

## Review Article

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

Charu Sharma,<sup>1</sup> Bassem Sadek,<sup>2</sup> Sameer N. Goyal,<sup>3</sup> Satyesh Sinha,<sup>4</sup>  
Mohammad Amjad Kamal,<sup>5,6</sup> and Shreesh Ojha<sup>2</sup>

<sup>1</sup>Department of Internal Medicine, College of Medicine and Health Sciences, United Arab Emirates University,  
P.O. Box 17666, Al Ain, Abu Dhabi, UAE

<sup>2</sup>Department of Pharmacology and Therapeutics, College of Medicine and Health Sciences, United Arab Emirates University,  
P.O. Box 17666, Al Ain, Abu Dhabi, UAE

<sup>3</sup>Department of Pharmacology, R. C. Patel Institute of Pharmaceutical Education & Research, Shirpur, Maharashtra 425405, India

<sup>4</sup>Department of Internal Medicine, College of Medicine, Charles R. Drew University of Medicine and Science, Los Angeles,  
CA 90059, USA

<sup>5</sup>King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

<sup>6</sup>Enzymoics, 7 Peterlee Place, Hebersham, NSW 2770, Australia

Correspondence should be addressed to Shreesh Ojha; [shreeshojha@uaeu.ac.ae](mailto:shreeshojha@uaeu.ac.ae)

Received 24 April 2015; Accepted 24 August 2015

Academic Editor: Ki-Wan Oh

Copyright © 2015 Charu Sharma et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The cannabinoid molecules are derived from *Cannabis sativa* plant which acts on the cannabinoid receptors types 1 and 2 (CB<sub>1</sub> and CB<sub>2</sub>) 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  $\Delta^9$ -tetrahydrocannabinol mediates its action through CB<sub>1</sub>/CB<sub>2</sub> 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<sub>1</sub> and CB<sub>2</sub>); 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<sub>1</sub> and CB<sub>2</sub> receptors are well characterized members of the GPCR which couple to G-proteins in the G<sub>i/o</sub> family. The activation of the CB<sub>1</sub> and CB<sub>2</sub> receptors causes the numerous intracellular effects which may be cell type and ligand specific and involve the inhibition of various voltage gated Ca<sup>2+</sup> channels and adenylate cyclase activity and the activation of K<sup>+</sup> channels, resulting in lower levels of cAMP along with activation of MAPK pathways [5]. The CB<sub>1</sub> receptors regulate the activities of adenylyl cyclase, ERK, glycogen synthase kinase 3, and calcium and potassium channels [5]. The CB<sub>2</sub> receptor couples to G<sub>i</sub> 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 neuro-modulator 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<sub>1</sub> or CB<sub>2</sub> 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  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -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<sub>1</sub> 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<sub>1</sub> receptors that mimic the effects of  $\Delta^9$ -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<sub>1</sub> receptor agonist, which is apparently higher for synthetic cannabinoids: JWH-018 and JWH-073 compared with  $\Delta^9$ -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<sub>1</sub> or CB<sub>2</sub> receptors are at present in the clinic such as Cesamet (nabilone), Marinol (dronabinol;  $\Delta^9$ -THC), and Sativex (cannabidiol and  $\Delta^9$ -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<sub>1</sub> receptors [6]. Recently, availability of different tools such as radioligand and [<sup>35</sup>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<sub>1</sub> and CB<sub>2</sub> receptors show little selectivity; however the CB<sub>1</sub> and CB<sub>2</sub> receptor antagonists are highly selective usually in nanomolar affinity

at the respective receptor. This allows differentiating the CB<sub>1</sub> or CB<sub>2</sub> mediated mechanism and responses of *in vitro* and *in vivo* studies. In addition to the selective CB<sub>1</sub> and CB<sub>2</sub> antagonists that are used to block agonist effects, there are also genetic tools (CB<sub>1</sub>/CB<sub>2</sub> receptor knockout mice) available to the research community. There are several nonselective agonists which are available which prefer either CB<sub>1</sub> or CB<sub>2</sub> 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<sub>1</sub> and CB<sub>2</sub> receptors have been recognized. Among the phytocannabinoids,  $\beta$ -caryophyllene is one which has been identified as a full agonist for CB<sub>2</sub> 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<sub>2</sub> receptors are primarily found in immune cells and participate in immune regulation [16, 17, 23, 24]. Thus, interactions of alkylamides with CB<sub>2</sub> 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<sub>2</sub> receptors more potently than endogenous cannabinoids with the  $K_i$  values (CB<sub>2</sub> approximately 60 nM; CB<sub>1</sub> > 1500 nM) and act as full agonist on CB<sub>2</sub> receptors in nanomolar range. Also, the molecular modeling studies have shown that alkylamide compounds bind in the solvent-accessible cavity in CB<sub>2</sub> receptors which is directed by the H-bonding and pi-pi interactions [27]. Furthermore, these compounds raised total intracellular Ca<sup>2+</sup> in CB<sub>2</sub>-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-dienoic acid isolated from *Echinacea* spp., stimulates 3T3-L1 differentiation mediated by PPAR- $\gamma$  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<sub>2</sub> receptors in homology modeling [31]. The root extract showed high binding affinity

to CB<sub>1</sub> and CB<sub>2</sub> receptors with  $K_i$  values of 2.2  $\mu$ g/mL and 1.3  $\mu$ g/mL, respectively, and moderate affinity to  $\mu$ - and  $\delta$ -opioid receptors in radioligand assay. Among the several identified compounds from extract, a tertiary alkylamide, 1-[(2E,4E,8Z)-tetradecatrienoyl] piperidine, showed most potent binding affinity with both CB<sub>1</sub> and CB<sub>2</sub> receptors with a  $K_i$  value of 0.8  $\mu$ M and 0.16  $\mu$ M, respectively. It showed CB<sub>2</sub> selectivity with a  $K_i$ CB<sub>1</sub>/ $K_i$ CB<sub>2</sub> = 5, with significant potency ( $K_i$  = 160 nM) [31]. Other isolated alkylamides as dodeca-2E,4E-dienoic acid isobutylamide, tetradeca-2E,4E-dienoic acid isobutylamide, tetradeca-2E,4E,8Z-trienoic acid isobutylamide, and 1-[(2E,4E,8Z)-tetradecatrienoyl] piperidine showed highest affinity for CB<sub>2</sub> receptors and show less affinity to opioid receptors. In regard to CB<sub>2</sub> 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<sub>2</sub> receptor interaction [32]. However, it is observed that the tertiary amide 1-[(2E,4E,8Z)-tetradecatrienoyl] piperidine which contains a piperidinyl moiety linked to a C14 acyl chain appears to have more affinity and potency on CB<sub>2</sub> than dodeca-2E,4E-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<sub>2</sub> receptors ligands in drug discovery.

**2.2.  $\alpha,\beta$ -Amyrin.** The pentacyclic triterpene and mixture (1:1) of two isomers,  $\alpha,\beta$ -amyrin, are mainly constituent of the resin of *Protium kleinii* and *Protium heptaphyllum*. The CB receptor mediated anti-inflammatory and antinociceptive effect of  $\alpha,\beta$ -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<sub>1</sub> or CB<sub>2</sub> receptor antagonists (AM251 and AM630, resp.) as well as knockdown of the CB<sub>1</sub>/CB<sub>2</sub> gene demonstrate CB activity. It binds to CB<sub>1</sub> receptors with a high affinity ( $K_i$  = 0.133 nM) and to CB<sub>2</sub> receptors with a lower affinity ( $K_i$  = 1989 nM) along with absence of behavioral disturbances. The binding to CB<sub>1</sub> receptors was 200–300-fold more potent than  $\Delta^9$ -THC. However, in contrast to  $\Delta^9$ -THC and 2-AG,  $\alpha,\beta$ -amyrin showed an unusual 15000-fold more binding selectivity for CB<sub>1</sub> receptors over CB<sub>2</sub>. Furthermore,  $\alpha,\beta$ -amyrin decreased proinflammatory cytokines and chemokines and prevented activation of the transcriptional factors: NF- $\kappa$ B 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<sub>2</sub>R mRNA but failed to affect CB<sub>1</sub> receptor mRNA upregulation as well as cortical levels of both CB<sub>1</sub> and CB<sub>2</sub> receptors. In another study, Chicca et al. [34] showed CB receptor binding interactions of  $\alpha,\beta$ -amyrin using hCB<sub>1</sub>/hCB<sub>2</sub> receptors transfected CHO-K1 cells and its effects on the endocannabinoid transport in U937 cells.

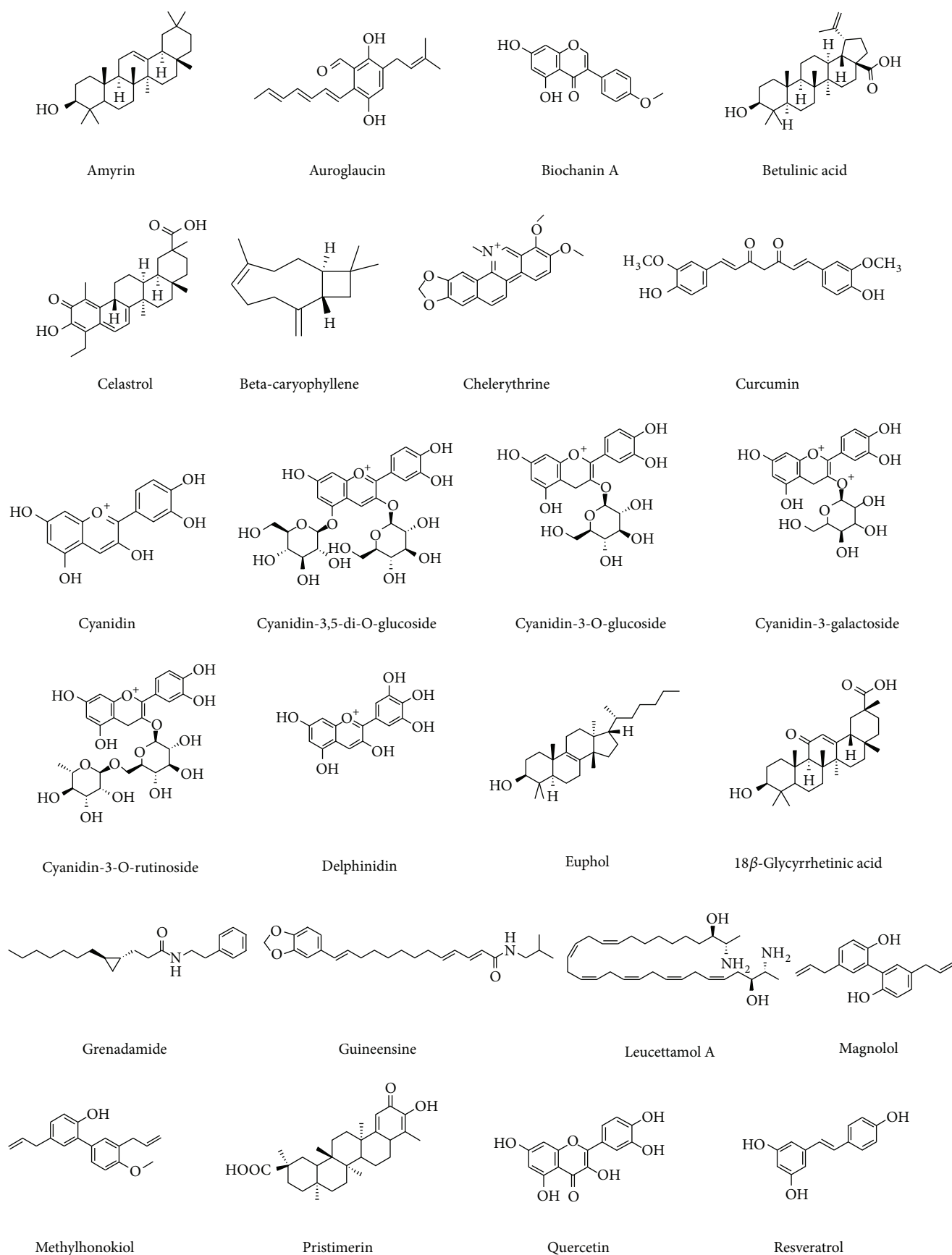


FIGURE 2: Continued.

