)

† These authors contributed equally to this work.

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Abstract: Medicinal use of *Cannabis sativa* L. has an extensive history and it was essential in the discovery of phytocannabinoids, including the *Cannabis* major psychoactive compound—Δ9-tetrahydrocannabinol (Δ9-THC)—as well as the G-protein-coupled cannabinoid receptors (CBR), named cannabinoid receptor type-1 (CB1R) and cannabinoid receptor type-2 (CB2R), both part of the now known endocannabinoid system (ECS). Cannabinoids is a vast term that defines several compounds that have been characterized in three categories: (i) endogenous, (ii) synthetic, and (iii) phytocannabinoids, and are able to modulate the CBR and ECS. Particularly, phytocannabinoids are natural terpenoids or phenolic compounds derived from *Cannabis sativa*. However, these terpenoids and phenolic compounds can also be derived from other plants (non-cannabinoids) and still induce cannabinoid-like properties. Cannabimimetic ligands, beyond the *Cannabis* plant, can act as CBR agonists or antagonists, or ECS enzyme inhibitors, besides being able of playing a role in immune-mediated inflammatory and infectious diseases, neuroinflammatory, neurological, and neurodegenerative diseases, as well as in cancer, and autoimmunity by itself. In this review, we summarize and critically highlight past, present, and future progress on the understanding of the role of cannabinoid-like molecules, mainly terpenes, as prospective therapeutics for different pathological conditions.

Keywords: phytocannabinoid; terpenoids; cannabinoid receptors; *Cannabis* plant; endocannabinoids; inflammation.

1. The Era of *Cannabis sativa*, Cannabinoids, and the Endocannabinoid System: A Long Journey Traveled

The *Cannabis sativa* era has a long and remarkable history dating from prehistoric Xinjiang, an ancient Chinese place, where users consumed *Cannabis* not only for religious/spiritual or hedonic purposes but also for its medicinal effects [1–3]. The first report of hemp medicinal use comes from Chinese medicine, around 2300 B.C. In India, *Cannabis* became part of the Hindu religion, being subsequently introduced to Europe between 1000 and 2000 B.C. Long after *Cannabis* reached

the Americas, South America (mainly Chile) in 1545, and over 60 years later (1606), its cultivation was introduced to North America. Western medicine slowly progressed from the understanding and moderate use in the early and mid-19th century, to its wider use, based on its medicinal properties in the 20th century. Nevertheless, due to prejudice and misinformation, the use of this plant has been marginalized, which has hindered research progress regarding its medicinal beneficial effects [1,2].

Currently, *Cannabis* is the most commonly cultivated, trafficked, and abused drug worldwide, potentially causing a substantial public health impact since it can alter sensory perception and induce elation and euphoria [4,5]. Recent use rates among the population in general show a concentration to adolescents and young adults (20 to 24 years-old), ranging from 2%–5% of the global population (an estimated 13 million cannabis-dependent individuals in 2010); yet, the highest numbers (~10%–13%) are reported in North America [5–7]. A study published by Hasin and colleagues revealed a significant rise in marijuana use prevalence in 2001–2002 and 2012–2013, accompanied by a large increase of marijuana-induced disorders in this same time period [8,9]. Conversely, another study showed that *Cannabis*-induced disorders declined among young users during 2013-2014, in the USA [10,11]. According to United States Code, "marijuana/cannabis" comprises "all parts" of the plant *Cannabis sativa L.* and every compound derivative of such plant. By the year 2016, 28 states in the USA have voted to authorize or implement medicinal cannabis programs. Among these, eight states and the district of Columbia have legalized the recreational use of *Cannabis* [12]. In other countries, including the United Kingdom (UK), Denmark, Czech Republic, Austria, Sweden, Germany, and Spain, it is formally approved; thus, decriminalizing the therapeutic use of *Cannabis* and cannabis-based products [13,14]. Pioneering in Latin America, Uruguay, became the first country to legalize the sale, cultivation, and distribution of *Cannabis* [15,16]. Wilkinson and D'Souza have previously described that the medicalization and/or incorporation of *Cannabis* into a medicine is complex for a number of reasons, including that (i) it is a plant rather than a pharmaceutical product, and (ii) knowledge of its properties and effects is still limited [17]. However, in light of the recently and largely reported pharmacological discoveries and therapeutic benefits of *Cannabis*, the controlled and medicinal use of *Cannabis* for some pathological conditions have been enforced.

Era of cannabinoids started when Mechoulam and Gaoni isolated and characterized the main psychoactive component of *Cannabis sativa*, the Δ9- tetrahydrocannabinol (Δ9-THC). Subsequently, in 1988, Howlett's group established the presence of a specific cannabinoid receptor in the rat brain by using a tritium labeled cannabinoid [18], followed by the cloning of the cannabinoid receptor type-1 (CB1R) [19]. Then, Matsuda and coworkers (1990) described a second receptor, named the cannabinoid receptor type-2 (CB2R), which was cloned by Munro and coworkers in 1993 [18,19]. These receptors can be activated by endogenous molecules produced normally by our bodies, and likewise by external synthetic and natural molecules. The number of natural compounds identified or isolated from *Cannabis sativa* has been increasing in the last decade, with 565 identified substances between cannabinoids and non-cannabinoid constituents [20]. The genus *Cannabis* comprises closely related species, mainly, *Cannabis indica*, *Cannabis ruderalis* (identified in 1924), *Cannabis sativa L.*, which is widely known as "hemp" and not psychoactive, as well as *Cannabis sativa*, which induces psychoactive effects [1]. Cannabinoids are defined as a group of molecules that modulate cannabinoid receptors (CBR) and are characterized by three varieties, such as endogenous or endocannabinoids, synthetic cannabinoids, and phytocannabinoids. The latter variety comprehends natural terpenoids or phenolic compounds derived from *Cannabis sativa* or other species, and will be further explored later in this review [21]. Altogether, 120 cannabinoids have been isolated from the *Cannabis sativa* plant and classified into 11 general types, as described below (Table 1) [20].

Table 1. *Cannabis sativa* L. constituents by chemical class.

Chemical Class	Compounds
Δ9-THC types	23
Δ8-THC types	5
CBG types	16
CBC types	9
CBD types	7
CBND types	2
CBE types	5
CBL types	3
CBN types	11
CBT types	9
Miscellaneous types	30
Total cannabinoids	120
Total non-cannabinoids	445
Grand Total	565

THC, tetrahydrocannabinol; CBG, cannabigerol; CBC, cannabichromene; CBD, cannabidiol; CBND, cannabinodiol; CBE, cannabielsoin; CBL, cannabicyclol; CBN, cannabinol; CBT, cannabitriol, as previously described [20].

Pharmacologically approaching, three compounds have been isolated and identified as the most important, namely the Δ9-tetrahydrocannabinol (Δ9-THC), cannabidiol (CBD), and cannabinol (CBN). Relevantly, preclinical and clinical research has shown that cannabinoids, especially CBD, play key a role in different pathological conditions (Table 2).

When we talk about the era of the "endocannabinoid system", we have to keep in mind that this biological system was named over the response of its receptors to cannabinoid drugs, such as the previously mentioned and well-studied Δ9-THC and biologically active synthetic analogs, just like it has happened with the opioids in the past. In addition to its receptors, the system is highly modulated by the enzymes involved in the endogenous cannabinoids synthesis and inactivation (endocannabinoid metabolism). Furthermore, some other receptors have been reported to be activated by cannabinoid drugs and related molecules, including GPR55, GPR18, and GPR119 [40–42]. CB1R is a key component of the endocannabinoid system (ECS), since it interacts with endogenous and exogenous cannabinoids, including Δ9-THC, and it is considered the most abundant metabotropic receptor in the brain [43]. It has been cloned from humans and it is accountable for the *Cannabis* effects on mood, as well as negative psychotomimetic effects, including anxiety, paranoia, and dysphoria [4,44]. While CB1R plays a role as a neurotransmission regulator in different brain regions and for this reason mediates the *Cannabis* psychoactive effects, CB2R, in particular, mediates anti-inflammatory and immunomodulatory actions [45]. An accumulating body of evidence suggests that both CB1R and CB2R, and their ligands, play a significant role in physiologic and pathologic processes [46]. In this context, both receptors have been widely studied regarding their relevance in the modulation of immune-mediated inflammatory diseases, neuroinflammation, neurological and neurodegenerative diseases, cancer, and autoimmunity.

Beyond the CBR, mammalian tissues can both synthesize and release cannabinoid receptor ligands [44,47,48]. The era of ECS started when Devane and colleagues (1992) described for the first time, the N-arachidonoylethanolamine molecule, named anandamide from porcine brain. Interestingly, anandamide interact to CBR and induces behavioral actions similar to the ones induced by Δ9-THC, when administered in rodents [4,49]. The mainly endogenous cannabinoids are the anandamide (AEA) and the 2-arachidonoyl glycerol (2-AG). It is now ordinarily accepted that the mammalian tissues contain an ECS composed by: (i) CB1R and CB2R cannabinoid receptors [19,44], (ii) endogenous cannabinoids ligands [49–51], and (iii) enzymes involved in the cannabinoids ligands synthesis and inactivation. Regarding these enzymes, the fatty acid amide hydrolase (FAAH) breaks amide bond and releases arachidonic acid and ethanolamine from AEA, and the monoacylglycerol lipase (MAGL) is responsible for a more efficiently 2-AG degradation [52]. Endocannabinoids are produced on demand from membrane lipids using the machinery of the enzymes responsible for their synthesis,

transport, and degradation. For instance, the N-arachidonoyl phosphatidylethanolamine (NArPE) originates a phosphatidic acid by a reaction mediated by a specific phospholipase D (NAPE-PLD); most importantly, it is hydrolyzed to AEA, in a reaction catalyzed by N-acyltransferase (NAT). The latter reaction happens out of an acyl group from the arachidonoylphosphatidylcholine (diArPC) sn-1 position converted to a phosphatidylethanolamine (PE) amino group. Following, AEA is degraded by FAAH. Synthesis of 2-AG depends on the phosphatidylinositol (PI) conversion to diacylglycerol (DAG) by the phospholipase C (PLC) enzyme, and subsequent DAG transformation to 2-AG by the action of the diacylglycerol lipase (DAGL) [53]. The ECS is involved with multiple biological functions, such as immune-mediated inflammatory and autoimmune diseases [53], as well as neuroinflammatory and neurodegenerative conditions [54]. Moreover, the ECS participates in the immune control at the CNS [55], maintaining overall "fine-tuning" of immune response balance [56], and influencing the neuroendocrine reaction to inflammation and infection [57].

Importantly, the ECS (i.e., CBR, endogenous cannabinoids, and anabolic/catabolic enzymes) are present in the cardiovascular tissues (myocardium, smooth muscle, and vascular endothelial cells), as well as in the circulating blood cells [58]. CB1R are expressed in the peripheral nervous system, including vagal afferent neurons, while CB2R are expressed in cardiomyocytes, coronary artery endothelial cells, and smooth muscle cells. For this reason, the endocannabinoid signaling exerts complex cardiac and vascular effects ranging from vasodilatation to vasoconstriction, and decreased myocardial contractility [58]. Those are important biological effects, as they could play an essential role in side effects promoted by potential molecules that are able to modulate this system. For instance, in healthy individuals, CB1R activation decreased myocardial contractility and blood pressure, possibly by peripheral inhibition of noradrenaline release from postganglionic sympathetic axons that leads to regulation of cardiac output [59]. In an opposite way, CB2R may exert a cardioprotective role associated to its immunomodulatory properties during tissue inflammation and tissue injury in cardiovascular diseases. The endogenous cannabinoids (2-AG and AEA) also have vascular effects, which are mediated by perivascular transient receptor potential vanilloid 1 (TRPV1) and transient receptor potential vanilloid 4 (TRPV4) activation in smooth muscle cells, promoting dilatory response [60]. Between the common clinical adverse effects associated with the *Cannabis* plant use, the increased cardiovascular activity and heart rate, as well as decreased blood pressure have been described [60]. In addition, the uses of *Cannabis* plant or synthetic cannabinoids have been linked to myocardial infarction, cardiomyopathy, arrhythmias, and stroke [58,61,62]. It occurs, possibly due to dose-dependent effects of phytocannabinoids and consequent modulation of the autonomic nervous system, at least partly via CB1R activation [60], since the CB1R antagonist Rimonabant® ameliorate the cannabis-induced tachycardia [63,64]. It is important to be aware of the harmful consequences that come along with the use of *Cannabis* plant and/or synthetic cannabinoids, as they could contribute to development of cardiovascular disorders, since the ECS has an essential role in the cardiovascular signaling.

The future, shedding light to a new era, is promising and based on the cloning of CBR associated with the possibility of manipulation of endocannabinoid levels in tissues, by using endocannabinoid enzymes-targeted pharmacology. This represents an opening of a possible gateway to the discovery and/or development of cannabimimetic ligands, beyond the *Cannabis* plant, which could still show therapeutic effects and possibly rule out many of the important adverse effects. A previous review has already stated that some plants, not belonging to the *Cannabis* genus, produce molecules chemically similar to the phytocannabinoids, named cannabimimetic ligands [65] (Figure 1). Cannabinoid-like molecules (mainly terpenes) of either plant or synthetic origin that are non-psychotropic have been studied. Terpenes and terpenoids are a widespread group of secondary metabolites found in numerous plant families, including Cannabaceae and others. Herein, we discuss the role of cannabinoid-like molecules, mainly terpenes, as prospective therapeutics for a variety of pathological conditions.

Table 2. CBD pharmacological actions on pathological conditions.

Research Themes	Main Findings	References
Alzheimer's disease (AD)	CBD prevented expression of proteins involved with *tau* phosphorylation and AD progression. CBD showed therapeutic potential for AD-associated cognitive impairment.	[22,23]
Anti-inflammatory properties	CBD induced apoptosis and inhibited lipopolysaccharide-activated NF-κB and interferon-β/STAT inflammatory pathways in microglial cells; CBD protected oligodendrocytes progenitor cells from inflammatory-induced apoptosis.	[24]
Anxiety	CBD modulated anxiety responses partially through 5-HT_{1A}-mediated neurotransmission, and demonstrated anxiolytic effects during a stimulated public speaking test; CBD action on limbic and paralimbic regions contributed to reduced autonomic arousal and subjective anxiety; CBD blocked anxiety-induced REM sleep alteration through anxiolytic properties.	[25,26]
Diabetes	CBD showed beneficial effects on glycemic control and cardiovascular dysfunction during diabetes.	[27]
Immunomodulatory effects	CBD modulated T-cell function and apoptotic signaling pathway.	[28]
Inflammatory bowel disease (IBD)	CBD attenuated intestinal inflammation and normalized motility in patients with IBD.	[29]
Cognitive impairments	CBD interacted with components of emotional memory processing and memory-rescuing, as well as attenuated THC-induced memory impairment effects.	[30]
Neuropathic pain	CBD inhibited chemotherapy-induced neuropathic pain.	[31,32]
Parkinson's disease (PD)	CBD administration showed neuroprotective effects during PD progression.	[33]
Schizophrenia	CBD showed antipsychotic-like properties in schizophrenia, as well as prevented clinical social dysfunction, and inhibited psychomotor agitation.	[34,35]
Seizure/Epilepsy	CBD showed anticonvulsant effects in animal models of seizure and patients with refractory epilepsy. CBD was also described as safe and beneficial for the treatment of epileptic disorders.	[36–39]

CBD, cannabidiol; NF-κB, nuclear factor kappa B; STAT, signal transducer and activator of transcription protein family; 5-HT_{1A}, serotonin 1A receptor; REM, rapid eye movement sleep; THC, tetrahydrocannabinol.

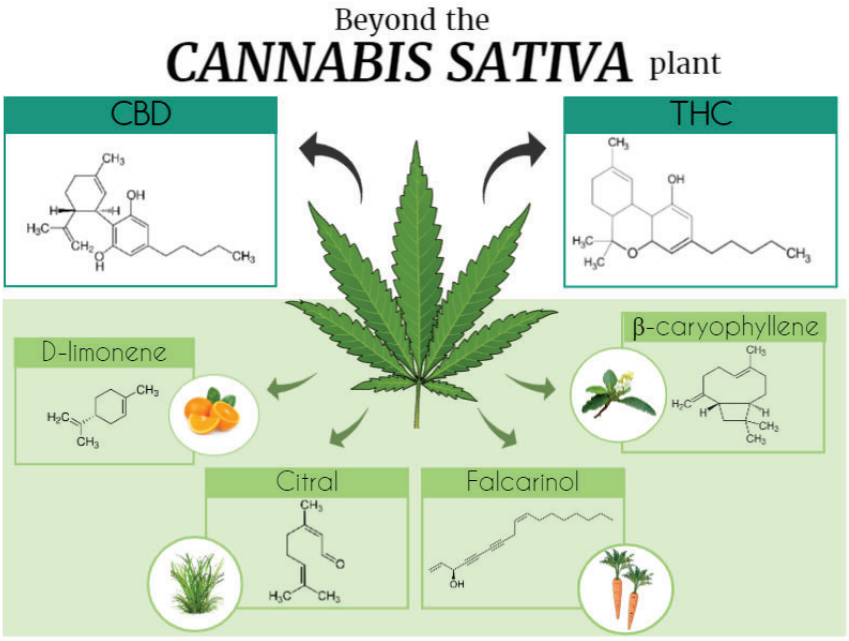

Figure 1. Beyond the *Cannabis sativa* plant. The Era of cannabinoids started with the description and isolation of the main *Cannabis sativa* psychoactive component, Δ9-tetrahydrocannabinol (THC). However, many other natural compounds were also identified, totalizing 565 substances among cannabinoids and non-cannabinoids constituents. This figure illustrates some of the *Cannabis sativa* compounds (ᴅ-limonene, β-caryophyllene, citral, and falcarinol) and its molecular structures that can be also found in other plants, such as *Cordia verbenacea*, lemon, *Cymbopogon citratus*, and carrot. CBD, cannabidiol. Figure created using the Mind the Graph platform.

2. Cannabis Phytocannabinoids: Focus on Tetrahydrocannabinol and Cannabidiol

The phytocannabinoid class includes more than a 100 compounds that are present in the *Cannabis sativa* plant [66], which interact with components of the human ECS, briefly addressed in this section. Phytocannabinoids production is dependent on plant internal factors (synthesized hormone levels, plant kind, and parts of the plant) and on external factors (humidity, light, type of soil, and temperature). The most elucidated compounds among the main phytocannabinoids are CBN, CBD, Δ8- e Δ9-THC, cannabigerol, and cannabivarin. The Δ9-THC is the major psychotropic compound found in high concentrations in the *Cannabis sativa* plants. It is classified as a CB1R and CB2R partial agonist, showing preference for the CB1R. The agonist activity on CBR triggers adenylyl cyclase (AC) inhibition and, thereby, the ability of modulating different neurotransmitters release as dopamine, acetylcholine, glutamate, and gamma-aminobutyric acid (GABA) [66]. Of note, phytocannabinoids not only bind to CBR, but also show potential actions on different kinds of receptors, such as peroxisome proliferator-activated receptors (PPAR), glycine receptors, and the transient receptor potential (TRP) cation channels. The CBD, unlike the tetrahydrocannabinol (THC), is a non-psychotropic cannabinoid that has been widely investigated regarding its potential therapeutic use. It has been already established in the literature that CBD shows anti-inflammatory, anti-epileptic, analgesic, anxiolytic, and neuroprotective properties, as well as it can be used to mitigate Parkinson's disease (PD) symptoms [67–69]—Table 2. CBD acts as a negative allosteric modulator of CB1R [65] and as an inverse agonist in CB2R, besides being a FAAH enzyme inhibitor.

To briefly highlight, many other phytocannabinoids (e.g., cannabigerol, cannabichromene, and cannabinol) showed significant therapeutic value. The cannabigerol (CBG) showed agonist and antagonist activity on TRP channels and it was also able to produce 5-HT_1 and CB1R antagonism [70]. Additionally, CBG is an AEA reuptake inhibitor [71], and it showed colon anti-tumor activity by

inhibiting transient receptor potential melastatin 8 (TRPM8) channels [72]. Relevantly, when associated with CBD, it demonstrated anti-inflammatory activity reducing tumor necrosis factor (TNF) expression and upregulating Interleukin–10 (IL-10) and Interleukin–37 (IL-37) levels [70]. Cannabichromene (CBC) showed agonist activity on CB2R [73]. Besides, it interacts with TRP channels, being suggested as a potential therapeutic resource for the treatment of pain and inflammation [71]. Lastly, CBN showed similar therapeutic properties to other phytocannabinoids, such as anticonvulsant, anti-inflammatory, and antibacterial [71]. In addition, CBN showed inhibitory activity on cyclooxygenase (COX), lipoxygenase (LOX), and P450 cytochrome enzymes [71], as well as on keratinocyte proliferation, supporting a possible potential therapeutic for psoriasis cases [74]. As it can be appreciated with the major phytocannabinoids, the wide ranges of possible interactions of these molecules with multiple targets in our body, demonstrates the magnitude and the complexity of phytocannabinoids acting in living organisms.

We just established that phytocannabinoids demonstrate different pharmacological effects, and it can get even more intriguing and complex when we focus on previous data describing that the combined use of some phytocannabinoids can possibly increase the positive effects proportionate by them. For instance, the use of CBD associated with Δ9-THC promoted downregulation of the neuroinflammatory process in animal models of multiple sclerosis (MS) [75], besides, reducing pain [76] and muscle spasticity in MS patients [75]. Importantly, CBD attenuated the psychotropic effects of THC when used in a combined form [75]. This last piece of data supports the hypothesis that CBD binds to an allosteric site on CB1R that is functionally distinct from the active site for 2-AG and THC [77]. In this same context, a recent study reported that a botanical drug preparation (BDP) was more potent than pure THC to produce antitumor responses in cell culture and animal models of breast cancer. While pure THC mainly activated CB2R and generated reactive oxygen species (ROS), the BDP modulated different targets and mechanisms of action [78]. This combined effect, observed with the association of phytocannabinoids and other compounds present in the *Cannabis sativa* plant, such as terpenoids, is known as the entourage effect [79] (Figure 2).

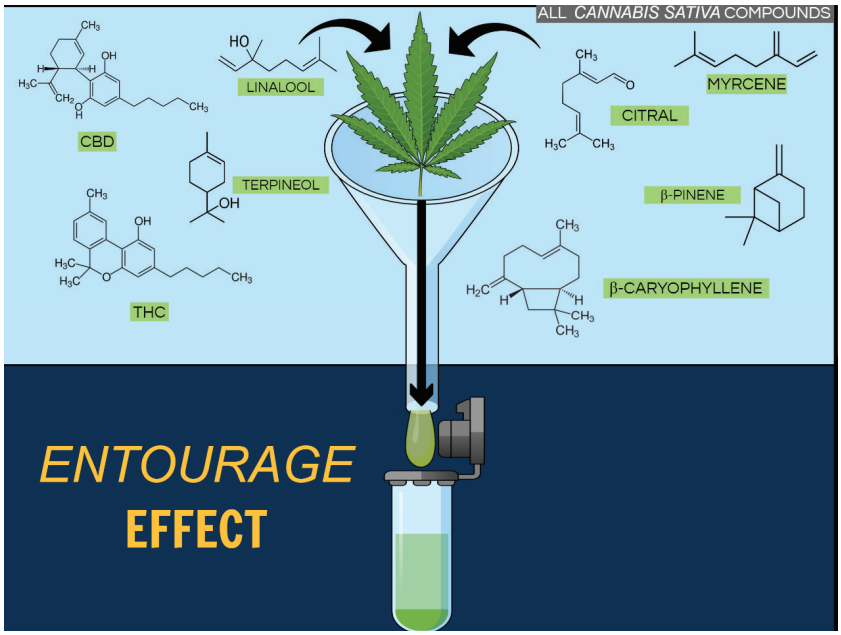

Figure 2. Entourage effect. Beyond the Δ9- tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD), there are many compounds present in *Cannabis sativa*, including terpenoids (such as linalool, terpineol, and citral), which could contribute to beneficial effects related to this plant. However, the underlying mechanism of these medicinal effects is largely unknown when molecules are associated. Figure created using the Mind the Graph platform.

Cannabis Terpenoids

Beyond the phytocannabinoids, the *Cannabis* plant is able to produce a diversity of compounds. Thirty-one-years ago, Mechoulam and Ben-Shabat described what they named the ''entourage effect'', suggesting interactions between *Cannabis* "inactive" metabolites and closely related molecules could markedly increase the activity of the "primary" cannabinoids (Figure 2). From this, it was possible to hypothesize that could be a contribution of "minor cannabinoids" and *Cannabis* terpenoids to the plant overall pharmacological effect. Therefore, a recent study evaluated the effect of common terpenoids, by themselves and in combination with THC, in AtT20 cells expressing CB1R or CB2R. Surprisingly, none of the analyzed terpenoids modulated the THC phytocannabinoid agonist signaling. Thus, the authors suggested that if the phytocannabinoids–terpenoids entourage effect exists, it is not at the CB1R or CB2R receptor level [80]. Corroborating, when rats were submitted to an abdominal writhing model and treated only with terpenoids they demonstrated increased abdominal writhing, while the animals treated with THC showed robust analgesia, even better than the rats that received the *Cannabis* full extract. In this case, *Cannabis* antinociceptive property was linked to Δ9-THC, since terpenes alone do not alter the nociceptive behavior [81]. Using a different approach, Nandal and co-authors exposed cancerous cell lines to treatment with phytocannabinoids combined with low concentrations of co-related terpenoids. They observed increased cell mortality at ratios similar to the ones obtained with the natural plant extracts [82]. According to the authors, their results differed from Santiago et al. findings because they evaluated terpenoids without statistical correlation to THC, meaning that terpenoids concentrations in their preparations where higher than the natural-occurred in the plants [80,82]. Thus, the possible "entourage effect" and the positive contribution derived from the addition of terpenoids to cannabinoids could be interpreted as uncertain. However, the study of terpenoids represents an open window that goes beyond its actions *(i)* in the endocannabinoid system solely, or *(ii)* as mere phytocannabinoids passive co-authors, and even beyond the *Cannabis* plant.

3. Terpenoids in and beyond the *Cannabis* Plant

Cannabis contains a large number of monoterpene and sesquiterpene compounds, together called terpenoids or terpenes, which are aromatic compounds synthesized in trichomes [71]. In the plant, these compounds (i.e., more than 120 terpenes) synthesized alongside phytocannabinoids are important volatile constituents that are responsible for the plant's characteristic smell and also serve for different organic functions, such as insect repellent, repellent to herbivore attack, and attractive to pollinators [71]. Booth and Bohlmann described the terpenes- and cannabinoid-rich resin as the most valuable cannabis products, with different psychoactive and medicinal properties [83]. Studies regarding terpenoid compounds (i.e., D-limonene, β-myrcene, α-pinene, α-terpineol, β-pinene, β-caryophyllene, and others) have been growing in the last decades due to their large number and extensive employability [71,84]. However, the presence of terpenoids has not been restricted to the *Cannabis sativa* plant. These compounds normally occur in several other plant species, such as *Mirabilis jalapa*, *Lithophragm glabrum*, *Cordia verbenacea*, *Eucalyptus globus*, *Syzygium aromaticum*, *Senna didymobotrya*, *Cymbopogon citratus*, and in some *Citrus* genus plants, as *Citrus limon* and others. To date, there are more than 10,000 articles versing about phytocannabinoids or cannabimimetics, and its actions described in the literature. There are many *Cannabis* terpenoid compounds that are not majorly found in the *Cannabis* plant but are highly expressed in other plants. Its actions are varied and complex, being many compounds studied deep down to the mechanisms of action, pharmacokinetics, toxicity, and pharmacodynamics, whereas others are still to be addressed regarding these aspects. The study about terpenoids beyond the *Cannabis* plant has been earning ground in the research field due to the fact that they can be utilized as tools for the improvement of therapeutic research for several diseases. Herein, we can have a sense of how literature stands at this end regarding some of these compounds, and we discuss the role of terpenoids as prospective therapeutics of different pathological conditions.

3.1. Beta (β)- and α-Caryophyllene

Beta and alpha-Caryophyllene are the major sesquiterpenes encountered in the *Cannabis* plant [85]. Importantly, a comparative study showed that regardless the type of extraction used supercritical fluid extraction, steam distillation, or hydrodistillation, the major sesquiterpene compound to be extracted was β-Caryophyllene (BCP) [86]. Caryophyllenes are considered phytocannabinoids with strong affinity to CB2R but not CB1R [87], and are produced not only by *Cannabis* but also by a number of plants, as a mechanism of defense to insects, for instance. The vast literature describes a number of plants that contain this compounds such as *Cordia verbenacea*, *Pterodon emarginatus*, *Artemisia campestris*, *Lantana camara*, *Centella asiatica*, *Cyanthillium cinereum*, and *Croton bonplandianus*, just to name a few of the more than 30 species previously described. Heretofore published original articles described seven main actions to caryophyllenes. These actions are reported to be repellent, antimicrobial or antibacterial, anticancer or antiproliferative, antifungal, AChE inhibitor, antioxidant, and anti-inflammatory. Regarding the antifungal and antimicrobial action, Sabulal and co-workers showed that *Zingiber nimmonii* rhizome oil, which is a unique isomeric caryophyllene-rich natural source, has inhibitory activity against fungi (e.g., *Candida glabrata*, *Candida albicans*, and *Aspergillus niger*) as well as against both *Bacillus subtilis* and *Pseudomonas aeruginosa* bacteria [88]. More recently, a study has shown that *Phoebe formosana* leaf extract has antifungal activity as well; BCP being one of the active compounds identified [89]. In this same study, authors have reported that the oil exhibited cytotoxic activity against human lung, liver, and oral cancer cells while the major active compound was BCP. Corroborating, BCP was the major compound found in the tree bark essential oil from *Pinus eldarica*, which showed antiproliferative activity in a concentration dependent manner against MCF-7 breast cancer cell line [90]. Likewise, anticancer activity against MCF-7 cells was also reported for the essential oil of *Cyperus longus* mainly constituted of β- and α- caryophyllenes [91]. Regarding analgesic effects, BCP has been demonstrated to attenuate paclitaxel (PTX)-induced peripheral neuropathy in mice by a mechanism dependent on mitogen-activated protein kinase (MAPK) inhibition [92]. Recently, a review has summarized, very well, the anticancer and analgesic properties of this compound [87].

The anti-inflammatory properties of BCP have been extensively shown in different mouse models of disease. Bento and co-workers have demonstrated the beneficial effect of BCP treatment in an inflammatory bowel disease mouse model, in which BCP oral treatment mitigated TNF and Interleukin-1β (IL-1β) expression, reduced colon damage, and ameliorated disease score. To a mechanistic level, they showed these effects were at some degree dependent on peroxisome proliferator-activated receptor gamma (PPAR-γ) and CB2R activation [93]. In a very interesting study, Gertsch and co-workers reported that BCP selectively binds to CB2R acting as a full agonist, highlighting its potential therapeutic effects for inflammatory and painful states [94]. In an experimental autoimmune encephalomyelitis (EAE) mouse model, Alberti and co-workers have reported anti-inflammatory actions (i.e., reduced microglial activation and inducible nitric oxide synthase (iNOS) expression) of *Pterodon emarginatus* essential oil that is mainly enriched with BCP. Anti-inflammatory actions, in this case, contributed to attenuate neurological score and disease progression, being dependent on the control of T helper 1 (Th1) and Treg activity [95]. Later, the same authors demonstrated the effect of BCP in the experimental model of multiple sclerosis [96]. In fact, BCP extracted from *Cordia verbenacea* essential oil induced a markedly anti-inflammatory effect in panoply models in rats involving the attenuation of the abovementioned inflammatory molecules iNOS, TNF, and IL-2, as well as prostaglandin E2 (PGE2), and COX-2 [97]. Likewise, through anti-inflammatory pathways, BCP demonstrated a neuroprotective effect in a rat model of PD [98]. These are few very important examples of the beneficial and useful properties of caryophyllene. We agree with Sut and co-workers' point-of-view that some of the considered old molecules, as sesquiterpenes, could possibly play an important role in drug discovery towards new discoveries [99].

3.2. D-Limonene

Limonene, (4R)-1-methyl-4-prop-1-en-2-ylcyclohexene, is the most common monoterpene found in nature; for instance, in *Cannabis sativa* oilseed hemp named *Finola* and also in citrus oils, from orange, lemon, and tangerine [84]. Despite being found in *Cannabis sativa*, limonene does not interact with CB1R or CB2R [100]. Interestingly, D-limonene absorption and metabolism in animals is accelerated, and consequently it has a high rate of distribution and excretion. D-limonene metabolites have been detected in adipose tissue and mammary glands in a high concentration, although it has low toxicity [101]. This compound shows different pharmacological properties, which include anti-inflammatory, gastro-protective, anti-nociceptive, anti-tumor, and neuroprotective [102–104]. A recent study has demonstrated D-limonene anti-tumor activity (i.e., tumor cells decreased in proliferation and growth) in an animal model of chronic myeloid leukemia [102]. Moreover, D-limonene also showed anti-inflammatory activity by inhibiting pro-inflammatory mediators, leukocyte migration, and vascular permeability [105]. Regarding its activity on the gastrointestinal tract, there are different articles described in the literature. For instance, the same group described a gastric protection effect in rats with colon inflammation [103], and in an animal model of an ulcer induced by ethanol and indomethacin [106]. In addition, D-limonene-induced mucus production and IL-6, IL-1β, and TNF inhibition has been previously described [107]. Corroborating this data, Wang and colleagues demonstrated that limonene affected the intestinal microbiota of mice and enhanced the relative abundance of Lactobacillus, suggesting limonene direct effects on intestinal bacteria [108].