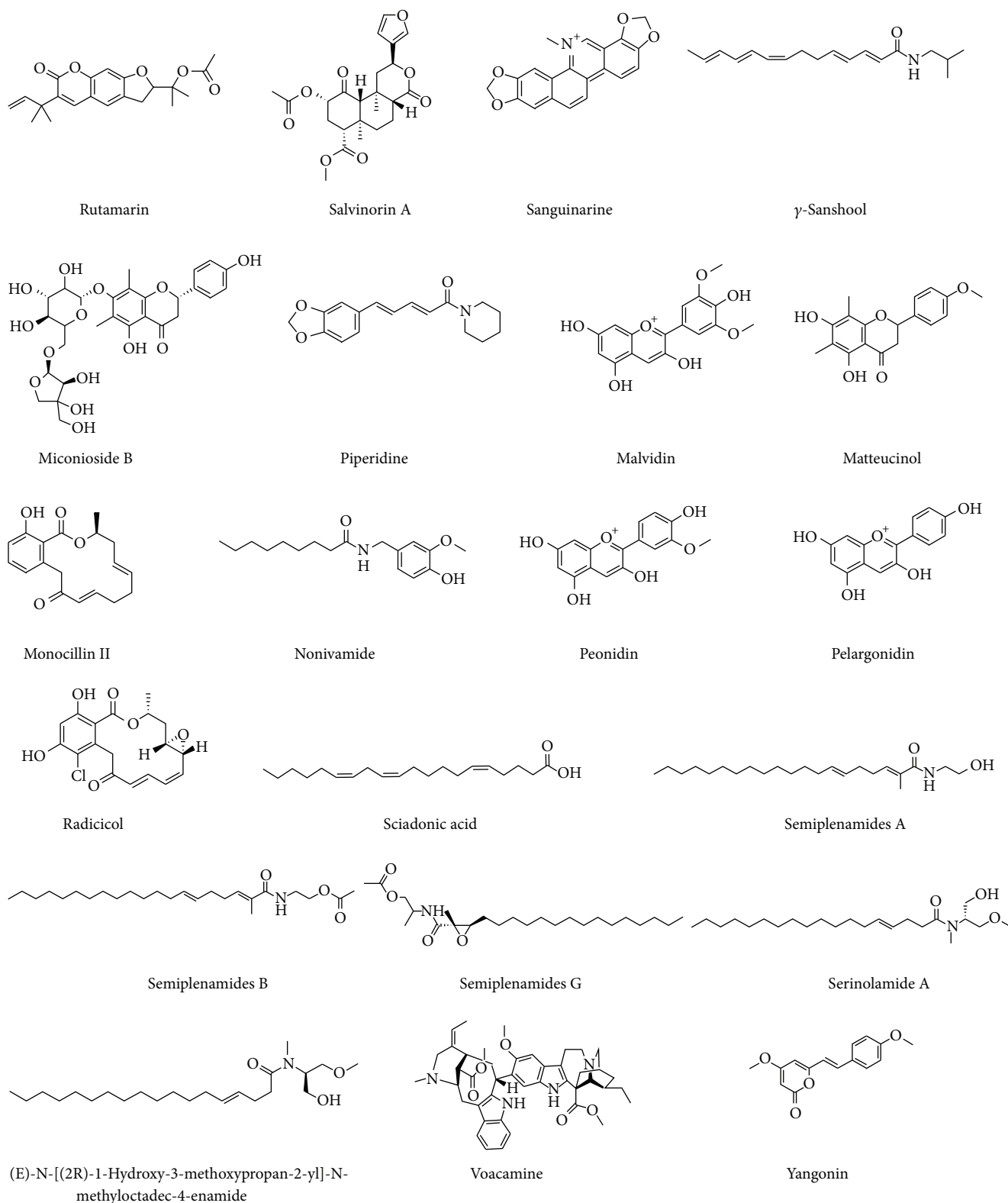


FIGURE 2: Chemical structure of isolated phytochemicals targeting endocannabinoid system.

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-tetradecahydricen-3-ol	M. Wt.: 426.71 [g/mol] M. formula: C <sub>30</sub> H <sub>50</sub> O XLogP3-AA: 9.2 H-bond donor/acceptor: 1/1	Olean-12-en-3-beta-ol
Auroglauin 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 <sub>19</sub> H <sub>22</sub> O <sub>3</sub> XLogP3-AA: 5.4 H-bond donor/acceptor: 2/3	Auroglauine
Biochanin A 5,7-Dihydroxy-3-(4-methoxyphenyl)chromen-4-one	M. Wt.: 284.26 [g/mol] M. formula: C <sub>16</sub> H <sub>12</sub> O <sub>5</sub> 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 <sub>30</sub> H <sub>48</sub> O <sub>3</sub> 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-octahydricen-2-carboxylic acid	M. Wt.: 450.60962 [g/mol] M. formula: C <sub>29</sub> H <sub>38</sub> O <sub>4</sub> XLogP3: 5.9 H-bond donor/acceptor: 2/4	Celastrol; tripterine; tripterin; celastrol, <i>Celastrus scandens</i>
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 <sub>15</sub> H <sub>24</sub> 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 <sub>21</sub> H <sub>18</sub> NO <sub>4</sub> <sup>+</sup> 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 <sub>21</sub> H <sub>20</sub> O <sub>6</sub> 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 <sub>15</sub> H <sub>11</sub> O <sub>6</sub> <sup>+</sup> 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 <sub>27</sub> H <sub>31</sub> O <sub>16</sub> <sup>+</sup> 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 <sub>21</sub> H <sub>20</sub> O <sub>11</sub> 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 <sub>21</sub> H <sub>21</sub> O <sub>11</sub> <sup>+</sup> 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 <sub>27</sub> H <sub>31</sub> ClO <sub>15</sub> 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 <sub>15</sub> H <sub>11</sub> O <sub>7</sub> <sup>+</sup> 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 <sub>30</sub> H <sub>50</sub> O XLogP3-AA: 8.9 H-bond donor/acceptor: 1/1	Eupha-8,24-dienol
18β-Glycyrrhetic 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 <sub>30</sub> H <sub>46</sub> O <sub>4</sub> XLogP3-AA: 6.4 H-bond donor/acceptor: 2/4	18β-Glycyrrhetic acid, glycyrrhetic 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 <sub>21</sub> H <sub>35</sub> Cl <sub>2</sub> NO <sub>2</sub> 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 <sub>24</sub> H <sub>33</sub> NO <sub>3</sub> 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 <sub>30</sub> H <sub>52</sub> N <sub>2</sub> O <sub>2</sub> 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 <sub>18</sub> H <sub>18</sub> O <sub>2</sub> 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 <sub>19</sub> H <sub>20</sub> O <sub>2</sub> 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-octahydronicene-2-carboxylate	M. Wt.: 464.63 [g/mol] M. formula: C <sub>30</sub> H <sub>40</sub> O <sub>4</sub> 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 <sub>15</sub> H <sub>10</sub> O <sub>7</sub> 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 <sub>14</sub> H <sub>12</sub> O <sub>3</sub> 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 <sub>21</sub> H <sub>24</sub> O <sub>5</sub> 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 <sub>23</sub> H <sub>28</sub> O <sub>8</sub> XLogP3-AA: 2.5 H-bond donor/acceptor: 0/8	Divinorin A
Sanguinarine	M. Wt.: 332.32 [g/mol] M. formula: C <sub>20</sub> H <sub>14</sub> NO <sub>4</sub> <sup>+</sup> XLogP3-AA: 4.4 H-bond donor/acceptor: 0/4	Dimethylene dioxybenzphenanthridine

TABLE 1: Continued.

Molecule & IUPAC name	Chemical properties	Common name(s)
$\gamma$ -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 <sub>17</sub> H <sub>25</sub> 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-(hydroxymethyl)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 <sub>28</sub> H <sub>34</sub> O <sub>14</sub> 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 <sub>17</sub> H <sub>19</sub> NO <sub>3</sub> 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 <sub>17</sub> H <sub>15</sub> O <sub>7</sub> <sup>+</sup> 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 <sub>18</sub> H <sub>18</sub> O <sub>5</sub> 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 <sub>18</sub> H <sub>20</sub> O <sub>4</sub> 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 <sub>17</sub> H <sub>27</sub> NO <sub>3</sub> 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 <sub>16</sub> H <sub>13</sub> O <sub>6</sub> <sup>+</sup> 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 <sub>15</sub> H <sub>11</sub> O <sub>5</sub> <sup>+</sup> H-bond donor/acceptor: 4/4	3,4',5,7-Tetrahydroxy flavylium chloride
Radicalol	M. Wt.: 364.77698 [g/mol] M. formula: C <sub>18</sub> H <sub>17</sub> ClO <sub>6</sub> 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 <sub>20</sub> H <sub>34</sub> O <sub>2</sub> 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 <sub>23</sub> H <sub>43</sub> NO <sub>2</sub> 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 <sub>25</sub> H <sub>45</sub> NO <sub>3</sub> 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 <sub>24</sub> H <sub>45</sub> NO <sub>4</sub> 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 <sub>23</sub> H <sub>45</sub> NO <sub>3</sub> 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 <sub>43</sub> H <sub>52</sub> N <sub>4</sub> O <sub>5</sub> 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 <sub>15</sub> H <sub>14</sub> O <sub>4</sub> XLogP3-AA: 2.7 H-bond donor/acceptor: 0/4	4-Methoxy-6-( $\beta$ -(p-anisyl)vinyl)- $\alpha$ -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
$\gamma$ -Sanshool	Diabetes	CB <sub>2</sub> agonist	Dossou et al. 2013 [106]
4'-O-Methylhonokiol	Alzheimer's diseases	CB <sub>2</sub> agonist	Gertsch and Anavi-Goffer 2012 [87] Schuehly et al. 2011 [85]
Yangonin	Anxiety	CB <sub>1</sub> receptor antagonist	Ligresti et al. 2012 [112]
Amyrin	Neuropathic pain	CB <sub>1</sub> /CB <sub>2</sub> agonist, MAGL inhibitor	Simão da Silva et al. 2011 [33]
Betulinic acid	Cancer	CB <sub>1</sub> antagonist/CB <sub>2</sub> agonist	Liu et al. 2012 [38]
$\beta$ -Caryophyllene	Ulcerative colitis Alzheimer's diseases Insulin resistance Alcohol addiction Anxiety Depression Nephrotoxicity Cerebral ischemia	CB <sub>2</sub> 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 <sub>2</sub> agonist	Yang et al. 2014 [55]
Chelerythrine	Neuropathic pain Neuroblastoma	CB <sub>1</sub> antagonist	Lim et al. 2003 [57]
Curcumin	Neuroprotective liver fibrosis	CB <sub>1</sub> antagonist/CB <sub>2</sub> agonist	Hassanzadeh and Hassanzadeh, 2012 [64]
Euphol	Neuropathic pain	CB <sub>1</sub> /CB <sub>2</sub> agonist, MAGL inhibitor	Dutra et al. 2012 [71]
18 $\beta$ -Glycyrrhetic acid	Obesity	CB <sub>1</sub> 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 <sub>1</sub> agonist, FAAH inhibitor	Fichna et al. 2012 [102] Aviello et al. 2011 [100] Capasso et al. 2008 [98] Braidia et al. 2009 [95] Braidia et al. 2007 [99]
Malyngamide B	Inflammation	CB <sub>1</sub> /CB <sub>2</sub> agonist	Montaser et al. 2012 [82]
Rutin	Depression	CB <sub>1</sub> agonist	Su et al. 2014 [83]
Serinolamide B	Inflammation Cancer	CB <sub>1</sub> and CB <sub>2</sub> 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			(i) Dodeca-2E,4E,8Z,10Z-tetraenoic acid isobutylamide
(ii) Chelerythrine		(i) Biochanin A	(ii) Dodeca-2E,4E-dienoic acid isobutylamide
(iii) Guineensine	(i) Amyrin	(ii) Curcumin and derivatives	(iii) 1-[(2E,4E,8Z)-Tetradecatrienoyl] piperidine
(iv) Bibenzyls	(ii) Betulinic acid	(iii) Cyanidin derivatives	(iv) Dodeca-2E,4E-dienoic acid isobutylamide
(v) Isoperrottetin A	(iii) $\beta$ -Caryophyllene	(iv) Desmodianones	(v) Tetradeca-2E,4E-dienoic acid isobutylamide
(vi) Sanguinarine	(iv) Celastrol	(v) Delphinidin	(vi) Tetradeca-2E,4E,8Z-trienoic acid isobutylamide
(vii) $\gamma$ -Sanshool	(v) Euphol	(vi) (+)-Catechin derivatives	(vii) 1-[(2E,4E,8Z)-Tetradecatrienoyl] piperidine
(viii) Voacamine	(vi) Falcarinol	(vii) Honokiol derivatives	(viii) Malyngamides
(ix) 3,6-Oxidovoacangine	(vii) 18 $\beta$ -Glycyrrhetic acid	(viii) Peonidin	(ix) Serinolamides
(x) 5-Hydroxy-3,6-oxidovoacangine	(viii) Isoperrottetin A	(ix) Pelargonidin	(x) Sciadonic acid
(xi) Haplosamates	(ix) Pristimerin	(x) Magnolol	(xi) Semiplenamides
(xii) Desulfohaplosamates	(x) Salvinorin A	(xi) Malvidin	
(xiii) Piperine	(xi) Thujone	(xii) Rutin	
(xiv) Neocosmosins	(xii) Yangonin	(xiii) 6-Methyltetrapterol A	
(xv) Monocillins	(xiii) Thujone	(xiv) Magnolol	
(xvi) Radicicol		(xv) Miconioside	
(xvii) Yangonin		(xvi) Resveratrol	

The study showed that it did not bind to cannabinoid receptors ( $K_i > 10 \mu\text{M}$ ) whereas it inhibited 2-AG hydrolysis in pig brain homogenates and failed to inhibit AEA. Additionally,  $\beta$ -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  $\alpha,\beta$ -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  $\alpha,\beta$ -amyrin by CB<sub>1</sub> receptor blocker, but not by CB<sub>2</sub> receptor blocker, demonstrates the CB<sub>1</sub> receptor mediated mechanism. Additionally,  $\alpha,\beta$ -amyrin treatment reduced the MAGL and FAAH enzymes. Integrating the ECS modulatory properties  $\alpha,\beta$ -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 ( $K_i = 16.2 \mu\text{M}$ ) and delphinidin ( $K_i = 21.3 \mu\text{M}$ ) for hCB<sub>1</sub> receptors whereas similar affinities for CB<sub>2</sub> receptors have been shown by cyanidin ( $K_i = 33.5 \mu\text{M}$ ), delphinidin ( $K_i = 34.3 \mu\text{M}$ ), and peonidin ( $K_i = 46.4 \mu\text{M}$ ) [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<sub>1</sub> and CB<sub>2</sub> receptors. Additionally, cyanidin-3-O- $\beta$ -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<sub>1</sub> (62.6%) and CB<sub>2</sub> 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<sub>50</sub> for CB<sub>1</sub> and CB<sub>2</sub> receptor was 15.2 and 19.9  $\mu\text{M}$ , 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, anti-angiogenic, immunomodulatory, and anticancer. Liu et al. [38] investigated the effects of CB<sub>1</sub> and CB<sub>2</sub> 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  $36.7 \pm 4.1$  and  $41.2 \pm 12.1 \mu\text{mol/L}$  for mCB<sub>1</sub> and hCB<sub>2</sub> receptors, respectively, in

radioligand binding assay. The role of CB receptor mediated activity was further confirmed in CB<sub>1</sub> and CB<sub>2</sub> 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<sub>1</sub> and CB<sub>2</sub> receptor dependent. Further, it has also been shown to activate PPAR- $\gamma$ , 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<sub>1</sub> and CB<sub>2</sub> receptors in [<sup>3</sup>H]CP55,940 assay and inhibited brain CB<sub>1</sub> receptors (27%) and recombinant CB<sub>2</sub> receptors (33%) [39]. No studies are available to demonstrate its other activities such as PPAR- $\gamma$  modulation. It has been reported to inhibit FAAH (IC<sub>50</sub> = 0.62  $\mu$ M) at micromolar potencies in RBL2H3 cells [39].

**2.7.  $\beta$ -Caryophyllene.**  $\beta$ -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  $\beta$ -caryophyllene which possesses an affinity for CB<sub>2</sub> receptors. In radioligand assays, (*E*)- $\beta$ -caryophyllene and its isomer (*Z*)- $\beta$ -caryophyllene dose-dependently displaced CP55,940 from hCB<sub>2</sub> receptors significantly expressed in HEK293 cells ( $K_i$  = 155  $\pm$  4 nM) in the nanomolar range and exhibit selective full agonism on CB<sub>2</sub> receptors. (*E*)- $\beta$ -caryophyllene exerts potent cannabimimetic anti-inflammatory effects in mice. Several studies have shown the CB<sub>2</sub> 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<sub>2</sub> receptor knockout mice [47]. Taking together the cannabimimetic [18], opioidergic [52], and PPARs mediated activity [53],  $\beta$ -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 health 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<sub>1</sub> and CB<sub>2</sub> 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<sub>1</sub> and CB<sub>2</sub> receptors in a dose-dependent manner. However, the weaker binding to CB<sub>2</sub> 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<sub>1</sub> and CB<sub>2</sub> receptors. The study demonstrates that catechins possess a moderate affinity for CB<sub>1</sub> receptors whereas binding to CB<sub>2</sub> receptor was not very prominent. In SAR studies, the ungalated catechins were found to have negligible bioactivities for CB<sub>1</sub> and CB<sub>2</sub> and the 3<sup>1</sup>,4<sup>1</sup>,5<sup>1</sup>-trihydroxyl substitution in the catechin B-ring partially contributing to antioxidant, apoptosis-inducer, and  $\beta$ -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<sub>2</sub> receptor antagonist, but not by SR141716, a specific CB<sub>1</sub> receptor antagonist, demonstrates the analgesia effects of celastrol through CB<sub>2</sub> signaling. Although celastrol shows an effect on CB<sub>2</sub> 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<sub>1</sub> receptors [56]. The CB<sub>1</sub> 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<sub>1</sub> receptors mainly within the ipsilateral superficial spinal cord dorsal horn mediating tyrosine kinase receptors. Chelerythrine also inhibits desacetyl levonantradol-dependent activation of CB<sub>1</sub> receptor in the neuroblastoma cells (N18TG2) and this was supported with modulation of a downstream PKC by CB<sub>1</sub> receptor [58]. The pseudobase forms of chelerythrine and sanguinarine inhibit CB<sub>1</sub> receptors similar to  $\Delta^9$ -THC at low micromolar concentrations in mouse brain membrane [59]. In [<sup>3</sup>H]CP55,940 binding assay, the IC<sub>50</sub> of sanguinarine and chelerythrine appears in the 1–2  $\mu$ M range, which has similar potency like cannabidiol, virodhamine, various  $\Delta^8$ -THC derivatives, and certain bicyclic resorcinols [60]. However, these were found weaker than  $\Delta^9$ -THC and  $\Delta^9$ -tetrahydrocannabivarin, which inhibit the binding of [<sup>3</sup>H]CP55,940 at low nanomolar concentrations [61]. Chelerythrine and sanguinarine showed lesser potency in comparison with several conventional CB<sub>1</sub> receptor blockers but act differently to AM251 by the reverse modulation of CB<sub>1</sub> receptors [56]. A recent study showed that chelerythrine produces the sequential activation of muscarinic (M<sub>3</sub>) receptors and CB<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> receptors with nanomolar affinities and in micromolar affinities with CB<sub>2</sub> receptors. Structurally, curcumin also shares structural motifs with some cannabinoid receptor ligands. Further, curcumin has been shown 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<sub>1</sub> receptor neutral antagonist, but not with SR144528, a CB<sub>2</sub> receptor antagonist, prevents induction of brain derived nerve growth factors and suggests CB<sub>1</sub> receptor mediated ECS as novel targets for curcumin. Recently, Witkin et al. [65] reported that curcumin did not potently alter GTP- $\gamma$ -35S binding, which suggests its functional CB<sub>1</sub> 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<sub>1</sub> knockout (CB<sub>1</sub><sup>-/-</sup>) 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<sub>2</sub> receptors and downregulated CB<sub>1</sub> 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<sub>1</sub> receptors contributed to curcumin inhibition of ECM expression. Further, *in silico* studies showed its binding to CB<sub>1</sub> receptors with two hydrogen bonds. In a very recent study, bisdemethoxycurcumin, a derivative of curcumin, has been shown to induce apoptosis in activated hepatic stellate stem cells by impairing cellular energetics and downregulating cytoprotective proteins, likely through a mechanism that involves CB<sub>2</sub> receptors as evidenced by reversal of the BDMC-induced apoptosis with cotreatment of SR144528, a CB<sub>2</sub> antagonist, and confirmed with genetic downregulation of the receptor using siCB<sub>2</sub> 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<sub>1</sub> receptor, whereas desulfohaplosamate showed higher affinity for CB<sub>2</sub> 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<sub>1</sub> and CB<sub>2</sub> 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 bioisoster 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<sub>2</sub> receptors play a vital role in pathogenesis [71]. The antihyperalgesic effect of euphol appears similar to the effects caused by ACEA, a CB<sub>1</sub> receptor agonist, and JWH-133, a CB<sub>2</sub> receptor agonist. The reversal of the antinociceptive effects of euphol on pretreatment with CB<sub>1</sub> antagonist AM251 or with CB<sub>2</sub> selective antagonist AM630 showed CB<sub>1</sub> and CB<sub>2</sub> receptor dependent mechanisms. Euphol was found efficacious in preventing the neuropathic behavior mediated through the modulation of both CB<sub>1</sub> and CB<sub>2</sub> 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 polyene 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<sub>1</sub> receptors and induces CB<sub>1</sub> receptor mediated functional signals by covalent and irreversible interaction with the CB<sub>1</sub> receptors ( $K_i = 0.59 \mu\text{M}$ ) [72]. Though, falcarinol is not a functional ligand at CB<sub>2</sub> receptor as it did not interfere with constitutive or forskolin-stimulated cAMP but appears as a weak partial agonist on CB<sub>2</sub> receptor and acting through G<sub>o</sub> 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<sub>1</sub> 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. 18 $\beta$ -Glycyrrhetic Acid.** 18 $\beta$ -Glycyrrhetic acid and its diastereomer 18 $\alpha$ -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<sub>1</sub> receptor-expressing Chem-1 cells showed a dose-dependent decrease in intracellular Ca<sup>2+</sup> levels ( $\text{IC}_{50} = 1.96 \pm 0.05 \mu\text{M}$ ) [73]. Other active constituents of licorice like liquiritin, glabridin, and 18 $\alpha$ -glycyrrhetic acid also exhibited inhibitory activity against Ca<sup>2+</sup> flux induced by AEA, whereas 18 $\beta$ -glycyrrhetic acid showed stronger potency evidenced by more than 90% inhibition in responses to CB<sub>1</sub> receptor agonist. The 18 $\beta$ -glycyrrhetic acid was also found to regulate CB<sub>1</sub> 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<sub>1</sub> 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- $\alpha$  and PPAR- $\gamma$ . The 18 $\beta$ -glycyrrhetic acid in licorice extract appears to be an active constituent possessing CB<sub>1</sub> receptor downregulatory effect and confers therapeutic effects against obesity.