Limonene also inhibited nociceptive behavior induced by intraperitoneal acetic acid injection and plantar formalin [109]. In a complementary way, combined administration of limonene and β-ciclodextrin inhibited hyperalgesia in a chronic musculoskeletal pain model by downregulation c-FOS expression in the spinal cord [84]. Reinforcing this information, treatment with *Schinus terebinthifolius* essential oil—which is highly-concentrated in limonene—showed anti-hyperalgesic and anti-depressive effects in a neuropathic pain animal model [110]. At a different point-of-view, Smeriglio and colleagues reported the antioxidant and free radical scavenging properties of *Citrus lumia* oil, which is highly-concentrated in monoterpenes (e.g., 48.9% D-limonene and 18.2% linalool), suggesting an important preventive role in the genesis of oxidative stress-related pathologies [111]. In this context, a study conducted by Shin et al. showed that limonene decreased cell death, ROS levels, extracellular signal-regulated kinase phosphorylation, and overall inflammation in the brains and eyes of drosophila during Aβ42-induced neurotoxicity, a model of Alzheimer's disease (AD) [104]. These and other authors have been studying limonene effects in the context of its impacts in the CNS. For instance, limonene has shown to exhibit anxiolytic effect increasing hippocampal dopamine levels and serotonin in the prefrontal cortex [75]. Considering the information above exposed, this is just one of the many compounds to be still addressed in this review that are natural and abundant in different plants, which could be used as potential therapeutics for diseases dependent on the inflammatory and oxidative-stress processes.

3.3. Linalool

Similar to limonene, linalool, 3,7-dimethylocta-1,6-dien-3-ol, is a monoterpene compound present in several medicinal plants and fruits, including *Cannabis sativa*, which has been widely used in the cosmetics and flavoring ingredients [112]. Linalool showed anti-inflammatory, anti-cancer, and anxiolytic effects [113–115]. The use of aromatherapy for the treatment of anxiety is disseminated among folk medicine. Accordingly, a study showed that linalool induced anxiolytic effects in mice by modulating GABAergic synaptic transmission [115]. Similarly to others terpenes, linalool showed anti-inflammatory activity, it prevented eosinophil migration, Th2-cytokines profile, and IgE concentration, in an asthma animal model. In addition, linalool inhibited iNOS expression, NF-κB (Nuclear factor kappa B) activation, inflammatory cells infiltration, and mucus hyper production during asthma progression [113]. Inflammation as well as oxidative stress are processes closely related to the progression of different CNS diseases, such as AD. In this context, a recent study demonstrated that

linalool decreased ROS and lipid peroxidation levels, as well as improved mitochondrial morphology, membrane potential, and respiration, directly reducing the cell death rate due to oxidative stress [114]. Additionally, linalool showed neuroprotective effects on Aβ1–40-induced cognitive impairment in mice, which it was suggested to be mediated by inhibition of apoptosis and oxidative stress induced by Aβ-dependent Nrf2/HO-1 pathway activation [116].

Regarding to its potential anti-tumor activity, linalool induced apoptosis of cancer cells in vitro following the cancer-specific induction of oxidative stress, which was measured based on spontaneous hydroxyl radical production and delayed lipid peroxidation. Besides, mice in the high-dose linalool group exhibited a 55% reduction in average xenograft tumor weight compared to the control group [117]. Linalool has also reported to be protective against ultraviolet B (UVB)-induced tumor through inhibition of inflammation and angiogenesis signaling, as well as induction of apoptosis in the mouse skin [118]. Finally, a study showed that linalool reduced paclitaxel-induced acute pain in mice, which was antagonized by the direct injection of naloxone hydrochloride, suggesting opioid signaling modulation [119]. What can be appreciated so far, and will continue to be addressed, is the general ability of different terpenes to modulate inflammation and oxidative stress through different pathways, which in turn could be very useful to shed light to novel treatments for pain, cancer, autoimmune diseases, and CNS diseases that rely greatly on the impact of these processes.

3.4. Terpineol

Terpineol (2-(4-methylcyclohex-3-en-1-yl)propan-2-ol) is a volatile monoterpene alcohol present in the essential oil of *Cannabis sativa* [120], but also in several medicinal plants, such as *Punica granatum L.*, *Rosmarinus officinalis L.*, and *Psidium guajava L.* Until this moment, there is no evidence in the literature about the interaction of terpineol with CBR. Nonetheless, this compound shows different pharmacological properties that include antinociceptive [121], antifungal [122], anti-inflammatory [123], and antidiarrheal [124]. Likewise, terpineol analgesic activity has been investigated in different animal models of pain. In this context, Oliveira and colleagues evaluated the effect of terpineol combined to β-cyclodextrin (βCD) (family of cyclic oligosaccharides with a wide variety of practical applications, including pharmacy, medicine, and foods) in an animal model of fibromyalgia. According to the authors, α-terpineol-βCD complex reduced nociceptive behavior induced by a chronic muscle pain model [121]. Still, this effect was mediated by activation of descending inhibitory pain system, since analgesic effect was reversed by systemic administration of naloxone (opioid antagonist), or ondansetron (5-HT3 antagonist) [121]. Additionally, terpineol has also been demonstrated to be a safe and effective drug for control of sarcoma-induced cancer pain in mice [125]. In a complementary way, terpineol could be investigated as preventive treatment for the development of dependence and of tolerance to opioid analgesics, since it attenuated the analgesic effect of morphine [126]. Thus, it is possible to suggest that terpineol alone, or combined to other drugs, could be an interesting target for development of new analgesics to control chronic pain symptoms. Besides, it could work as adjunctive therapy to morphine in order to reduce side effects related to treatment with opioid drugs.

Terpineol showed not only antinociceptive but also neuroprotective properties, since improved memory impairment in rats exposed to transient bilateral common carotid artery occlusion. The underlying mechanisms described comprise the facilitation of LTP and suppression of lipid peroxidation, in the hippocampus [127]. In accordance, *Abies koreana* essential oil (terpenoids-rich oil, including terpineol) enhanced memory of mice submitted to scopolamine-induced amnesia [128]. Regarding it anti-inflammatory properties, terpineol has also been investigated for the treatment of allergic inflammation and asthma because decreased leucocyte migration and TNF levels. Furthermore, terpinen-4-ol and α-terpineol were found to suppress the production of inflammatory mediators (e.g., NF-κB, p38, ERK, and MAPK signaling pathways) in lipopolysaccharide (LPS)-stimulated human macrophages [129]. Altogether, data supports that terpineol should be better investigated in order to characterize its neuroprotective effects found in cerebral ischemia-related memory impairment and

possibly be extended to other neurological conditions, such as seizures, migraine, Parkinson's disease, as well as to clarify its anti-inflammatory potential.

Terpineol properties go beyond, it has previously been shown antifungal properties against *Penicillium digitatum* because it disrupts fungi cell wall allowing the leakage of intracellular components [130]. In agreement with this, tea tree oil's antibacterial and antifungal properties were attributed mainly to 1,8-cineol, methyl eugenol, and terpinen-4-ol [131]. Recently, Chaudhari and co-authors reported the efficacy of α-terpineol loaded chitosan nanoemulsion (α-TCsNe) to control AFB1, a secondary metabolite produced by *Aspergillus flavus* and *Aspergillus parasiticus* fungi [122]. Included in miscellaneous actions, in addition to bactericidal and antifungal activities, terpineol has been recognized as algaecide [132] and by its natural repellent activity against *Tribolium castaneum* (H.) [133]. Finally, this monoterpenoid exhibited strong anti-proliferative activity on cancer cell lines [134], as well as it inhibited growth of tumor cells trough modulation of NF-κB signaling pathway [135]. Thus, it is possible hypothesize that terpineol as a versatile compound with a wide variety of beneficial effects could be a possible venue for the development of new antibiotics, antifungal, and anticancer agents.

3.5. Terpinene

Gamma-terpinene, 1-methyl-4-propan-2-ylcyclohexa-1,4-diene, is a monoterpene structurally similar to 1.8-cineol, being both found in the essential oils of *Cannabis sativa* and several other plants including the *Eucalyptus genus* (Myrtaceae), *Cupressus cashmeriana*, *Lippia microphylla*, *Lavandula angustifolia*, and *Citrus myrtifolia* [136–141]. Gamma-terpinene is very well described in the literature as an anti-inflammatory, antimicrobial, analgesic, and anticancer agent [136,137,142–144]. A recent study demonstrated that γ-terpinene reduced some inflammatory parameters, such as edema and inflammatory cell infiltration during tests in experimental models of inflammation, namely phlogistic agent-induced paw edema, acetic acid-induced microvascular permeability, carrageenan-induced peritonitis, and lipopolysaccharide-induced acute lung injury [145]. In addition, another study assessed the effect of γ-terpinene on pro- and anti-inflammatory macrophage production of cytokines in an animal model. The authors reported that γ-terpinene significantly increased the production of IL-10, which was dependent on PGE2 production since effects were reversed by COX-2 inhibitor nimesulide [146].

Besides the anti-inflammatory action, Assmann and colleagues described the anti-tumor activity and some of the possible underlying mechanisms of the *Melaleuca alternifolia* essential oil, which is composed of three major compounds terpinen-4-ol (41.98%), γ-terpinene (20.15%), and α-terpinene (9.85%), on MCF-7 breast cancer cells [147,148]. Authors reported γ-terpinene potential cytotoxic activity by decreasing breast cancer cells viability. Effects were observed in the early stages of apoptosis, such as increased BAX/BCL-2 genes ratio and increased cell arresting to S phase of the cycle [148]. Antimicrobial activity has been tested as well; *Melaleuca* spp. plants demonstrated effects against a wide range of gram-positive and gram-negative bacteria, fungi, and yeasts. Impressively, *Melaleuca thymifolia* volatile oil exhibits higher antimicrobial activity than *gentamicin* and *streptomycin* against *Staphylococcus aureus* [131]. Considering the exposed, it is feasible to suggest that γ-terpinene could server as natural immunomodulatory agent with antioxidant, antimicrobial, and anticancer properties that could be useful therapeutically.

3.6. Alpha (α)- and β-Pinene

Alpha-pinene is considered a natural compound present not only in *Cannabis sativa* but also in essential oils of many aromatic plants, such as *Lavender angustifolia*, *Rosmarinus officinalis*, and coniferous trees [149]. Alpha-pinene is a bicyclo[3.1.1]hept-2-ene that contains a reactive 4-membered ring structure and exhibits antioxidant, antimicrobial, anti-tumor, hypnotic, and anxiolytic activities [83,120,150–152]. There are different biological properties described to α-pinene, as well as essential oils containing this compound have been used to treat several diseases [153], although no affinity towards CBRs

have been described [154]. Alpha-pinene has been extensively investigated in the last years for its medicinal properties that include sedative, hypnotic, and anxiolytic [152,155]. In this context, Yang and colleagues demonstrated that α-pinene interacts with $GABA_A$/benzodiazepine receptors prolonging its synaptic transmission, significantly increasing the duration of non-rapid eye movement sleep (NREMS), and reducing sleep latency [151]. The beneficial effects of α-pinene are also extended to convulsions [80,81], ischemic stroke [82], and schizophrenia [156]. Besides, α-pinene also showed neuroprotective effects that might be related to its antioxidant properties, which include being able to decrease malondialdehyde and hydrogen peroxide levels while increasing catalase and peroxidase activity. A study has reported that rats exposed to pentylenetetrazol (PTZ)-induced convulsions submitted to α-pinene intraperitoneal (i.p.) administration presented both initiation time delayed and reduced duration of myoclonic and tonic-clonic seizures, following PTZ injection [81]. Another study suggested that α-pinene appears to be devoid of anticonvulsant action, since only β-pinene affected the intensity of seizures and time of death of PTZ-treated mice [80]. Further, it was suggested that α-pinene might serve as potential therapeutics for schizophrenia since it possibly suppresses neuronal activity. However, it has also been demonstrated that inhalation of α-pinene inhibits dizocilpine (MK-801)-induced schizophrenia-like behavioral abnormalities in mice [156]. Lastly, α-pinene mitigated learning and memory loss induced by scopolamine in mice. The underlying mechanisms reported were increased choline acetyltransferase messenger RNA (mRNA) expression in the cortex and increased antioxidant enzyme levels (e.g., HO-1 and manganese superoxide dismutase (MnSOD)) in the hippocampus through activation of Nrf2 [157].

Beyond neuroprotection, the cytoprotective and antinociceptive properties of α-pinene have been previously described. Regarding the former, studies were conducted using peptic ulcer, ultraviolet A radiation (UVA) irradiation, and aspirin-induced cytotoxicity models [158–160]. In details, α-pinene was able to prevent UVA-induced loss of mitochondrial membrane potential, lipid peroxidation, DNA damages, and ROS generation [158]. Likewise, α-pinene inhibited UVA-induced activation of pro-angiogenesis factors (e.g., iNOS and vascular endothelial growth factor (VEGF)), as well as blocked expression of inflammatory mediators (e.g., TNF, IL-6, and COX-2) and apoptotic mediators (e.g., Bax, Bcl-2, caspase-3, and caspase- 9) in mouse skin submitted to UVA-irradiation at the rate of 10 J/cm2/day, for 10 days [159]. In contrast, α-pinene promoted cytoxicity, and consequently cancer cells apoptosis by increasing activity of caspase-3 in human ovarian cancer cells (PA-1) [161]. In this sense, another study showed that α-pinene was also able to inhibit human hepatoma tumor progression by inducing G2/M phase cell cycle arrest [162]. Regarding α-pinene antinociceptive effects, it was previously demonstrated its beneficial potential in capsaicin-induced dental pulp nociception [163], xylene-induced ear edema, and formalin-inflamed hind paw models [164]. In this context, α-pinene exhibited significantly anti-inflammatory and analgesic effects through inhibition of COX-2. Moreover, the analgesic effect of α-pinene on capsaicin-induced pulp nociception was blocked by co-administration with bicuculline or naloxone, thus suggesting that this effect could be mediated, at least in part, by interaction with GABA-A and μ-opioid receptors [163].

Related to α-pinene, another important monoterpene present in different *Cannabis sativa L.* varieties is β-pinene, which can also be found in many plants essential oils and obtained commercially by distillation or by α-pinene conversion [165,166]. Literature describes β-pinene antimicrobial and antioxidant activity [167], as well as its derivatives have been associated to anticancer, anticoagulation, and antimalarial effects. Additionally, β-pinene showed repellent activity against *Tribolium castaneum*, which is a beetle species from the *Tenebrionidae* family that is also a powerful invertebrate system for molecular genetics studies. Looking for the mechanism by which β-pinene mediated this repellent activity; authors reported that exposition to this compound alters the gene expression, namely Grd (which encodes GABA receptor), Ace1 (which encodes class A acetylcholinesterase) and Hiscl2 (which encodes histamine-gated chloride channel subunit 2) [168]. However, according to Pajaro-Castro and colleagues, β-pinene showed little ability to dock on proteins associated with neurotransmission process in the *Tribolium castaneum* [168]. Even though the β-pinene-induced repellent effect still remains to be

fully addressed, it seems feasible to be considered that β-pinene monoterpene could act on different insect and mammalian receptors associated with neurotransmission. For instance, Guzmán-Gutiérrez and co-authors attributed to *Litsea glaucescens* essential oil (being β-pinene and linalool the two main active principles) antidepressant-like and sedative-like properties [169]. Posteriorly, the same group evaluated the mechanisms related to antidepressant effect of the essential oil compounds. In brief and focused on β-pinene, adult male ICR mice were pre-treated with (1*S*)-(−)-β-pinene (100 mg/kg) and exposed to forced swimming test (FST). Results showed that β-pinene, as well as imipramine (control drug), decreased the immobility time of mice when compared with control in the FST. Furthermore, administration of 5-HT_{1A} receptor antagonist prevented the antidepressant-like of β-pinene, demonstrating that this compound could interact with the serotonergic system. Likewise, β-pinene anti-immobility effects were also prevented by propranolol (β-receptor antagonist), neurotoxin DSP-4 (noradrenergic neurotoxin), and SCH23390 (a D1 receptor antagonist), suggesting its possible interactions with the adrenergic and dopaminergic system as well [170].

The use of β-pinene as an antitumor, as well as antiviral and antifungal agent has also been explored. Regarding the former, β-pinene-based thiazole derivatives were investigated as antineoplastic agents in vitro. Twenty-four β-pinene-based thiazole derivatives were synthesized and 5 g compound showed cytotoxic against three different cancer cell lines (Hela, CT-26, and SMMC-7721). Cytotoxic effect have been described to be mediated by action in the following signaling pathways: i) increased ROS activity, ii) loss of mitochondrial membrane potential, and iii) altered expression of Bax/Bcl-2, ultimately provoking cell injury and even cell death [171]. Concerning its antiviral and antifungal activity, it was shown its beneficial effects against *Rhizopus stolonifer* (the common bread mold) and *Absidia coerulea* fungi, as well as against herpes simplex virus type 1 (HSV-1), in vitro [172,173]. In fact, β-pinene reduced HSV-1 viral infectivity through interaction with free virus particles by 100% in a dose-dependent manner [174]. Similarly, β-pinene was able to reduce *Candida* biofilm adhesion through molecular interaction mainly with delta-14-sterol reductase–enzyme, which is related to metabolic pathway leading to cholesterol biosynthesis; thus, an effective target for antifungal drugs development [175,176]. Interestingly, when combined with commercial antimicrobial ciprofloxacin, both β-pinene and α-pinene demonstrated synergistic activity against methicillin-resistant *Staphylococcus aureus* [177]. Summarizing, here we describe, the antioxidant, anti-inflammatory, and immunomodulatory activity of both pinenes. Importantly, the neuromodulatory role that α-pinene and β-pinene are able to play could be used to shed light on innovative approaches to treat a variety of neurological conditions.

3.7. β-Elemene

β-elemene (1-methyl-1-vinyl-2,4-diisopropenyl-cyclohexane) is a derivative terpenoid found in *Cannabis sativa*, which may arise due to oxidation or due to thermal- or UV-induced rearrangements during processing or storage [85,178,179]. However, β-elemene is present not only in *Cannabis sativa* but also from *Curcuma rhizome,* and it is commonly used in traditional Chinese medicine due to its anticancer properties with no reported severe side effects [180]. In this way, this compound has been extensively studied as an anticancer agent in vitro and in vivo and has been demonstrated to be a promising drug for the treatment of a wide variety of tumors [181–186]. Among the challenges associated to cancer treatment, it is the development of multidrug resistance (MDR), which negatively impacts the effect of chemotherapy drugs, and consequently treatment success. It was previously proposed that one of the viable solutions to overcome MDR is to combine two chemotherapeutic drugs, acting synergistically to target multiple key pathways to inhibit tumor progression [187,188]. In this context, the combination of β-elemene with other chemotherapeutic agents (i.e., cisplatin and doxorubicin) and other therapeutic adjuvant has demonstrated great potential to inhibit tumor cells and tumor growth. According to Li and colleagues, β-elemene and cisplatin combined chemotherapy treatment is one of the most important approaches available for lung cancer therapy in China. Besides, the China Food and Drug Administration has approved it for the treatment of different tumors, such as brain, ovary, prostate, breast, lung, liver, and colon [189–191]. Additionally, when associated to hyperthermia β-elemene

significantly inhibited growth of adenocarcinoma human alveolar basal epithelial cells A549 cells in a dose-dependent manner, when compared to β-elemene treatment alone [182]. Mechanistically, the exposition of A549 cells to hyperthermia plus β-elemene significantly increased mRNA expression of cyclin-dependent kinase inhibitor p21 that ultimately induced cell apoptosis [182]. Another approach to try overcoming unsuccessful chemotherapy is the nanotechnology-based drug delivery system, which could improve pharmacokinetics of chemotherapeutic agents [192]. These carriers encompass a broad range of dispersion systems (i.e., polymeric micelles, liposomes, and dendrimers) that protect against drug degradation, promote sustained release, and reduce side effects [192]. Thus, different studies evaluated the therapeutic effects of β-elemene co-loaded with chemotherapy drugs: i) cisplatin in co-loaded liposomes [193]; ii) doxorubicin (DOX) in pH-sensitive nanostructured lipid carriers (DOX/β-elemene Hyd NLCs) [194]; iii) cabazitaxel in complex liposome [195]. In summary, these reports described that β-elemene co-loaded with lower doses of chemotherapy drugs was able to induce toxicity effects against tumor while retaining a similar therapeutic effect of the drug by itself, demonstrating synergistic effect of the compounds. Corroborating, β-elemene was also described as a radiosensitizer producing DNA damage and inhibition of DNA repair, as well as increased apoptosis. Beta-elemene was also able to inhibit the activation of the Prx1-NFκB-HIF-1α axis, a key regulator whereby tumor cells adapt to radiation therapy and hypoxia [196]. Beta-elemene was also shown to inhibited monocyte chemoattractant protein-1 (MCP-1) secretion, a macrophage recruitment chemokine that contributes to cancer cells metastasis [197]. Altogether, these reports demonstrate the possible mechanisms behind β-elemene anticancer activity and suggest different ways to incorporate this compound into current clinical therapies.

Besides the very promising anticancer activity, it has been reported in the literature a variety of other beneficial effects attributed to β-elemene. Li and co-authors, for instance, provided evidence of β-elemene beneficial effects for atherosclerosis treatment [198]. In this study, apoE homozygous deficient mice were fed a high-fat diet during four weeks followed by β-elemene (135 mg/kg) oral gavage administration for another 12 weeks. Beta-elemene treatment significantly reduced lipid areas of atherosclerotic plaques and aortic root lesion sizes and necrotic core, basically by boosting antioxidant enzymes while decreasing inflammatory cytokines levels. [198]. In a different study, β-elemene exerted retino-protective effect by downregulation of hypoxia-inducible factor–1alpha (HIF-1α), VEGF, iNOS, and pro-inflammatory mediators during diabetes progression in a streptozotocin (STZ)-induced rat model [199]. Finally, the potential application of β-elemene in an EAE animal model was tested, in which mice were treated from day one after induction with β-elemene (20 mg/kg, i.p.) until the end of experiment. Beta-elemene reduced IFN-γ and IL-17 levels and completely blocked EAE onset and the severity of clinical symptoms. Furthermore, β-elemene inhibited IL-17, IFN-γ, ROR-γT, and T-bet mRNA expression in the optic nerve of EAE mice [200]. If we start to appreciate the bigger picture, it is possible to note that as the other terpenes here described so far, β-elemene shows the ability to modulate essential biological functions, such as inflammation, oxidative stress, immunology response, cell division, as well as endothelial regulation. Beneficial properties of this compound have been studied to a mechanistically level highlighting it as a promising tool for the treatment of relevant diseases, but there are many venues that still remain to be explored.

3.8. β-Ocimene and Camphene

Beta-ocimene (3,7-dimethyl-1,3,6-octatriene) is acyclic monoterpene that serves as a chemical cue to attract natural enemies of phytophagous insect in several plant species, including *Cannabis sativa* [85]. Booth et al. demonstrated using the variety '*Finola*' of *Cannabis sativa* oilseeds that the most abundant monoterpenes found were myrcene, (+)-α-pinene, (−)-limonene, (+)-β-pinene, terpinolene, and (*E*)-β-ocimene [85]. Farré-Armengol and colleagues demonstrated that the emissions of β-ocimene in flowers follow marked temporal and spatial patterns of emission, which are typical from floral volatile organic compound (VOC) emissions that are involved in pollinator attraction [201]. Another study reported that a monoecious cultivar (*Futura 75*) and a dioecious one (*Finola*) of *Cannabis sativa*

tested in a mountain area in Alps, Italy (elevation: 1100 meters above sea level, during the growing season 2018) showed particular phytochemical behavior. For instance, inflorescences from *Finola* variety were characterized by higher concentrations of β-ocimene and α-terpinolene, while α- and β-pinene accompanied by extremely high β-myrcene were found as predominant in *Futura* variety indicating that geographical provenience should be considered for a specific medicinal use of *Cannabis sativa* [202]. Currently, at least three beneficial properties have been described in the literature for this compound, such as antitumor, antifungal, and anticonvulsant [203,204], but mechanisms underlying the biological activity of this compound remain poorly explored.

Camphene (2,2-dimethyl-3-methylidenebicyclo(2.2.1)heptane) is a cyclic monoterpene present in *Cannabis* inflorescence in low titer but abundant in the essential oil of *Thymus vulgaris* that showed some pharmacological activities, such as expectorant, spasmolytic, and antimicrobial [205]. Camphene showed fumigant and contact toxicity against *Liposcelis bostrychophila* and *Tribolium castaneum* insects. Furthermore, it presented moderate repellent effect to *T. castaneum* while showed attractant effect to *Liposcelis bostrychophila*, [206]. Extending these observations, Benelli et al. showed that camphene inhibited *Helicoverpa armigera* and *Spodoptera litura*—key polyphagous insects pest—with a lethal dose (LC50) of 10.64 and 6.28 μg/mL, respectively, confirming the promising potential as a botanical insecticide [207,208]. Altogether, these findings strongly support the use of camphene as an eco-friendly and effective insecticidal agent. More recently, Souza and co-authors evaluated the anti-*Mycobacterium tuberculosis* activity of 17 novel synthesized thiosemicarbazones derived from (−)-camphene, *in vitro*. Overall, the majority of the tested compounds exhibited significant inhibitory effects on the *Mycobacterium tuberculosis* growth, with minimal inhibitory concentrations (MIC) values ranged from 3.9 to > 250 μg/mL [209]. Although there are not as much reports about β-ocimene and camphene as was described to the other compounds here reviewed thus far, their repellent and/or insecticide activity seem to be promising.

3.9. Nerolidol

Nerolidol ((6E)-3,7,11-trimethyldodeca-1,6,10-trien-3-ol), also known as peruviol, is a noncyclic sesquiterpene alkene alcohol common to citrus peels, *Piper claussenianum*, *Baccharis dracunculifolia*, and *Cannabis* plant [210]. Previously, it was demonstrated its inhibitory effect on the growth of *Leishmania braziliensis* promastigotes. Importantly, ultra-structural observation of nerolidol-treated parasites by STM showed mitochondria morphological alterations in the, nuclear chromatin and flagellar pocket along with cell shrinkage. In this same study, authors demonstrated some nerolidol mechanisms of action that included loss of mitochondrial membrane potential, phosphatidylserine exposure, and DNA degradation [211]. These evidences have been further exploited and extended in a study showing that nerolidol also inhibited *Leishmania amazonensis* amastigotes and promastigotes (with IC50 values between 2.6 and 3.0 M), indicating substantial accumulation of nerolidol in the cell membrane [212]. What is also relevant to this topic are the findings demonstrating the antiparasitic activity of nerolidol in mice infected with adult stages of *Schistosoma mansoni*. Authors showed that nerolidol (100, 200, or 400 mg/kg oral route) inhibited worm burden and egg production, directly associated with tegumental damage, although nerolidol showed low efficacy in mice harboring juvenile schistosomes. [213]. Substantiating, Baldissera et al. reported that nerolidol-loaded nanospheres mitigated the *Trypanosoma evansi*-induced cytotoxic and genotoxic effects in the rodent brain tissue during infection by upregulating NO levels; thus, preventing DNA damage and cell death [210]. Such results strongly support that nerolidol (a food additive and safe molecule) is an effective antiparasitic agent and could potentially display anti-inflammatory properties.

Regarding its potential anti-inflammatory and/or immunomodulatory activity, there are a number of studies using different cell-based and rodent models, which here we summarize. A study has shown that nerolidol blocked LPS-induced acute kidney injury by inhibiting the TLR4/NF-κB signaling pathway. Specifically, nerolidol markedly prevented the rise of nitrogen and creatinine levels in LPS-treated rats, and also inhibited the increase of inflammatory mediators, like TNF, IL-1β, and

NF-κB in LPS-treated NRK-52E cells [214]. Further, de Souza et al. demonstrated that nerolidol nanoencapsulation improved its anti-inflammatory effect on zymosan-induced arthritis in mice. Importantly, under the conditions assessed the formulation did not demonstrated cytotoxicity in J774 cell line [215]. A study has also shown the immunomodulatory actions of trans-nerolidol on the efficacy of doxorubicin in breast cancer cells and in a breast tumor mouse model. The compound increased doxorubicin accumulation into MDA-MB-231 and MCF7 breast cancer cells while blocked cell migration ability, in vitro [216]. In addition, nerolidol demonstrated positive effects on cyclophosphamide (CYP)-induced neuroinflammation, oxidative stress, and cognitive impairment, as well as prevented structural abnormalities in the hippocampus and cortex regions of rodents [217]. The same authors also showed using in silico approach that nerolidol binds into Nrf2 pocket domain—a key nuclear factor that regulates the expression of antioxidant proteins [217], as previously addressed in this review. In summary, authors concluded that nerolidol could be a prospective therapeutic molecule that can mitigate CYP-induced neurotoxic signs through regulation of Nrf2 and NF-κB pathway [217], although further studies are needed to confirm this neuroprotective hypothesis. Lastly, cardioprotective effects have been suggested to this compound by the same research group. They previously evaluated nerolidol cardioprotective potential as an oral treatment against CYP-induced cardiotoxicity in mice. Nerolidol inhibited cardiac inflammation, oxidative stress, cardiac apoptosis, and cardiac fibrosis, as well as ultra-structural changes leading to cardiac dysfunction induced by cyclophosphamide [218]. Corroborating, Asaikumar et al. showed that nerolidol inhibited isoproterenol-induced myocardial damage in rats [219]. Here we reviewed the most described and better-explored activities of the nerolidol, which are antiparasitic, anti-inflammatory and/or immunomodulatory, and cardioprotective.

3.10. Euphol

Euphol is a tetracyclic triterpene usually extracted in alcoholic preparations due to its chemical structure and therefore affinity for this solvent. Even though it is not a major compound of the *Cannabis* plant, one could find a few chemically structure similarities in between the euphol molecule and a couple of cannabinoids derivate, such as CBD and CBN [220]. In fact, euphol is the major compound found in different plant species from the Euphorbiaceae family [221], including *Euphorbia resinifera, Euphorbia nerifolia, Euphorbia bivonae, Euphorbia umbellata,* and *Euphorbia tirucalli.* Regarding the latest cited *Euphorbia tirucalli*, it is a common plant found in Brazil and by far the most studied species from the Euphorbia family in concern to its major compound: euphol. Studies on euphol chemical structure using x-ray crystallographic, Fourier transform-ion cyclotron resonance mass spectrometry, tandem mass spectrometry, and gas chromatography coupled mass spectrometry, as well as its quantitative determination in the rat plasma by liquid chromatography-tandem mass spectrometry allowed a better understanding of this compound chemical and biological behavior [222–224]. Importantly, ethnopharmacology evidences have lead and contributed to studies on the anticancer and anti-inflammatory effects of this triterpene compound, as by many years the plants from this family have been used as folk phytomedicine to treat tumors and inflammation states [221]. Although, limited studies on antiviral, antiparasitic [225,226], antimicrobial, and antifungal activities of euphol have been recently reported. In our point, the most interesting aspect of a recent study is the finding that euphol can modulate the immune system by inducing cytokine production, namely IL-4, IL-3, and IL-2; thereby, influencing the Th1/Th2 balance [227]. These results could help to explain and support many of the previous described actions of euphol as an anti-inflammatory compound that will be discussed later. That being established, the two most described activities of this compound are the antitumor and the anti-inflammatory. The former is the primary and the most reported activity in the literature, being described for different *Euphorbia* species as well as cancer cell types while the latter is more recent; however, better studied in terms of mechanism of action. For instance, *Euphorbia tirucalli*-derived euphol beneficial effects against many cancer cell lines was previously tested and described. These cell lines included tumor cells from breast, head and neck, colon, glioma, prostate, epidermis, lung, bladder, melanoma, esophagus, ovary, and pancreas. Euphol cytotoxicity effect was observed against

all cancer cell lines being very pronounced in this last cited, in which inhibited proliferation, motility, and colony formation as well [228]. Likewise, *Euphorbia umbellata*-derived euphol exhibited cytotoxic effects against K-562 leukemia cell line; being suggested that the main mechanism of action was apoptosis induction [229]. Other mechanisms of action proposed to euphol cytotoxic activity against breast and glioblastoma tumor cell lines included CDK2 downregulation whilst upregulates p21- and p27-CDK inhibitors and autophagy induction/facilitation, respectively [230,231]. Despite of its beneficial anticancer effect, very recently a study has suggested that euphol, along with sitosterol and lupeol, could cause hepatotoxicity by inducing significant increase in alanine aminotransferase, aspartate aminotransferase, and total bilirubin levels in rats treated sub-chronically with *Euphorbia bivonae* extract [232]. That consists of one report showing potential toxic actions of this compound in one species while there are many other enlightening reports describing its safety and its beneficial use to treat inflammatory diseases. Reports from a group in the south of Brazil coordinated by Professor Calixto in the early 2010s have described many of this compound uses towards inflammatory diseases management, as well as possible mechanisms of action. The earliest report described its anti-inflammatory actions on a mouse model of colitis, in which this compound inhibited important inflammatory cytokine production in the colon tissue (e.g., IL-1β, MCP-1, TNF, and IL-6); besides, the inhibition of adhesion molecules (i.e., selectins and integrins) [233]. A second study reported that euphol also inhibits inflammatory mediators and lymphocyte function-associated antigen-1 (LFA-1) integrin in the CNS, as it did in the periphery. At this time, euphol blocked Th17 myelin-specific cell migration with an overall benefic effect of reducing the severity and development of EAE, a multiple sclerosis model [234]. Later, it was described its beneficial action in a skin-inflammation mouse model induced by 12-O-tetradecanoylphorbol-13-acetate (TPA), corroborating early 2000s findings described by a Japanese group, and further extending the understanding about euphol mechanisms of action by showing that it inhibits TPA-induced protein kinase C (PKC) isoforms [235,236]. Later, PKC inhibition was again implicated in mediating euphol anti-inflammatory effects, as well as CB1R and CB2R in mouse models of inflammatory (e.g., PGE2-, carrageenan-, and complete Freund's Adjuvant (CFA)-induced) and neuropathic (e.g., spared nerve injury (SNI)-, paclitaxel-, and B16F10 melanoma cells-induced hypersensitivity) pain [237,238]. Notably, cannabinoid-mediated anti-inflammatory actions involve suppression of inflammatory cytokines, MAPKs pathway activation, and modulation of TNF and NF-κB [220], all pathways in which euphol has been demonstrated to effective. Euphol has the potential to be a very attractive anti-inflammatory molecule that works through the cannabinoid system but evidence shows that it definitely can go beyond that.

3.11. Citral

Citral, (2E)-3,7-dimethylocta-2,6-dienal, is the main compound of essential oils that have been used mainly in popular medicine in eastern countries. It is the major compound extracted from *Cymbopogon citratus*, popularly known as lemongrass, but it can also be extracted from different plants including lemon myrtle and *Lindera citriodora* [239]. This essential oil has been used as ingredient in foods because of its lemon-like fragrance. However, citral has gained attention in the last years due to its antimicrobial properties against *Cronobacter sakazakii*, a foodborne pathogen clinically associated to neonatal infections such as meningitis, septicemia, and/or necrotizing enteritis [240,241]. Its reported antimicrobial activity also extends to *Staphylococcus aureus* [242], *Candida albicans* [243], *Enterobacter cloacae* [244], *Listeria monocytogenes* [245], *Aeromonas spp*. [246], and *Streptococcus pyogenes* [247]. In this context, Yang and colleagues recently demonstrated that when combined with cinnamaldehyde, citral changed cecal microbiota composition of non-vaccinated and vaccinated broiler chickens, reducing the incidence and severity of necrotic enteritis induced by coccidiosis [239]. This is in accordance with another finding, in which citral was able to affect mouse intestinal microbiota, enhancing the relative abundance of *Lactobacillus* [108]. From these evidences, it was possible suggest that citral could be an important molecule for development of new antibiotic and antifungal drugs, especially because until the moment there is no evidence of relevant toxicity and side effects related to its accumulation in tissues and

delayed excretion [248]. However, Sharma and co-authors have well highlighted that strategies are required to increase citral stability, which could facilitate its applications [249].

Citral has also been recognized by its anti-inflammatory actions in animal models of acute lung injury [250], carrageenan-induced paw edema and croton oil-induced ear edema [251], segmental glomerulosclerosis [252], pleurisy [253], and peritonitis [254]. In this context, citral inhibited LPS-induced myeloperoxidase (MPO) activity, TNF, COX-2, and IL-8 expression, as well as NF-κB activation via PPAR-γ [254,255]. In accordance, Shen and colleagues demonstrated that GW9662 PPAR-γ antagonist reversed the anti-inflammatory response mediated by citral. Additionally, citral showed antioxidant properties linked to inhibition of Nrf2 pathway early activation, oxidative stress, and apoptosis [252]. More recently, Gonçalves and colleagues demonstrated that citral immunomodulatory property appears to be related to its ability to modulate CB2R, TLR4 and TLR2/dectin-1, as well as signaling pathways downstream of CBR and TLRs activation, including ATP-dependent K+ channels [256]. The antioxidant activity of this compound was also shown when co-administrated with aspirin in rat small intestine epithelial cells, in which it regulated superoxide dismutase (SOD) and glutathione (GSH) enzymes, significantly decreasing the aspirin-induced cell death [257]. Importantly, a link between its antioxidant and antinociceptive activity has been shown in an animal model of rheumatoid arthritis. Citral has promoted a decrease in oxidative stress parameters and induced antinociceptive effects through serotonergic communication at spinal the spinal cord level [227]. In fact, the citral antinociceptive activity is among the broad variety of beneficial effects already contemplated in the literature. When combined to other analgesics as naproxen, citral increased their antinociceptive activity as well significantly inhibited naproxen-induced gastric injury [258]. However, citral showed high volatility, low solubility in water, and consequent low bioavailability, which could limit its use. One possible solution could be the combination of citral with β-cyclodextrin and hydroxypropyl-β-cyclodextrin, which in turn demonstrated antihyperalgesic and anti-inflammatory activity [253]. Here we could suggest that citral should be better investigated in order to identify its possible clinical application for the treatment of chronic pain conditions, such as peripheral neuropathy, fibromyalgia, complex regional pain syndrome (CRPS) and lumbar chronic pain.

Beyond, citral attracted scientists' attention towards its anticancer properties in a variety of cancer types, such as melanoma [259], colon cancer [260], and breast cancer [261]. Bayala and co-authors provided evidence about *Cymbopogon citratus and Cymbopogon gigantescus* essential oil cytotoxic activity, which have citral as its major component and significantly decreased prostate and glioblastoma cancer cell survival [262]. In addition, citral showed cytotoxic effect in non-tumoral HaCaT and tumoral A431 cells, inhibiting NO production even at the lowest concentration tested [263]. Regarding the possible mechanisms underlying its antiproliferative effects, it has been reported MARK4 and a Ser/Thr kinase inhibition. Of note, aberrant expression or dysregulation of these proteins are linked with cancer development, such as hepatocellular carcinoma, glioma, and metastatic breast carcinomas [264,265]. Other mechanisms also comprise apoptosis induction and downregulation of the aldehyde dehydrogenase activity—a reactive protein overexpressed during cancer progression and therapy resistance [266,267]. From this, it was previously suggested that citral could work as aldehyde dehydrogenase inhibitor, and consequently as adjuvant therapy for treatment of some types of cancer [268]. In order to improve citral solubility and delivery without enhancing toxic effects in vivo, Nordin and colleagues incorporated citral into a nanostructured lipid carrier (NLC) and evaluated its in vitro anti-cancer effects. Initially, they showed that NLC as a drug delivery system for citral has the potential to sustain drug release without inducing any toxicity [269]. Then, they showed that NLC-citral regulated apoptosis, cell cycle, and metastasis signaling, all key signaling pathways related to cancer development [261]. In addition, citral was pointed as a potential effective additive to chemotherapeutic treatment [270,271]. Thus, when combined with hyperthermia intraperitoneal chemotherapy (HIPEC) and pirarubicin for colorectal cancer, citral increased the HIPEC efficacy by enhancing chemo-drug penetration and consequently its intracellular concentration. Furthermore, it was described a safe alternative that decreased the chemo-drug dose necessary to induce antiproliferative effect reducing

possible side effects [271]. Still, this natural compound showed chemoprotective actions in hairless (HRS/J) mice exposed to UVB irradiation for 24 weeks, a model of skin carcinogenesis. Mechanisms involved in citral chemoprotective effect not surprisingly included oxidative stress and inflammatory cytokines inhibition and increased skin cell apoptosis [272]. It has been previously described that citral mediated antiproliferative effects through p53activation, ROS- and mitochondrial-mediated apoptosis, as well as by NO depletion and interference with cell proliferation-related signaling pathways [259,260]. Collectively, these set of data here gathered suggests that citral represents an important molecule for the management of different types of cancer and highlights the possibility of translational application as a novel treatment alone or in combination with other chemotherapeutic drugs.

3.12. Celastrol

Celastrol, 2R,4aS,6aR,6aS,14aS,14bR-10-hydroxy-2,4a,6a,6a,9,14a-hexamethyl-11-oxo-1,3,4,5,6,13,14, 14b-octahydropicene-2-carboxylic acid, is a pentacyclic triterpenoid isolated from *Tripterygium wilfordii* root extracts and used in traditional Chinese medicine for treatment of chronic diseases, including neurodegenerative disorders (e.g., amyotrophic lateral sclerosis, AD, and PD), type 2 diabetes, obesity, atherosclerosis, cancer, inflammatory and autoimmune diseases (e.g., systemic lupus erythematosus, multiple sclerosis, inflammatory bowel disease (IBD), psoriasis, and rheumatoid arthritis (RA) [273–275]. In fact, this natural compound has been cited in a wide variety of reports describing its antioxidant [276,277], and anti-inflammatory action [278,279] through inhibition of NF-κB signaling pathway [280]. In details, this last study demonstrated that celastrol significantly blocked COX-2 expression, IL-8 and ICAM-1, as well as IL-1β-induced PGE2 through inhibition of NF-κB in a Graves' ophthalmopathy model using orbital fibroblasts [281]. Here are a few more examples of this extent literature about celastrol anti-inflammatory effects. Kim and co-authors demonstrated that celastrol inhibited LPS-stimulated NO generation, PGE2, iNOS, and COX-2, in RAW264·7 cells. In this same study, authors have reported that celastrol inhibited LPS-induced inflammatory cytokines production and also protected mice from TPA-induced ear edema by inhibiting MPO activity and the production of inflammatory mediators [278]. In addition, celastrol inhibited CFA-induced arthritis rat model via modulation of *i)* inflammatory cytokines (i.e., IL-17, IL-6, and IFN-γ) in response to the disease-related antigens, *ii)* IL-6/IL-17-related transcription factor STAT3, *iii)* cyclic citrullinated- and Bhsp65-peptides directed antibodies, and *iv)* MMP-9 and phospho-ERK activity, supporting the use of celastrol as an adjunct (along with conventional drugs) or alternative approach for the RA treatment [279]. Aside from the anti-inflammatory effect, also relevant are the findings demonstrating celastrol antitumor activity in a variety of human tumor cell types. Data previously suggested that celastrol represents a promising agent for the management of human tumor cell lines, such as triple negative breast cancer [282], leukemia [283,284], carcinoma [285] and lung cancer [286]. In terms of mechanisms, a study based on pharmacological and biochemical approaches has shown that celastrol inhibited cell proliferation and induce apoptosis through JNK activation, AKT suppression, and anti-apoptotic proteins downregulation [287].

Celastrol potential beneficial effects on the CNS have also been previously reported. Kiaei et al. described that celastrol improved weight loss, motor performance, and delayed the onset of motor neuron degeneration in the G93A SOD1 transgenic amyotrophic lateral sclerosis (ALS) mouse model. Celastrol increased HSP70 while mitigated iNOS, TNF, cluster of differentiation 40 (CD40), and GFAP proteins expression in the lumbar spinal cord of G93A mice [288]. Celastrol effects on HSPs have also been reported to play a key neuroprotective role in defense against misfolded proteins and aggregation-prone proteins [289]. Speaking of protein aggregation, celastrol was reported to inhibit amyloid beta aggregation, the main toxin to be accounted for AD initiation and progression [276,281]. Based on these facts, we could suggest that celastrol might represent a useful molecule to treat neurodegenerative diseases with an inflammatory background. In spite of that, celastrol use is still limited by its low water solubility, reduced oral bioavailability, and side effects reducing its therapeutic

potential [290]. Different structure modifications or encapsulation solutions must be studied to overcome this problem.

3.13. Falcarinol

Falcarinol—(3R,9Z)-heptadeca-1,9-dien-4,6-diyn-3-ol)—also named panaxynol or carotatoxin is found in carrots, parsley, celery, and *Panax ginseng* [291]. This natural compound has been cited in a wide variety of reports describing its antineoplastic [292] and anti-inflammatory properties [293]. Besides, falcarinol has been also investigated as pharmacological tool for treatment of cardiovascular and metabolic diseases. Regarding the latter, it is know that serum high molecular weight (HMW) adiponectin values are inversely correlated with the presence of metabolic syndrome, and consequently linked to pathogenesis of insulin resistance, type 2 diabetes, and cardiovascular diseases [294]. In this sense, Takagi and colleagues demonstrated that falcarinol restored FoxO1 and increased C/EBPα levels (transcription factors that positively regulate adiponectin gene transcription), resulting in HMW adiponectin secretion by 3T3-L1 adipocytes treated with palmitic acid, an obesity model in vitro [295]. In addition, falcarinol also reduced endoplasmic reticulum (ER) stress, C/EBP homologous protein (CHOP) protein and ROS levels, as well as decreased inflammatory adipokine-induced MCP-1 [295]. Still in this scenario, the association of chronic inflammatory disorders and/or systemic diseases to microbiota dysbiosis has been gaining attention [296]. Importantly, a study previously showed that the beneficial effects of falcarinol and falcarindiol rely on its ability of changing the composition of low abundant gut-microbiota members. In this study, the ability of falcarinol to regulate microbiota was allied to its ability to reduce the incidence of neoplastic lesions [292]. In this cancer scenario, the mucosa-associated bacterial population as the fecal microbiota plays an important role in colon carcinogenesis, the second most commonly diagnosed cancer with high incidence, morbidity, and mortality [296,297]. That being established, Kobaek-Larson and co-authors have reported that daily diet supplementation with falcarinol and falcarindiol decreased the number of neoplastic lesions and polyps growth rate in the colon of azoxymethane-treated rats [298]. Recently, this same group demonstrated the chemopreventive effect of a special diet supplemented with falcarinol and falcarindiol on colorectal precancerous lesions in a dose-dependent manner; besides, this effect was mainly mediated by inhibition of NF-κB and its downstream inflammatory markers, especially COX-2 [299]. Anticarcinogenic properties of falcarinol were also demonstrated in cancer stem-like cells (CSCs), in which it played an essential role in tumor occurrence, evolution, metastasis, recurrence, and therapeutic resistance [300], as well as in non-small cell lung cancer (NSCLC) [301]. Essentially, falcarinol eliminated CSC population in NSCLC and abolished lung tumor formation in mice via HSP90 (a molecular chaperone of numerous oncoproteins) modulation [302]. Falcarinol anticancer activity also extends to leukemia [303], breast cancer [304], hepatocarcinoma [305], renal carcinoma [306], and glioma [307]. For instance, mechanisms pointed to explain its ability to induce cell cycle arrest, thus, its anticarcinogenic properties on human promyelocytic leukemia cell growth are PKCδ proteolytic cleavage, caspase-3 activation, and PARP degradation [303].

In a different context, falcarinol has been also reported to be a facilitator of type 1 hypersensitivity and atopic dermatitis [308]. On the other hand, Leonti and colleagues showed that falcarinol is not an allergen itself; however, it facilitates sensitization by other allergens, since it aggravated histamine-induced edema reactions in skin prick tests. In this study, similar effects were obtained with Rimonabant® (a CB1R inverse antagonist), implying that falcarinol-induced dermatitis could be related to CB1R antagonism in keratinocytes [291]. Despite that falcarinol has been related to allergic reactions, it has also been shown to induce anti-inflammatory responses in a couple of different models. Falcarinol promoted a reduced cell infiltration in a LPS-induced reduction in intestinal barrier context [293]. In addition, falcarinol was able to induce Nrf2-mediated resolution of inflamed macrophage-induced cardiomyocyte hypertrophy [309]. Collectively, data here presented provided information about falcarinol crucial positive effects on pathological conditions, such as metabolic diseases, cardiovascular

diseases, and cancer. However, we consider that for the development of possible therapeutic tools underlying mechanisms as well as toxicity, and bioavailability needs be better investigated.

3.14. Salvinorin A

The trans-neoclerodane diterpenoid salvinorin A is a short-acting highly-selective kappa opioid receptor agonist and consequently the primary psychoactive component of *Salvia divinorum* (psychoactive herb used in magic-ritual contexts by Mazateca Indians in Mexico) [310]. In agreement, eight healthy hallucinogen-using adults exposed to inhalation of 16 doses of *Salvia divinorum* showed dose-related dissociative effects and impairments in recall/recognition memory tests [311]. Given the fact that salvinorin A highly interacts with opioid receptors, it has been considered an emerging target for next-generation of analgesics. In addition, salvinorin A showed hallucinogen effects similarly to lysergic acid diethylamide (LSD) [312,313]. Walentiny and colleagues demonstrated that salvinorin A administration induced pronounced hypolocomotion and antinociception (and to a lesser extent, hypothermia) effects in the tetrad assay, which were reverted by the administration of kappa opioid receptor (KOR) selective antagonist but not by CB1R antagonist Rimonabant® [310]. Moreover, rats exposed to sciatic nerve ligature neuropathic pain model and treated with salvinorin A directly in the insular cortex showed antinociceptive behavior. However, in contrast with Walentiny and colleagues, the analgesic effect of salvinorin A in this case was reverted by selective KOR and CB1R antagonists [314]. In accordance with this finding, daily treatment with salvinorin A significantly decreased formalin-induced mechanical allodynia at days three and seven in a KOR and CB1R dependent manner, without inducing CB1R-related adverse effects. Electrophysiological experiments in vivo also showed that repeated salvinorin A treatment completely normalized neuronal activity following formalin injection, as well as it reduced formalin-evoked glial and microglial activation at the spinal cord level [315]. Nonetheless, unlike other opioid ligands, salvinorin A showed short duration of action and centrally mediated side-effects limiting its usefulness [316–318], justifying the development of new salvinorin A analogues [319]. In this context, novel analogue β-tetrahydropyran salvinorin B attenuated acute nociceptive and inflammatory pain, as well as mechanical and cold allodynia in the PTX-induced neuropathic pain model [319]. On the other hand, mesyl salvinorin B (a KOR agonist) showed moderated antinociceptive effect when compared to salvinorin A in warm-water (50 °C) tail withdrawal and intraplantar formaldehyde (2%) tests. However, it mitigated cocaine-induced hyperactivity and behavioral sensitization, without affecting aversion, sedation, anxiety, or learning and memory impairment in rats [320]. Additionally, mesyl salvinorin B alone or associated with naltrexone prevented alcohol-induced deprivation effect in mice [321], which could represent an alternative tool for treatment of alcoholism in humans. Other salvinorin A analogues, such as p38, could also be effective for the treatment of gastrointestinal inflammation, since it demonstrated anti-inflammatory and analgesic effects in an experimental model of colitis [322]. Thus, these findings support the use of novel salvinorin A-like compounds and its analogues as possible pharmacological alternatives for pain relief, control of cocaine-seeking behavior, and alcoholism, as it seems to have potent CNS and anti-inflammatory actions.

Regarding these actions, the anti-inflammatory effects associated with salvinorin A also extend to cerebral hypoxia/ischemia [323–326]. Salvinorin A attenuated brain edema and inhibited neuronal death in hippocampal CA1 region, cortex, and striatum during forebrain ischemia model [325]. According to Dong and colleagues, rats submitted to middle cerebral artery occlusion and treated with salvinorin A one hour after reperfusion showed improvement of neurological severity score when compared to control groups. Additionally, salvinorin A reduced infarct volume and effectively protected cerebral vessels after ischemia/reperfusion. Importantly, human brain microvascular endothelial cells exposed to the oxygen glucose deprivation model and treated with salvinorin A were protected against ROS damage and decreased mitochondrial function (i.e., mitochondrial morphological changes and loss of membrane potential). The latter, highly regulated by AMPK and phosphorylation mitofusin-2 expression, both upregulated in response to salvinorin A treatment [327]. Salvinorin A also mitigated

cerebral vasospasm through endothelial nitric oxide synthase (eNOS) and NO upregulation and ET-1 downregulation. At the same time, salvinorin A inhibited AQP4 protein expression—a member of a family of channel proteins that facilitate water transport and contribute to brain edema and neuro-disorders development [326,328]. Concerning still its actions in the CNS, salvinorin A effects on the mood were also investigated and linked to anxiolytic and antidepressant properties mediated by KOR, as well as the ECS [329]. In lieu of antidepressant properties, another study associated salvinorin A to depressive-like effects through dopamine signaling inhibition in the *nucleus accumbens* of rats [330]. Extending, dysphoria as well as depressant-like effects of salvinorin A were attributed to KOR-linked ERK activation, which in turn promoted dopamine transporter (DAT) phosphorylation, modulating dopamine neurotransmission [331]. Recently, Keasling and colleagues evaluated the effects of salvinolin, a new semisynthetic analog of salvinorin A, with mu opioid receptor affinity. In summary, salvinolin demonstrated good oral bioavailability and showed antidepressant-like effect that was blocked by the selective $5HT_{1A}$ antagonist WAY100635 [332]. Another derivative of salvinorin A, the 22-azido salvinorin A, also promoted an antidepressant-like effect linked to its ability of inhibiting monoamine oxidase (MAO) enzyme, as well as to its affinity for α1A, α1B, α1D adrenergic receptors beyond KOR [333]. Here, we could sense the staggering effects of salvinorin A and its analogues to modulate a variety of neurotransmission systems in the CNS.

The pharmacological effects of salvinorin A are not limited to CNS but also related to the respiratory system. Salvinorin A inhibited mast cell degranulation in the lung and consequently blocked airway hyperactivity induced by ovalbumin sensitization. Thus, the authors suggested that salvinorin A could represent a promising tool for the treatment of type 1 hypersensitivity and immune-mediated diseases [334]. Moreover, salvinorin A inhibited leukotriene production in inflammatory exudates, as well as it showed antipruritic effects mediated by KOR on compound 48/80-induced scratching behaviors in mice [335]. Findings here summarized provide evidence about the anti-inflammatory action of salvinorin A, and highlight this natural compound as a possible new tool for the treatment of inflammatory diseases.

3.15. Pristimerin

Pristimerin (20α-3-hydroxy-2-oxo-24-nor-friedela-1-10,3,5,7-tetraene-carboxylic acid-29-methyl ester) is a natural quinonoid triterpene isolated from the shrub families *Celastraceae* and *Hippocrateaceae*. It is a natural compound with cannabimimetic effects without direct interacting with CBR. For instance, pristimerin inhibited MAGL with high potency through a reversible mechanism [336]. It has been extensively investigated mainly by its inhibitory activity against cancer cell growth. Pristimerin inhibited Wnt/β-catenin signaling via GSK3β activation and Wnt gene suppression in colorectal cancer cells [337]. In addition, Yousef and colleagues demonstrated pristimerin anticancer activity on colon tumor cells associated to NF-κB signaling inhibition during the carcinogenic process [338,339]. Corroborating, this triterpenoid has also been shown to attenuated colitis-associated colon cancer by modulating NF-κB positive cells, as well as AKT/FOXO3a signaling pathway [340]. The transcription factor FOXO3 represents important target for cellular homeostasis, since it was able to regulate apoptosis, proliferation, cell cycle progression, and consequently tumorigenesis [341,342]. Pristimerin was also previously demonstrated to downregulate the PI3K/AKT/mTOR pathway playing a critical cytotoxic and anti-metastatic role in the progression of HCT-116 colorectal cancer cells in vitro and in vivo [343]. Finally, another study from Yousef and co-authors suggest that pristimerin downregulates phospho-EGF and -EGRF2 and its downstream signaling pathways, which represent a key mechanism involved in the proliferation of cancer malignant phenotypes [344,345].

The antiproliferative activity of pristimerin goes beyond colon-related cancers, it is extended to breast [346–350], melanomas [351], osteosarcoma [352], pancreatic [353,354], and prostate cancers [355–358]. Herein, we describe a few examples focusing on articles that have demonstrated potential mechanisms of action. Pristimerin anticancer activity against breast cancer cells was associated to ROS production and ASK1/JNK signaling pathway activation [346], as well as AKT

signaling suppression [349,359]. Additionally, when combined to paclitaxel, pristimerin induced cell autophagy through inhibition of ERK1/2/p90RSK signaling—involved in cancer cell proliferation, differentiation, and migration [347,360]. Pristimerin-induced glioma overgrowth was dependent on AGO2 upregulation (a critical protein for tumorigenesis) and PTPN1 downregulation (a metabolism regulator oncogene reported to be aberrantly expressed in cancer cells) [361–363]. Furthermore, pristimerin induced glioma cell necrosis by promoting mitochondrial dysfunction, c-Jun activation, and consequently ROS overproduction [364]. It also inhibited the epidermal growth factor receptor (EGFR) protein expression during glioma cancer development [365,366]. Antiproliferative effects of pristimerin were investigated in oral squamous cell carcinoma cell lines as well. In this way, pristimerin showed more potent antiproliferative activity than chemotherapy drugs cisplatin and 5-fluorouracil. This effect was associated with inhibition of MAPK1/2 and PKB signaling pathways [367]. Pristimerin-induced apoptosis activity was also demonstrated in ovarian cancer cells via inhibition of AKT/NF-κB/mTOR signaling pathway [368]. Besides, few articles have reported pristimerin beneficial effects on prostate cancer. Its progression was reported to be prevented by pristimerin-induced inhibition on HIF-1α and SPHK-1, which stimulates different cellular processes including cell proliferation, cell survival, and angiogenesis [355,369]. Pristimerin also induced apoptosis of prostate cancer cells through activation of mitochondrial apoptotic pathway [358], ubiquitin-proteasomal degradation [357], and inhibition of proteasomal chymotrypsin-like activity (a complex associated with cell proliferation, apoptosis, and cancer progression) [370,371]. These summarized findings provide evidences regarding pristimerin antiproliferative and cytotoxic activity as well as clinical benefits for treatment of different types of cancer.

Finally, yet importantly, Tong and co-authors showed that pristimerin inhibited arthritic and cartilage inflammation, as well as bone damage in the joints of rats submitted to adjuvant arthritis. Pristimerin inhibited inflammatory cytokines and pSTAT3 and ROR-γt transcription factors, as well as Th17/Treg ratio favoring immune suppression [372]. In addition, anti-inflammatory properties of pristimerin included inhibition of inflammatory cytokine levels (e.g., IL-6, IL-17, IL-18, and IL-23), increase IL-10 expression, and mitigate NF-κB and MAPK signaling, showed during rheumatoid arthritis model and murine macrophages exposed to LPS [373]. In this sense, pristimerin seems able to interact with essential targets of the inflammatory and/or immune-mediated processes; and for this reason, it should further investigated regarding its potential ability to serve as a treatment of disorders related to the imbalance in the immune system, including autoimmune diseases.

4. Conclusions

The reports here highlighted showed the complex and varied pharmacology of *Cannabis sativa*, particularly phytocannabinoids—typical terpenophenolic compounds—as well as plenty of non-cannabinoids second metabolites, such as monoterpene, sesquiterpene, and stilbenoids. Interestingly enough, there are an increasing number of studies on cannabimimetic ligands beyond the *Cannabis* plant, which can act as CBR agonists or antagonist, or ECS enzyme inhibitors. They are mainly terpenes including β-caryophyllene, D-limonene, terpineol, β-elemene, euphol, pristimerin, citral, and many others (Figure 3), which can play a key role in the modulation of different pathological conditions.

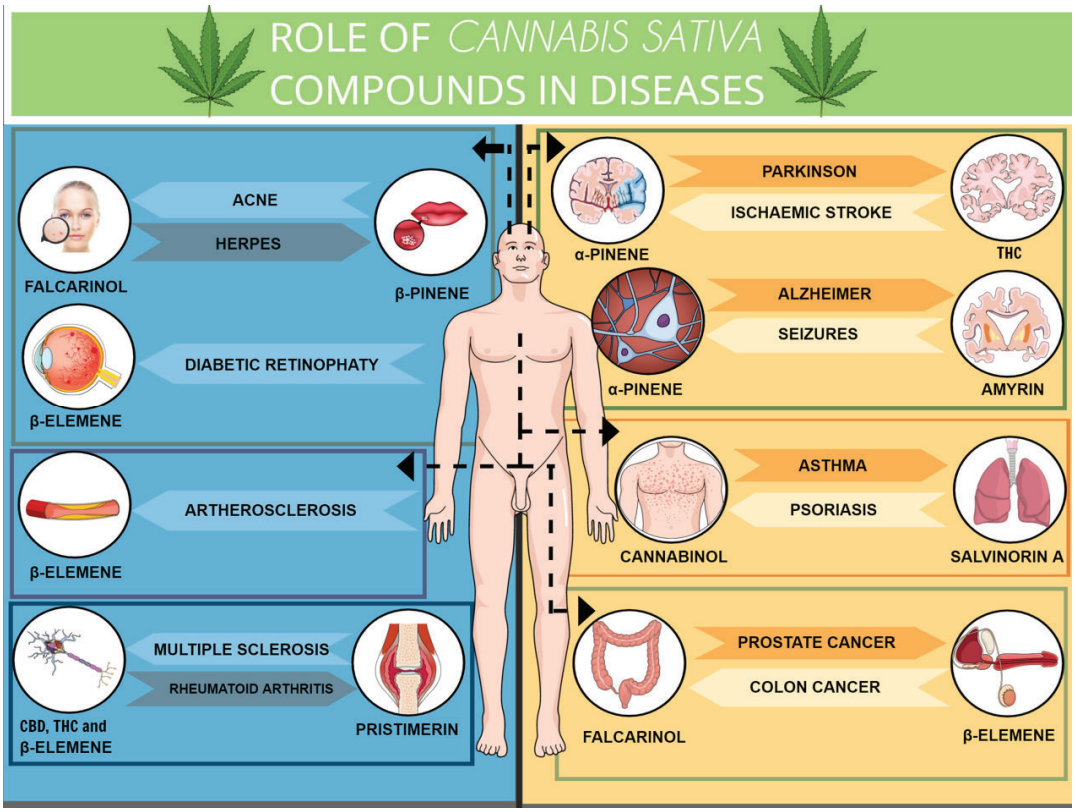

Figure 3. Role of *Cannabis sativa* compounds in diseases. The *Cannabis sativa* compounds have been proved useful for treatment of different diseases in the periphery and the CNS, as illustrated above. The CBD and THC actions in the CNS include immunomodulatory, neuroprotective, anxiolytic, and anticonvulsant, in addition to its potential effects on PD and multiple sclerosis control. Anticancer effects can be attributed to almost all *Cannabis sativa* compounds. This figure further illustrates the effect of terpenoids, cannabimimetic ligands, beyond the *Cannabis* plant in different pathological conditions, such as *Herpes* infection, diabetic retinopathy, psoriasis, asthma, AD, seizures, ischemic stroke, and others. Figure created using the Mind the Graph platform.

Herein, we describe that many of them share common properties, namely anti-inflammatory, analgesic, immunomodulatory, antiproliferative, and neuromodulatory. More specifically, the majority of these compounds seem to be acting on the same targets even though if in different pathological contexts (Table 3). We highlight the NF-κB, Nfr2, PPAR, COX-2, and CDKs proteins, just to name a few. Although there are many published preclinical studies demonstrating the beneficial effects of terpenes, there is an urge for detailed pharmacokinetic and pharmacodynamics characterization of these compounds. As the cannabinoids and the *Cannabis* plant appear to be the most recent great hope for the treatment of uncured diseases, the particular phytocannabinoid–terpenoid interaction—the so-called entourage effect—must be continuously investigated. Besides, clinical studies are sorely needed to confirm its efficacy and safety in humans; thus, we could finally have novel potential treatments for a number of diseases that for the time being remain poorly managed.

Table 3. The main findings about terpenoid compounds reviewed in the article.

Compound	Main Findings
β- and α-Caryophyllene	Antidepressant, anxiolytic, analgesic, anticonvulsant properties. Acetylcholinesterase (AChE) inhibitor.
D-Limonene	Anti-inflammatory, antinociceptive, gastroprotective, and neuroprotective effects.
Linalool	Anxiolytic, anticancer properties; neuroprotective effects against AD.
Terpineol	Analgesic activity in chronic pain conditions, such as fibromyalgia and cancer pain. Adjunctive therapy to morphine adopted in order to reduce its adverse effects. Preventive treatment for opioid analgesic dependence and tolerance.
Terpinene	Analgesic, antiproliferative, anti-inflammatory, and antimicrobial properties.
α-Pinene	Sedative, hypnotic, anti-seizure, anxiolytic, anticancer, and analgesic activities. Neuroprotective effects against memory loss.
β-Pinene	Antiviral, antifungal, anticancer, antimalarial, antidepressant properties.
β-Elemene	Anticancer and hypolipidemic compound. Potential treatment for demyelinating disease.
β-Ocimene	Antiproliferative, antifungal, and anticonvulsant properties.
Camphene	Eco-friendly botanical insecticide.
Nerolidol	Anti-inflammatory, anticancer, neuroprotective and antimicrobial effects.
Euphol	Antiviral, antiparasitic, antimicrobial, and antifungal activities.
Citral	Antimicrobial, anti-inflammatory, antinociceptive, and anticancer properties.
Celastrol	Anti-inflammatory and anticancer compound.
Falcarinol	Possible tool for treatment of cardiovascular diseases. Anticarcinogenic compound.
Salvinorin A	Psychoactive herb; anxiolytic, anti-inflammatory, and antidepressant effects. Alternative treatment for control of cocaine-seeking behavior and alcoholism. Promising tool for treatment of type 1 hypersensitivity.
Pristimerin	MGL inhibitor; anticancer and anti-metastatic effects.

AD, Alzheimer's disease; MGL, monoacylglycerol lipase.