**2.16. Guineensine.** Guineensine possesses potent cytotoxic, insect repellents, anti-inflammatory, insecticidal, and anti-feedant activities from black pepper, *Piper nigrum* (family: Piperaceae). It appears as a potent novel inhibitor ( $\text{EC}_{50} = 290 \text{ nM}$ ) 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> receptor antagonist. Piperine appears to reduce upper gastrointestinal motility independent of CB<sub>1</sub> 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  $\beta$ -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<sub>1</sub> receptors was  $0.02 \pm 0.015 \mu\text{M}$ ,  $1.85 \pm 0.275 \mu\text{M}$ , and  $1.46 \pm 0.33$  and for CB<sub>2</sub> receptors was  $0.11 \pm 0.025 \mu\text{M}$ ,  $6.43 \pm 0.7710 \mu\text{M}$ , and  $4.85 \pm 0.38 \mu\text{M}$ , 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<sub>1</sub>

and hCB<sub>2</sub> 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<sub>2</sub> receptor, while honokiol was less potent but showed full agonistic activity at CB<sub>1</sub> and antagonistic properties at CB<sub>2</sub> 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<sub>2</sub> receptor and antagonist at GPR55, a CB-related orphan receptor in  $\beta$ -arrestin translocation assays.

Fuchs et al. [80] synthesized analogs of magnolol and investigated affinity at hCB<sub>1</sub>/CB<sub>2</sub> 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<sub>2</sub> receptors; however depending on which of the two phenolic groups was methylated the resulting compounds exhibited an enhanced affinity to CB<sub>1</sub> receptors. Full agonism on CB<sub>1</sub> and CB<sub>2</sub> 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<sub>1</sub> antagonist and partial CB<sub>2</sub> receptors agonist activity, emphasizing the importance of the free phenolic hydroxyl group for high intrinsic activity. Further, activity of new analogs at G<sub>i</sub>-coupled CB<sub>1</sub> and CB<sub>2</sub> 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<sub>1</sub> and partial agonist activity at CB<sub>2</sub> receptors can be achieved. Magnolol also exhibited dual agonism of RXR $\alpha$  and PPAR $\beta/\gamma$  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<sub>1</sub> and CB<sub>2</sub> 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<sub>50</sub> of 6.2  $\mu$ M without affecting cellular viability up to 25  $\mu$ M. 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 involucreata* 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<sub>1</sub> receptors in mouse brain tissue demonstrating antifatigue activity and CB<sub>1</sub> receptor-interacting proteins. Further, in brain tissues, an increase in expression of peroxisome proliferator-activated receptor- $\alpha$  coactivator (PGC-1 $\alpha$ ) and sirtuin 1 (SIRT1) was also demonstrated [83]. Integrating together the cannabinoid, PPAR- $\gamma$ , 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<sub>1</sub> receptors with moderate binding affinity [84], whereas serinolamide B appeared to inhibit forskolin-stimulated cAMP accumulation mediating both CB<sub>1</sub> and CB<sub>2</sub> receptors with moderate potencies along with more CB<sub>2</sub> 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<sub>2</sub> ( $K_i$  = 5.2  $\mu$ M) over CB<sub>1</sub> receptor ( $K_i$  = 16.4  $\mu$ M) [82]. Serinolamide B like other cannabimimetic compounds exerted anti-inflammatory effects in lipopolysaccharide-(LPS-) induced murine macrophages RAW 264.7 with an IC<sub>50</sub> > 25  $\mu$ M. 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<sub>2</sub> 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<sub>2</sub> receptors but no effects on GPR55 and CB<sub>1</sub> 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<sub>2</sub> receptors significantly expressed in astrocytes and microglia. Its structural similarity with HU308, a synthetic CB<sub>2</sub> 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- $\gamma$ , and AChE modulatory, methylhonokiol seems to be a novel agent to target CB<sub>2</sub> 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<sub>1</sub> and CB<sub>2</sub> receptors. They showed weak inhibition for CB<sub>2</sub> receptors, but no activity on CB<sub>1</sub> 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<sup>208</sup>. Chicca et al. [34] also showed that pristimerin and JZL184 both produce potent inhibition of MAGL activity. Pristimerin produced inhibition of [<sup>3</sup>H]-glycerol formation and accumulation of intracellular [<sup>3</sup>H]2-AG and was found less potent than  $\beta$ -amyryn, 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<sub>1</sub> and CB<sub>2</sub> receptors was investigated in CHO cells expressing cannabinoid receptors and it was found that trans-resveratrol and all analogs bind to CB<sub>1</sub> receptors, whereas isoprenylated trans-resveratrol derivatives tA1 and tA3 bind to CB<sub>2</sub>

receptors [63]. The study showed affinity of trans-resveratrol and trans-piceatannol for CB<sub>2</sub> receptors is 5- to 10-fold lower than that observed for CB<sub>1</sub> receptors. All compounds except for tA3 exhibit approximately 2- to 10-fold selectively for binding to CB<sub>1</sub> receptors relative to CB<sub>2</sub> receptors. In molecular docking, trans-arachidins, trans-resveratrol, trans-piceatannol, and their glucuronidated metabolites bind with CB<sub>2</sub> receptors while isoprenylated analogs tA1 and tA3 bind with both CB<sub>1</sub> and CB<sub>2</sub> receptors. Trans-resveratrol and Trans-piceatannol also bind to mCB<sub>1</sub> receptors; however they lack affinity for hCB<sub>2</sub> 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<sub>1</sub> receptors and were confirmed by the antagonistic actions produced by CB<sub>1</sub> receptor agonists. However, the analogs possess an isoprenyl group, trans-arachidin 1 and trans-arachidin 3, showed affinity for CB<sub>2</sub> 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<sub>1</sub> receptors using CP55,940 as standard. However, compounds, neocosmosin A, neocosmosin B, neocosmosin C, monocillin II, and radicicol, exhibited binding affinity to CB<sub>2</sub> 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  $\kappa$ -opioid receptors [94–96], but not  $\mu$ - or  $\delta$ -opioid receptors. Other studies have shown that salvinorin A possesses ECS mediated activity and interaction with  $\kappa$ -opioid in rats and Zebra fish models [94, 96–101]. It provides a new lead compound for developing antiallodynic agents via opioid and CB<sub>1</sub> receptors activation. Fichna et al. [102] demonstrated that salvinorin A impedes gastrointestinal motility and ion transport, mediated by  $\kappa$ -opioid receptors in mice. Further, it significantly attenuated chemical-induced colitis in mice and the antinociceptive

action was blocked by opioid and CB<sub>1</sub> 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<sub>1</sub> and  $\kappa$ -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  $\kappa$ -opioid receptors and CB<sub>1</sub> 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<sub>1</sub> receptors but effects are mediated by its activation of  $\kappa$ -opioid receptors [96]. Braida et al. [95] reported the anxiolytic- and antidepressant-like effects of salvinorin A which are mediated by both  $\kappa$ -opioid and CB<sub>1</sub> receptors. In addition to a weak affinity for CB<sub>1</sub> receptors, it also reduced FAAH activity in amygdala. 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.  $\gamma$ -Sanshool.**  $\gamma$ -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<sub>2</sub> receptor activity. Subsequently, a novel plate-based assay was developed in order to determine both CB<sub>1</sub> and CB<sub>2</sub> receptors antagonist and agonist activity and the ligand effect on internalization of the CB<sub>1</sub>/CB<sub>2</sub> receptors in different extracts of the plant genus *Zanthoxylum* [106].

Later, it was found that  $\gamma$ -Sanshool isolated from *Zanthoxylum bungeanum* shows potent agonism on the CB<sub>2</sub> receptor and antagonism on CB<sub>1</sub> receptors. In addition to its interactions with CB<sub>1</sub> and CB<sub>2</sub> 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,  $\gamma$ -Sanshool with a dual function on CB<sub>1</sub> receptors inhibition in combination with CB<sub>2</sub> 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<sup>2+</sup> levels in neuroblastoma glioma hybrid cells (NG108-15) expressing CB<sub>1</sub> receptors. This was the first study showing the occurrence of a cannabimimetic monoacylglycerol

in higher plants exhibiting CB<sub>1</sub> receptor dependent mechanism.