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Abbreviations

AC: Adenylyl Cyclase; AChE, Acetylcholinesterase; AFB1, Aflatoxin B1; Ago2, Argonaute 2; ALS, Amyotrophic lateral sclerosis; AMPK, AMP-activated protein kinase; apoE, Apolipoprotein E; Aβ, Amyloid-beta; BAX, BCL2 associated X protein; BCL2, B-cell lymphoma 2 protein; CD40, Cluster of differentiation 40; CHOP, C/EBP

Homologous Protein; COX, Cyclooxygenase; COX-2, Cyclooxygenase-2; CNS, Central nervous system; CYP, Cyclophosphamide; DAT-1, Dopamine transporter; EAE, Experimental autoimmune encephalomyelitis; EGFR, Epidermal growth factor receptor; eNOS, Endothelial nitric oxide synthase; ET-1, Endothelin-1; FOXO3, Forkhead box O3; GABA, Gamma-aminobutyric acid; GPR18, G protein-coupled receptor 18; GPR55, G protein-coupled receptor 55; GPR119, G protein-coupled receptor 19; GSH, Glutathione; GSK-3β, Glycogen synthase kinase-3 beta; HIF-1α, Hypoxia-inducible factor – 1alpha; HMW, High molecular weight; HO1, Heme oxygenase-1; Hsp90, Heat shock protein 90; HSPs, Heat-shock proteins; i.p., Intraperitoneal; IBD, Inflammatory bowel disease; IFN-γ, Interferon-gamma; IgE, Immunoglobulin E; IL-1β, Interleukin -1β; IL-2, Interleukin – 2; IL-3, Interleukin – 3; IL-4, Interleukin – 4; IL-6, Interleukin – 6; IL-8, Interleukin – 8; IL-10, Interleukin – 10; IL-12, Interleukin – 12; IL-17, Interleukin – 17; IL-37, Interleukin – 37; iNOS, Inducible nitric oxide synthase; LFA-1, Lymphocyte function-associated antigen-1; LPS, Lipopolysaccharide; LOX, Lipoxygenase; LTP, Long term potentiation; MAO, Monoamine oxidase; MAPK, Mitogen-activated protein kinase; MARK4, Microtubule Affinity-Regulating Kinase 4; MCP-1, Monocyte chemoattractant protein-1; MMP-9, Matrix metallopeptidase – 9; MnSOD, Manganese superoxide dismutase; MPO, Myeloperoxidase; mTOR, Mammalian target of rapamycin; NF-κB, Nuclear factor kappa B; NO, Nitric oxide; NREMS, Non-rapid eye movement sleep; p90RSK, 90 kDa ribosomal S6 kinase; PARP, Poly ADP-ribose polymerase; PGE2, Prostaglandin E2; PI3K, Phosphoinositide 3-kinase; PKB, Protein kinase B; PKC, Protein kinase C; PPAR, Peroxisome proliferator-activated receptors; PPAR-γ, Peroxisome proliferator-activated receptor gamma; pSTAT3, Phosphorylated STAT3; PTX, Paclitaxel; PTZ, Pentylenetetrazole; RA, Rheumatoid arthritis; ROR-γt, Retinoid-related orphan receptor-γt; ROS, Reactive oxygen species; SNI, Spared nerve injury; SOD1, Superoxide dismutase – 1; SPHK1, Sphingosinekinase 1; Th1, T helper 1; Th2, T helper 2; TLR4, Toll-Like Receptor 4; TPA, 12-O-tetradecanoylphorbol-13-acetate; TRPM8, Transient receptor potential melastatin 8; TRP, Transient receptor potential; TRPV1, Transient receptor potential vanilloid 1; TRPV4, Transient receptor potential vanilloid 4; TNF, Tumor necrosis factor; UVA, Ultraviolet A radiation; VEGF, Vascular endothelial growth factor.

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REVIEW

Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects

Ethan B Russo

GW Pharmaceuticals, Salisbury, Wiltshire, UK

Correspondence
Ethan Russo, MD, 20402 81st Avenue SW, Vashon, WA 98070, USA. E-mail: ethanrusso@comcast.net

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Tetrahydrocannabinol (THC) has been the primary focus of cannabis research since 1964, when Raphael Mechoulam isolated and synthesized it. More recently, the synergistic contributions of cannabidiol to cannabis pharmacology and analgesia have been scientifically demonstrated. Other phytocannabinoids, including tetrahydrocannabivarin, cannabigerol and cannabichromene, exert additional effects of therapeutic interest. Innovative conventional plant breeding has yielded cannabis chemotypes expressing high titres of each component for future study. This review will explore another echelon of phytotherapeutic agents, the cannabis terpenoids: limonene, myrcene, α-pinene, linalool, β-caryophyllene, caryophyllene oxide, nerolidol and phytol. Terpenoids share a precursor with phytocannabinoids, and are all flavour and fragrance components common to human diets that have been designated Generally Recognized as Safe by the US Food and Drug Administration and other regulatory agencies. Terpenoids are quite potent, and affect animal and even human behaviour when inhaled from ambient air at serum levels in the single digits ng·mL^{-1}. They display unique therapeutic effects that may contribute meaningfully to the entourage effects of cannabis-based medicinal extracts. Particular focus will be placed on phytocannabinoid-terpenoid interactions that could produce synergy with respect to treatment of pain, inflammation, depression, anxiety, addiction, epilepsy, cancer, fungal and bacterial infections (including methicillin-resistant *Staphylococcus aureus*). Scientific evidence is presented for non-cannabinoid plant components as putative antidotes to intoxicating effects of THC that could increase its therapeutic index. Methods for investigating entourage effects in future experiments will be proposed. Phytocannabinoid-terpenoid synergy, if proven, increases the likelihood that an extensive pipeline of new therapeutic products is possible from this venerable plant.

LINKED ARTICLES

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Abbreviations

2-AG, 2-arachidonoylglycerol; 5-HT, 5-hydroxytryptamine (serotonin); AD, antidepressant; AEA, arachidonoylethanolamide (anandamide); AI, anti-inflammatory; AMPA, α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; Ca^{++}, calcium ion; CB_1/CB_2, cannabinoid receptor 1 or 2; CBC, cannabichromene; CBCA, cannabichromenic acid; CBD, cannabidiol; CBDA, cannabidiolic acid; CBDV, cannabidivarin; CBG, cannabigerol; CBGA, cannabigerolic acid; CBGV, cannabigerivarin; CNS, central nervous system; COX, cyclo-oxygenase; DAGL, diacylglycerol lipase; ECS, endocannabinoid system; EO, essential oil; FAAH, fatty acid amidohydrolase; FDA, US Food and Drug Administration; FEMA, Food and Extract Manufacturers Association; fMRI, functional magnetic resonance imaging; GABA, gamma aminobutyric acid; GPCR, G-protein coupled receptor; GPR, G-protein coupled receptor; HEK, human embryonic kidney; IC_{50}, 50% inhibitory concentration; i.p., intraperitoneal; MAGL, monoacylglycerol lipase; MIC, minimum inhibitory concentration; MS, multiple sclerosis; NGF, nerve growth factor; NIDA, US National Institute on Drug Abuse; PG, prostaglandin; PTSD, post-traumatic stress disorder; RCT, randomized clinical trial; SPECT, single photon emission computed tomography; SSADH, succinic semialdehyde dehydrogenase; Sx, symptoms; $T_{1/2}$, half-life; TCA, tricyclic antidepressant; THC, tetrahydrocannabinol; THCA, tetrahydrocannabinolic acid; THCV, tetrahydrocannabivarin; TNF-α, tumour necrosis factor-alpha, TRPV, transient receptor potential vanilloid receptor

The roots of cannabis synergy

Cannabis has been a medicinal plant of unparalleled versatility for millennia (Mechoulam, 1986; Russo, 2007; 2008), but whose mechanisms of action were an unsolved mystery until the discovery of tetrahydrocannabinol (THC) (Gaoni and Mechoulam, 1964a), the first cannabinoid receptor, CB_1 (Devane *et al.*, 1988), and the endocannabinoids, anandamide (arachidonoylethanolamide, AEA) (Devane *et al.*, 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam *et al.*, 1995; Sugiura *et al.*, 1995). While a host of phytocannabinoids were discovered in the 1960s: cannabidiol (CBD) (Mechoulam and Shvo, 1963), cannabigerol (CBG) (Gaoni and Mechoulam, 1964b), cannabichromene (CBC) (Gaoni and Mechoulam, 1966), cannabidivarin (CBDV) (Vollner *et al.*, 1969) and tetrahydrocannabivarin (THCV) (Gill *et al.*, 1970), the overwhelming preponderance of research focused on psychoactive THC. Only recently has renewed interest been manifest in THC analogues, while other key components of the activity of cannabis and its extracts, the cannabis terpenoids, remain understudied (McPartland and Russo, 2001b; Russo and McPartland, 2003). The current review will reconsider essential oil (EO) agents, their peculiar pharmacology and possible therapeutic interactions with phytocannabinoids. Nomenclature follows conventions in Alexander *et al.* (2009).

Phytocannabinoids and terpenoids are synthesized in cannabis, in secretory cells inside glandular trichomes (Figure 1) that are most highly concentrated in unfertilized female flowers prior to senescence (Potter, 2004; Potter, 2009). Geranyl pyrophosphate is formed as a precursor via the deoxyxylulose pathway in cannabis (Fellermeier *et al.*, 2001), and is a parent compound to both phytocannabinoids and terpenoids (Figure 2). After coupling with either olivetolic acid or divarinic acid, pentyl or propyl cannabinoid acids are produced, respectively, via enzymes that accept either substrate (de Meijer *et al.*, 2003), a manifestation of Mechoulam's postulated 'Nature's Law of Stinginess'. Although having important biochemical properties in their own right, acid forms of phytocannabinoids are most commonly decarboxylated via heat to produce the more familiar neutral phytocannabinoids (Table 1). Alternatively, geranyl pyrophosphate may form limonene and other monoterpenoids in secretory cell plastids, or couple with isopentenyl pyrophosphate in the cytoplasm to form farnesyl pyrophosphate, parent compound to the sesquiterpenoids, that co-localizes with transient receptor potential vanilloid receptor (TRPV) 1 in human dorsal root ganglion, suggesting a role in sensory processing of noxious stimuli (Bradshaw *et al.*, 2009), and which is the most potent endogenous ligand to date on the G-protein coupled receptor (GPR) 92 (Oh *et al.*, 2008).

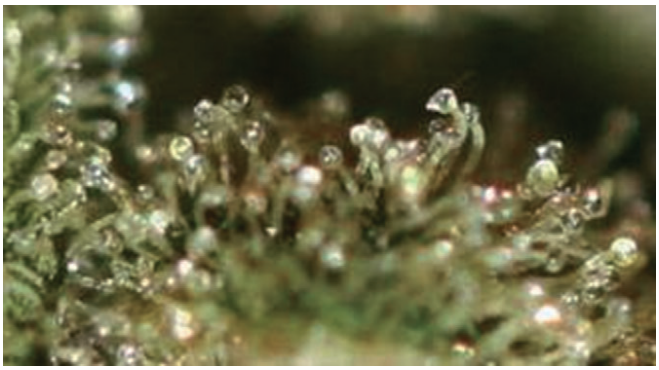


Figure 1
Cannabis capitate glandular (EBR by permission of Bedrocan BV, Netherlands).

An obvious question pertains to the chemical ecology of such syntheses that require obvious metabolic demands on the plant (Gershenzon, 1994), and these will be considered.

Is cannabis merely a crude vehicle for delivery of THC? Might it rather display herbal synergy (Williamson, 2001) encompassing potentiation of activity by active or inactive components, antagonism (evidenced by the ability of CBD to reduce side effects of THC; Russo and Guy, 2006), summation, pharmacokinetic and metabolic interactions? Recently, four basic mechanisms of synergy have been proposed (Wagner and Ulrich-Merzenich, 2009): (i) multi-target effects; (ii) pharmacokinetic effects such as improved solubility or bioavailability; (iii) agent interactions affecting bacterial resistance; and (iv) modulation of adverse events. Cannabis was cited as an illustration.

Could phytocannabinoids function analogously to the endocannabinoid system (ECS) with its combination of active and 'inactive' synergists, first described as an entourage (Ben-Shabat *et al.*, 1998), with subsequent refinement (Mechoulam and Ben-Shabat, 1999) and qualification (p. 136): 'This type of synergism may play a role in the widely held (but not experimentally based) view that in some cases plants are better drugs than the natural products isolated from them'. Support derives from studies in which cannabis extracts demonstrated effects two to four times greater than THC (Carlini *et al.*, 1974); unidentified THC antagonists and synergists were claimed (Fairbairn and Pickens, 1981), anticonvulsant activity was observed beyond the cannabinoid fraction (Wilkinson *et al.*, 2003), and extracts of THC and CBD modulated effects in hippocampal neurones distinctly from pure compounds (Ryan *et al.*, 2006). Older literature also presented refutations: no observed differences were noted by humans ingesting or smoking pure THC versus herbal cannabis (Wachtel *et al.*, 2002); pure THC seemed to account for all tetrad-type effects in mice (Varvel *et al.*, 2005); and smoked cannabis with varying CBD or CBC content failed to yield subjective differences combined with THC (Ilan *et al.*, 2005). Explanations include that the cannabis employed by Wachtel yielded 2.11% THC, but with only 0.3% cannabinol (CBN) and 0.05% CBD (Russo and McPartland, 2003), and Ilan's admission that CBN and CBD content might be too low to modulate THC. Another factor is apparent in that terpenoid yields from vaporization of street cannabis were 4.3–8.5 times of those from US National Institute on Drug Abuse cannabis (Bloor *et al.*, 2008). It is undisputed that the black market cannabis in the UK (Potter *et al.*, 2008), Continental Europe (King *et al.*, 2005) and the USA (Mehmedic *et al.*, 2010) has become almost exclusively a high-THC preparation to the almost total exclusion of other phytocannabinoids. If – as many consumers and experts maintain (Clarke, 2010) – there are biochemical, pharmacological and

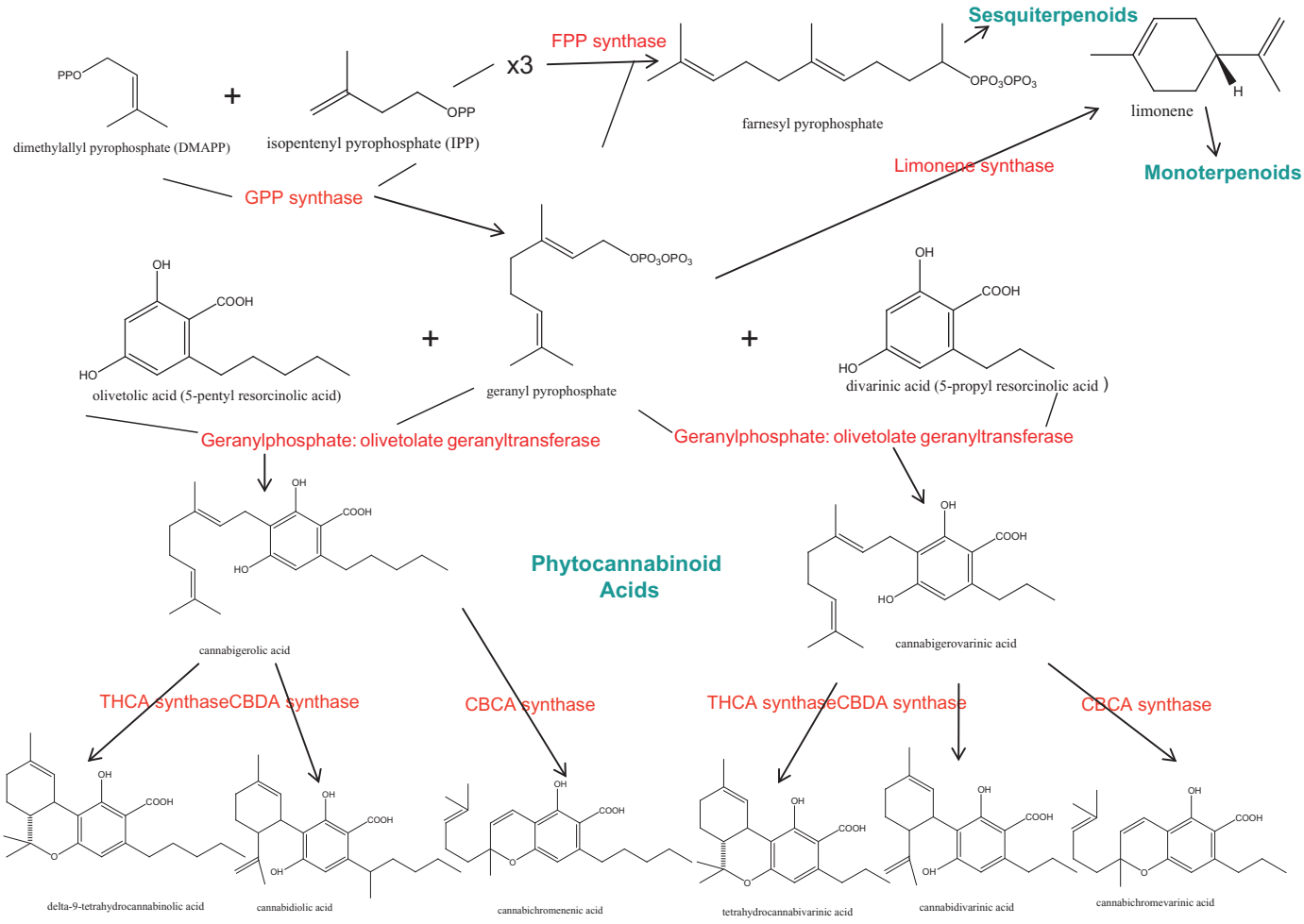


Figure 2
Phytocannabinoid and cannabis terpenoid biosynthesis.

phenomenological distinctions between available cannabis 'strains', such phenomena are most likely related to relative terpenoid contents and ratios. This treatise will assess additional evidence for putative synergistic phytocannabinoid-terpenoid effects exclusive of THC, to ascertain whether this botanical may fulfil its promise as, 'a neglected pharmacological treasure trove' (Mechoulam, 2005).

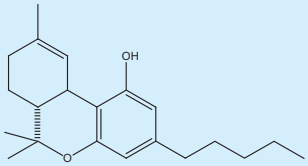
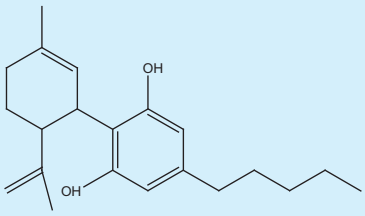
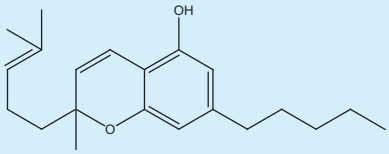
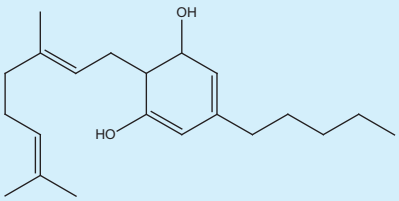
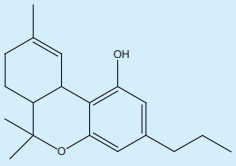
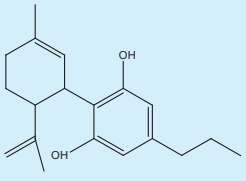
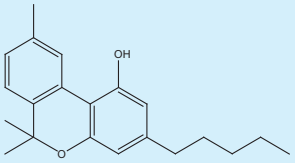
Phytocannabinoids, beyond THC: a brief survey

Phytocannabinoids are exclusively produced in cannabis (*vide infra* for exception), but their evolutionary and ecological *raisons d'être* were obscure until recently. THC production is maximized with increased light energy (Potter, 2009). It has been known for some time that CBG and CBC are mildly antifungal (ElSohly *et al.*, 1982), as are THC and CBD against a cannabis pathogen (McPartland, 1984). More pertinent, however, is the mechanical stickiness of the trichomes, capable of trapping insects with all six legs (Potter, 2009). Tetrahydrocannabinolic acid (THCA) and cannabichromenic acid (Morimoto *et al.*, 2007), as well as cannabidiolic acid and cannabigerolic acid (CBGA; Shoyama *et al.*, 2008) produce necrosis in plant cells. Normally, the cannabinoid acids are sequestered in trichomes away from the flower tissues. Any trichome breakage at senescence may contribute to natural pruning of lower fan leaves that otherwise utilize energy that the plant preferentially diverts to the flower, in continued efforts to affect fertilization, generally in vain when subject to human horticulture for pharmaceutical production. THCA and CBGA have also proven to be insecticidal in their own right (Sirikantaramas *et al.*, 2005).

Over 100 phytocannabinoids have been identified (Brenneisen, 2007; Mehmedic *et al.*, 2010), but many are artefacts of analysis or are produced in trace quantities that have not permitted thorough investigation. The pharmacology of the more accessible phytocannabinoids has received excellent recent reviews (Pertwee *et al.*, 2007; Izzo *et al.*, 2009; De Petrocellis and Di Marzo, 2010; De Petrocellis *et al.*, 2011), and will be summarized here, with emphasis on activities with particular synergistic potential.

Table 1

Phytocannabinoid activity table

Phytocannabinoid structure	Selected pharmacology (reference)	Synergistic terpenoids
 delta-9-tetrahydrocannabinol (THC)	Analgesic via CB_1 and CB_2 (Rahn and Hohmann, 2009)	Various
	AI/antioxidant (Hampson *et al.*, 1998)	Limonene *et al.*
	Bronchodilatory (Williams *et al.*, 1976)	Pinene
	↓ Sx. Alzheimer disease (Volicer *et al.*, 1997; Eubanks *et al.*, 2006)	Limonene, pinene, linalool
	Benefit on duodenal ulcers (Douthwaite, 1947)	Caryophyllene, limonene
	Muscle relaxant (Kavia *et al.*, 2010)	Linalool?
	Antipruritic, cholestatic jaundice (Neff *et al.*, 2002)	Caryophyllene?
 cannabidiol	AI/antioxidant (Hampson *et al.*, 1998)	Limonene *et al.*
	Anti-anxiety via 5-HT_{1A} (Russo *et al.*, 2005)	Linalool, limonene
	Anticonvulsant (Jones *et al.*, 2010)	Linalool
	Cytotoxic versus breast cancer (Ligresti *et al.*, 2006)	Limonene
	↑ adenosine A_{2A} signalling (Carrier *et al.*, 2006)	Linalool
	Effective versus MRSA (Appendino *et al.*, 2008)	Pinene
	Decreases sebum/sebocytes (Biro *et al.*, 2009)	Pinene, limonene, linalool
	Treatment of addiction (see text)	Caryophyllene
 cannabichromene	Anti-inflammatory/analgesic (Davis and Hatoum, 1983)	Various
	Antifungal (ElSohly *et al.*, 1982)	Caryophyllene oxide
	AEA uptake inhibitor (De Petrocellis *et al.*, 2011)	–
	Antidepressant in rodent model (Deyo and Musty, 2003)	Limonene
 cannabigerol	TRPM8 antagonist prostate cancer (De Petrocellis *et al.*, 2011)	Cannabis terpenoids
	GABA uptake inhibitor (Banerjee *et al.*, 1975)	Phytol, linalool
	Anti-fungal (ElSohly *et al.*, 1982)	Caryophyllene oxide
	Antidepressant rodent model (Musty and Deyo, 2006); and via 5-HT_{1A} antagonism (Cascio *et al.*, 2010)	Limonene
	Analgesic, α-2 adrenergic blockade (Cascio *et al.*, 2010)	Various
	↓ keratinocytes in psoriasis (Wilkinson and Williamson, 2007)	adjunctive role?
	Effective versus MRSA (Appendino *et al.*, 2008)	Pinene
 tetrahydrocannabivarin	AI/anti-hyperalgesic (Bolognini *et al.*, 2010)	Caryophyllene *et al.* . . .
	Treatment of metabolic syndrome (Cawthorne *et al.*, 2007)	–
	Anticonvulsant (Hill *et al.*, 2010)	Linalool
 cannabidivarin	Inhibits diacylglycerol lipase (De Petrocellis *et al.*, 2011)	–
	Anticonvulsant in hippocampus (Hill *et al.*, 2010)	Linalool
 cannabinol (CBN)	Sedative (Musty *et al.*, 1976)	Nerolidol, myrcene
	Effective versus MRSA (Appendino *et al.*, 2008)	Pinene
	TRPV2 agonist for burns (Qin *et al.*, 2008)	Linalool
	↓ keratinocytes in psoriasis (Wilkinson and Williamson, 2007)	adjunctive role?
	↓ breast cancer resistance protein (Holland *et al.*, 2008)	Limonene

5-HT, 5-hydroxytryptamine (serotonin); AEA, arachidonoylethanolamide (anandamide); AI, anti-inflammatory; CB1/CB2, cannabinoid receptor 1 or 2; GABA, gamma aminobutyric acid; TRPV, transient receptor potential vanilloid receptor; MRSA, methicillin-resistant *Staphylococcus aureus*; Sx, symptoms.

THC (Table 1) is the most common phytocannabinoid in cannabis drug chemotypes, and is produced in the plant via an allele co-dominant with CBD (de Meijer *et al*., 2003). THC is a partial agonist at CB_1 and cannabinoid receptor 2 (CB_2) analogous to AEA, and underlying many of its activities as a psychoactive agent, analgesic, muscle relaxant and antispasmodic (Pacher *et al*., 2006). Additionally, it is a bronchodilator (Williams *et al*., 1976), neuroprotective antioxidant (Hampson *et al*., 1998), antipruritic agent in cholestatic jaundice (Neff *et al*., 2002) and has 20 times the anti-inflammatory power of aspirin and twice that of hydrocortisone (Evans, 1991). THC is likely to avoid potential pitfalls of either COX-1 or COX-2 inhibition, as such activity is only noted at concentrations far above those attained therapeutically (Stott *et al*., 2005).

CBD is the most common phytocannabinoid in fibre (hemp) plants, and second most prevalent in some drug chemotypes. It has proven extremely versatile pharmacologically (Table 1) (Pertwee, 2004; Mechoulam *et al*., 2007), displaying the unusual ability to antagonize CB_1 at a low nM level in the presence of THC, despite having little binding affinity (Thomas *et al*., 2007), and supporting its modulatory effect on THC-associated adverse events such as anxiety, tachycardia, hunger and sedation in rats and humans (Nicholson *et al*., 2004; Murillo-Rodriguez *et al*., 2006; Russo and Guy, 2006). CBD is an analgesic (Costa *et al*., 2007), is a neuroprotective antioxidant more potent than ascorbate or tocopherol (Hampson *et al*., 1998), without COX inhibition (Stott *et al*., 2005), acts as a TRPV1 agonist analogous to capsaicin but without noxious effect (Bisogno *et al*., 2001), while also inhibiting uptake of AEA and weakly inhibiting its hydrolysis. CBD is an antagonist on GPR55, and also on GPR18, possibly supporting a therapeutic role in disorders of cell migration, notably endometriosis (McHugh *et al*., 2010). CBD is anticonvulsant (Carlini and Cunha, 1981; Jones *et al*., 2010), anti-nausea (Parker *et al*., 2002), cytotoxic in breast cancer (Ligresti *et al*., 2006) and many other cell lines while being cyto-preservative for normal cells (Parolaro and Massi, 2008), antagonizes tumour necrosis factor-alpha (TNF-α) in a rodent model of rheumatoid arthritis (Malfait *et al*., 2000), enhances adenosine receptor A_{2A} signalling via inhibition of an adenosine transporter (Carrier *et al*., 2006), and prevents prion accumulation and neuronal toxicity (Dirikoc *et al*., 2007). A CBD extract showed greater anti-hyperalgesia over pure compound in a rat model with decreased allodynia, improved thermal perception and nerve growth factor levels and decreased oxidative damage (Comelli *et al*., 2009). CBD also displayed powerful activity against methicillin-resistant *Staphylococcus aureus* (MRSA), with a minimum inhibitory concentration (MIC) of 0.5–2 $\mu g \cdot mL^{-1}$ (Appendino *et al*., 2008). In 2005, it was demonstrated that CBD has agonistic activity at 5-hydroxytryptamine $(5\text{-HT})_{1A}$ at 16 μM (Russo *et al*., 2005), and that despite the high concentration, may underlie its anti-anxiety activity (Resstel *et al*., 2009; Soares Vde *et al*., 2010), reduction of stroke risk (Mishima *et al*., 2005), anti-nausea effects (Rock *et al*., 2009) and ability to affect improvement in cognition in a mouse model of hepatic encephalopathy (Magen *et al*., 2009). A recent study has demonstrated that CBD 30 $mg \cdot kg^{-1}$ i.p. reduced immobility time in the forced swim test compared to imipramine ($P < 0.01$), an effect blocked by pre-treatment with the 5-HT_{1A} antagonist WAY100635 (Zanelati *et al*., 2010), supporting a prospective role for CBD as an antidepressant. CBD also inhibits synthesis of lipids in sebocytes, and produces apoptosis at higher doses in a model of acne (*vide infra*). One example of CBD antagonism to THC would be the recent observation of lymphopenia in rats (CBD 5 $mg \cdot kg^{-1}$) mediated by possible CB_2 inverse agonism (Ignatowska-Jankowska *et al*., 2009), an effect not reported in humans even at doses of pure CBD up to 800 mg (Crippa *et al*., 2010), possibly due to marked interspecies differences in CB_2 sequences and signal transduction. CBD proved to be a critical factor in the ability of nabiximols oromucosal extract in successfully treating intractable cancer pain patients unresponsive to opioids (30% reduction in pain from baseline), as a high-THC extract devoid of CBD failed to distinguish from placebo (Johnson *et al*., 2010). This may represent true synergy if the THC–CBD combination were shown to provide a larger effect than a summation of those from the compounds separately (Berenbaum, 1989).

CBC (Table 1) was inactive on adenylate cyclase inhibition (Howlett, 1987), but showed activity in the mouse cannabinoid tetrad, but only at 100 $mg \cdot kg^{-1}$, and at a fraction of THC activity, via a non-CB_1, non-CB_2 mechanism (Delong *et al*., 2010). More pertinent are anti-inflammatory (Wirth *et al*., 1980) and analgesic activity (Davis and Hatoum, 1983), its ability to reduce THC intoxication in mice (Hatoum *et al*., 1981), antibiotic and antifungal effects (ElSohly *et al*., 1982), and observed cytotoxicity in cancer cell lines (Ligresti *et al*., 2006). A CBC-extract displayed pronounced antidepressant effect in rodent models (Deyo and Musty, 2003). Additionally, CBC was comparable to mustard oil in stimulating TRPA1-mediated Ca^{++} in human embryonic kidney 293 cells (50–60 nM) (De Petrocellis *et al*., 2008). CBC recently proved to be a strong AEA uptake inhibitor (De Petrocellis *et al*., 2011). CBC production is normally maximal, earlier in the plant's life cycle (de Meijer *et al*., 2009a). An innovative technique employing cold water extraction of immature leaf matter from selectively bred cannabis chemotypes yields a high-CBC 'enriched trichome preparation' (Potter, 2009).

CBG (Table 1), the parent phytocannabinoid compound, has a relatively weak partial agonistic effect at CB_1 (K_i 440 nM) and CB_2 (K_i 337 nM) (Gauson *et al*., 2007). Older work supports gamma aminobutyric acid (GABA) uptake inhibition greater than THC or CBD (Banerjee *et al*., 1975) that could suggest muscle relaxant properties. Analgesic and anti-erythemic effects and the ability to block lipooxygenase were said to surpass those of THC (Evans, 1991). CBG demonstrated modest antifungal effects (ElSohly *et al*., 1982). More recently, it proved to be an effective cytotoxic in high dosage on human epithelioid carcinoma (Baek *et al*., 1998), is the next most effective phytocannabinoid against breast cancer after CBD (Ligresti *et al*., 2006), is an antidepressant in the rodent tail suspension model (Musty and Deyo, 2006) and is a mildly anti-hypertensive agent (Maor *et al*., 2006). Additionally, CBG inhibits keratinocyte proliferation suggesting utility in psoriasis (Wilkinson and Williamson, 2007), it is a relatively potent TRPM8 antagonist for possible application in prostate cancer (De Petrocellis and Di Marzo, 2010) and detrusor over-activity and bladder pain (Mukerji *et al*., 2006). It is a strong AEA uptake inhibitor (De Petrocellis *et al*., 2011) and a powerful agent against MRSA (Appendino *et al*., 2008; *vide infra*). Finally, CBG behaves as a potent α-2 adrenorecep-

tor agonist, supporting analgesic effects previously noted (Formukong *et al.*, 1988), and moderate 5-HT_{1A} antagonist suggesting antidepressant properties (Cascio *et al.*, 2010). Normally, CBG appears as a relatively low concentration intermediate in the plant, but recent breeding work has yielded cannabis chemotypes lacking in downstream enzymes that express 100% of their phytocannabinoid content as CBG (de Meijer and Hammond, 2005; de Meijer *et al.*, 2009a).

THCV (Table 1) is a propyl analogue of THC, and can modulate intoxication of the latter, displaying 25% of its potency in early testing (Gill *et al.*, 1970; Hollister, 1974). A recrudescence of interest accrues to this compound, which is a CB_1 antagonist at lower doses (Thomas *et al.*, 2005), but is a CB_1 agonist at higher doses (Pertwee, 2008). THCV produces weight loss, decreased body fat and serum leptin concentrations with increased energy expenditure in obese mice (Cawthorne *et al.*, 2007; Riedel *et al.*, 2009). THCV also demonstrates prominent anticonvulsant properties in rodent cerebellum and pyriform cortex (Hill *et al.*, 2010). THCV appears as a fractional component of many southern African cannabis chemotypes, although plants highly predominant in this agent have been produced (de Meijer, 2004). THCV recently demonstrated a CB_2-based ability to suppress carageenan-induced hyperalgesia and inflammation, and both phases of formalin-induced pain behaviour via CB_1 and CB_2 in mice (Bolognini *et al.*, 2010).

CBDV (Table 1), the propyl analogue of CBD, was first isolated in 1969 (Vollner *et al.*, 1969), but formerly received little investigation. Pure CBDV inhibits diacylglycerol lipase [50% inhibitory concentration (IC_{50}) 16.6 μM] and might decrease activity of its product, the endocannabinoid, 2-AG (De Petrocellis *et al.*, 2011). It is also anticonvulsant in rodent hippocampal brain slices, comparable to phenobarbitone and felbamate (Jones *et al.*, 2010).

Finally, CBN is a non-enzymatic oxidative by-product of THC, more prominent in aged cannabis samples (Merzouki and Mesa, 2002). It has a lower affinity for CB_1 (K_i 211.2 nM) and CB_2 (K_i 126.4 nM) (Rhee *et al.*, 1997); and was judged inactive when tested alone in human volunteers, but produced greater sedation combined with THC (Musty *et al.*, 1976). CBN demonstrated anticonvulsant (Turner *et al.*, 1980), anti-inflammatory (Evans, 1991) and potent effects against MRSA (MIC 1 $\mu g \cdot mL^{-1}$). CBN is a TRPV2 (high-threshold thermosensor) agonist (EC 77.7 μM) of possible interest in treatment of burns (Qin *et al.*, 2008). Like CBG, it inhibits keratinocyte proliferation (Wilkinson and Williamson, 2007), independently of cannabinoid receptor effects. CBN stimulates the recruitment of quiescent mesenchymal stem cells in marrow (10 μM), suggesting promotion of bone formation (Scutt and Williamson, 2007) and inhibits breast cancer resistance protein, albeit at a very high concentration (IC_{50} 145 μM) (Holland *et al.*, 2008).

Cannabis terpenoids: neglected entourage compounds?

Terpenoids are EO components, previously conceived as the quintessential fifth element, 'life force' or spirit (Schmidt, 2010), and form the largest group of plant chemicals, with 15–20 000 fully characterized (Langenheim, 1994). Terpenoids, not cannabinoids, are responsible for the aroma of cannabis. Over 200 have been reported in the plant (Hendriks *et al.*, 1975; 1977; Malingre *et al.*, 1975; Davalos *et al.*, 1977; Ross and ElSohly, 1996; Mediavilla and Steinemann, 1997; Rothschild *et al.*, 2005; Brenneisen, 2007), but only a few studies have concentrated on their pharmacology (McPartland and Pruitt, 1999; McPartland and Mediavilla, 2001a; McPartland and Russo, 2001b). Their yield is less than 1% in most cannabis assays, but they may represent 10% of trichome content (Potter, 2009). Monoterpenes usually predominate (limonene, myrcene, pinene), but these headspace volatiles (Hood *et al.*, 1973), while only lost at a rate of about 5% before processing (Gershenzon, 1994), do suffer diminished yields with drying and storage (Turner *et al.*, 1980; Ross and ElSohly, 1996), resulting in a higher relative proportion of sesquiterpenoids (especially caryophyllene), as also often occurs in extracts. A 'phytochemical polymorphism' seems operative in the plant (Franz and Novak, 2010), as production favours agents such as limonene and pinene in flowers that are repellent to insects (Nerio *et al.*, 2010), while lower fan leaves express higher concentrations of bitter sesquiterpenoids that act as anti-feedants for grazing animals (Potter, 2009). Evolutionarily, terpenoids seem to occur in complex and variable mixtures with marked structural diversity to serve various ecological roles. Terpenoid composition is under genetic control (Langenheim, 1994), and some enzymes produce multiple products, again supporting Mechoulam's 'Law of Stinginess'. The particular mixture of mono- and sesquiterpenoids will determine viscosity, and in cannabis, this certainly is leveraged to practical advantage as the notable stickiness of cannabis exudations traps insects (McPartland *et al.*, 2000), and thus, combined with the insecticidal phytocannabinoid acids (Sirikantaramas *et al.*, 2005), provides a synergistic mechano-chemical defensive strategy versus predators.

As observed for cannabinoids, terpenoid production increases with light exposure, but decreases with soil fertility (Langenheim, 1994), and this is supported by the glasshouse experience that demonstrates higher yields if plants experience relative nitrogen lack just prior to harvest (Potter, 2004), favouring floral over foliar growth. EO composition is much more genetically than environmentally determined, however (Franz and Novak, 2010), and while cannabis is allogamous and normally requires repeat selective breeding for maintenance of quality, this problem may be practically circumvented by vegetative propagation of high-performance plants under controlled environmental conditions (light, heat and humidity) (Potter, 2009), and such techniques have proven to provide notable consistency to tight tolerances as Good Manufacturing Practice for any pharmaceutical would require (Fischedick *et al.*, 2010).

The *European Pharmacopoeia*, Sixth Edition (2007), lists 28 EOs (Pauli and Schilcher, 2010). Terpenoids are pharmacologically versatile: they are lipophilic, interact with cell membranes, neuronal and muscle ion channels, neurotransmitter receptors, G-protein coupled (odorant) receptors, second messenger systems and enzymes (Bowles, 2003; Buchbauer, 2010). All the terpenoids discussed herein are Generally Recognized as Safe, as attested by the US Food and Drug Admin-

istration as food additives, or by the Food and Extract Manufacturers Association and other world regulatory bodies. Germane is the observation (Adams and Taylor, 2010) (p. 193), 'With a high degree of confidence one may presume that EOs derived from food are likely to be safe'. Additionally, all the current entries are non-sensitizing to skin when fresh (Tisserand and Balacs, 1995; Adams and Taylor, 2010), but may cause allergic reactions at very low rates when oxidized (Matura *et al.*, 2005). For additional pharmacological data on other common cannabis terpenoids not discussed herein (1,8-cineole, also known as eucalyptol, pulegone, α-terpineol, terpineol-4-ol, ρ-cymene, borneol and Δ-3-carene), please see McPartland and Russo (2001b).

Are cannabis terpenoids actually relevant to the effects of cannabis? Terpenoid components in concentrations above 0.05% are considered of pharmacological interest (Adams and Taylor, 2010). Animal studies are certainly supportive (Buchbauer *et al.*, 1993). Mice exposed to terpenoid odours inhaled from ambient air for 1 h demonstrated profound effects on activity levels, suggesting a direct pharmacological effect on the brain, even at extremely low serum concentrations (examples: linalool with 73% reduction in motility at 4.22 ng·mL^{-1}, pinene 13.77% increase at trace concentration, terpineol 45% reduction at 4.7 ng·mL^{-1}). These levels are comparable to those of THC measured in humans receiving cannabis extracts yielding therapeutic effects in pain, or symptoms of multiple sclerosis in various randomized controlled trials (RCTs) (Russo, 2006; Huestis, 2007). Positive effects at undetectable serum concentrations with orange terpenes (primarily limonene, 35.25% increase in mouse activity), could be explainable on the basis of rapid redistribution and concentration in lipophilic cerebral structures. A similar rationale pertains to human studies (Komori *et al.*, 1995), subsequently discussed. Limonene is highly bioavailable with 70% human pulmonary uptake (Falk-Filipsson *et al.*, 1993), and a figure of 60% for pinene with rapid metabolism or redistribution (Falk *et al.*, 1990). Ingestion and percutaneous absorption is also well documented in humans (Jäger *et al.*, 1992): 1500 mg of lavender EO with 24.7% linalool (total 372 mg) was massaged into the skin of a 60 kg man for 10 min, resulting in a peak plasma concentration of 100 ng·mL^{-1} at 19 min, and a half-life of 13.76 min in serum (Jäger *et al.*, 1992). EO mixtures (including limonene and pinene) also increase permeation of estradiol through mouse skin (Monti *et al.*, 2002).

Government-approved cannabis supplied to patients in national programmes in the Netherlands and Canada is gamma-irradiated to sterilize coliform bacteria, but the safety of this technique for a smoked and inhaled product has never been specifically tested. Gamma-radiation significantly reduced linalool titres in fresh cilantro (Fan and Sokorai, 2002), and myrcene and linalool in orange juice (Fan and Gates, 2001).

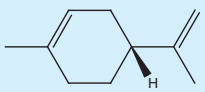
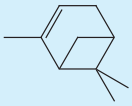
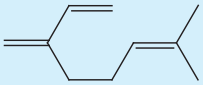
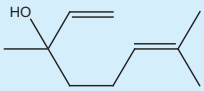
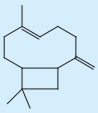
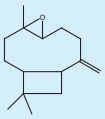
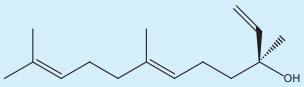
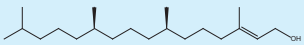
D-limonene, common to the lemon and other citrus EOs (Table 2), is the second most widely distributed terpenoid in nature (Noma and Asakawa, 2010), and is the precursor to other monoterpenoids (Figure 2) through species-specific synthetic schemes. Unfortunately, these pathways have not yet been investigated in cannabis. The ubiquity of limonene serves, perhaps, as a demonstration of convergent evolution that supports an important ecological role for this monoterpene. Studies with varying methodology and dosing in citrus oils in mice suggest it to be a powerful anxiolytic agent (Carvalho-Freitas and Costa, 2002; Pultrini Ade *et al.*, 2006), with one EO increasing serotonin in the prefrontal cortex, and dopamine (DA) in hippocampus mediated via 5-HT$_{1A}$ (Komiya *et al.*, 2006). Compelling confirmatory evidence in humans was provided in a clinical study (Komori *et al.*, 1995), in which hospitalized depressed patients were exposed to citrus fragrance in ambient air, with subsequent normalization of Hamilton Depression Scores, successful discontinuation of antidepressant medication in 9/12 patients and serum evidence of immune stimulation (CD4/8 ratio normalization). Limonene also produces apoptosis of breast cancer cells, and was employed at high doses in Phase II RCTs (Vigushin *et al.*, 1998). Subsequent investigation in cancer treatment has centred on its immediate hepatic metabolite, perillic acid, which demonstrates anti-stress effects in rat brain (Fukumoto *et al.*, 2008). A patent has been submitted, claiming that limonene effectively treats gastro-oesophageal reflux (Harris, 2010). Citrus EOs containing limonene proved effective against dermatophytes (Sanguinetti *et al.*, 2007; Singh *et al.*, 2010), and citrus EOs with terpenoid profiles resembling those in cannabis demonstrated strong radical scavenging properties (Choi *et al.*, 2000). As noted above, limonene is highly bioavailable (Falk-Filipsson *et al.*, 1993), and rapidly metabolized, but with indications of accumulation and retention in adipose tissues (e.g. brain). It is highly non-toxic (estimated human lethal dose 0.5–5 g·kg^{-1}) and non-sensitizing (Von Burg, 1995)

β-Myrcene is another common monoterpenoid in cannabis (Table 2) with myriad activities: diminishing inflammation via prostaglandin E-2 (PGE-2) (Lorenzetti *et al.*, 1991), and blocking hepatic carcinogenesis by aflatoxin (De-Oliveira *et al.*, 1997). Interestingly, myrcene is analgesic in mice, but this action can be blocked by naloxone, perhaps via the α-2 adrenoreceptor (Rao *et al.*, 1990). It is non-mutagenic in the Ames test (Gomes-Carneiro *et al.*, 2005). Myrcene is a recognized sedative as part of hops preparations (*Humulus lupulus*), employed to aid sleep in Germany (Bisset and Wichtl, 2004). Furthermore, myrcene acted as a muscle relaxant in mice, and potentiated barbiturate sleep time at high doses (do Vale *et al.*, 2002). Together, these data would support the hypothesis that myrcene is a prominent sedative terpenoid in cannabis, and combined with THC, may produce the 'couch-lock' phenomenon of certain chemotypes that is alternatively decried or appreciated by recreational cannabis consumers.

α-Pinene is a bicyclic monoterpene (Table 2), and the most widely encountered terpenoid in nature (Noma and Asakawa, 2010). It appears in conifers and innumerable plant EOs, with an insect-repellent role. It is anti-inflammatory via PGE-1 (Gil *et al.*, 1989), and is a bronchodilator in humans at low exposure levels (Falk *et al.*, 1990). Pinene is a major component of *Sideritis* spp. (Kose *et al.*, 2010) and *Salvia* spp. EOs (Ozek *et al.*, 2010), both with prominent activity against MRSA (*vide infra*). Beyond this, it seems to be a broad-spectrum antibiotic (Nissen *et al.*, 2010). α-Pinene forms the biosynthetic base for CB_2 ligands, such as HU-308 (Hanus *et al.*, 1999). Perhaps most compelling, however, is its activity as an acetylcholinesterase inhibitor aiding memory (Perry *et al.*, 2000), with an observed IC_{50} of 0.44 mM (Miyazawa

Table 2

Cannabis Terpenoid Activity Table

Terpenoid	Structure	Commonly encountered in	Pharmacological activity (Reference)	Synergistic cannabinoid
Limonene		 Lemon	Potent AD/immunostimulant via inhalation (Komori *et al.*, 1995)	CBD
			Anxiolytic (Carvalho-Freitas and Costa, 2002; Pultrini Ade *et al.*, 2006) via 5-HT_{1A} (Komiya *et al.*, 2006)	CBD
			Apoptosis of breast cancer cells (Vigushin *et al.*, 1998)	CBD, CBG
			Active against acne bacteria (Kim *et al.*, 2008)	CBD
			Dermatophytes (Sanguinetti *et al.*, 2007; Singh *et al.*, 2010)	CBG
			Gastro-oesophageal reflux (Harris, 2010)	THC
α-Pinene		 Pine	Anti-inflammatory via PGE-1 (Gil *et al.*, 1989)	CBD
			Bronchodilatory in humans (Falk *et al.*, 1990)	THC
			Acetylcholinesterase inhibitor, aiding memory (Perry *et al.*, 2000)	THC?, CBD
β-Myrcene		 Hops	Blocks inflammation via PGE-2 (Lorenzetti *et al.*, 1991)	CBD
			Analgesic, antagonized by naloxone (Rao *et al.*, 1990)	CBD, THC
			Sedating, muscle relaxant, hypnotic (do Vale *et al.*, 2002)	THC
			Blocks hepatic carcinogenesis by aflatoxin (de Oliveira *et al.*, 1997)	CBD, CBG
Linalool		 Lavender	Anti-anxiety (Russo, 2001)	CBD, CBG?
			Sedative on inhalation in mice (Buchbauer *et al.*, 1993)	THC
			Local anesthetic (Re *et al.*, 2000)	THC
			Analgesic via adenosine A_{2A} (Peana *et al.*, 2006)	CBD
			Anticonvulsant/anti-glutamate (Elisabetsky *et al.*, 1995)	CBD, THCV, CBDV
			Potent anti-leishmanial (do Socorro *et al.*, 2003)	?
β-Caryophyllene		 Pepper	AI via PGE-1 comparable phenylbutazone (Basile *et al.*, 1988)	CBD
			Gastric cytoprotective (Tambe *et al.*, 1996)	THC
			Anti-malarial (Campbell *et al.*, 1997)	?
			Selective CB_2 agonist (100 nM) (Gertsch *et al.*, 2008)	THC
			Treatment of pruritus? (Karsak *et al.*, 2007)	THC
			Treatment of addiction? (Xi *et al.*, 2010)	CBD
Caryophyllene Oxide		 Lemon balm	Decreases platelet aggregation (Lin *et al.*, 2003)	THC
			Antifungal in onychomycosis comparable to ciclopiroxolamine and sulconazole (Yang *et al.*, 1999)	CBC,CBG
			Insecticidal/anti-feedant (Bettarini *et al.*, 1993)	THCA, CBGA
Nerolidol		 Orange	Sedative (Binet *et al.*, 1972)	THC, CBN
			Skin penetrant (Cornwell and Barry, 1994)	–
			Potent antimalarial (Lopes *et al.*, 1999, Rodrigues Goulart *et al.*, 2004)	?
			Anti-leishmanial activity (Arruda *et al.*, 2005)	?
Phytol		 Green tea	Breakdown product of chlorophyll	–
			Prevents Vitamin A teratogenesis (Arnhold *et al.*, 2002)	–
			↑GABA via SSADH inhibition (Bang *et al.*, 2002)	CBG

Representative plants containing each terpenoid are displayed as examples to promote recognition, but many species contain them in varying concentrations. 5-HT, 5-hydroxytryptamine (serotonin); AD, antidepressant; AI, anti-inflammatory; CB_1/CB_2, cannabinoid receptor 1 or 2; GABA, gamma aminobutyric acid; PGE-1/PGE-2, prostaglandin E-1/prostaglandin E-2; SSADH, succinic semialdehyde dehydrogenase.

and Yamafuji, 2005). This feature could counteract short-term memory deficits induced by THC intoxication (*vide infra*).

D-Linalool is a monoterpenoid alcohol (Table 2), common to lavender (*Lavandula angustifolia*), whose psychotropic anxiolytic activity has been reviewed in detail (Russo, 2001). Interestingly, linalyl acetate, the other primary terpenoid in lavender, hydrolyses to linalool in gastric secretions (Bickers *et al*., 2003). Linalool proved sedating to mouse activity on inhalation (Buchbauer *et al*., 1991; Jirovetz *et al*., 1992). In traditional aromatherapy, linalool is the likely suspect in the remarkable therapeutic capabilities of lavender EO to alleviate skin burns without scarring (Gattefosse, 1993). Pertinent to this, the local anaesthetic effects of linalool (Re *et al*., 2000) are equal to those of procaine and menthol (Ghelardini *et al*., 1999). Another explanation would be its ability to produce hot-plate analgesia in mice ($P < 0.001$) that was reduced by administration of an adenosine A_{2A} antagonist (Peana *et al*., 2006). It is also anti-nociceptive at high doses in mice via ionotropic glutamate receptors (Batista *et al*., 2008). Linalool demonstrated anticonvulsant and antiglutamatergic activity (Elisabetsky *et al*., 1995), and reduced seizures as part of *Ocimum basilicum* EO after exposure to pentylenetetrazole, picrotoxin and strychnine (Ismail, 2006). Furthermore, linalool decreased K^+-stimulated glutamate release and uptake in mouse synaptosomes (Silva Brum *et al*., 2001). These effects were summarized (Nunes *et al*., 2010, p. 303): 'Overall, it seems reasonable to argue that the modulation of glutamate and GABA neurotransmitter systems are likely to be the critical mechanism responsible for the sedative, anxiolytic and anticonvulsant properties of linalool and EOs containing linalool in significant proportions'. Linalool also proved to be a powerful anti-leishmanial agent (do Socorro *et al*., 2003), and as a presumed lavender EO component, decreased morphine opioid usage after inhalation versus placebo ($P = 0.04$) in gastric banding in morbidly obese surgical patients (Kim *et al*., 2007).

β-Caryophyllene (Table 2) is generally the most common sesquiterpenoid encountered in cannabis (Mediavilla and Steinemann, 1997), wherein its evolutionary function may be due to its ability to attract insect predatory green lacewings, while simultaneously inhibiting insect herbivory (Langenheim, 1994). It is frequently the predominant terpenoid overall in cannabis extracts, particularly if they have been processed under heat for decarboxylation (Guy and Stott, 2005). Caryophyllene is common to black pepper (*Piper nigrum*) and Copaiba balsam (*Copaifera officinalis*) (Lawless, 1995). It is anti-inflammatory via PGE-1, comparable in potency to the toxic phenylbutazone (Basile *et al*., 1988), and an EO containing it was on par with etodolac and indomethacin (Ozturk and Ozbek, 2005). In contrast to the latter agents, however, caryophyllene was a gastric cytoprotective (Tambe *et al*., 1996), much as had been claimed in the past in treating duodenal ulcers in the UK with cannabis extract (Douthwaite, 1947). Caryophyllene may have contributed to antimalarial effects as an EO component (Campbell *et al*., 1997). Perhaps the greatest revelation regarding caryophyllene has been its demonstration as a selective full agonist at CB_2 (100 nM), the first proven phytocannabinoid beyond the cannabis genus (Gertsch *et al*., 2008). Subsequent work has demonstrated that this dietary component produced anti-inflammatory analgesic activity at the lowest dose of 5 $mg \cdot kg^{-1}$ in wild-type, but not CB_2 knockout mice (Gertsch, 2008). Given the lack of attributed psychoactivity of CB_2 agonists, caryophyllene offers great promise as a therapeutic compound, whether systemically, or in dermatological applications such as contact dermatitis (Karsak *et al*., 2007). Sensitization reactions are quite rare, and probably due to oxidized product (Skold *et al*., 2006).

Nerolidol is a sesquiterpene alcohol with sedative properties (Binet *et al*., 1972), present as a low-level component in orange and other citrus peels (Table 2). It diminished experimentally induced formation of colon adenomas in rats (Wattenberg, 1991). It was an effective agent for enhancing skin penetration of 5-fluorouracil (Cornwell and Barry, 1994). This could be a helpful property in treating fungal growth, where it is also an inhibitor (Langenheim, 1994). It seems to have anti-protozoal parasite control benefits, as a potent antimalarial (Lopes *et al*., 1999; Rodrigues Goulart *et al*., 2004) and anti-leishmanial agent (Arruda *et al*., 2005). Nerolidol is non-toxic and non-sensitizing (Lapczynski *et al*., 2008).

Caryophyllene oxide (Table 2) is a sesquiterpenoid oxide common to lemon balm (*Melissa officinalis*), and to the eucalyptus, *Melaleuca stypheloides*, whose EO contains 43.8% (Farag *et al*., 2004). In the plant, it serves as an insecticidal/anti-feedant (Bettarini *et al*., 1993) and as broad-spectrum antifungal in plant defence (Langenheim, 1994). Analogously, the latter properties may prove therapeutic, as caryophyllene oxide demonstrated antifungal efficacy in a model of clinical onychomycosis comparable to ciclopiroxalamine and sulconazole, with an 8% concentration affecting eradication in 15 days (Yang *et al*., 1999). Caryophyllene oxide is non-toxic and non-sensitizing (Opdyke, 1983). This agent also demonstrates anti-platelet aggregation properties *in vitro* (Lin *et al*., 2003). Caryophyllene oxide has the distinction of being the component responsible for cannabis identification by drug-sniffing dogs (Stahl and Kunde, 1973).

Phytol (Table 2) is a diterpene (McGinty *et al*., 2010), present in cannabis extracts, as a breakdown product of chlorophyll and tocopherol. Phytol prevented vitamin A-induced teratogenesis by inhibiting conversion of retinol to a harmful metabolite, all-*trans*-retinoic acid (Arnhold *et al*., 2002). Phytol increased GABA expression via inhibition of succinic semialdehyde dehydrogenase, one of its degradative enzymes (Bang *et al*., 2002). Thus, the presence of phytol could account for the alleged relaxing effect of wild lettuce (*Lactuca sativa*), or green tea (*Camellia sinensis*), despite the latter's caffeine content.

Selected possibilities for phytocannabinoid-terpenoid synergy

Cannabis and acne

AEA simulates lipid production in human sebocytes of sebaceous glands at low concentrations, but induces apoptosis at higher levels, suggesting that this system is under ECS control (Dobrosi *et al*., 2008). CBD 10–20 μM did not affect basal lipid synthesis in SZ95 sebocytes, but did block such stimulation by AEA and arachidonate (Biro *et al*., 2009). Higher doses of CBD (30–50 μM) induced sebocyte apoptosis, which was augmented in the presence of AEA. The effect of CBD to increase

Ca^{++} was blocked by ruthenium red, a TRP-inhibitor. RNA-mediated silencing of TRPV1 and TRPV3 failed to attenuate CBD effects, but experiments did support the aetiological role of TRPV4, a putative regulator of systemic osmotic pressure (T. Bíró, 2010, pers. comm.). Given the observed ability of CBD to be absorbed transcutaneously, it offers great promise to attenuate the increased sebum production at the pathological root of acne.

Cannabis terpenoids could offer complementary activity. Two citrus EOs primarily composed of limonene inhibited *Propionibacterium acnes*, the key pathogen in acne (MIC 0.31 $\mu L \cdot mL^{-1}$), more potently than triclosan (Kim *et al.*, 2008). Linalool alone demonstrated an MIC of 0.625 $\mu L \cdot mL^{-1}$. Both EOs inhibited *P. acnes*-induced TNF-α production, suggesting an adjunctive anti-inflammatory effect. In a similar manner, pinene was the most potent component of a tea-tree eucalyptus EO in suppression of *P. acnes* and *Staph* spp. in another report (Raman *et al.*, 1995).

Considering the known minimal toxicities of CBD and these terpenoids and the above findings, new acne therapies utilizing whole CBD-predominant extracts, via multi-targeting (Wagner and Ulrich-Merzenich, 2009), may present a novel and promising therapeutic approach that poses minimal risks in comparison to isotretinoin.

MRSA

MRSA accounted for 10% of cases of septicaemia and 18 650 deaths in the USA in 2005, a number greater than that attributable to human immunodeficiency virus/acquired immunodeficiency syndrome (Bancroft, 2007). Pure CBD and CBG powerfully inhibit MRSA (MIC 0.5–2 $\mu g \cdot mL^{-1}$) (Appendino *et al.*, 2008).

Amongst terpenoids, pinene was a major component of *Sideritis erythrantha* EO that was as effective against MRSA and other antibiotic-resistant bacterial strains as vancomycin and other agents (Kose *et al.*, 2010). A *Salvia rosifolia* EO with 34.8% pinene was also effective against MRSA (MIC 125 $\mu g \cdot mL^{-1}$). The ability of monoterpenoids to enhance skin permeability and entry of other drugs may further enhance antibiotic benefits (Wagner and Ulrich-Merzenich, 2009).

Given that CBG can be produced in selected cannabis chemotypes (de Meijer and Hammond, 2005; de Meijer *et al.*, 2009a), with no residual THC as a possible drug abuse liability risk, a whole plant extract of a CBG-chemotype also expressing pinene would seem to offer an excellent, safe new antiseptic agent.

Psychopharmacological applications: depression, anxiety, insomnia, dementia and addiction

Scientific investigation of the therapeutic application of terpenoids in psychiatry has been hampered by methodological concerns, subjective variability of results and a genuine dearth of appropriate randomized controlled studies of high quality (Russo, 2001; Bowles, 2003; Lis-Balchin, 2010). The same is true of phytocannabinoids (Fride and Russo, 2006). Abundant evidence supports the key role of the ECS in mediating depression (Hill and Gorzalka, 2005a,b), as well as anxiety, whether induced by aversive stimuli, such as post-traumatic stress disorder (Marsicano *et al.*, 2002) or pain (Hohmann *et al.*, 2005), and psychosis (Giuffrida *et al.*, 2004). With respect to the latter risk, the presence of CBD in smoked cannabis based on hair analysis seems to be a mitigating factor reducing its observed incidence (Morgan and Curran, 2008). A thorough review of cannabis and psychiatry is beyond the scope of this article, but several suggestions are offered with respect to possible therapeutic synergies operative with phytocannabinoids-terpenoid combinations. While the possible benefits of THC on depression remain controversial (Denson and Earleywine, 2006), much less worrisome would be CBD- or CBG-predominant preparations. Certainly the results obtained in human depression solely with a citrus scent (Komori *et al.*, 1995), strongly suggest the possibility of synergistic benefit of a phytocannabinoid-terpenoid preparation. Enriched odour exposure in adult mice induced olfactory system neurogenesis (Rochefort *et al.*, 2002), an intriguing result that could hypothetically support plasticity mechanisms in depression (Delgado and Moreno, 1999), and similar hypotheses with respect to the ECS in addiction treatment (Gerdeman and Lovinger, 2003). Phytocannabinoid-terpenoid synergy might theoretically apply.

The myriad effects of CBD on $5\text{-}HT_{1A}$ activity provide a strong rationale for this and other phytocannabinoids as base compounds for treatment of anxiety. Newer findings, particularly imaging studies of CBD in normal individuals in anxiety models (Fusar-Poli *et al.*, 2009; 2010; Crippa *et al.*, 2010) support this hypothesis. Even more compelling is a recent randomized control trial of pure CBD in patients with social anxiety disorder with highly statistical improvements over placebo in anxiety and cognitive impairment (Crippa *et al.*, 2011). Addition of anxiolytic limonene and linalool could contribute to the clinical efficacy of a CBD extract.

THC was demonstrated effective in a small crossover clinical trial versus placebo in 11 agitated dementia patients with Alzheimer's disease (Volicer *et al.*, 1997). THC was also observed to be an acetylcholinesterase inhibitor in its own right, as well as preventing amyloid β-peptide aggregation in that disorder (Eubanks *et al.*, 2006). Certainly, the anti-anxiety and anti-psychotic effects of CBD may be of additional benefit (Zuardi *et al.*, 1991; 2006; Zuardi and Guimaraes, 1997). A recent study supports the concept that CBD, when present in significant proportion to THC, is capable of eliminating induced cognitive and memory deficits in normal subjects smoking cannabis (Morgan *et al.*, 2010b). Furthermore, CBD may also have primary benefits on reduction of β-amyloid in Alzheimer's disease (Iuvone *et al.*, 2004; Esposito *et al.*, 2006a,b). Psychopharmacological effects of limonene, pinene and linalool could putatively extend benefits in mood in such patients.

The effects of cannabis on sleep have been reviewed (Russo *et al.*, 2007), and highlight the benefits that can accrue in this regard, particularly with respect to symptom reduction permitting better sleep, as opposed to a mere hypnotic effect. Certainly, terpenoids with pain-relieving, anti-anxiety or sedative effects may supplement such activity, notably, caryophyllene, linalool and myrcene.

The issue of cannabis addiction remains controversial. Some benefit of oral THC has been noted in cannabis withdrawal (Hart *et al.*, 2002; Haney *et al.*, 2004). More intriguing, perhaps, are claims of improvement on other substance dependencies, particularly cocaine (Labigalini *et al.*, 1999; Dreher, 2002). The situation with CBD is yet more promising. CBD and THC at doses of 4 $mg \cdot kg^{-1}$ i.p. potentiated extinction of cocaine- and amphetamine-induced conditioned place preference in rats, and CBD produced no hedonic effects of its own (Parker *et al.*, 2004). CBD 5 $mg \cdot kg^{-1} \cdot d^{-1}$ in rats attenuated heroin-seeking behaviour by conditioned stimuli, even after a lapse of 2 weeks (Ren *et al.*, 2009). A suggested mechanism of CBD relates to its ability to reverse changes in α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate glutamate and CB_1 receptor expression in the nucleus accumbens induced by heroin. The authors proposed CBD as a treatment for heroin craving and addiction relapse. A recent study demonstrated the fascinating result that patients with damage to the insula due to cerebrovascular accident were able to quit tobacco smoking without relapse or urges (Naqvi *et al.*, 2007), highlighting this structure as a critical neural centre mediating addiction to nicotine. Further study has confirmed the role of the insula in cocaine, alcohol and heroin addiction (Naqvi and Bechara, 2009; Naqvi and Bechara, 2010). In a provocative parallel, CBD 600 mg p.o. was demonstrated to deactivate functional magnetic resonance imaging (fMRI) activity in human volunteers in the left insula versus placebo ($P < 0.01$) without accompanying sedation or psychoactive changes (Borgwardt *et al.*, 2008), suggesting the possibility that CBD could act as a pharmaceutical surrogate for insular damage in exerting an anti-addiction therapeutic benefit. Human studies have recently demonstrated that human volunteers smoking cannabis with higher CBD content reduced their liking for drug-related stimuli, including food (Morgan *et al.*, 2010a). The authors posited that CBD can modulate reinforcing properties of drugs of abuse, and help in training to reduce relapse to alcoholism. A single case report of a successful withdrawal from cannabis dependency utilizing pure CBD treatment was recently published (Crippa *et al.*, 2010).

Perhaps terpenoids can provide adjunctive support. In a clinical trial, 48 cigarette smokers inhaling vapour from an EO of black pepper (*Piper nigrum*), a mint-menthol mixture or placebo (Rose and Behm, 1994). Black pepper EO reduced nicotine craving significantly ($P < 0.01$), an effect attributed to irritation of the bronchial tree, simulating the act of cigarette smoking, but without nicotine or actual burning of material. Rather, might not the effect have been pharmacological? The terpenoid profile of black pepper suggests possible candidates: myrcene via sedation, pinene via increased alertness, or especially caryophyllene via CB_2 agonism and a newly discovered putative mechanism of action in addiction treatment.

CB_2 is expressed in dopaminergic neurones in the ventral tegmental area and nucleus accumbens, areas mediating addictive phenomena (Xi *et al.*, 2010). Activation of CB_2 by the synthetic agonist JWH144 administered systemically, intranasally, or by microinjection into the nucleus accumbens in rats inhibited DA release and cocaine self-administration. Caryophyllene, as a high-potency selective CB_2 agonist (Gertsch *et al.*, 2008), would likely produce similar effects, and have the advantage of being a non-toxic dietary component. All factors considered, CBD, with caryophyllene, and possibly other adjunctive terpenoids in the extract, offers significant promise in future addiction treatment.

Taming THC: cannabis entourage compounds as antidotes to intoxication

Various sources highlight the limited therapeutic index of pure THC, when given intravenously (D'Souza *et al.*, 2004) or orally (Favrat *et al.*, 2005), especially in people previously naïve to its effects. Acute overdose incidents involving THC or THC-predominant cannabis usually consist of self-limited panic reactions or toxic psychoses, for which no pharmacological intervention is generally necessary, and supportive counselling (reassurance or 'talking down') is sufficient to allow resolution without sequelae. CBD modulates the psychoactivity of THC and reduces its adverse event profile (Russo and Guy, 2006), highlighted by recent results above described. Could it be, however, that other cannabis components offer additional attenuation of the less undesirable effects of THC? History provides some clues.

In 10th century Persia, Al-Razi offered a prescription in his *Manafi al-agdhiya wa-daf madarri-ha* (p. 248), rendered (Lozano, 1993, p. 124; translation EBR) ' – and to avoid these harms {from ingestion of cannabis seeds or hashish}, one should drink fresh water and ice or eat any acid fruits'. This concept was repeated in various forms by various authorities through the ages, including ibn Sina (ibn Sina (Avicenna), 1294), and Ibn al-Baytar (ibn al-Baytar, 1291), until O'Shaughnessy brought Indian hemp to Britain in 1843 (O'Shaughnessy, 1843). Robert Christison subsequently cited lemon (Figure 3A) as an antidote to acute intoxication in numerous cases (Christison, 1851) and this excerpt regarding morning-after residua (Christison, 1848) (p. 973):

> Next morning there was an ordinary appetite, much torpidity, great defect and shortness of memory, extreme apparent protraction of time, but no peculiarity of articulation or other effect; and these symptoms lasted until 2 P.M., when they ceased entirely in a few minutes after taking lemonade.