**2.30. Semiplenamides.** Semiplenamides (semiplenamides 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<sub>1</sub> 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<sub>A</sub> 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<sub>1</sub> receptor in radioligand assay and found that thujone affinity with the CB<sub>2</sub> receptor is approximately similar to the CB<sub>1</sub> receptor. In bioassays and forskolin-stimulated adenylate cyclase assays, thujone did not show any activity on CB<sub>1</sub> 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<sup>2+</sup> functional assay using CP55,940 or rimonabant as a positive control for cannabinoid receptors ligands [111]. These compounds exhibited potent CB<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> receptor ( $K_i = 0.72 \mu\text{M}$ ). 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<sub>1</sub> 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<sup>+</sup>/Ca<sup>2+</sup> 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<sub>1</sub>, CB<sub>2</sub>, 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 *Renalmia 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  $\mu$ -opioid receptor and CB<sub>1</sub> 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<sub>1</sub>/CB<sub>2</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>A</sub> receptors [127]. The analgesic and anti-inflammatory effect mediated by CB<sub>1</sub> receptors along with anxiolytic activity is an advantage over synthetic CB<sub>1</sub> 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
<i>Corydalis yanhusuo</i>	Neuropathic pain	CB <sub>1</sub> antagonist	Huang et al. 2010 [119]
<i>Echinacea purpurea</i>	Immunomodulation	CB <sub>2</sub> agonist	Chicca et al. 2009 [25]
<i>Linum usitatissimum</i>	Inflammation	CB <sub>2</sub> agonist	Styrzewska et al. 2012 [120]
<i>Melilotus suaveolens</i>	Lung injury	CB <sub>2</sub> agonist	Liu et al. 2014 [121]
<i>Morinda citrifolia</i>	Immunomodulation	CB <sub>1</sub> antagonist/CB <sub>2</sub> agonist	Palu et al. 2008 [122]
<i>Nelumbo nucifera</i>	Obesity	CB <sub>2</sub> agonist	Velusami et al. 2013 [123]
<i>Olea europaea</i>	Colon cancer	CB <sub>1</sub> agonist	Cotrim et al. 2012 [124]
<i>Rubus coreanus</i>	Osteoporosis	CB <sub>1</sub> antagonist/CB <sub>2</sub> agonist	Lim et al. 2015 [125]
<i>Ruta graveolens</i>	Diabetes	CB <sub>2</sub> 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, alkaloids, and ketoalkenes/alkynes. The alkaloids were the first compounds identified in plants besides cannabis to possess cannabimimetic properties on both the cannabinoid CB<sub>1</sub> and CB<sub>2</sub> receptors, revealing their structural similarity to the endogenous cannabinoid ligand anandamide [20, 128]. The extract of *Echinacea* roots was studied in <sup>[35S]</sup>GTPγS-binding experiments on rat brain membrane preparations using arachidonyl-20-chloroethylamide (ACEA), a full agonist ligand at the CB<sub>1</sub> receptor [128]. Among the isolated compounds, some displayed partial agonist property while others exhibit inverse agonist effects to CB<sub>1</sub> 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<sub>2</sub> receptor activity of alkaloids, demonstrated by binding assays, is believed to be the most probable mechanism of action of alkaloids 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<sub>1</sub> 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 alkaloids [29, 30]. All together the studies convincingly suggest that *Echinacea* could provide scaffolds for future CB<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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- $\kappa$ B65. The CB<sub>2</sub> expression was shown to be correlated negatively with NF- $\kappa$ B 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<sub>1</sub> and CB<sub>2</sub> 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<sub>2</sub> receptor but inhibit CB<sub>1</sub> receptors.

Tahitian Noni Juice produced inhibition of CP55,940 for CB<sub>1</sub> receptors and enhancement for CB<sub>2</sub> receptors. Noni fruit juice concentrate caused stimulation of [<sup>3</sup>H]WIN-55,212-2 binding. At different concentrations the CB<sub>1</sub> receptor was inhibited whereas CB<sub>2</sub> receptor showed stimulations at the same concentrations.

The binding activity of Tahitian Noni Juice for CB<sub>1</sub> receptors was similar at each concentration, even at fivefold increased concentration. However, at both concentrations a remarkable selectivity for CB<sub>2</sub> binding/activation was observed for CB<sub>2</sub> receptor [122]. In mice orally administered Tahitian Noni Juice decreased IL-4 and increased IFN- $\gamma$  suggesting that Noni juice favorably alters the immune system and exhibits immunomodulatory effects by activating the CB<sub>2</sub> receptors which are involved in the immune regulation. The dual activity of Noni juice as CB<sub>1</sub> receptor inhibitor and CB<sub>2</sub> 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, nelumbo-side, 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<sub>2</sub> activity in the G<sub>i</sub>/G<sub>o</sub>-coupled CHO-K1 cell line [123]. The methanolic extract showed antagonism against CP55,940 activity towards CB<sub>2</sub> receptor, whereas water extract was found inactive. A potent antagonist activity towards CP55,940 activated CB<sub>2</sub> receptor with an IC<sub>50</sub> 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<sub>2</sub> 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<sub>1</sub> receptor was induced by exposure of Caco-2 cells to the oil. However, the other ECS components such as CB<sub>2</sub>, 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> endogenous ligands and PPAR- $\alpha$  as potential targets [124]. Taken together,

the findings demonstrate modulation of CB<sub>1</sub> 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 sanguin H-4 and sanguin 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 ovariectomy [132]. The upregulation of CB<sub>1</sub> and CB<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> receptor with a K<sub>i</sub> of 2.64  $\pm$  0.2  $\mu$ g/mL or 7.4  $\pm$  0.6  $\mu$ M in radioligand displacement assay. The findings reveal that rutamarin may provide a novel scaffold for the discovery of CB<sub>2</sub> 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<sub>1</sub> 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<sub>1</sub> 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<sub>1</sub> and CB<sub>2</sub> 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 multi-targeted 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<sub>1</sub>

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.

#### Acknowledgments

The author, Shreesh Ojha, is supported by the research grant from National Research Foundation, United Arab Emirates, and College of Medicine and Health Sciences (31M195), United Arab Emirates University, Al Ain, Abu Dhabi, UAE, and Satyesh Sinha was supported by NIH Grant (U54-MD-007598) at Charles R. Drew University of Medicine and Science, CA, USA.

#### References

- [1] R. Mechoulam, S. Ben-Shabat, L. Hanus et al., "Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors," *Biochemical Pharmacology*, vol. 50, no. 1, pp. 83–90, 1995.
- [2] T. Sugiura, S. Kondo, A. Sukagawa et al., "2-arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain," *Biochemical and Biophysical Research Communications*, vol. 215, no. 1, pp. 89–97, 1995.

- [3] V. Di Marzo and M. Maccarrone, "FAAH and anandamide: is 2-AG really the odd one out?" *Trends in Pharmacological Sciences*, vol. 29, no. 5, pp. 229–233, 2008.
- [4] L. Console-Bram, J. Marcu, and M. E. Abood, "Cannabinoid receptors: nomenclature and pharmacological principles," *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, vol. 38, no. 1, pp. 4–15, 2012.
- [5] C. C. Felder, K. E. Joyce, E. M. Briley et al., "Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors," *Molecular Pharmacology*, vol. 48, no. 3, pp. 443–450, 1995.
- [6] J. Gertsch, R. G. Pertwee, and V. Di Marzo, "Phytocannabinoids beyond the Cannabis plant—do they exist?" *British Journal of Pharmacology*, vol. 160, no. 3, pp. 523–529, 2010.
- [7] P. Pacher and G. Kunos, "Modulating the endocannabinoid system in human health and disease—successes and failures," *FEBS Journal*, vol. 280, no. 9, pp. 1918–1943, 2013.
- [8] S. Geiger, K. Nickl, E. H. Schneider, R. Seifert, and J. Heilmann, "Establishment of recombinant cannabinoid receptor assays and characterization of several natural and synthetic ligands," *Naunyn-Schmiedeberg's Archives of Pharmacology*, vol. 382, no. 2, pp. 177–191, 2010.
- [9] S. Steffens and P. Pacher, "Targeting cannabinoid receptor CB(2) in cardiovascular disorders: promises and controversies," *British Journal of Pharmacology*, vol. 167, no. 2, pp. 313–323, 2012.
- [10] J. L. Blankman and B. F. Cravatt, "Chemical probes of endocannabinoid metabolism," *Pharmacological Reviews*, vol. 65, no. 2, pp. 849–871, 2013.
- [11] R. Mechoulam, "Interview with Prof. Raphael Mechoulam, codiscoverer of THC.. Interview by Stanley Einstein," *The International Journal of the Addictions*, vol. 21, no. 4-5, pp. 579–587, 1986.
- [12] M. Rinaldi-Carmona, A. Le Duigou, D. Oustric et al., "Modulation of CB1 cannabinoid receptor functions after a long-term exposure to agonist or inverse agonist in the Chinese hamster ovary cell expression system," *Journal of Pharmacology and Experimental Therapeutics*, vol. 287, no. 3, pp. 1038–1047, 1998.
- [13] X.-Q. Xie, L. S. Melvin, and A. Makriyannis, "The conformational properties of the highly selective cannabinoid receptor ligand CP-55,940," *Journal of Biological Chemistry*, vol. 271, no. 18, pp. 10640–10647, 1996.
- [14] L. Hrubá, B. C. Ginsburg, and L. R. McMahon, "Apparent inverse relationship between cannabinoid agonist efficacy and tolerance/cross-tolerance produced by  $\Delta^9$ -tetrahydrocannabinol treatment in rhesus monkeys," *Journal of Pharmacology and Experimental Therapeutics*, vol. 342, no. 3, pp. 843–849, 2012.
- [15] S. Ruiu, N. Anzani, A. Orrù et al., "N-Alkyl dien- and trien-amides from the roots of *Otanthus maritimus* with binding affinity for opioid and cannabinoid receptors," *Bioorganic and Medicinal Chemistry*, vol. 21, no. 22, pp. 7074–7082, 2013.
- [16] J. Gertsch, "Anti-inflammatory cannabinoids in diet: towards a better understanding of CB<sub>2</sub> receptor action?" *Communicative & Integrative Biology*, vol. 1, no. 1, pp. 26–28, 2008.
- [17] J. Gertsch, "Immunomodulatory lipids in plants: plant fatty acid amides and the human endocannabinoid system," *Planta Medica*, vol. 74, no. 6, pp. 638–650, 2008.
- [18] J. Gertsch, M. Leonti, S. Raduner et al., "Beta-caryophyllene is a dietary cannabinoid," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 26, pp. 9099–9104, 2008.
- [19] K. Woelkart, O. M. H. Salo-Ahen, and R. Bauer, "CB receptor ligands from plants," *Current Topics in Medicinal Chemistry*, vol. 8, no. 3, pp. 173–186, 2008.
- [20] K. Woelkart and R. Bauer, "The role of alkamides as an active principle of *Echinacea*," *Planta Medica*, vol. 73, no. 7, pp. 615–623, 2007.
- [21] K. Woelkart, W. Xu, Y. Pei, A. Makriyannis, R. P. Picone, and R. Bauer, "The endocannabinoid system as a target for alkamides from *Echinacea angustifolia* roots," *Planta Medica*, vol. 71, no. 8, pp. 701–705, 2005.
- [22] R. G. Pertwee, "Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 367, no. 1607, pp. 3353–3363, 2012.
- [23] S. Zoppi, J. L. Madrigal, J. R. Caso et al., "Regulatory role of the cannabinoid CB2 receptor in stress-induced neuroinflammation in mice," *British Journal of Pharmacology*, vol. 171, no. 11, pp. 2814–2826, 2014.
- [24] S. Rom and Y. Persidsky, "Cannabinoid receptor 2: potential role in immunomodulation and neuroinflammation," *Journal of Neuroimmune Pharmacology*, vol. 8, no. 3, pp. 608–620, 2013.
- [25] A. Chicca, S. Raduner, F. Pellati et al., "Synergistic immunopharmacological effects of N-alkylamides in *Echinacea purpurea* herbal extracts," *International Immunopharmacology*, vol. 9, no. 7-8, pp. 850–858, 2009.
- [26] J. Gertsch, R. Schoop, U. Kuenzle, and A. Suter, "*Echinacea* alkylamides modulate TNF- $\alpha$  gene expression via cannabinoid receptor CB2 and multiple signal transduction pathways," *FEBS Letters*, vol. 577, no. 3, pp. 563–569, 2004.
- [27] S. Raduner, A. Majewska, J.-Z. Chen et al., "Alkylamides from *Echinacea* are a new class of cannabinomimetics: cannabinoid type 2 receptor-dependent and -independent immunomodulatory effects," *The Journal of Biological Chemistry*, vol. 281, no. 20, pp. 14192–14206, 2006.
- [28] M. Egger, P. Pellett, K. Nickl et al., "Synthesis and cannabinoid receptor activity of ketoalkenes from *Echinacea pallida* and nonnatural analogues," *Chemistry—A European Journal*, vol. 14, no. 35, pp. 10978–10984, 2008.
- [29] K. Spelman, K. Iiams-Hauser, N. B. Cech, E. W. Taylor, N. Smirnov, and C. A. Wenner, "Role for PPAR $\gamma$  in IL-2 inhibition in T cells by *Echinacea*-derived undeca-2E-ene-8,10-dienoic acid isobutylamide," *International Immunopharmacology*, vol. 9, no. 11, pp. 1260–1264, 2009.
- [30] K. Spelman, M. H. Wetschler, and N. B. Cech, "Comparison of alkylamide yield in ethanolic extracts prepared from fresh versus dry *Echinacea purpurea* utilizing HPLC-ESI-MS," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 49, no. 5, pp. 1141–1149, 2009.
- [31] T. Rühl, W. Deuther-Conrad, S. Fischer et al., "Cannabinoid receptor type 2 (CB<sub>2</sub>)-selective N-aryl-oxadiazolyl-propionamides: synthesis, radiolabelling, molecular modelling and biological evaluation," *Organic and Medicinal Chemistry Letters*, vol. 2, article 32, 2012.
- [32] N. Matovic, A. Matthias, J. Gertsch et al., "Stereoselective synthesis, natural occurrence and CB<sub>2</sub> receptor binding affinities of alkylamides from herbal medicines such as *Echinacea* sp.," *Organic and Biomolecular Chemistry*, vol. 5, no. 1, pp. 169–174, 2007.