Literary icons on both sides of the Atlantic espoused similar support for the citrus cure in the 19th century, notably Bayard Taylor after travels in Syria (Taylor, 1855), and Fitzhugh Ludlow after his voluntary experiments with ever higher cannabis extract doses in the USA (Ludlow, 1857). The sentiment was repeated by Calkins (1871), who noted the suggestion of a friend in Tunis that lemon retained the confidence of cure of overdoses by cannabis users in that region. This is supported by the observation that lemon juice, which normally contains small terpenoid titres, is traditionally enhanced in North Africa by the inclusion in drinks of the limonene-rich rind, as evidenced by the recipe for *Agua Limón* from modern Morocco (Morse and Mamane, 2001). In his comprehensive review of cannabis in the first half of the 20th century, Walton once more supported its prescription (Walton, 1938).

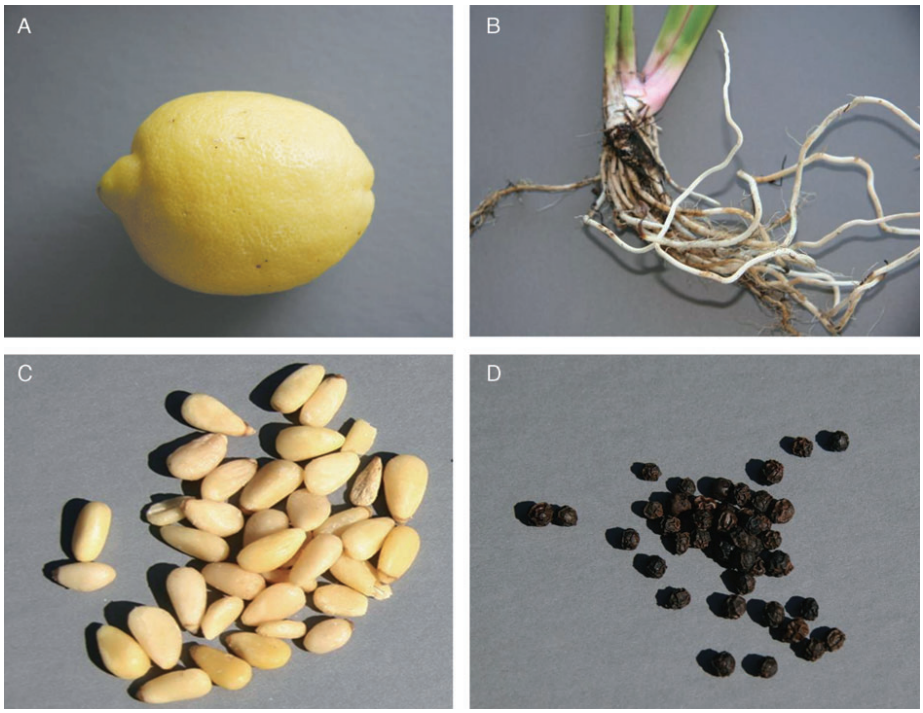


Figure 3

Ancient cannabis antidotes. (A) Lemon (*Citrus limon*). (B) Calamus plant roots (*Acorus calamus*). (C) Pine nuts (*Pinus* spp.). (D) Black pepper (*Piper nigrum*).

Another traditional antidote to cannabis employing *Acorus calamus* (Figure 3B) is evident from the Ayurvedic tradition of India (Lad, 1990, p. 131):

> Calamus root is the best antidote for the ill effects of marijuana. . . . if one smokes a pinch of calamus root powder with the marijuana, this herb will completely neutralize the toxic side effects of the drug.

This claim has gained credence, not only through force of anecdotal accounts that abound on the Internet, but with formal scientific case reports and scientific analysis (McPartland *et al.*, 2008) documenting clearer thinking and improved memory with the cannabis–calamus combination, and with provision of a scientific rationale: calamus contains beta-asarone, an acetylcholinesterase inhibitor with 10% of the potency of physotigmine (Mukherjee *et al.*, 2007). Interestingly, the cannabis terpenoid, α-pinene, also has been characterized as a potent inhibitor of that enzyme (Miyazawa and Yamafuji, 2005), bolstering the hypothesis of a second antidote to THC contained in cannabis itself. Historical precedents also support pinene in this pharmacological role.

In the firstt century, Pliny wrote of cannabis in his *Natural History, Book XXIV* (Pliny, 1980, p. 164):

> The gelotophyllis ['leaves of laughter' = cannabis] grows in Bactria and along the Borysthenes. If this be taken in myrrh and wine all kinds of phantoms beset the mind, causing laughter which persists until the kernels of pine-nuts are taken with pepper and honey in palm wine.

Of the components, palm wine is perhaps the most mysterious. Ethanol does not reduce cannabis intoxication (Mello and Mendelson, 1978). However, ancient wines were stored in clay pots or goatskins, and required preservation, usually with addition of pine tar or terebinth resin (from *Pistacia* spp.; McGovern *et al.*, 2009). Pine tar is rich in pinene, as is terebinth resin (from *Pistacia terebinthus*; Tsokou *et al.*, 2007), while the latter also contains limonene (Duru *et al.*, 2003). Likewise, the pine nuts (Figure 3C) prescribed by Pliny the Elder harbour pinene, along with additional limonene (Salvadeo *et al.*, 2007). Al-Ukbari also suggested pistachio nuts as a cannabis antidote in the 13th century (Lozano, 1993), and the ripe fruits of *Pistacia terebinthus* similarly contain pinene (Couladis *et al.*, 2003). The black pepper (Figure 3D), might offer the mental clarity afforded by pinene, sedation via myrcene and helpful contributions by β-caryophyllene. The historical suggestions for cannabis antidotes are thus supported by modern scientific rationales for the claims, and if proven experimentally would provide additional evidence of synergy (Berenbaum, 1989; Wagner and Ulrich-Merzenich, 2009).

Conclusions and suggestions for future study

Considered ensemble, the preceding body of information supports the concept that selective breeding of cannabis chemotypes rich in ameliorative phytocannabinoid and terpenoid content offer complementary pharmacological activities that may strengthen and broaden clinical applications and improve the therapeutic index of cannabis extracts containing THC, or other base phytocannabinoids. Psychopharmacological and dermatological indications show the greatest promise.

One important remaining order of business is the elucidation of mono- and sesquiterpenoid biosynthetic pathways in cannabis, as has been achieved previously in other species of plants (Croteau, 1987; Gershenzon and Croteau, 1993; Bohlmann *et al*., 1998; Turner *et al*., 1999; Trapp and Croteau, 2001).

Various cannabis component combinations or cannabis extracts should be examined via high throughput pharmacological screening where not previously accomplished. Another goal is the investigation of the biochemical targets of the cannabis terpenoids, along with their mechanisms of action, particularly in the central nervous system. Possible techniques for such research include radio-labelling of select agents in animals with subsequent necropsy. On a molecular level, investigation of terpenoid changes to phytocannabinoid signal transduction and trafficking may prove illuminating. While it is known that terpenoids bind to odorant receptors in the nasal mucosa (Friedrich, 2004) and proximal olfactory structures (Barnea *et al*., 2004), it would be essential to ascertain if direct effects in limbic or other cerebral structures are operative. Given that farnesyl pyrophosphate is a sesquiterpenoid precursor and the most potent endogenous agonist yet discovered for GPR92 (McHugh *et al*., 2010), *in silico* studies attempting to match minor cannabinoids and terpenoids to orphan GPCRs may prove fruitful. Behavioural assays of agents in animal models may also provide clues. Simple combinations of phytocannabinoids and terpenoids may demonstrate synergy as antibiotics if MICs are appreciable lowered (Wagner and Ulrich-Merzenich, 2009). Ultimately, fMRI and single photon emission computed tomography studies in humans, with simultaneous drug reaction questionnaires and psychometric testing employing individual agents and phytocannabinoid-terpenoid pairings via vaporization or oromucosal application, would likely offer safe and effective methods to investigate possible interactions and synergy.

Should positive outcomes result from such studies, phytopharmaceutical development may follow. The development of zero-cannabinoid cannabis chemotypes (de Meijer *et al*., 2009b) has provided extracts that will facilitate discernment of the pharmacological effects and contributions of different fractions. Breeding work has already resulted in chemotypes that produce 97% of monoterpenoid content as myrcene, or 77% as limonene (E. de Meijer, pers. comm.). Selective cross-breeding of high-terpenoid- and high-phytocannabinoid-specific chemotypes has thus become a rational target that may lead to novel approaches to such disorders as treatment-resistant depression, anxiety, drug dependency, dementia and a panoply of dermatological disorders, as well as industrial applications as safer pesticides and antiseptics. A better future via cannabis phytochemistry may be an achievable goal through further research of the entourage effect in this versatile plant that may help it fulfil its promise as a pharmacological treasure trove.

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Conflict of Interest

The author is a Senior Medical Advisor to GW Pharmaceuticals and serves as a consultant.

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MINI-REVIEW Open Access

An Update on Non-CB_1, Non-CB_2 Cannabinoid Related G-Protein-Coupled Receptors

Paula Morales* and Patricia H. Reggio

Abstract

The endocannabinoid system (ECS) has been shown to be of great importance in the regulation of numerous physiological and pathological processes. To date, two Class A G-protein-coupled receptors (GPCRs) have been discovered and validated as the main therapeutic targets of this system: the cannabinoid receptor type 1 (CB_1), which is the most abundant neuromodulatory receptor in the brain, and the cannabinoid receptor type 2 (CB_2), predominantly found in the immune system among other organs and tissues. Endogenous cannabinoid receptor ligands (endocannabinoids) and the enzymes involved in their synthesis, cell uptake, and degradation have also been identified as part of the ECS. However, its complex pharmacology suggests that other GPCRs may also play physiologically relevant roles in this therapeutically promising system. In the last years, GPCRs such as GPR18 and GPR55 have emerged as possible missing members of the cannabinoid family. This categorization still stimulates strong debate due to the lack of pharmacological tools to validate it. Because of their close phylogenetic relationship, the Class A orphan GPCRs, GPR3, GPR6, and GPR12, have also been associated with the cannabinoids. Moreover, certain endo-, phyto-, and synthetic cannabinoid ligands have displayed activity at other well-established GPCRs, including the opioid, adenosine, serotonin, and dopamine receptor families. In addition, the cannabinoid receptors have also been shown to form dimers with other GPCRs triggering cross-talk signaling under specific conditions. In this mini review, we aim to provide insight into the non-CB_1, non-CB_2 cannabinoid-related GPCRs that have been reported thus far. We consider the physiological relevance of these molecular targets in modulating the ECS.

Keywords: cannabinoid receptors; endocannabinoid system; GPCRs; orphan receptors

Introduction

The Class A G-protein-coupled receptors (GPCRs), cannabinoid receptor type 1 (CB_1) and cannabinoid receptor type 2 (CB_2), have been widely confirmed as cannabinoid targets. These receptors have been shown to be involved in numerous physiopathological processes, including pain, inflammation, cancer, metabolic syndromes, hypertension, and neurodegenerative disorders.[1] Nonetheless, the complex pharmacology of the endocannabinoid system (ECS) and its wide implication in numerous biological functions suggest the existence of other receptors playing important physiological roles. Consequently, extensive research is currently focused on the identification of potential missing cannabinoid receptors.

Diverse Class A orphans or lately deorphanized GPCRs have been proposed and evaluated as possible ECS members. Nonetheless, the lack of selective ligands for these receptors along with their intricate signaling pathways is delaying a clear elucidation of their relationship with the ECS. Therefore, thus far no other GPCR has been categorized as the cannabinoid receptor type 3 by the International Union of Pharmacology.[2]

Chemistry and Biochemistry Department, UNC Greensboro, Greensboro, North Carolina.

*Address correspondence to: Paula Morales, PhD, Chemistry and Biochemistry Department, UNC Greensboro, 1400 Spring Garden Street, Greensboro, NC 27412, E-mail: p_morale@uncg.edu

Herein, we intend to provide an overview of the GPCRs that have been postulated as cannabinoid molecular targets and the current available evidence of their relationship with the ECS. Non-GPCR targets of the cannabinoids such as the peroxisome proliferator-activated receptors, ligand-gated ion channels, or transient receptor potential channels have been revised elsewhere and are beyond the scope of this review.[3,4]

GPR55 and GPR18

Several GPCRs have been postulated to be putative cannabinoid receptors, but so far, only GPR18 and GPR55 have been demonstrated to be targets of a wide variety of endogenous, phytogenic, and synthetic cannabinoid ligands.[4] Despite this fact, inconsistencies in pharmacological data in the literature are hampering their categorization.[5,6]

The cannabinoid-related class A GPCR GPR55 displays low sequence identity with CB_1 and CB_2 (~13% and 14%, respectively). GPR55 is widely expressed in the brain, as well as in the peripheral system, co-localizing with the cannabinoid receptors in diverse tissues.[7–9] This receptor displays G-protein coupling promiscuity associating with $G_{\alpha 13}$,[8,10] $G_{\alpha q/11}$,[11] $G_{\alpha 12}$,[11] or $G_{\alpha 12/13}$[8,12] depending on the cell line or tissue. GPR55 has been implicated in different physiopathological conditions such as cancer,[13–15] pain,[11,16,17] metabolic disorders,[18,19] vascular functions,[20,21] bone physiology,[22] and motor coordination.[23]

The phospholipid lysophosphatidylinositol (LPI) is considered the endogenous GPR55 ligand.[8,24,25] In fact, GPR55 has also been named the LPI1 receptor.[26] Numerous CB_1 and CB_2 ligands have also been reported to act as GPR55 modulators.[6,27–29] However, significant pharmacological discrepancies have been found depending on the tested functional outcome.[6] For instance, the well-known phytocannabinoid Δ^9-tetrahydrocannabinol (Δ^9-THC) displayed activation of GPR55 according to certain reports,[10,11] while it was unable to exert any effect in other functional assays.[24,30] Cannabinoid ligands reported to be recognized by GPR55 and their intriguing pharmacology have been recently reviewed elsewhere.[31]

Although its sequence presents low identity with CB_1 and CB_2 (~13% and 8%), GPR18 has also been tightly associated with the ECS.[4,32] High expression of GPR18 has been found in the lymphoid tissues, while it is moderately expressed in other organs such as lungs, brain, testis, or ovary.[33,34] Initially, GPR18 was found to couple to $G_{\alpha i/o}$; however, subsequent findings suggested the participation of the $G_{\alpha q/11}$ transduction pathway as well.[34–36] Different reports have shown the therapeutic potential of this target in the treatment of pathologies such as intraocular pressure,[37] cancer,[38] or metabolic disorders[39] among others.

N-arachidonoyl glycine (NAGly) has been suggested to be the endogenous GPR18 ligand by several research groups.[32,34] However, other researchers were not able to confirm these data.[40] Recent investigations point to the existence of another endogenous GPR18 activator: the polyunsaturated fatty acid metabolite, Resolvin D2 (RvD2), which is mainly involved in inflammatory processes.[41] In addition, and despite the pharmacological divergences observed among some reports, GPR18 has been shown to recognize an array of CB_1 and/or CB_2 ligands of endogenous, phytogenic, or synthetic nature (reviewed by others).[39,42]

The pharmacological discrepancies on the appraisal of cannabinoids in these two receptors, as well as the lack of selective ligands targeting them, are delaying an insightful understanding of the relation of GPR55 and GPR18 with the ECS. These inconsistencies, which may rely on intrinsic properties of these GPCRs, or on the cell type or functional assay, need to be further studied. Intensive efforts are also focused on the structural understanding of these receptors,[43] as well as the development of more potent and selective pharmacological tools for the study of these promising therapeutic targets.

GPR3, GPR6, and GPR12

GPR3, GPR6, and GPR12 are three orphan Class A GPCRs that exhibit a very close phylogenetic relationship with the cannabinoid receptors CB_1 and CB_2 (Fig. 1). Indeed, they belong to the same cluster of receptors, the so-called MECA cluster (which consists of the melanocortin receptors, the endothelial differentiation GPCRs, the cannabinoid receptors, the adenosine binding receptors, and the orphan receptor subset GPR3, –6, and –12).[44,45] Because of their phylogenetic proximity, these orphan receptors share common conserved residues and unique sequence motifs with CB_1 and CB_2.[46] According to Fredriksson et al. these orphan receptors may share a common ancestor with the cannabinoid receptors since they share the same chromosomal positions.[45]

GPR3, GPR6, and GPR12, which share over 60% of sequence similarity, were first cloned in 1995.[47,48] These receptors constitutively activate adenylate cyclase by coupling to $G_{\alpha s}$ proteins. In fact, different

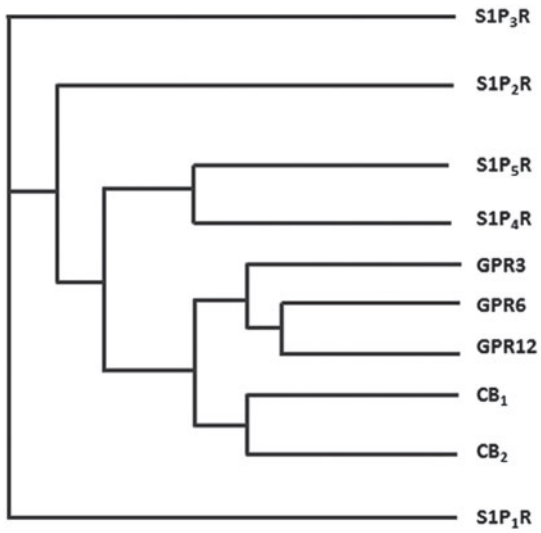


FIG. 1. Phylogenetic tree of cannabinoid receptors and the closely related Class A GPCRs (S1PR family and the orphan receptors GPR3, GPR6, and GPR12). Data were obtained from GPCRdb.org. CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; GPCR, G-protein-coupled receptor; S1P, sphingosine-1-phosphate.

groups have reported that when expressed in diverse cell lines, they can stimulate adenylate cyclase to levels similar in amplitude to agonist-activated GPCRs.[47,49,50] In addition to $G_{\alpha s}$, GPR6 and GPR12 have also been suggested to couple to $G_{\alpha i/o}$,[51,52] but further investigations are required to confirm this G-protein promiscuity.

GPR3, GPR6, and GPR12 are predominantly expressed in the brain and the reproductive system.[49] This family of constitutively active GPCRs is involved in neuronal differentiation and growth, as well as in the formation of synaptic contacts.[49] Therefore, their role in different neurological processes such as neurite outgrowth,[49] Alzheimer's disease,[53–57] development of cerebellar granule neurons,[58,59] neuropathic pain,[60] early phases of cocaine reinforcement,[61] emotional-like responses,[62] instrumental learning,[63] or Parkinson's disease[64,65] has been studied. Other pathophysiological conditions such as oocyte maturation,[66,67] dyslipidemia,[68] and cell proliferation[69] may also be impacted by the modulation of some of these receptors.

The bioactive lipids, sphingosine-1-phosphate[50,52] and/or sphingosylphosphorylcholine,[51] have been proposed as endogenous ligands of these receptors (Fig. 2). However, other groups were not able to confirm this claim, and consequently, GPR3, GPR6, and GPR12 are still categorized as orphans.[30,70,71] Interestingly, among the very few ligands discovered so far for these receptors, the nonpsychoactive phytocannabinoid cannabidiol (CBD) stands out as being able to target GPR3 and GPR6,[72] acting as a β-arrestin2 inverse agonist of both receptors. This functionality is of high interest in the GPR3 field because β-arrestin2 signaling at GPR3 has been directly linked to the manufacture of beta-amyloid plaque ($A\beta_{1-40}$ and $A\beta_{1-42}$) in Alzheimer's disease through complex formation with γ-secretase.[56,57] Because CBD is an inverse agonist of this signaling pathway at GPR3, it may represent a potential tool for the reduction of amyloid pathology. Other phytocannabinoids and several endocannabinoids were also tested but so far none of them were found to modulate this family of orphan receptors.[30,72]

So, a relationship between the cannabinoids and the orphan receptors GPR3, GPR6, and GPR12 has been evidenced. Nonetheless, extensive research and more pharmacological tools are needed to extract significant conclusions about the association of these receptors with the ECS and its ligands.

Alkylindole-Sensitive Receptors

As reported by different research groups, the well-known aminoalkylindole cannabinoid agonist WIN55,212-2 (Fig. 3) displays pharmacological functions independent of the cannabinoid receptors CB_1 and CB_2.[73–75] This fact led to the identification of novel targets commonly referred to as the alkylindole (AI)-sensitive receptors.[74,76,77] These cannabinoid-related receptors are modulated by AI derivatives, but not by other classes of cannabinoid ligands.[76] Diverse evidence suggests that the AI-sensitive receptors are $G_{\alpha s}$-protein coupled receptors that are mainly expressed in microglia and astrocytomas.[76–79] However, their biological functions, pharmacology, and therapeutic value remain to be unraveled due to the lack of selective pharmacological tools.

Recent studies from Stella and coworkers revealed the role of AI-sensitive receptors in the modulation of microglial cell migration and proliferation highlighting their potential in the treatment of gliomas.[77,78] Moreover, these authors have identified a series of naphthoyl AI derivatives, ST-11, ST-23, ST-25, and ST-48 (Fig. 3) among them, that bind to the AI-sensitive receptors.[78] These compounds display affinities in the nanomolar range when competing with [^{3}H]WIN55,212-2 in DBT

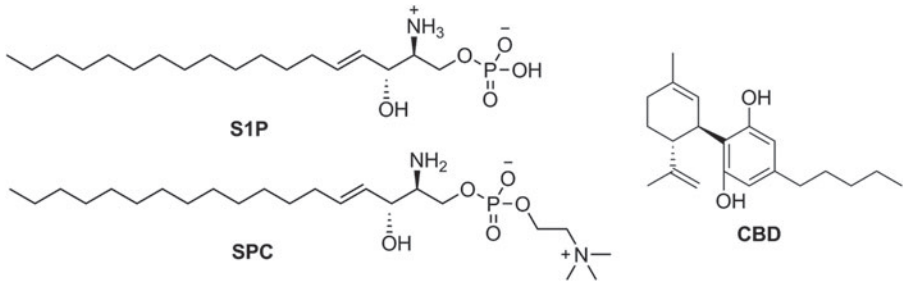


FIG. 2. Structures of the putative GPR3, GPR6, and GPR12 endogenous ligands S1P and SPC and the GPR3 and GPR6 inverse agonist CBD. SPC, sphingosylphosphorylcholine.

(Delayed Brain Tumor) cells which endogenously express AI-sensitive receptors, while lacking CB_1 and CB_2 receptors.[80] Compound ST-11 stands out from this study because of its potency at AI-sensitive receptors, while not interacting with CB_1 and CB_2 receptors. In addition, *in vitro* assays revealed that this compound inhibits cell migration and proliferation in the aforementioned mouse glioma cell line, DBT. Further studies revealed that ST-11 can reduce glioblastoma growth in a syngeneic mouse model.[81]

Even though extensive research is clearly needed to understand the pathophysiological function of these receptors, reported data suggest that AI-sensitive receptor agonists could represent a novel class of potential brain cancer antitumor drugs.

Cannabinoid-Related Oligomers

Numerous studies have shown that GPCRs, cannabinoid receptors among them, can exist and function as dimers or complexes of higher order.[82–85] This oligomerization may affect receptor signaling, receptor trafficking, and ligand binding. The physiological relevance of such dimerization has not yet been fully established for the cannabinoid receptors; nonetheless, the presence of cannabinoid homo- and heterodimers in specific tissues has been intensely reported over the last years.

For the CB_1 receptor, heteromers have been suggested to exist under certain physiological conditions with serotonin,[86] angiotensin,[87] opioid,[88–90] GPR55,[91] somatostatin,[92] orexin,[93,94] dopamine,[95–97] and adenosine[98] receptors among others (Table 1). Although CB_2 has been less investigated, recent research revealed that it may form heterodimers with CB_1,[99] with GPR55,[100,101] with the serotonin receptor $5HT_{1A}$,[102] or with the chemokine receptor CXCR4.[103] The expression of these heterodimers has been associated with different pathologies. For instance, the CB_2−CXCR4 and the CB_2−GPR55 dimers have been associated with cancer progression, while the CB_1−A_{2A} and the CB_1−D2 heteromers have been suggested to have physiological implications in neurodegenerative disorders such as

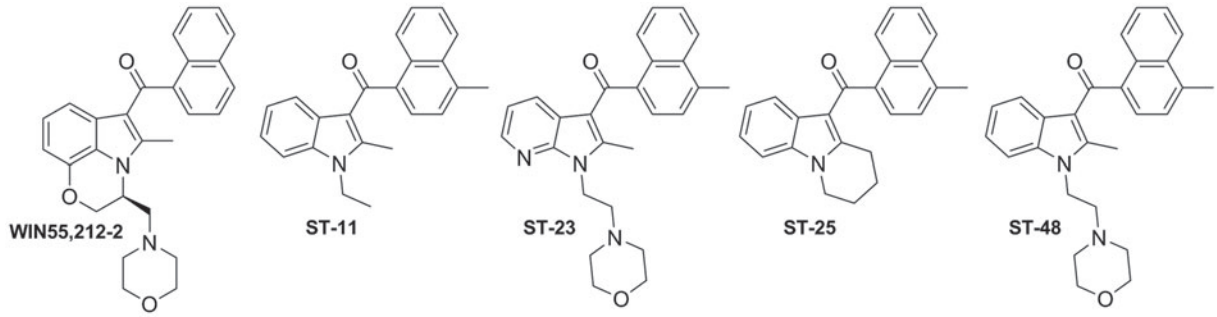
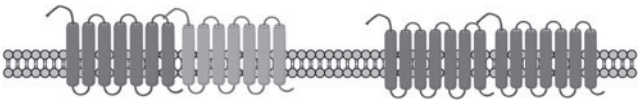


FIG. 3. Alkylindole derivatives WIN55,212–2, ST-11, ST-23, ST-25, and ST-48.

Table 1. Cannabinoid-Related G-Protein-Coupled Receptor Dimers Reported So Far



Heterodimers		Homodimers	
CB_1—D2	95,97	CB_1—CB_1	104–106
CB_1—A_{2A}	98	CB_2—CB_2	107–109
CB_1—$5HT_{2A}$	86		
CB_1—AT_1	87		
CB_1—GPR55	91		
CB_1—SST5	92		
CB_1—OX1	94		
CB_1—OX2	93		
CB_1—μOR	90		
CB_1—δOR	88		
CB_1—CB_2	99		
CB_2—GPR55	100,101		
CB_2—$5HT_{1A}$	102		
CB_2—CXCR4	103		

CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2.

Huntington's or Parkinson's diseases. All these data suggest that the ECS interacts in a significant manner with several other endogenous systems.

With regard to cannabinoid receptor homodimerization, more data have been published on CB_1 homodimers than on their CB_2 counterparts. The presence of CB_1 receptor homodimers has been reported in different biological tissues,[104–106] but their functional role has not been determined. In contrast, CB_2 homodimers have been evidenced,[107–109] but their pharmacological potential has not been explored yet.

In this field, bivalent ligands have emerged as promising new pharmacological entities and potential tools for the biological study of their respective dimeric receptors.[110–113] Despite their poor pharmacokinetic properties,[114] bivalent ligands can exhibit enhanced activity and selectivity over their respective corresponding parent ligands offering unique pharmacological strategies. Bivalent ligands have been synthesized and evaluated for several GPCRs. Opioid,[115,116] dopamine,[117,118] and histamine[119] are some of the receptors for which a bivalent compound provided higher activity than their monomer counterparts. CB_1 homobivalent[120–122] and heterobivalent[123–125] ligands have been also reported and explored. However, currently available receptor structural information challenges the fact that bivalent ligands can simultaneously bind to both receptors within the dimer, especially in the case of lipid receptors as the cannabinoids.[126] Therefore, novel drug design approaches to target dimers, as well as new techniques to determine bivalent binding, remain to be explored.

Homo- and heterodimerization likely influences the manner in which the ECS responds to ligands. Nevertheless, unambiguous data about their physical association in native tissues, as well as their pharmacology, are needed to clearly identify what biological functions are impacted by cannabinoid dimers.

Well-Established GPCRs Related to the Cannabinoids

Certain endo-, phyto-, and synthetic cannabinoid ligands have been shown to modulate well-known GPCRs. These GPCRs include members of established families such as the opioid, serotonin, muscarinic, dopamine, and adenosine families. For instance, the endocannabinoid anandamide has been shown to act at the adenosine receptor A_3,[127] the muscarinic acetylcholine receptors M1 and M4,[128] and the serotonin receptors 5-HT_{1A} and 5-HT_{2A}[129] among others. In addition, phytocannabinoids such as Δ^9-THC and CBD have been shown to modulate the μ— and δ—opioid receptors,[130] while other plant-derived compounds such as CBG (cannabigerol) and Δ^9-THCV (tetrahydrocannabivarin) display activity at the 5-HT_{1A} receptor.[131,132] Likewise, synthetic cannabinoids, such as the CB_1 inverse agonists taranabant (MK-0364) and rimonabant (SR141716), have also displayed activity in well-established targets. These include the adenosine A_3 and the tachykinin NK2 receptors.[133]

Some of these cannabinoid ligands have been proposed to interact allosterically with the aforementioned targets. It is worth mentioning that the efficacy that most of these cannabinoids exhibit toward these GPCRs is lower than the one displayed at the CB_1 and/or CB_2 receptors. Therefore, there is no evidence indicating a necessary recategorization of these receptors.

Other GPCRs

Because of their ability to recognize lipids and their relatively close phylogenetic relationship with CB_1 and CB_2, several other Class A orphan or recently deorphanized GPCRs such as GPR40, GPR43, GPR41, GPR120 (currently classified as free fatty acid receptors FFA1, FFA2, FFA3, and FFA4, respectively), GPR23, GPR92 (recently categorized as lysophosphatidic acid receptors LPA4 and LPA5), GPR84, GPR119, or GPR35 have been postulated as possible cannabinoid receptor candidates.[4] However, there is no available evidence since they do not meet some of the criteria established by the International Union of Pharmacology.[4,70]

Conclusions

Two cannabinoid receptors, CB_1 and CB_2, have been validated at the molecular level as the main targets of the ECS. These two GPCRs have been widely explored in the development of numerous pathophysiological processes, and their therapeutic potential for the treatment of different diseases has been extensively confirmed.[1] Great efforts are being made to structurally understand these receptors; in fact, CB_1 in its inactive[134,135] and active[136] states has been recently crystallized. Despite possible crystallization artifacts, these structures will help shedding light into the complex pharmacology of the cannabinoid receptors.

Growing evidence suggests that other cannabinoid or cannabinoid-like receptors remain to be identified as important players of the ECS. Different endogenous, phytogenic, and/or synthetic cannabinoid ligands have been reported to modulate GPCRs such as GPR18, GPR55, GPR3, GPR6, or the AI-sensitive receptors, among others. Pharmacological discrepancies and the lack of selective ligands for these receptors are delaying the characterization of their relationship with the ECS. Consequently, no CB_3 receptor has yet been confirmed.[2]

Adding more complexity to the ECS scenario, molecular interactions of the cannabinoid receptors with other GPCRs have been reported. Co-localization or co-immunoprecipitation data suggest the presence of cannabinoid homo- and heterodimers in specific native tissues. Cannabinoid receptor dimerization may not only influence the pharmacology of these receptors but also it may provide new signaling pathways through the interacting protomers. However, due to the lack of appropriate tools, there is still limited *in vivo* information about the expression of cannabinoid dimers. Hence, it remains a challenge to elucidate their therapeutic relevance under specific physiological conditions.

Currently, appropriate characterization of cannabinoid ligands should take into account the activity at the aforementioned GPCRs. Possible biased agonism of ligands, allosterism, or cross-talk signaling could be determining the intricate GPCR pharmacology. In addition, differential coupling and regulation of G-proteins or the formation of oligomers are among GPCR intrinsic properties that might be delaying the validation of novel potential cannabinoid targets. Therefore, further research is needed to fully understand the physiopathological role of these non-CB_1, non-CB_2 GPCRs in the modulation of the ECS.

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Author Disclosure Statement

No competing financial interests exist.

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Abbreviations Used

AI = alkylindole
CB_1 = cannabinoid receptor type 1
CB_2 = cannabinoid receptor type 2
CBD = cannabidiol
ECS = endocannabinoid system
GPCR = G-protein-coupled receptor
LPI = lysophosphatidylinositol
THC = tetrahydrocannabinol

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Some Prospective Alternatives for Treating Pain: The Endocannabinoid System and Its Putative Receptors GPR18 and GPR55

*Raquel Guerrero-Alba[1], Paulino Barragán-Iglesias[2], Abimael González-Hernández[3], Eduardo E. Valdez-Morales[4], Vinicio Granados-Soto[5], Miguel Condés-Lara[3], Martín G. Rodríguez[1] and Bruno A. Marichal-Cancino[1]**

[1] Departamento de Fisiología y Farmacología, Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Aguascalientes, Mexico, [2] School of Behavioral and Brain Sciences and Center for Advanced Pain Studies, University of Texas at Dallas, Richardson, TX, United States, [3] Departamento de Neurobiología del Desarrollo y Neurofisiología, Instituto de Neurobiología, Universidad Nacional Autónoma de México, Santiago de Querétaro, Mexico, [4] Cátedras CONACYT, Departamento de Cirugía, Centro de Ciencias Biomédicas, Universidad Autónoma de Aguascalientes, Aguascalientes, Mexico, [5] Neurobiology of Pain Laboratory, Departamento de Farmacobiología, Cinvestav, Mexico City, Mexico

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Thomas Heinbockel, Howard University, United States

***Correspondence:**
Bruno A. Marichal-Cancino
bruno.marichal@edu.uaa.mx;
br.marichal@gmail.com

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Background: Marijuana extracts (cannabinoids) have been used for several millennia for pain treatment. Regarding the site of action, cannabinoids are highly promiscuous molecules, but only two cannabinoid receptors (CB_1 and CB_2) have been deeply studied and classified. Thus, therapeutic actions, side effects and pharmacological targets for cannabinoids have been explained based on the pharmacology of cannabinoid CB_1/CB_2 receptors. However, the accumulation of confusing and sometimes contradictory results suggests the existence of other cannabinoid receptors. Different orphan proteins (e.g., GPR18, GPR55, GPR119, etc.) have been proposed as putative cannabinoid receptors. According to their expression, GPR18 and GPR55 could be involved in sensory transmission and pain integration.