- [33] K. A. B. Simão da Silva, A. F. Paszcuk, G. F. Passos et al., "Activation of cannabinoid receptors by the pentacyclic triterpene  $\alpha,\beta$ -amyirin inhibits inflammatory and neuropathic persistent pain in mice," *Pain*, vol. 152, no. 8, pp. 1872–1887, 2011.
- [34] A. Chicca, J. Marazzi, and J. Gertsch, "The antinociceptive triterpene beta-amyirin inhibits 2-arachidonoylglycerol (2-AG) hydrolysis without directly targeting cannabinoid receptors," *British Journal of Pharmacology*, vol. 167, no. 8, pp. 1596–1608, 2012.
- [35] I. Matos, A. F. Bento, R. Marcon, R. F. Claudino, and J. B. Calixto, "Preventive and therapeutic oral administration of the pentacyclic triterpene  $\alpha,\pm$ -amyirin ameliorates dextran sulfate sodium-induced colitis in mice: the relevance of cannabinoid system," *Molecular Immunology*, vol. 54, no. 3–4, pp. 482–492, 2013.
- [36] G. Korte, A. Dreiseitel, P. Schreier et al., "An examination of anthocyanins' and anthocyanidins' affinity for cannabinoid receptors," *Journal of Medicinal Food*, vol. 12, no. 6, pp. 1407–1410, 2009.
- [37] J. Gao, F. León, M. M. Radwan et al., "Benzyl derivatives with in vitro binding affinity for human opioid and cannabinoid receptors from the fungus *Eurotium repens*," *Journal of Natural Products*, vol. 74, no. 7, pp. 1636–1639, 2011.
- [38] X. Liu, I. Jutooru, P. Lei et al., "Betulinic acid targets YY1 and ErbB2 through cannabinoid receptor-dependent disruption of microRNA-27a:ZBTB10 in breast cancer," *Molecular Cancer Therapeutics*, vol. 11, no. 7, pp. 1421–1431, 2012.
- [39] L. Thors, J. J. Burston, B. J. Alter et al., "Biochanin A, a naturally occurring inhibitor of fatty acid amide hydrolase," *British Journal of Pharmacology*, vol. 160, no. 3, pp. 549–560, 2010.
- [40] A. F. Bento, R. Marcon, R. C. Dutra et al., " $\beta$ -caryophyllene inhibits dextran sulfate sodium-induced colitis in mice through CB2 receptor activation and PPAR $\gamma$  pathway," *American Journal of Pathology*, vol. 178, no. 3, pp. 1153–1166, 2011.
- [41] S. Al Mansouri, S. Ojha, E. Al Maamari, M. Al Ameri, S. M. Nurulain, and A. Bahi, "The cannabinoid receptor 2 agonist, beta-caryophyllene, reduced voluntary alcohol intake and attenuated ethanol-induced place preference and sensitivity in mice," *Pharmacology Biochemistry and Behavior*, vol. 124, pp. 260–268, 2014.
- [42] K. Guo, X. Mou, J. Huang, N. Xiong, and H. Li, "Trans-caryophyllene suppresses hypoxia-induced neuroinflammatory responses by inhibiting NF- $\kappa$ B activation in microglia," *Journal of Molecular Neuroscience*, vol. 54, no. 1, pp. 41–48, 2014.
- [43] I.-Y. Choi, C. Ju, A. M. A. Anthony Jalin, D. I. Lee, P. L. Prather, and W.-K. Kim, "Activation of cannabinoid CB2 receptor-mediated AMPK/CREB pathway reduces cerebral ischemic injury," *The American Journal of Pathology*, vol. 182, no. 3, pp. 928–939, 2013.
- [44] W. Suijun, Y. Zhen, G. Ying, and W. Yanfang, "A role for trans-caryophyllene in the moderation of insulin secretion," *Biochemical and Biophysical Research Communications*, vol. 444, no. 4, pp. 451–454, 2014.
- [45] L. C. Assis, M. R. Straliootto, D. Engel, M. A. Hort, R. C. Dutra, and A. F. de Bem, "Beta-Caryophyllene protects the C6 glioma cells against glutamate-induced excitotoxicity through the Nrf2 pathway," *Neuroscience*, vol. 279, pp. 220–231, 2014.
- [46] X. Zheng, T. Sun, and X. Wang, "Activation of type 2 cannabinoid receptors (CB2R) promotes fatty acid oxidation through the SIRT1/PGC-1 $\alpha$  pathway," *Biochemical and Biophysical Research Communications*, vol. 436, no. 3, pp. 377–381, 2013.
- [47] B. Horváth, P. Mukhopadhyay, M. Kechrid et al., " $\beta$ -caryophyllene ameliorates cisplatin-induced nephrotoxicity in a cannabinoid 2 receptor-dependent manner," *Free Radical Biology and Medicine*, vol. 52, no. 8, pp. 1325–1333, 2012.
- [48] M. F. Mahmoud, S. E. Swefy, R. A. Hasan, and A. Ibrahim, "Role of cannabinoid receptors in hepatic fibrosis and apoptosis associated with bile duct ligation in rats," *European Journal of Pharmacology*, vol. 742, pp. 118–124, 2014.
- [49] A. Bahi, S. Al Mansouri, E. Al Memari, M. Al Ameri, S. M. Nurulain, and S. Ojha, " $\beta$ -caryophyllene, a CB2 receptor agonist produces multiple behavioral changes relevant to anxiety and depression in mice," *Physiology and Behavior*, vol. 135, pp. 119–124, 2014.
- [50] A.-L. Klauke, I. Racz, B. Pradier et al., "The cannabinoid CB<sub>2</sub> receptor-selective phytocannabinoid beta-caryophyllene exerts analgesic effects in mouse models of inflammatory and neuropathic pain," *European Neuropsychopharmacology*, vol. 24, no. 4, pp. 608–620, 2014.
- [51] Y. Cheng, Z. Dong, and S. Liu, " $\beta$ -Caryophyllene ameliorates the Alzheimer-like phenotype in APP/PS1 Mice through CB2 receptor activation and the PPAR $\gamma$  pathway," *Pharmacology*, vol. 94, pp. 1–12, 2014.
- [52] S. Katsuyama, H. Mizoguchi, H. Kuwahata et al., "Involvement of peripheral cannabinoid and opioid receptors in  $\beta$ -caryophyllene-induced antinociception," *European Journal of Pain*, vol. 17, no. 5, pp. 664–675, 2013.
- [53] A. Chicca, D. Caprioglio, A. Minassi et al., "Functionalization of  $\beta$ -caryophyllene generates novel polypharmacology in the endocannabinoid system," *ACS Chemical Biology*, vol. 9, no. 7, pp. 1499–1507, 2014.
- [54] G. Korte, A. Dreiseitel, P. Schreier et al., "Tea catechins' affinity for human cannabinoid receptors," *Phytomedicine*, vol. 17, no. 1, pp. 19–22, 2010.
- [55] L. Yang, Y. Li, J. Ren et al., "Celastrrol attenuates inflammatory and neuropathic pain mediated by cannabinoid receptor type 2," *International Journal of Molecular Sciences*, vol. 15, no. 8, pp. 13637–13648, 2014.
- [56] A. S. Dhopeshwarkar and R. A. Nicholson, "Benzophenanthridine alkaloid, piperonyl butoxide and (S)-methoprene action at the cannabinoid-1 receptor (CB<sub>1</sub>-receptor) pathway of mouse brain: interference with [<sup>3</sup>H]CP55940 and [<sup>3</sup>H]SR141716A binding and modification of WIN55212-2-dependent inhibition of synaptosomal l-glutamate release," *European Journal of Pharmacology*, vol. 723, no. 1, pp. 431–441, 2014.
- [57] G. Lim, B. Sung, R.-R. Ji, and J. Mao, "Upregulation of spinal cannabinoid-1-receptors following nerve injury enhances the effects of win 55,212-2 on neuropathic pain behaviors in rats," *Pain*, vol. 105, no. 1–2, pp. 275–283, 2003.
- [58] V. Rubovitch, M. Gafni, and Y. Sarne, "The involvement of VEGF receptors and MAPK in the cannabinoid potentiation of Ca<sup>2+</sup> flux into N18TG2 neuroblastoma cells," *Molecular Brain Research*, vol. 120, no. 2, pp. 138–144, 2004.
- [59] A. S. Dhopeshwarkar, S. Jain, C. Liao, S. K. Ghose, K. M. Bisset, and R. A. Nicholson, "The actions of benzophenanthridine alkaloids, piperonyl butoxide and (S)-methoprene at the G-protein coupled cannabinoid CB<sub>1</sub> receptor *in vitro*," *European Journal of Pharmacology*, vol. 654, no. 1, pp. 26–32, 2011.
- [60] M. Steffens, J. Zentner, J. Honegger, and T. J. Feuerstein, "Binding affinity and agonist activity of putative endogenous cannabinoids at the human neocortical CB1 receptor," *Biochemical Pharmacology*, vol. 69, no. 1, pp. 169–178, 2005.

- [61] A. Thomas, L. A. Stevenson, K. N. Wease et al., "Evidence that the plant cannabinoid  $\Delta^9$ -tetrahydrocannabivarin is a cannabinoid CB<sub>1</sub> and CB<sub>2</sub> receptor antagonist," *British Journal of Pharmacology*, vol. 146, no. 7, pp. 917–926, 2005.
- [62] M. R. Romano and M. D. Lograno, "Signaling cross-talk between cannabinoid and muscarinic systems activates Rho-kinase and increases the contractile responses of the bovine ciliary muscle," *European Journal of Pharmacology*, vol. 702, no. 1–3, pp. 174–179, 2013.
- [63] K. A. Seely, M. S. Levi, and P. L. Prather, "The dietary polyphenols trans-resveratrol and curcumin selectively bind human CB<sub>1</sub> cannabinoid receptors with nanomolar affinities and function as antagonists/inverse agonists," *Journal of Pharmacology and Experimental Therapeutics*, vol. 330, no. 1, pp. 31–39, 2009.
- [64] P. Hassanzadeh and A. Hassanzadeh, "The CB<sub>1</sub> receptor-mediated endocannabinoid signaling and NGF: the novel targets of curcumin," *Neurochemical Research*, vol. 37, no. 5, pp. 1112–1120, 2012.
- [65] J. M. Witkin, S. Leucke, L. K. Thompson et al., "Further evaluation of the neuropharmacological determinants of the antidepressant-like effects of curcumin," *CNS & Neurological Disorders—Drug Targets*, vol. 12, no. 4, pp. 498–505, 2013.
- [66] Z. Zhang, Y. Guo, S. Zhang et al., "Curcumin modulates cannabinoid receptors in liver fibrosis in vivo and inhibits extracellular matrix expression in hepatic stellate cells by suppressing cannabinoid receptor type-1 in vitro," *European Journal of Pharmacology*, vol. 721, no. 1–3, pp. 133–140, 2013.
- [67] P. J. Lee, S. J. Woo, J. G. Jee, S. H. Sung, and H. P. Kim, "Bisdemethoxycurcumin induces apoptosis in activated hepatic stellate cells via cannabinoid receptor 2," *Molecules*, vol. 20, no. 1, pp. 1277–1292, 2015.
- [68] G. Chianese, E. Fattorusso, O. Tagliatela-Scafati et al., "Desulfohaplosamate, a new phosphate-containing steroid from *Dasychalina* sp., is a selective cannabinoid CB<sub>2</sub> receptor ligand," *Steroids*, vol. 76, no. 10–11, pp. 998–1002, 2011.
- [69] A. Pereira, T. A. Pfeifer, T. A. Grigliatti, and R. J. Andersen, "Functional cell-based screening and saturation transfer double-difference NMR have identified haplosamate A as a cannabinoid receptor agonist," *ACS Chemical Biology*, vol. 4, no. 2, pp. 139–144, 2009.
- [70] A. R. King, E. Y. Dotsey, A. Lodola et al., "Discovery of potent and reversible monoacylglycerol lipase inhibitors," *Chemistry & Biology*, vol. 16, no. 10, pp. 1045–1052, 2009.
- [71] R. C. Dutra, K. A. B. Simão da Silva, A. F. Bento et al., "Euphol, a tetracyclic triterpene produces antinociceptive effects in inflammatory and neuropathic pain: the involvement of cannabinoid system," *Neuropharmacology*, vol. 63, no. 4, pp. 593–605, 2012.
- [72] M. Leonti, L. Casu, S. Raduner et al., "Falcarinol is a covalent cannabinoid CB<sub>1</sub> receptor antagonist and induces pro-allergic effects in skin," *Biochemical Pharmacology*, vol. 79, no. 12, pp. 1815–1826, 2010.
- [73] M. Park, J.-H. Lee, J. K. Choi et al., "18 $\beta$ -glycyrrhetic acid attenuates anandamide-induced adiposity and high-fat diet induced obesity," *Molecular Nutrition and Food Research*, vol. 58, no. 7, pp. 1436–1446, 2014.
- [74] S. Nicolussi, A. Chicca, M. Rau et al., "Correlating FAAH and anandamide cellular uptake inhibition using *N*-alkylcarbamate inhibitors: from ultrapotent to hyperpotent," *Biochemical Pharmacology*, vol. 92, no. 4, pp. 669–689, 2014.
- [75] S. Nicolussi, J. M. Viveros-Paredes, M. S. Gachet et al., "Guineensine is a novel inhibitor of endocannabinoid uptake showing cannabimimetic behavioral effects in BALB/c mice," *Pharmacological Research*, vol. 80, pp. 52–65, 2014.
- [76] R. Capasso, A. A. Izzo, F. Borrelli et al., "Effect of piperine, the active ingredient of black pepper, on intestinal secretion in mice," *Life Sciences*, vol. 71, no. 19, pp. 2311–2317, 2002.
- [77] A. A. Izzo, R. Capasso, L. Pinto, G. D. Carlo, N. Mascolo, and F. Capasso, "Effect of vanilloid drugs on gastrointestinal transit in mice," *British Journal of Pharmacology*, vol. 132, no. 7, pp. 1411–1416, 2001.
- [78] M. van der Stelt, J. A. van Kuik, M. Bari et al., "Oxygenated metabolites of anandamide and 2-arachidonoylglycerol: conformational analysis and interaction with cannabinoid receptors, membrane transporter, and fatty acid amide hydrolase," *Journal of Medicinal Chemistry*, vol. 45, no. 17, pp. 3709–3720, 2002.
- [79] V. Rempel, A. Fuchs, S. Hinz et al., "Magnolia extract, magnolol, and metabolites: activation of cannabinoid CB<sub>2</sub> receptors and blockade of the related GPR55," *ACS Medicinal Chemistry Letters*, vol. 4, no. 1, pp. 41–45, 2013.
- [80] A. Fuchs, V. Rempel, and C. E. Müller, "The natural product magnolol as a lead structure for the development of potent cannabinoid receptor agonists," *PloS one*, vol. 8, no. 10, Article ID e77739, 2013.
- [81] H. Zhang, X. Xu, L. Chen et al., "Molecular determinants of magnolol targeting both RXR $\alpha$  and PPAR $\gamma$ ," *PLoS ONE*, vol. 6, no. 11, Article ID e28253, 2011.
- [82] R. Montaser, V. J. Paul, and H. Luesch, "Marine cyanobacterial fatty acid amides acting on cannabinoid receptors," *ChemBioChem*, vol. 13, no. 18, pp. 2676–2681, 2012.
- [83] K.-Y. Su, C. Y. Yu, Y.-W. Chen et al., "Rutin, a flavonoid and principal component of *Saussurea involucre*, attenuates physical fatigue in a forced swimming mouse model," *International Journal of Medical Sciences*, vol. 11, no. 5, pp. 528–537, 2014.
- [84] M. Gutiérrez, A. R. Pereira, H. M. Debonsi, A. Ligresti, V. Di Marzo, and W. H. Gerwick, "Cannabinomimetic lipid from a marine cyanobacterium," *Journal of Natural Products*, vol. 74, no. 10, pp. 2313–2317, 2011.
- [85] W. Schuehly, J. M. V. Paredes, J. Kleyer et al., "Mechanisms of osteoclastogenesis inhibition by a novel class of biphenyl-type cannabinoid CB<sub>2</sub> receptor inverse agonists," *Chemistry and Biology*, vol. 18, no. 8, pp. 1053–1064, 2011.
- [86] J. H. Oh, L. L. Kang, J. O. Ban et al., "Anti-inflammatory effect of 4-O-methylhonokiol, a novel compound isolated from *Magnolia officinalis* through inhibition of NF- $\kappa$ B," *Chemico-Biological Interactions*, vol. 180, no. 3, pp. 506–514, 2009.
- [87] J. Gertsch and S. Anavi-Goffer, "Methylhonokiol attenuates neuroinflammation: a role for cannabinoid receptors?" *Journal of Neuroinflammation*, vol. 9, article 135, 2012.
- [88] O. Ofek, M. Karsak, N. Leclerc et al., "Peripheral cannabinoid receptor, CB<sub>2</sub>, regulates bone mass," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 3, pp. 696–701, 2006.
- [89] A. H. Tarawneh, F. León, M. A. Ibrahim, S. Pettaway, C. R. McCurdy, and S. J. Cutler, "Flavanones from *Miconia prasina*," *Phytochemistry Letters*, vol. 7, pp. 130–132, 2014.
- [90] J. L. Bolton, S. B. Turnipseed, and J. A. Thompson, "Influence of quinone methide reactivity on the alkylation of thiol and amino groups in proteins: studies utilizing amino acid and peptide models," *Chemico-Biological Interactions*, vol. 107, no. 3, pp. 185–200, 1997.