Methods: This article reviews select relevant information about the potential role of GPR18 and GPR55 in the pathophysiology of pain.

Results: This work summarized novel data supporting that, besides cannabinoid CB_1 and CB_2 receptors, GPR18 and GPR55 may be useful for pain treatment.

Conclusion: There is evidence to support an antinociceptive role for GPR18 and GPR55.

Keywords: GPR18, GPR55, endocannabinoid system, cannabinoid receptors, pain

PHYSIOLOGY OF PAIN

Adaptive Function of Pain

Pain involves unpleasant sensations in response to real or potential tissue damage (Basbaum et al., 2009). Usually, pain unleashes a signal alert to prevent extensive injury by promoting defensive (passive and/or active) actions against the noxious (nociceptive) stimuli. Thus, pain is considered a protective and adaptive mechanism. However, pain may become persistent

and pathological without a recognized protective or adaptive mechanism. When this happens, it affects the quality of life of patients and their social environment. Hence, pathological pain is an important medical problem causing distress and disability that requires prompt clinical investigation and treatment (Julius and Basbaum, 2001; Moffat and Rae, 2011). On the other hand, considering that tissue damage is not always the main origin of pain, cognitive perception and somatic sensation should be considered as related but different phenomena. Cognitive perception involves a psychological component frequently related with emotional experiences. Therefore, pain may be cataloged as a subjective event that requires patient awareness (Basbaum and Woolf, 1999; Julius and Basbaum, 2001; Walker and Hohmann, 2005).

Sensory System: Anatomical and Functional View

The terminal endings of primary afferent neurons whose cell bodies are located in the dorsal root ganglia (DRG) and trigeminal ganglia (TG) are responsible for the transmission of multiple peripheral stimuli (proprioceptive or nociceptive) to the central nervous system (Julius and Basbaum, 2001; Walker and Hohmann, 2005). In the case of nociceptive transmission, two main types of pseudo-unipolar nociceptive neurons are found in those ganglia: (1) non-myelinated small diameter and multimodal C-fibers, which conduct electrical impulses at low speed (∼1 m/s), sensing and transducing thermal, chemical and mechanical stimuli; and (2) thinly myelinated Aδ-fibers that show fast conduction velocity (∼5–30 m/s), sensing mechanical and thermal stimuli (Moffat and Rae, 2011). These primary afferent nociceptive fibers sense the peripheral nociceptive environment and send the nociceptive information to the spinal dorsal horn where they make a synapse with second order neurons, which convey neuronal firing to supraspinal sites where the action potentials are decoded and perceived as pain. At the peripheral level, there are several channels and receptors involved in the initiation of nociceptive transmission, such as the transient receptor potential vanilloid type 1 (TRPV1) channel, tetrodotoxin-resistant (Na^+-TTXr) voltage-gated sodium (Na^+) channels, purinergic P_2X receptors, serotonin ($5\text{-}HT_3$) channel receptor, and calcium (Ca^{2+}) channels, among others.

The nociceptive signal from the peripheral nociceptive fibers is directed toward a second order neuron into the spinal cord, and then the electrical signal is conducted to the brain cortex mainly through the antero-lateral pathway tract where the signal is interpreted as a painful sensation (Snider and McMahon, 1998; Steeds, 2009; Fabbro and Crescentini, 2014). In fact, several sensorial components such as stimuli identification, location, and emotional components are codified in the cortex (Albe-Fessard et al., 1985). The diversity of peripheral and central regions and mechanisms implicated made the control of nociception and pain a complex challenge. Finally, we must keep in mind that nociceptive transmission could be endogenously modulated. For instance, the spinal cord, which is the first relay of nociceptive transmission, could be modulated by diverse neuromodulators (noradrenergic, serotonergic, opioidergic, and oxytocinergic) (for references see Mason, 2001; Vanegas and Schaible, 2004; Loyd and Murphy, 2009; Condés-Lara et al., 2015; Llorca-Torralba et al., 2016) that may diminish or increase the noxious sensation. Nevertheless, these modulatory systems exist along the noxious pathways, including the cortical station. So, the modulation of nociceptive transmission is complex and involves an array of neurotransmitters, neuromodulators and a wide variety of specific and non-specific receptors, which are dysregulated during pathological pain states (Heinricher, 2016).

Classic Treatments for Pain

Pain treatment can be categorized as pharmacologic and non-pharmacologic. In the first case, there are a variety of druggable targets in both central and peripheral nervous system commonly used for pain treatment. Analgesics are classified as: (i) non-opioid analgesics; (ii) opioid analgesics; and (iii) adjuvant analgesics (**Figure 1**). The most frequently non-opioid analgesics used are non-steroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen and celecoxib. The primary mechanism of action of NSAIDs is through the inhibition of the cyclooxygenase enzymes (COX) by consequently decreasing the action of prostaglandins and their sensitizing properties. Opioid-like drugs, such as morphine, ameliorate pain by modulating the cellular excitability at the supraspinal, spinal and peripheral level through activation of opioid receptors (μ-, δ-, and κ-opioid receptors). Furthermore, opioids could enhance descending inhibitory pathways and modify the sensory and affective components of pain. In the case of adjuvants, local anesthetics (e.g., lidocaine) stop the electrical impulse by blocking voltage-gated sodium (Na^+) channels. Tricyclic and noradrenaline-reuptake inhibitors act by maintaining and/or augmenting the monoamine levels in descending tracts and anticonvulsants decrease the synaptic transmission affecting neuronal excitability (Basbaum and Woolf, 1999; Sinha et al., 2017).

Non-steroidal Anti-inflammatory Drugs

Non-steroidal anti-inflammatory drugs are substances that inhibit a component of the inflammatory cascade and, thence, are an important therapeutic option for non-steroid-based pain treatment. Briefly, these compounds (with exception of acetaminophen) have anti-inflammatory, antipyretic, and analgesic effects by inhibiting COX activity. At this point, we must keep in mind that the COX enzymes have at least three isoforms (COX-1, COX-2 and COX-3) and the non-selective NSAIDs act to block COX-1 and COX-2 indistinctly, favoring gastrointestinal and renal side effects (mediated by COX-1 inhibition). These side effects are particularly common in the elderly, who are most likely to experience chronic pain (Griffin et al., 1991; Buffum and Buffum, 2000; Horl, 2010). To minimize the side effects, selective COX-2 inhibitors have arrived at clinical practice. Unfortunately, several clinical trials have shown that these inhibitors also increase harmful cardiovascular effects (Bhosale et al., 2015).

Opioid-Based Treatments

Opioid analgesics act in the central nervous system and are typically prescribed to patients suffering chronic pain

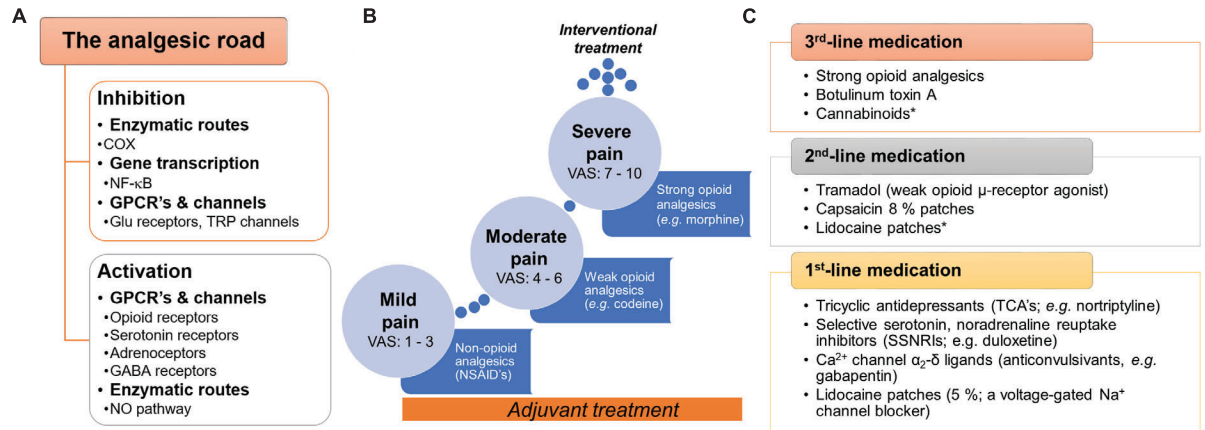

FIGURE 1 | General view of the current pharmacotherapy and guidelines used to treat pain. **(A)** Snapshot of the two main pathways (inhibition or activation) by which analgesic drugs induce pain relief at peripheral, spinal and supraspinal levels. **(B)** To treat pain, the WHO proposed the three-step analgesic ladder. Although primarily for the management of cancer pain, it is also used as a general guideline for the management of acute and chronic non-malignant pain. A key characteristic in this approach is the use of adjuvants** with the primary drug along the pain treatment. **(C)** In the case of neuropathic pain, specific clinical guidelines have been proposed by several international and regional professional associations. Although several recent clinical trials support these guidelines, we need to keep in mind that several factors could limit the applicability in real-world settings (i.e., neuropathic pain is a syndrome caused by diverse etiologies and different clinical manifestations). In general terms, three-line medication has been proposed by several professional associations (including the IASP, EFNS, NICE, and CPS). In all cases, the use of strong opioids is recommended as a 3rd-line medication agent considering the potential risk for abuse, overdose, mortality or misuse. *No conclusive efficacy on neuropathic pain treatment. **Adjuvants or co-analgesics are drugs non-specifically designed (or marketed) to treat pain; some examples: glucocorticoids, antidepressants (some SNRIs and TCAs), α_2-adrenergic agonists (e.g., clonidine) and cannabinoids (including cannabis). COX, cyclooxygenase or prostaglandin-endoperoxide synthase; CPS, Canadian Pain Society; EFNS, European Federation of Neurological Sciences; GPCRs, G protein-coupled receptors, IASP, International Association for the Study of Pain; NICE, National Institute for Health and Care Excellence (of the United Kingdom); WHO, World Health Organization.

refractory to non-opioid treatment. Despite their well-known side effects (sedation, nausea, vomiting, constipation, pruritus and respiratory depression), opioids are widely accepted as effective for acute pain as well as cancer pain. This group of drugs have high abuse liability and are also toxic in elevated doses. For instance, from 1999 to 2014, more than 165,000 persons died of overdose related to opioids in the Unites States. In 2013, an estimated of 1.9 million people abused or were dependent on opioid pain medication (Dowell et al., 2016). Moreover, placebo-controlled trials indicate that, on average, opioids do not result in a clinically significant reduction of chronic pain symptoms (Martell et al., 2007), and even in cases where opioid analgesia is adequate for the individual patient, analgesic effects are typically not maintained during the long-term opioid pharmacotherapy due to pharmacokinetic or pharmacodynamic tolerance (Ballantyne and Shin, 2008; Dumas and Pollack, 2008). Eventually, chronic exposure to opioids results in hyperalgesia (Chu et al., 2008).

Antidepressants

Antidepressant drugs have been used as analgesics in chronic pain disorders for decades (Mico et al., 2006). Their pharmacological mechanisms have been associated with the ability to block 5-hydroxytriptamine (serotonin or 5-HT) and noradrenaline re-uptake and consequently with an increase of the activity of the endogenous analgesic system. Tricyclic antidepressants (TCAs) (e.g., amitriptyline and imipramine), tetracyclic antidepressants (TeCAs) (e.g., amoxapine, maprotiline) and the selective serotonin-norepinephrine reuptake inhibitors (SNRIs) (e.g., duloxetine and venlafaxine) are traditionally used to treat chronic pain (Mika et al., 2013). TCAs have been shown to be effective for different neuropathic pain conditions in randomized controlled trials (Finnerup et al., 2010). TCAs are generally reasonably well-tolerated but high doses are associated with a high risk of sudden cardiac death (Ray et al., 2004). The SNRIs duloxetine and venlafaxine have a well-documented efficacy in painful poly-neuropathy (Finnerup et al., 2010). SNRIs are generally well tolerated. However, the most common side-effects reported are nausea, somnolence, dizziness, constipation, anorexia, dry mouth, hyperhidrosis, and sexual dysfunction (Stahl et al., 2005).

Anticonvulsants

Gabapentin and pregabalin are anticonvulsants with therapeutic activity against neuropathic pain (Rajapakse et al., 2015). Their analgesic mechanism has been associated to their binding to the $\alpha_2\delta_1$ subunit, which in turn blocks voltage-gated calcium (Ca^{2+})-channels at presynaptic sites (Gee et al., 1996) or NMDA receptors at post-synaptic neurons (Chen et al., 2018; Ma et al., 2018). Both drugs are well tolerated but the most common side-effects are somnolence and dizziness, peripheral edema, weight gain, nausea, vertigo, asthenia, dry mouth, and ataxia (Quintero, 2017). Other anticonvulsants used for pain relief are carbamazepine and its analog oxcarbazepine, lamotrigine and valproate. Lamotrigine is effective for central post-stroke pain (Vestergaard et al., 2001) and diabetic neuropathy (Eisenberg et al., 2001), but has failed to relieve pain in patients

with multiple sclerosis (Breuer et al., 2007) and neuropathic pain (Silver et al., 2007). Valproate also has a limited role in the treatment of neuropathic pain (Drewes et al., 1994; Otto et al., 2004; Agrawal et al., 2009).

Cannabinoids

One alternative for pain treatment came from Asia more than 3000 years ago: marijuana extracts (Li, 1974; Touw, 1981; Jensen et al., 2015). The utility of marijuana-based drugs for treating pain is explained by the existence of an ancient system of cellular control named the endocannabinoid system (ECS). Unfortunately, our knowledge about the physiology of the ECS is only partial (see below). In this review, we summarized novel data supporting that, apart from cannabinoid type-1 (CB_1) and cannabinoid type-2 (CB_2) receptors, some putative cannabinoid receptors (i.e., GPR18 and GPR55) may be useful for pain treatment. This should allow researchers to focus their studies on developing endocannabinoid-based options as analgesics and anti-inflammatory drugs.

ENDOCANNABINOIDS AND PAIN

Endocannabinoid System: Generalities

Despite the ancient and well-known use of cannabis derivatives for pain management, medically recognized use of these compounds has largely subsided due to the lack of knowledge of its molecular pharmacology, its abuse for recreational purposes and additional undesirable effects, such as hypomotility and hypothermia (Crawley et al., 1993), impairments in executive function (Crean et al., 2011) and memory consolidation (Ranganathan and D'Souza, 2006). However, the identification of the major psychoactive component Δ^9-tetrahydrocannabinol (Δ^9-THC) (Gaoni and Mechoulam, 1964), and the subsequent isolation of cannabinoid receptors (CB_1 and CB_2 receptors, both G-proteins-coupled receptors linked to $G_{i/o}$ proteins) with high expression levels in the nervous system, led to an explosion of studies exploring the ECS and its regulatory functions in health and disease. Briefly, the ECS consists of endogenous cannabinoids (endocannabinoids, eCBs), cannabinoid receptors, enzymes responsible for synthesis and degradation of eCBs and all genes related to them (Mackie, 2008a,b).

In this context, although several cannabinoids are available, current literature about their potential use for pain treatment remains controversial (Davis, 2014). Indeed, as reviewed by Nurmikko et al. (2007) and Martin-Sanchez et al. (2009), Δ^9-THC or Δ^9-THC plus cannabidiol induced relief in only one among six to nine patients (number needed to treat, NNT = 6–9). Moreover, the number needed to harm (NNH) (motor and cognitive dysfunction and altered perception) ranged between five and eight. These data suggest that, apart from its low efficacy, Δ^9THC could have a narrow therapeutic index. Nevertheless, the above cannabimimetic effects seem to be mainly mediated by CB_1 receptor activation, suggesting that other parts of the ECS could be druggable to treat pain. In addition, one of the physiological functions attributed to the eCBs is to suppress pain (Walker and Huang, 2002).

Endogenous Cannabinoids

The first eCB isolated in the brain was *N*-arachidonoyl ethanolamide (AEA), or anandamide (a name taken from the Sanskrit word Ananda, which means "bliss, joy," and amide) (Devane et al., 1992; **Figure 2**). AEA is a fatty acid neuromodulator derived from the non-oxidative metabolism of arachidonic acid (AA). The second endocannabinoid identified was 2-arachidonoyl glycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995). As the search for endogenous Δ^9-THC-like compounds continued, other bioactive lipids were extracted from animal tissues. These include noladin ether (Hanus et al., 2001), virodhamine (Porter et al., 2002) and *N*-arachidonoyl dopamine (NADA) (Huang et al., 2001).

The most widely investigated eCBs are anandamide and 2-AG. Indeed, anandamide is present in about 170-fold lower levels of brain tissue than 2-AG (Stella et al., 1997), and both lipidic derivatives activate cannabinoid CB_1 and CB_2 receptors. Certainly, anandamide shows preferential affinity for CB_1 (K_i = 89 nM) compared to CB_2 (K_i = 371 nM) receptors (Gauldie et al., 2001), whereas 2-AG is considered a full agonist at both CB_1 and CB_2 receptors (Sugiura and Waku, 2000). Nevertheless, it has been shown that AEA could activate the vanilloid type-1 receptor (TRPV1), which contributes to the many non-CB_1-mediated effects (Zygmunt et al., 1999; Smart et al., 2000). Furthermore, AEA and other eCBs (palmitoylethanolamide [PEA] and oleylethanolamide [OEA]) also are agonists of the peroxisome proliferator-activated receptor α (PPARα) (Fu et al., 2003; Bouaboula et al., 2005; Lo Verme et al., 2005). PEA also has a well-established role in pain modulation and inflammation in rodents (Jaggar et al., 1998; Calignano et al., 2001; Lo Verme et al., 2005; D'Agostino et al., 2007; González-Hernández et al., 2015), whereas in humans PEA treatment seems to relieve neuropathic pain (Calabro et al., 2010; Conigliaro et al., 2011; Gatti et al., 2012).

The eCBs are atypical neurotransmitters and/or neuromodulators. They are not stored in synaptic vesicles and are not released from presynaptic terminals via an exocytotic mechanism. In fact, their precursors exist in the cell membrane, are cleaved by specific enzymes "on demand" depending on intracellular calcium increase and are released from cells immediately after their production. The synthesis, release and deactivation of the endogenous cannabinoids are tightly regulated processes. As discussion of these processes is beyond the scope of this review, the interested reader is referred to several reviews on the topic (Howlett, 2002; Piomelli, 2003; Simon and Cravatt, 2006; Okamoto et al., 2007; Ueda et al., 2011; Luchicchi and Pistis, 2012).

Cannabinoid Receptors

To date, there are two known cannabinoid receptors that are part of the ECS, the CB_1 and CB_2 receptors. These receptors belong to the 7-transmembrane G-protein coupled receptors (GPCRs) primarily coupled to $G_{i/o}$ proteins that inhibit adenylyl cyclase (AC) and increase mitogen-activated protein kinase (MAPK) activity downstream of β-arrestin (Howlett, 2002; Vasileiou et al., 2013). Activation of these receptors triggers the inwardly

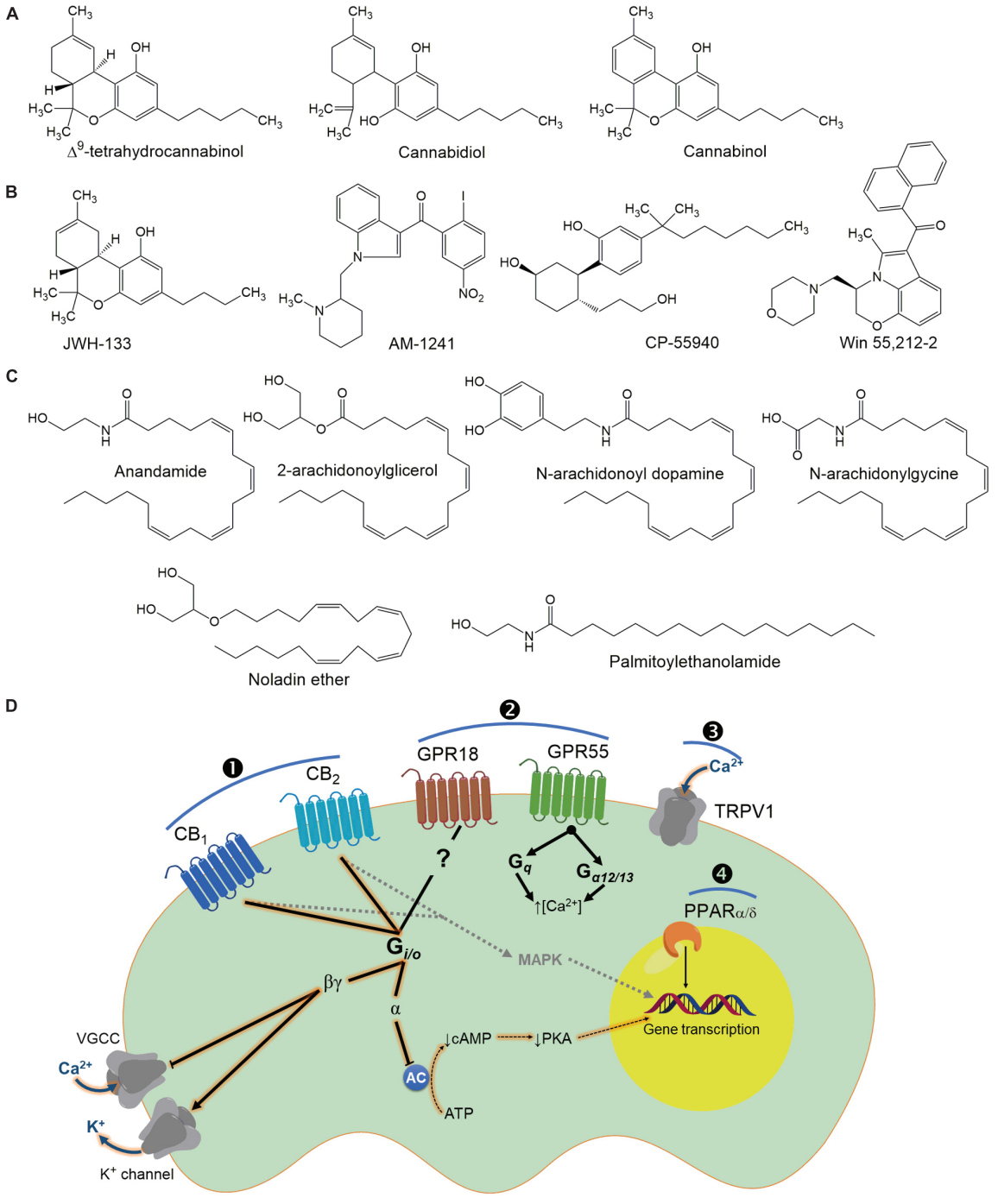

FIGURE 2 | Chemical structures of some plant **(A)**, synthetic cannabinoids **(B)** and endocannabinoids **(C)** that bind to cannabinoid receptors **(D)**. It is interesting to note that cannabinoids could activate intracellular pathways by direct activation of its receptors (\protect❶ and ❷) or modulate other family receptors (\protect❸ and ❹), which contribute to the biological effect of these molecules (particularly for the endocannabinoids). In general terms, classic cannabinoid receptors (CB_1 and CB_2) are GPCRs, which are canonically coupled to $G_{i/o}$ proteins. Consequently, under $CB_{1/2}$ receptors: (i) a decrease of adenylyl cyclase (AC) activity; (ii) an inactivation of Ca^{2+} channels; and (iii) activation of inwardly rectifying K^+ channels are achieved. These are signal transduction systems associated with inhibition of neurotransmitter release. The inhibition of AC occurs *via* activation of $G\alpha_i$-mediated signaling whereas $G\alpha_o$-activation results in inhibition of voltage-dependent Ca^{2+} channels (VDCCs) through the release of associated βγ subunits (apparently CB_2 receptors are ineffective, compared with CB_1, for shifting ionic currents via βγ subunits). In addition to PKA inhibition, $CB_{1/2}$ receptor signaling also leads to the downstream activation of MAPK which can regulate nuclear transcription factors and consequently expression of several genes. Note that GPR18 seems to be coupled to $G_{i/o}$ proteins, whereas GPR55 has been associated with an increase of intracellular Ca^{2+} via $G_{\alpha 12/13}$. In the case of TRPV1 channels (a non-selective cation channel for Ca^{2+}, Mg^{2+}, and Na^+ ions), it is well-known that agonist can be used rationally for the treatment of pain considering that this channel under constant activation desensitizes the nociceptive neuron. Finally, although not fully investigated, cannabinoid compounds could also activate $PPAR_{\alpha/\delta}$, which are involved in pain modulation and transmission.

rectifying potassium (K^+)-channels and A-type potassium (K^+)-channel currents and inhibits N-Type and P/Q type calcium (Ca^{2+})-channel activity (Demuth and Molleman, 2006). The CB_2 receptor is also negatively coupled to adenylyl cyclase but it seems not to be coupled to calcium (Ca^{2+})-channels (Felder et al., 1995). However, CB_1 receptors can also interact with G_s and $G_{q/11}$ under certain conditions and with certain agonists (Mackie, 2005, 2008b). In addition, a pair of orphan-related receptors (GPR18 and GPR55) is also described as cannabinoid putative receptors.

CB_1 receptor expression

The CB_1 receptor is highly expressed in the cortex, cerebellum and associational cortical regions of neocortex (Glass et al., 1997). It is also expressed in the spinal dorsal horn (Sanudo-Pena et al., 1999) and in DRG neurons (Hohmann and Herkenham, 1999; Salio et al., 2002; Walker and Hohmann, 2005). Autonomic nerve terminals express CB_1 receptors (Ishac et al., 1996; Vizi et al., 2001), which negatively modulate the sympathetic tone (Marichal-Cancino et al., 2013). Low levels of these receptors have been reported in the adrenal gland, thymus, heart, bone marrow, tonsils, prostrate, uterus, ovary and lung (Galiegue et al., 1995; Rice et al., 1997). A key characteristic of this receptor is the formation of heterodimers, suggesting that intracellular signaling could change under different conditions (Callen et al., 2012; Laprairie et al., 2012; Straiker et al., 2012).

CB_2 receptor expression

The CB_2 receptor is mostly expressed on cells of the immune system and spleen (Munro et al., 1993; Galiegue et al., 1995; Di Marzo et al., 2004). A few studies have found CB_2 immunoreactivity expression in glial and neuronal cells in some areas of the rodent brain (Gong et al., 2006; Onaivi et al., 2006), but this expression remains controversial (Hohmann and Herkenham, 1999; Salio et al., 2002; Walker and Hohmann, 2005). Notably, nerve injury and inflammation upregulate expression of CB_2 receptors in neurons and microglia (Beltramo et al., 2006; Rahn and Hohmann, 2009; Sagar et al., 2009; Hsieh et al., 2011). Furthermore, some studies have demonstrated the presence of CB_2 receptors in the DRG and afferent fibers in the spinal dorsal horn (Ross et al., 2001; Anand et al., 2008).

Role of CB_1 and CB_2 Receptors on Primary Afferent Neurons

DRG neurons express CB_1 receptors (Hohmann and Herkenham, 1999; Ross et al., 2001; Price et al., 2003). This receptor is synthesized in the cell neuronal bodies and inserted on both central and peripheral terminals (Hohmann and Herkenham, 1999; Hohmann et al., 1999). CB_1 receptors are mainly expressed in myelinated fibers of DRG neurons (Hohmann and Herkenham, 1999; Salio et al., 2002; Bridges et al., 2003) and also co-localize with CGRP, TRPV1 and IB4 (Hohmann and Herkenham, 1999; Hohmann et al., 1999; Ahluwalia et al., 2000; Bridges et al., 2003; Veress et al., 2013).

Nerve injury enhances CB_1 receptor expression in the DRG and spinal cord (Lim et al., 2003; Wang et al., 2007; Shiue et al., 2017) and other brain areas related with the emotional component of pain (Knerlich-Lukoschus et al., 2011). These data give an anatomical basis for the involvement of CB_1 receptors in modulating neuropathic pain. In this regard, it has been shown that systemic and local administration of CB_1 receptor agonists produce anti-nociceptive effects in neuropathic pain models (Herzberg et al., 1997; Fox et al., 2001; Bridges et al., 2003; Yu et al., 2010). Moreover, deletion of CB_1 receptors in peripheral (but not at spinal or supraspinal level) nociceptors reduced analgesia by local or systemic (but no intrathecal) CB_1 receptor agonists (Agarwal et al., 2007). Thus, CB_1 receptors located at primary afferent neurons constitute the prime target for producing cannabinoid analgesia.

Some of the peripheral antinociceptive effects of cannabinoids may occur through interaction with another receptor system. In this regard, an early work in rat nodose ganglion neurons showed that cannabinoid agonists inhibited 5-HT-induced currents in a concentration-dependent manner. The inward current was sensitive to the serotonin ($5\text{-}HT_3$) receptor antagonist MDL72222, suggesting a cannabinoid-mediated inhibition of serotonin ($5\text{-}HT_3$) currents (Fan, 1995). Later, *in vivo* experiments demonstrated that application of CB_1 and CB_2 receptor agonists attenuated the activity of rat peripheral ($5\text{-}HT_3$) receptors on the terminals of cardiopulmonary afferent C-fibers (Godlewski et al., 2003) through an allosteric interaction at a ($5\text{-}HT_3$) modulatory site (Barann et al., 2002). Moreover, the inhibitory effects of cannabinoids may occur through a synergistic action with opioid receptors and their signal transduction pathways (Pugh et al., 1996; Smith et al., 1998; Manzanares et al., 1999; Massi et al., 2003; Scavone et al., 2013) or by a cannabinoid-mediated increase in opioid peptide synthesis and release of endogenous opioids such as enkephalins and dynorphins (Corchero et al., 1997a,b; Valverde et al., 2001).

The use of cannabinoid agonists as analgesic drugs is limited due to adverse effects in the CNS (Clermont-Gnamien et al., 2002; Attal et al., 2004; Turcotte et al., 2010). However, since it has been demonstrated that CB_1 receptors are expressed at primary afferent neurons (Agarwal et al., 2007), the synthesis of CB_1 receptor agonists with limited CNS penetration is under development (Clapper et al., 2010; Yu et al., 2010).

The molecular mechanisms by which the CB_1 receptor has peripheral antinociceptive effects are not completely understood. It is known that CB_1 receptor, coupled to $G_{i/o}$ protein, can modulate several cellular mechanisms, all of which can reduce the excitability of neurons (e.g., opening of inward rectifying potassium (K^+)-channels and A-type potassium (K^+)-channels, and inhibiting N-Type and P/Q type calcium (Ca^{2+})-channels) (Demuth and Molleman, 2006). Moreover, there are several studies showing that cannabinoids can modulate the activity of transient receptor potential (TRP) channels, which are implicated in the modulation of pain processing. For example, multiple studies have shown that activation of the CB_1 receptor suppresses capsaicin-induced hyperalgesia in afferent neurons (Ko and Woods, 1999; Li et al., 1999; Johanek et al., 2001; Millns et al., 2001; Johanek and Simone, 2004; Santha et al., 2010). However, there are controversial findings regarding the effects of CB_1 receptor agonists on TRPV1 channels, because the CB_1 receptor agonist anandamide exerts dual effects on afferent

neurons, depending on the concentration used (Ross, 2003; Evans et al., 2004; Sousa-Valente et al., 2014). Specifically, anandamide produces a CB_1-mediated inhibitory effect at nM concentration, while it exerts a TRPV1-mediated stimulatory effect at higher concentrations (μM) in primary afferent neurons (Tognetto et al., 2001; Roberts et al., 2002; Ross, 2003; Fischbach et al., 2007). A recent study using mouse afferent neurons has shown that activation of CB_1 receptors inhibit nerve growth factor (NGF)-induced sensitization of TRPV1 (Wang et al., 2014), possibly through multiple signaling pathways, including ERK1/2 and PI3K (Zhuang et al., 2004; Stein et al., 2006; Zhu and Oxford, 2007).

The analgesic action of cannabinoids may be mediated by the presynaptic inhibition of neurotransmitter release in sensory neurons. For example, presynaptic CB_1 receptors inhibit CGRP and substance P (SP) release from trigeminal sensory nerves (Akerman et al., 2004; Oshita et al., 2005). Moreover, CB_1 receptor agonists reduce voltage-activated Ca^{2+} current in DRG neurons (Ross et al., 2001). On the other hand, it is possible that even more important than peripheral actions, cannabinoids induce analgesia by interfering with circuitry in the rostral ventromedial medulla (RVM) (Meng et al., 1998).

CB_2 receptors have also been found in nociceptive sensory neurons of rodents (Ross et al., 2001; Merriam et al., 2008; Schuelert et al., 2010) and humans (Anand et al., 2008). Like with CB_1 receptors, nerve damage upregulates CB_2 receptors in the superficial laminae of the dorsal horn of the spinal cord and isolated DRG of mice (Wotherspoon et al., 2005) and human beings (Anand et al., 2008).

Although the specific role of the CB_2 receptor in sensory neurons remains unclear, several functional studies in sensory neurons point to an antinociceptive role (Burston and Woodhams, 2014). For instance, the putative CB_2 receptor agonist JWH-133 inhibits capsaicin-induced depolarization of the vagus sensory nerve in guinea pigs and humans (Patel et al., 2003). Moreover, JWH-133 reduces the response of wide dynamic range dorsal horn neurons to both innocuous and noxious intensities of mechanical stimuli (Elmes et al., 2004). This compound also attenuates the capsaicin-evoked Ca^{2+} response in DRG neurons in neuropathic rats (Sagar et al., 2009), while GW818646X (other CB_2 receptor agonist) diminishes capsaicin-induced inward cation currents and elevation of cytoplasmic Ca^{2+} (Anand et al., 2008). Another CB_2 receptor agonist, A-836339, inhibits von Frey-evoked activity of WDR neurons in neuropathic rats (McGaraughty et al., 2009). Local peripheral injection of the selective CB_2 receptor agonist AM1241 into the hind paw produces antinociception to thermal stimulation (Malan et al., 2001). AM1241 also inhibits bradykinin-induced mesenteric afferent nerve activity (Hillsley et al., 2007). This effect was absent in CB_2 knock-out mice and blocked by AM630, a CB_2 receptor inverse agonist. Local injection of the PEA analog *N*-(4-methoxy-2-nitrophenyl)hexadecanamide induces CB_1- and CB_2-dependent antinociception in rats (Roa-Coria et al., 2012). Similar results were observed with GW833972A, another putative CB_2 receptor agonist (Belvisi et al., 2008). Interestingly, repeated systemic administration of the CB_2 receptor selective agonist AM1710 suppresses paclitaxel-induced allodynia (Deng et al., 2015). Taken together, the data strongly suggest that CB_1 and CB_2 receptors have an antinociceptive role. Despite this evidence, there are few cannabinoid-based drugs currently available for clinical use (see below).

CB_1 and CB_2-Based Treatment for Pain

A randomized, placebo-controlled, double-blinded crossover design was used to examine the effect of cannabinoids on pain. Low, medium, and high doses of smoked cannabis (respectively 2, 4, and 8% Δ^9-THC by weight) did not modify capsaicin-induced pain assessed in 15 healthy volunteers 5 min after exposure (Wallace et al., 2007). In contrast, the medium dose of Δ^9-THC diminished capsaicin-induced pain 45 min after cannabis exposure. Of note, these authors found that a high dose of cannabis increased capsaicin-induced pain (Wallace et al., 2007). Similar results have been reported with a high dose of nabilone (an oral synthetic cannabinoid Δ^9-THC analog) on 41 patients with postoperative pain (Beaulieu, 2006). Another study evaluated cannabis extract capsules (20 mg of Δ^9-THC) in 18 healthy female volunteers (Kraft et al., 2008). Treatment with Δ^9-THC was not able to reduce pain induced by capsaicin, electrical stimulation or sunburn. Taken together, it seems that Δ^9-THC is not effective for acute pain. A similar conclusion was reached after analyzing a total of 611 patients in seven well-designed studies (Stevens and Higgins, 2017).

Although the effects of cannabinoids in the acute pain setting seem to be disappointing, results of clinical trials evaluating cannabinoids in chronic pain are much more promising (see **Table 1**). The conditions causing chronic pain varied between studies and included neuropathy (chemotherapy, diabetes, human immunodeficiency virus [HIV]), cancer, fibromyalgia, multiple sclerosis and rheumatoid arthritis (Whiting et al., 2015). Sativex (containing Δ^9-THC:cannabidiol [CBD] in an approximate 1:1 ratio [oral spray]) reduced neuropathic pain in patients with unilateral neuropathic pain (Berman et al., 2004; Nurmikko et al., 2007; Langford et al., 2013; Serpell et al., 2014). Likewise, treatment with smoked cannabis diminished pain in patients with multiple sclerosis (Rog et al., 2005; Corey-Bloom et al., 2012), neuropathic pain (Wilsey et al., 2013) and diabetic neuropathy (Wallace et al., 2015). In contrast, sativex was ineffective in relieving chemotherapy-induced neuropathic pain (Lynch et al., 2014). Oral administration of dronabinol, a synthetic Δ^9-THC analog, modestly reduced central pain in patients with multiple sclerosis (Svendsen et al., 2004). Nabilone, another synthetic Δ^9-THC analog, diminished neuropathic pain in diabetic patients (Toth et al., 2012). Oral administration of Δ^9-THC (ECP002A) reduced pain in patients with progressive multiple sclerosis. Drug dosage was well tolerated and had a stable pharmacokinetic profile (van Amerongen et al., 2017). Nabilone is also effective in patients with medication overuse headache (Pini et al., 2012). In contrast, nabilone did not reduce pain in patients with fibromyalgia (Skrabek et al., 2008).

A limitation to clinical use of cannabinoids for pain is their unfavorable side-effect profile, such as drowsiness, dizziness, speech impediments, memory impairment and confusion. Results of clinical trials with these agents indicate that high

TABLE 1 | Studies about the antinociceptive effects of CB1 and CB2 receptor agonists in different pain models.

Pain model	Drug treatment and dose	Behavioral readout	Route	Results	Proposed mechanisms of action	Reference
Partial SNL	WIN 55,212-2 0.3–10 mg/kg CP-55,940 0.03–1 mg/kg HU-210 0.001–0.03 mg/kg	Mechanical hyperalgesia Thermal hyperalgesia Tactile allodynia	s.c. or i.t.	They produce complete reversal of mechanical hyperalgesia with catalepsy Only WIN 55,212-2 reversed tactile allodynia and thermal hyperalgesia in this model	Via activation of CB1 receptors in both CNS and in the periphery	Herzberg et al., 1997; Fox et al., 2001; Bridges et al., 2003
SNL or carrageenan model	AZ11713908 0.6–1.2 μmol/kg	Thermal and mechanical hyperalgesia	s.c.	Robust analgesia in both models	Likely via peripheral activation of CB1 receptor	Yu et al., 2010
Mechanical stimulation, formalin or capsaicin models, in mice that lacked CB1 receptor specifically in primary nociceptors	Endocannabinoids (AEA and arachidonic acid)	Thermal and mechanical hyperalgesia		The nociceptor-specific loss of CB1 receptor substantially reduced the analgesia produced by local and systemic but no intrathecal, delivery of cannabinoids	Via CB1 receptors expressed on the peripheral terminals of nociceptors	Agarwal et al., 2007
SNL, carrageenan, LPS or CIA model	URB937 1 mg/kg URB597 10 mg/kg PF-3845 0.1-10 μg/kg	Thermal and mechanical hyperalgesia, tactile allodynia	i.p. or i.t.	Attenuation of hyperalgesia and partial reduction of allodynia	Suppresses FAAH activity and increases AEA levels	Clapper et al., 2010; Kinsey et al., 2011; Booker et al., 2012
FCA, partial SNL, tail flick, hot plate or incision model of postoperative pain	GW405833 0.3–30 mg/kg	Mechanical hyperalgesia and tactile allodynia	i.p.	Elicits potent and efficacious antihyperalgesic effects in rodent models of neuropathic, incisional and chronic inflammatory pain	Via activation of CB2 receptors	Valenzano et al., 2005
FCA, chronic constriction injury, incision model of postoperative pain or knee joint osteoarthritic pain	A796260 11–35 mg/kg	Thermal and tactile allodynia	i.p.	Analgesic activity in all pain models	Via activation of CB2 receptors	Yao et al., 2008
Partial SNL or carrageenan model	JWH133 50–100 nmol/mouse	Tactile allodynia	i.t., i.p. or local	Reverses partial sciatic nerve ligation-induced mechanical allodynia in mice.	Via activation of central CB2 receptors	Patel et al., 2003; Elmes et al., 2004; Yamamoto et al., 2008; Sagar et al., 2009
SNL, Formalin, Carrageenan, FCA or intradermal capsaicin	AM1241 0.03–6 mg/kg	Tactile and thermal allodynia, mechanical hyperalgesia and nocifensive response	i.v., i.p. or i.pl.	Analgesic effects in all pain models	Via activation of peripheral CB2 receptors	Malan et al., 2001, 2002; Ibrahim et al., 2003; Quartilho et al., 2003; LaBuda et al., 2005; Beltramo et al., 2006; Hillsley et al., 2007; Yao et al., 2008
Formalin model or postoperative pain	HU308 30, 50 mg/kg	Nocifensive response and actile allodynia	i.p.	Reduces blood pressure, blocks defecation, and elicits anti-inflammatory and peripheral analgesic activity	Via activation of CB2 receptors	Hanus et al., 1999; LaBuda et al., 2005
FCA or chronic constriction injury	GW842166X 0.1–0.3, 15 mg/kg	Mechanical hyperalgesia	p.o.	Very potent analgesic in inflammatory and neuropathic pain models	Potent and highly selective full agonist at the CB2 receptor	Clayton et al., 2004; Giblin et al., 2007; Anand et al., 2008
SNL	A836339 1–3 μmol/kg	Tactile allodynia	i.v.	Reduces both spontaneous and von Frey-evoked firing of WDR neurons in neuropathic rats	Via activation of spinal and peripheral CB2 receptors	McGaraughty et al., 2009
Paclitaxel-neuropathic pain	AM1710 0.1–10 mg/kg	Mechanical and thermal allodynia	i.p.	Suppresses allodynia generated by paclitaxel without central side effects	Via activation of CB2 receptors	Rahn et al., 2011; Deng et al., 2015

AEA, anandamide; SNP, spinal nerve ligation; FCA, Freud's complete adjuvant; CIA, collagen-induced arthritis; LPS, lipopolysaccharide; s.c., subcutaneous; i.p, intraperitoneal; i.t., intrathecal; i.v., intravenous; p.o., oral administration; i.pl, intraplantar.

dosages are required to attain therapeutic effects and it is difficult to reach these dosages in clinical practice (Turcotte et al., 2010). At doses that prevent subjective effects, some cannabinoids seem to be ineffective for controlling acute pain (Kalliomäki et al., 2013). Several peripherally restricted CB_1 and CB_2 receptor agonists have been developed to avoid these side effects (Pertwee, 2009; Yu et al., 2010; Rahn et al., 2011; Yrjola et al., 2013). However, additional research is needed to improve study methodologies including the use of standard formulations and/or dosages, the increase in the number of subjects involved, and the general determination of the safe and effective use of cannabis for the treatment of human pain.

Another interesting area of research has recently focused on the evaluation of the possible synergy between cannabinoids and opioids in the management of pain. A combination of Δ^9-THC and morphine diminished experimental pain in healthy volunteers (Roberts et al., 2006). Furthermore, dronabinol combined with opioids relieved chronic pain in patients (Narang et al., 2008).

In the last years, pain research has focused on the inhibition of the enzymes playing a role in EC metabolism and the elevation of the EC tonus locally. Special emphasis is given on multi-target analgesia compounds, where one of the targets is the EC degrading enzyme. Dual FAAH[1] /TRPV1 blockers, such as *N*-arachidonoyl-serotonin (AA-5-HT) and OMDM198, are effective in animal studies, but this multi-target strategy has not yet reached the clinic (Maione et al., 2007, 2013; Morera et al., 2009; Costa et al., 2010; Malek et al., 2015).

Importantly, cannabinoids interact (apart from CB_1 and CB_2) with several other pharmacological receptors, including the cannabinoid putative receptors GPR18 and GPR55 (which have been even suggested as CB_x and CB_3 receptors). It is likely that the contradictory effects observed in clinical trials using *Cannabis sp*.-based treatments (e.g., Δ^9-THC) may be due to the high promiscuity of cannabinoids for their receptors. Before achieving a clinical benefit from an EC system-based therapy in pain (and other alterations), it is mandatory to detect and understand the physiological and/or pathophysiological role of the cellular targets involved. In this context, we provide an analysis of the potential participation of the putative cannabinoid receptors GPR18 and GPR55 in pain (see below).

GPR18 AND GPR55: POTENTIAL TARGETS FOR PAIN TREATMENT

GPR55 and GPR18: Generalities

Cannabinoids interact with multiple orphan receptors (Alexander, 2012). Different groups have discussed if G protein-coupled receptor 18 (GPR18) and 55 (GPR55) should be considered as novel cannabinoid receptors (Alexander, 2012; Alexander et al., 2017). Nevertheless, the nomenclature suggested by the Nomenclature Committee of the Union of Basic and Clinical Pharmacology (NC−IUPHAR) Subcommittee on Cannabinoid Receptors (Pertwee et al., 2010) decided that all criteria to consider these as novel cannabinoid receptors remain incomplete and, accordingly, they were classified again as orphan receptors (Alexander et al., 2017). Independently of the official decision, these receptors clearly interact with cannabinoids directly or indirectly. Expression of GPR18 seems to be rich in the testis, spleen, peripheral blood leucocytes and lymph nodes (Gantz et al., 1997; Vassilatis et al., 2003; Rosenkilde et al., 2006). Its expression suggests a potential role in the control of immune system activity (e.g., leucocytes migration) (Burstein et al., 2011) and accordingly inflammation. Moreover, activation of GPR18 by *N*-arachidonoylglycine leads to apoptosis of inflammatory leukocytes (Burstein et al., 2011; Takenouchi et al., 2012), which in turn reduces local inflammation. There is also evidence that activation of GPR18 lowers intraocular pressure in mice (Miller et al., 2016). All these findings suggest a physiological function of NAGly *via* GPR18 in different inflammatory processes.

Knowledge about GPR55 physiology in the nervous system has increased recently (Marichal-Cancino et al., 2017). This receptor has been suggested as a potential therapeutic target in Parkinson's disease due to a possible alteration on its expression in the basal nuclei (Celorrio et al., 2017), where it is related to procedural memories (Marichal-Cancino et al., 2016). GPR55 is also expressed in the hippocampus, where it has a role in spatial navigation (Marichal-Cancino et al., 2018). Furthermore, it is possible that some antiepileptic actions observed with phytocannabinoids involve the blocking of GPR55 (Kaplan et al., 2017). However, the above is a topic under study and findings are preliminary. Despite all advances in the physiology of GPR55, several actions in different areas of the CNS remain obscure (Marichal-Cancino et al., 2017). Interestingly, PEA (a cannabinoid related compound) is currently used to treat pain and inflammation. Like other cannabinoid related molecules, PEA has a very complex mechanism of action, which includes direct and/or indirect interaction with CB_1, TRPV1, PPAR, GPR55 and GPR18, among other receptors (Keppel Hesselink et al., 2014). Certainly, PEA has high affinity for GPR55 as a full agonist (Ryberg et al., 2007). Thus, it is necessary to investigate whether GPR55 is involved in the analgesic and anti-inflammatory actions of PEA.

Actions of GPR18 and GPR55 and Their Potential Role in the Pharmacology of Pain

GPR18 and GPR55 are differentially expressed in the central and peripheral nociceptive systems of rodents and humans, suggesting a potential role in the modulation of nociceptive pathways (DRG TXome Database)[2](Ray et al., 2018). In general, GPR18 is less studied compared to GPR55 (see below). This is partly due to the fact that signaling mechanisms and

[1]FAAH, Fatty Acid Amide Hydrolase Enzyme. FAAH is an integral membrane enzyme that hydrolyzes the endocannabinoid anandamide and related amidated signaling lipids. FAAH KO mice display elevated anandamide levels, showing reduced nociceptive transmission in several pain models. Journal of Neurobiology 61: 149–60.

[2]http://www.utdallas.edu/bbs/painneurosciencelab/DRGtranscriptome/search.php

endogenous ligands are still controversial (Alexander et al., 2017). GPR18 has been suggested to modulate, depending on the ligand, both $G_{\alpha i/o}$ and $G_{\alpha q/11}$ transduction pathways (Console-Bram et al., 2014). In this sense, NAGly is proposed as the endogenous GPR18 ligand (Kohno et al., 2006; McHugh et al., 2010). However, a recent study suggests that NAGly increases Ca^{2+} mobilization and MAPK activity in HAGPR55/CHO cells (Console-Bram et al., 2017). This response is attenuated by ML193 (GPR55 receptor antagonist) suggesting that NAGly-mediated effects depend on GPR55 activation. Moreover, an independent study reported that NAGly does not activate GPR18 receptors (Lu et al., 2013). In support of this, there is a previous observation showing that NAGly does not activate GPR18 (Yin et al., 2009). These discrepancies could be partially explained by the fact that NAGly is also a reversible and non-competitive inhibitor of the glycine transporter type 2 (GlyT2) (Wiles et al., 2006). In line with this, it has been shown that NAGly enhances inhibitory glycinergic transmission synaptic within the superficial dorsal horn by blocking glycine uptake *via* GlyT2 and decreasing excitatory NMDA-mediated synaptic transmission (Jeong et al., 2010).

It has been proposed that both GPR18 and GPR55 could play a role in the modulation of acute and chronic pain (**Table 2**). In animal models of inflammatory pain, intraplantar NAGly administration attenuates formalin-induced pain (Huang et al., 2001). Moreover, intrathecal administration of NAGly reduces complete Freund's adjuvant (CFA)-induced mechanical allodynia and thermal hyperalgesia by a CB_1-independent mechanism (Succar et al., 2007). Additionally, NAGly increases the production of 15-deoxy-$\Delta^{13,14}$-prostaglandin J2 and lipoxin A4, leading to a reduction in the migration of inflammatory cells into the area of acute inflammation (Burstein et al., 2011). GPR18 is expressed on human leukocytes, including polymorphonuclear neutrophils (PMN), monocytes, and macrophages and, furthermore, its activation regulates leukocyte trafficking during acute inflammation (Chiang et al., 2015). GPR18 and TRPV1 are expressed in chondrocytes within the deep zone of cartilage in patients with osteoarthritis (OA) (Dunn et al., 2016), suggesting that GPR18 presence in degenerate tissues could be a target for treatment with cannabinoids.

Nerve injury enhances expression of GPR18 mRNA in spinal cord and/or the DRG of rats, suggesting a potential role of GPR18 in the modulation of neuropathic pain (Malek et al., 2016). Accordingly, intrathecal administration of NAGly reduces mechanical allodynia in rats subjected to spinal nerve ligation and this effect is not prevented by pretreatment with either the CB_1 or CB_2 receptor antagonists AM251 and SR144528, respectively (Vuong et al., 2008). Although NAGly has been proposed as an endogenous GPR18 ligand, recent studies have found that resolvin D2 (RvD2) also activates GPR18 receptors (Chiang et al., 2015; Zhang et al., 2016). RvD2 activates recombinant human GPR18 in a receptor- and ligand-dependent manner and promotes the resolution of bacterial infections and organ protection (Chiang et al., 2015). Moreover, RvD2 enhances endothelial cell migration in a Rac-dependent manner *via* GPR18, and GPR18-deficient mice have an endogenous defect in perfusion recovery following hind limb ischemia (Zhang et al., 2016). In rodents, intrathecal administration of RvD2 reverses CFA-induced inflammatory pain, prevents formalin-induced spontaneous pain, and also reverses C-fiber stimulation-evoked long-term potentiation in the spinal cord (Park et al., 2011). However, RvD2 antinociceptive effects seem to be mediated by additional mechanisms involving the inhibition of transient receptor potential (TRPV1 and TRPA1) channels (Park et al., 2011). Undoubtedly, more studies to redefine the signaling pathways, ligands and physiological functions of GPR18 are needed.

GPR55 has been found highly expressed in large-diameter neurons, but present at low levels in small-diameter neurons of the mouse DRG (Lauckner et al., 2008). Indeed, reports suggest that GPR55 plays a role in modulating nociceptor excitability. Activation of GPR55 with lysophosphatidylinositol (LPI) promotes excitability in cultured large DRG neurons by increasing intracellular Ca^{2+} (Lauckner et al., 2008) and also produces mechanical hypersensitivity in mice after local peripheral administration (Gangadharan et al., 2013). Although there is a general consensus that LPI acts as an agonist for GPR55, it has been also reported that LPI modulates large-conductance Ca^{2+}-activated potassium (K^+) channels (BK_{Ca}) (Bondarenko et al., 2011a,b), 2-pore domain potassium (K^+)-channels (TREK-1) (Maingret et al., 2000; Danthi et al., 2003) and the potassium (K^+) channel subfamily K member 4 (KCNK4 or TRAAK) (Maingret et al., 2000), transient receptor potential (TRPV2; Monet et al., 2009; Harada et al., 2017), and transient receptor potential (TRPM8; Vanden Abeele et al., 2006; Andersson et al., 2007) channels. All these channels are expressed in the primary nociceptive pathway and their activation either modulates or amplifies sensory information (Basbaum et al., 2009). Therefore, the pharmacological data with LPI should be taken with caution. Furthermore, LPI is not the sole GPR55 activator. The hydrophilic glycerophospholipid lyso-phosphatidyl-β-D-glucoside (LysoPtdGlc) was recently reported as a regulator of the nociceptive central axon projections by activating GPR55 with high affinity (Guy et al., 2015). This indicates that glycerophospholipids could play a role modulating nociceptive inputs *in vivo*.

Nerve damage increases GPR55 mRNA expression in the spinal cord and DRG of rats (Malek et al., 2016) suggesting the participation of these receptors in neuropathic pain. It has been shown that the synthetic GPR55 agonist O-1602 reduces movement-evoked firing of nociceptive C fibers in a rat model of acute joint inflammation, and this effect is blocked by the GPR55 receptor antagonist O-1918 (Schuelert and McDougall, 2011). O-1602 also has protective effects in a murine model of experimentally induced colitis, but this anti-inflammatory effect could not be mediated by GPR55 (Schicho et al., 2011).

On the other hand, other studies have reported that GPR55 knockout mice show a reduced tumor-induced mechanical hypersensitivity (Gangadharan et al., 2013). GPR55 agonist O-1602 produces pronociceptive effects in neuropathic rats

TABLE 2 | Possible role of GPR18 and GPR55 receptors in different animal models of pain.

Pain model/specie	Drug treatment	Dose	Route	Outcome	Proposed mechanisms of action	Reference
Formalin /rat	NAGly	275 nmol	i.pl.	Suppression of phase II response	Non-CB1 mediated mechanism	Huang et al., 2001
	ClD16020046	10 μM	Intra-ACC	Attenuation of phase II response Reduction of p-ERK in the ACC Attenuation of spinal *c-fos* expression in the spinal cord	Endogenous activation of GPR55 signaling. Modulatory effects of GPR55 signaling in the ACC on the descending pain pathway	Okine et al., 2016
Formalin/mouse	N/T	N/T	N/T	No differences between WT and GPR55$^{-/-}$ mice in mechanical, cold and heat hypersensitivity	Non-GPR55 mediated mechanism	Carey et al., 2017
CFA /rat	NAGly	70–700 nmol	i.t.	Attenuation of mechanical and thermal hyperalgesia	Non-cannabinoid mediated mechanism	Succar et al., 2007
CFA/mouse	N/T	N/T	N/T	Absence of mechanical hyperalgesia in GPR55$^{-/-}$ mice	GPR55 signaling	Staton et al., 2008
Capsaicin/mouse	N/T	N/T	N/T	GPR55$^{-/-}$ and WT mice display comparative levels of capsaicin-evoked nocifensive behavior, mechanical and thermal hyperalgesia	Non-GPR55 mediated mechanism	Carey et al., 2017
PNL/rat PNL/Mouse PNL/Mouse	NAGly N/T N/T	70–700 nmol N/T N/T	i.t. N/T N/T	Reduction of mechanical allodynia Absence of mechanical hyperalgesia in GPR55$^{-/-}$ mice GPR55$^{-/-}$ and WT mice develop similar levels of hypersensitivity to mechanical, heat, and cold stimulation	CB1 and CB2 independent mechanism GPR55 signaling Non-GPR55 mediated mechanism	Vuong et al., 2008 Staton et al., 2008 Carey et al., 2017
CCI/rat	O-1602 AA-5-HT	1–10 mg/kg 100–1000 nM	i.p. i.t.	Pronociceptive properties in neuropathic pain induced by O-1602 (atypical cannabinoid) Upregulation of CB2, GPR18, and GPR55 mRNA in the spinal cord and/or DRG after CCI. Increased pain threshold to mechanical and thermal stimuli following AA-5HT	Pronociceptive role of GPR55. Possible role of GPR18 Involvement of CB2, GPR18 and GPR55 receptors	Breen et al., 2012 Malek et al., 2016
Paclitaxel/mouse	N/T	N/T	N/T	GPR55$^{-/-}$ and WT mice develop similar levels of paclitaxel-induced mechanical and cold allodynia	Non-GPR55 mediated mechanism	Carey et al., 2017
LPI-induced pain/mouse	LPI	2 pmol–6 nmol	i.pl.	WT mice: Sensitization against non-painful and painful mechanical stimuli. GPR55$^{-/-}$ mice: reduction of LPI-induced acute allodynia, attenuation of LPI-induced long-term mechanical hyperalgesia	GPR55, $G\alpha_{q/11}$, and Gα13 pathways, and their signaling via RhoA-ROCK as well as ERK1/2	Gangadharan et al., 2013
Hot plate test/rat	LPI	1 μg	Intra-PAG	Reduction in nociceptive threshold that is abolished by a pretreatment with ML-193, a GPR55 antagonist.	Pro-nociception mediated by GPR55 activation at central levels. Blockade of GPR55 signaling in the PAG may promote analgesia	Deliu et al., 2015

CFA, Complete Freund's Adjuvant; PNL, partial ligation of the sciatic nerve; CCI, chronic constriction injury; NAGly, N-arachidonylglycine; LPI, lysophosphatidylinositol; AA-5-HT, N-arachidonoyl-serotonin; WT, wild type; ACC, anterior cingulated cortex; PAG, periaqueductal gray; N/T, not tested.

(Breen et al., 2012). At the central nervous system, local injection of the GPR55 putative inverse agonist CID16020046 into the anterior cingulated cortex (ACC) produces antinociception in the formalin test by decreasing the extracellular signal-regulated kinase 1/2 (ERK1/2) phosphorylation in the ACC and c-*fos* mRNA expression in the spinal cord (Okine et al., 2016). Moreover, LPI administration into the periaqueductal gray (PAG) attenuates nociceptive latencies in a hot-plate test and also produces a concentration-dependent increase in intracellular Ca^{2+} levels in dissociated rat PAG neurons expressing GPR55 mRNA (Deliu et al., 2015). Although the exact mechanisms underlying the GPR55-mediated antinociceptive effects remain to be elucidated, it has been suggested that some cytokines (e.g., IL-4 and IL-10) are responsible for the modulatory effects observed during inflammatory pain conditions (Staton et al., 2008).

Using cell lines, other studies have shown that GPR55 couples to $G_{\alpha 13}$ and activates GTPases RhoA, Cdc42 and Rac1 (Ryberg et al., 2007; Henstridge et al., 2009). Some efforts have tried to elucidate the G-protein signaling pathway activated by GPR55 agonists *in vivo*. Using pharmacological and conditional genetic tools in mice, the research group headed by Rohini Kuner showed that LPI-mediated hypersensitivity depends on the activation of $G_{\alpha 13}$ and $G_{\alpha q/11}$, which in turn activate ERK1/2 (Gangadharan et al., 2013). In support of these results, it has been shown that LPI produces β-arrestin trafficking, MAPK, ERK1/2 phosphorylation and activates the G-protein signaling by a PKCβII-independent mechanism (Oka et al., 2007; Kapur et al., 2009). Interestingly, the effects on β-arrestin GPR55 complex formation, ERK1/2 phosphorylation and internalization of GPR55 are blocked by the GPR55 antagonist/partial agonist CP55,940 (Kapur et al., 2009), suggesting that a complex mechanism triggered upon GPR55 activation modulates G-coupled signaling pathways. Moreover, it has been documented that activation of GPR55 leads to additional p38 MAPK (Oka et al., 2010) and AKT phosphorylation (Pineiro et al., 2011). These events are related to the subsequent activation of several major transcription factors such as the nuclear factor of activated T-cells (NFAT) (Waldeck-Weiermair et al., 2008; Henstridge et al., 2009, 2010), CREB (Henstridge et al., 2010), NF-kB (Waldeck-Weiermair et al., 2008; Henstridge et al., 2010), and ATF2 (Oka et al., 2010).

Certainly, there is extensive literature indicating that signaling pathways involving MAPK and transcription factors such as NF-κB play an important role in pain (Niederberger and Geisslinger, 2008; Ji et al., 2009). However, it is worth emphasizing that most of the signaling mechanisms reported for GPR55 receptors have been obtained *in vitro* using cell lines and may not be completely translated to *in vivo* models. This is particularly important due to the recent discrepancies in the pain field using GPR55 knock-out mice. It was originally reported that mice lacking GPR55 show no differences in baseline pain responses compared to wild-type mice, but mechanical hyperalgesia is absent following either intraplantar CFA injection or partial nerve ligation (Staton et al., 2008). However, a recent study using knock-out mice suggests that GPR55 is dispensable for the development of inflammatory and neuropathic pain (Carey et al., 2017). According to these authors, GPR55 knock-out mice have no differences in mechanical, cold or heat hypersensitivity after intraplantar capsaicin, formalin or CFA injection. Likewise, development and maintenance of neuropathic pain after paclitaxel administration or partial nerve ligation is undistinguishable between GPR55 knock-out and wild-type mice. While the explanation for this discrepancy is not clear, Carey et al. have suggested that these differences could be due to multiple factors, including the way the GPR55 knock-out mice were made, the battery of tests used, freely moving animals versus restrained animals during the test, sex differences, body weight, and age of animals. Evidently, more behavioral studies using controlled experimental conditions will be necessary to define the importance of GPR55 receptors in modulating pain responses.

CONCLUSION

Cannabinoids, *via* CB_1 receptors, mainly induce inhibition of pain integration that seems to be useful particularly in the treatment of chronic pain, whereas CB_2 stimulation mainly causes antiinflammation *via* negative modulation of the immune system. GPR18 and GPR55 have a role in integrating, transmitting and/or alleviating pain. However, further studies using more selective pharmacological tools combined with genetic tools to generate cell-specific ablation or reactivation of GPR18/GPR55 receptors in specific cell populations will help to clarify the functional role of these receptors to take advantage of them in therapeutics.

AUTHOR CONTRIBUTIONS

RG-A, PB-I, and EV-M developed the manuscript and discussed central ideas of it. AG-H and MC-L adapted the manuscript, designed graphs, and discussed central ideas of it. VG-S corrected the style and reviewed and edited the manuscript. MR supervised the project, worked on the conceptualization and acquired funding. BM-C conceived of the presented idea, integrated and edited information, and developed some central themes.

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Quick guide

Cannabinoid receptors

Jane M. Sullivan

What are they? Cannabinoid receptors are G protein-coupled receptors with 7 transmembrane domains. They are expressed on the cell surface with their binding domain exposed to the extracellular space. To date, two cannabinoid receptors have been cloned, CB1 and CB2. Recent evidence suggests that a third 'CB3' receptor is out there, waiting to be cloned.

Where are they? CB1 receptors are found in many brain regions including cortex, hippocampus, nucleus accumbens, basal ganglia, hypothalamus, amygdala, cerebellum and retina. CB2 is localized to immune system cells. Experiments in the hippocampus suggest that 'CB3' is there, but the presence or absence of 'CB3' in other brain regions remains to be determined.

What turns them on? Δ9-tetrahydrocannabinol (THC), one of the psychoactive ingredients in marijuana, does a pretty good job, but more potent agonists such as the synthetic compound WIN55,212-2 (WIN), are available. Endocannabinoids also bind and activate cannabinoid receptors.

What are endocannabinoids? THC and WIN are examples of *exogenous* cannabinoid receptor ligands, but the body makes its own ligands, too, and these are referred to as endocannabinoids. Two of the best-characterized endocannabinoids are anandamide and 2-arachidonylglycerol (2-AG). Other candidate endocannabinoids have been identified but which, if any, of these compounds are physiologically relevant cannabinoid receptor ligands is still an open question.

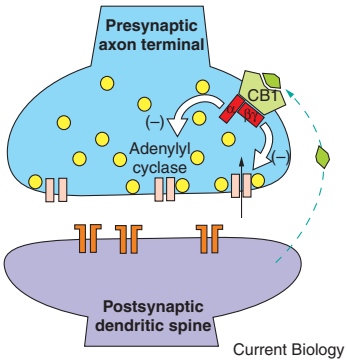

Figure 1. Depolarization of a postsynaptic neuron releases endocannabinoids. Binding of ligand to the CB1 receptor causes dissociation of the α and βγ subunits (red) of the G protein that is coupled to the receptor. The α subunit inhibits adenylate cyclase while the βγ subunits inhibit voltage-dependent calcium channels (pink) that control release of neurotransmitter-filled vesicles (yellow).

Events downstream of cannabinoid receptors... Like other G protein-coupled receptors, binding of ligand to cannabinoid receptors causes dissociation of the α and βγ G protein subunits from the cannabinoid receptor and from each other. Release of the α subunit leads to inhibition of adenylyl cyclase, reducing cAMP levels in the cell. In neurons dissociated βγ subunits directly inhibit calcium channels that control neurotransmitter release. Effects on other ion channels have also been reported. In addition, there is evidence of a direct inhibitory effect on the transmitter release machinery.

When do they get activated? Endocannabinoids are released in a calcium-dependent manner from dendrites, and maybe other parts of the cell, when neurons are activated. Endocannabinoids then travel backwards across the synaptic cleft, acting as retrograde messengers at cannabinoid receptors that are present on nearby presynaptic axon terminals.

What effect does this have? Action potential-evoked neurotransmitter release is suppressed when cannabinoid receptors are activated. In the hippocampus, axon terminals that release inhibitory neurotransmitter are much more sensitive to endocannabinoids than terminals that release excitatory neurotransmitter, so moderate neuronal activity may preferentially reduce inhibitory input, while stronger activity could suppress both excitatory and inhibitory inputs. In the cerebellum, excitatory and inhibitory inputs seem to have about the same sensitivity to endocannabinoids.

What happens if we don't have them? We don't know what happens in humans, but mice that have no CB1 receptors have improved memory, decreased appetite, a decreased tendency to become addicted to opiates, an increased sensitivity to pain, reduced locomotor activity, and shorter life spans than normal mice, suggesting a role for endocannabinoids in each of these systems.

Where can I find out more?

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Dept. of Physiology & Biophysics, University of Washington School of Medicine, Box 357290, Seattle, Washington 98195, USA.
Email: jmsull@u.washington.edu