- [91] L. K. Brents, F. Medina-Bolivar, K. A. Seely et al., "Natural prenylated resveratrol analogs arachidin-1 and -3 demonstrate improved glucuronidation profiles and have affinity for cannabinoid receptors," *Xenobiotica*, vol. 42, no. 2, pp. 139–156, 2012.
- [92] J. Gao, M. M. Radwan, F. Leon et al., "Correction to *Neocosmospora* sp.-derived resorcylic acid lactones with in vitro binding affinity for human opioid and cannabinoid receptors," *Journal of Natural Products*, vol. 76, no. 11, article 2174, 2013.
- [93] J. Gao, M. M. Radwan, F. León et al., "*Neocosmospora* sp.-derived resorcylic acid lactones with in vitro binding affinity for human opioid and cannabinoid receptors," *Journal of Natural Products*, vol. 76, no. 5, pp. 824–828, 2013.
- [94] B. L. Roth, K. Baner, R. Westkaemper et al., "Salvinorin A: a potent naturally occurring nonnitrogenous  $\kappa$  opioid selective agonist," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 18, pp. 11934–11939, 2002.
- [95] D. Braidia, V. Capurro, A. Zani et al., "Potential anxiolytic- and antidepressant-like effects of salvinorin A, the main active ingredient of *Salvia divinorum*, in rodents," *British Journal of Pharmacology*, vol. 157, no. 5, pp. 844–853, 2009.
- [96] D. M. Walentiny, R. E. Vann, J. A. Warner et al., "Kappa opioid mediation of cannabinoid effects of the potent hallucinogen, salvinorin A, in rodents," *Psychopharmacology*, vol. 209, no. 2, pp. 275–284, 2010.
- [97] D. Braidia, V. Limonta, V. Capurro et al., "Involvement of kappa-opioid and endocannabinoid system on Salvinorin A-induced reward," *Biological Psychiatry*, vol. 63, no. 3, pp. 286–292, 2008.
- [98] R. Capasso, F. Borrelli, M. G. Cascio et al., "Inhibitory effect of salvinorin A, from *Salvia divinorum*, on ileitis-induced hypermotility: cross-talk between kappa-opioid and cannabinoid CB<sub>1</sub> receptors," *British Journal of Pharmacology*, vol. 155, no. 5, pp. 681–689, 2008.
- [99] D. Braidia, V. Limonta, S. Pegorini et al., "Hallucinatory and rewarding effect of salvinorin A in zebrafish:  $\kappa$ -opioid and CB<sub>1</sub>-cannabinoid receptor involvement," *Psychopharmacology*, vol. 190, no. 4, pp. 441–448, 2007.
- [100] G. Aviello, F. Borrelli, F. Guida et al., "Ultrapotent effects of salvinorin A, a hallucinogenic compound from *Salvia divinorum*, on LPS-stimulated murine macrophages and its anti-inflammatory action in vivo," *Journal of Molecular Medicine*, vol. 89, no. 9, pp. 891–902, 2011.
- [101] F. Guida, L. Luongo, G. Aviello et al., "Salvinorin A reduces mechanical allodynia and spinal neuronal hyperexcitability induced by peripheral formalin injection," *Molecular Pain*, vol. 8, article 60, 2012.
- [102] J. Fichna, M. Dı́cay, K. Lewellyn et al., "Salvinorin A has antiinflammatory and antinociceptive effects in experimental models of colitis in mice mediated by KOR and CB<sub>1</sub> receptors," *Inflammatory Bowel Diseases*, vol. 18, no. 6, pp. 1137–1145, 2012.
- [103] J. Fichna, R. Schicho, C. N. Andrews et al., "Salvinorin A inhibits colonic transit and neurogenic ion transport in mice by activating kappa-opioid and cannabinoid receptors," *Neurogastroenterology & Motility*, vol. 21, no. 12, pp. 1326–e128, 2009.
- [104] J. Fichna, R. Schicho, A. Janecka, J. K. Zjawiony, and M. Storr, "Selective natural  $\kappa$  opioid and cannabinoid receptor agonists with a potential role in the treatment of gastrointestinal dysfunction," *Drug News and Perspectives*, vol. 22, no. 7, pp. 383–392, 2009.
- [105] J. Fichna, M. Dı́cay, S. A. Hirota et al., "Differential effects of salvinorin A on endotoxin-induced hypermotility and neurogenic ion transport in mouse ileum," *Neurogastroenterology and Motility*, vol. 23, no. 6, p. 583–e212, 2011.
- [106] K. S. S. Dossou, K. P. Devkota, C. Morton et al., "Identification of CB<sub>1</sub>/CB<sub>2</sub> ligands from *Zanthoxylum bungeanum*," *Journal of Natural Products*, vol. 76, no. 11, pp. 2060–2064, 2013.
- [107] S. Nakane, T. Tanaka, K. Satouchi, Y. Kobayashi, K. Waku, and T. Sugiura, "Occurrence of a novel cannabimimetic molecule 2-sciadonoylglycerol (2-eicosa-5',11',14'-trienoylglycerol) in the umbrella pine *Sciadopitys verticillata* seeds," *Biological and Pharmaceutical Bulletin*, vol. 23, no. 6, pp. 758–761, 2000.
- [108] B. Han, K. L. McPhail, A. Ligresti, V. Di Marzo, and W. H. Gerwick, "Semiplenamides A-G, fatty acid amides from a Papua New Guinea collection of the marine cyanobacterium *Lyngbya semiplena*," *Journal of Natural Products*, vol. 66, no. 10, pp. 1364–1368, 2003.
- [109] J. H. Greenberg, A. Mellors, and J. C. McGowan, "Molar volume relationships and the specific inhibition of a synaptosomal enzyme by psychoactive cannabinoids," *Journal of Medicinal Chemistry*, vol. 21, no. 12, pp. 1208–1212, 1978.
- [110] J. P. Meschler and A. C. Howlett, "Thujone exhibits low affinity for cannabinoid receptors but fails to evoke cannabimimetic responses," *Pharmacology Biochemistry and Behavior*, vol. 62, no. 3, pp. 473–480, 1999.
- [111] M. Kitajima, M. Iwai, R. Kikura-Hanajiri et al., "Discovery of indole alkaloids with cannabinoid CB<sub>1</sub> receptor antagonistic activity," *Bioorganic & Medicinal Chemistry Letters*, vol. 21, no. 7, pp. 1962–1964, 2011.
- [112] A. Ligresti, R. Villano, M. Allarà, I. Ujváry, and V. Di Marzo, "Kavalactones and the endocannabinoid system: the plant-derived yanonin is a novel CB<sub>1</sub> receptor ligand," *Pharmacological Research*, vol. 66, no. 2, pp. 163–169, 2012.
- [113] B. Botta, E. Gacs-Baitz, V. Vinciguerra, and G. Delle Monache, "Three isoflavanones with cannabinoid-like moieties from *Desmodium canum*," *Phytochemistry*, vol. 64, no. 2, pp. 599–602, 2003.
- [114] M. Toyota, T. Shimamura, H. Ishii, M. Renner, J. Braggins, and Y. Asakawa, "New bibenzyl cannabinoid from the New Zealand liverwort *Radula marginata*," *Chemical and Pharmaceutical Bulletin*, vol. 50, no. 10, pp. 1390–1392, 2002.
- [115] K. Soderstrom, T. F. Murray, H.-D. Yoo et al., "Discovery of novel cannabinoid receptor ligands from diverse marine organisms," in *Recent Advances in Prostaglandin, Thromboxane, and Leukotriene Research*, vol. 433 of *Advances in Experimental Medicine and Biology*, pp. 73–77, Springer, New York, NY, USA, 1997.
- [116] E. di Tomaso, M. Beltramo, and D. Piomelli, "Brain cannabinoids in chocolate," *Nature*, vol. 382, no. 6593, pp. 677–678, 1996.
- [117] I. Gómez-Betancur, N. Cortés, D. Benjumea, E. Osorio, F. León, and S. J. Cutler, "Antinociceptive activity of extracts and secondary metabolites from wild growing and micropropagated plants of *Renealmia alpinia*," *Journal of Ethnopharmacology*, vol. 165, pp. 191–197, 2015.
- [118] F. León, J. Gao, O. R. Dale et al., "Secondary metabolites from *Eupenicillium parvum* and their in vitro binding affinity for human opioid and cannabinoid receptors," *Planta Medica*, vol. 79, no. 18, pp. 1756–1761, 2013.
- [119] J.-Y. Huang, M. Fang, Y.-J. Li, Y.-Q. Ma, and X.-H. Cai, "Analgesic effect of *Corydalis yanhusuo* in a rat model of trigeminal neuropathic pain," *Nan Fang Yi Ke Da Xue Xue Bao*, vol. 30, no. 9, pp. 2161–2164, 2010.

- [120] M. Styrzcewska, A. Kulma, K. Ratajczak, R. Amarowicz, and J. Szopa, "Cannabinoid-like anti-inflammatory compounds from flax fiber," *Cellular and Molecular Biology Letters*, vol. 17, no. 3, pp. 479–499, 2012.
- [121] M.-W. Liu, M.-X. Su, Y.-H. Wang et al., "Effect of melilotus extract on lung injury by upregulating the expression of cannabinoid CB2 receptors in septic rats," *BMC Complementary and Alternative Medicine*, vol. 14, article 94, 2014.
- [122] A. K. Palu, A. H. Kim, B. J. West, S. Deng, J. Jensen, and L. White, "The effects of *Morinda citrifolia* L. (noni) on the immune system: its molecular mechanisms of action," *Journal of Ethnopharmacology*, vol. 115, no. 3, pp. 502–506, 2008.
- [123] C. C. Velusami, A. Agarwal, and V. Mookambeswaran, "Effect of nelumbo nucifera petal extracts on lipase, adipogenesis, adipolysis, and central receptors of obesity," *Evidence-Based Complementary and Alternative Medicine*, vol. 2013, Article ID 145925, 7 pages, 2013.
- [124] B. A. Cotrim, J. Joglar, M. J. L. Rojas et al., "Unsaturated fatty alcohol derivatives of olive oil phenolic compounds with potential low-density lipoprotein (LDL) antioxidant and antiobesity properties," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 4, pp. 1067–1074, 2012.
- [125] H.-K. Lim, H.-R. Lee, and S. H. Do, "Stimulation of cannabinoid receptors by using *Rubus coreanus* extracts to control osteoporosis in aged male rats," *The Aging Male*, vol. 18, no. 2, pp. 124–132, 2015.
- [126] J. M. Rollinger, D. Schuster, B. Danzl et al., "In silico target fishing for rationalized ligand discovery exemplified on constituents of *Ruta graveolens*," *Planta Medica*, vol. 75, no. 3, pp. 195–204, 2009.
- [127] H. Henkes, M. Franz, O. Kendall et al., "Evaluation of the anxiolytic properties of Tetrahydropalmatine, a *Corydalis yanhushuo* compound, in the male Sprague-Dawley rat," *AANA Journal*, vol. 79, no. 4, pp. S75–S80, 2011.
- [128] J. Hohmann, D. Rédei, P. Forgo et al., "Alkamides and a neolignan from *Echinacea purpurea* roots and the interaction of alkamides with G-protein-coupled cannabinoid receptors," *Phytochemistry*, vol. 72, no. 14-15, pp. 1848–1853, 2011.
- [129] S. Raduner, W. Bisson, R. Abagyan, K.-H. Altmann, and J. Gertsch, "Self-assembling cannabinomimetics: supramolecular structures of N-alkyl amides," *Journal of Natural Products*, vol. 70, no. 6, pp. 1010–1015, 2007.
- [130] A. Di Francesco, A. Falconi, C. Di Germanio et al., "Extravirgin olive oil up-regulates CB<sub>1</sub> tumor suppressor gene in human colon cancer cells and in rat colon via epigenetic mechanisms," *Journal of Nutritional Biochemistry*, vol. 26, no. 3, pp. 250–258, 2015.
- [131] J. E. Lee, E. Park, J. E. Lee et al., "Effects of a *Rubus coreanus* Miquel supplement on plasma antioxidant capacity in healthy Korean men," *Nutrition Research and Practice*, vol. 5, no. 5, pp. 429–434, 2011.
- [132] C. Choi, H. Lee, H. Lim, S. Park, J. Lee, and S. Do, "Effect of *Rubus coreanus* extracts on diabetic osteoporosis by simultaneous regulation of osteoblasts and osteoclasts," *Menopause*, vol. 19, no. 9, pp. 1043–1051, 2012.
- [133] A. Orrù, G. Marchese, G. Casu et al., "*Withania somnifera* root extract prolongs analgesia and suppresses hyperalgesia in mice treated with morphine," *Phytomedicine*, vol. 21, no. 5, pp. 745–752, 2014.
- [134] R. Capasso, F. Borrelli, F. Capasso, N. Mascolo, and A. A. Izzo, "Inhibitory effect of the antidepressant St. John's Wort (*hypericum perforatum*) on rat bladder contractility in vitro," *Urology*, vol. 64, no. 1, pp. 168–172, 2004.
- [135] N. D. Yuliana, M. Iqbal, M. Jahangir et al., "Screening of selected Asian spices for anti obesity-related bioactivities," *Food Chemistry*, vol. 126, no. 4, pp. 1724–1729, 2011.
- [136] A. T. El-Alfy, L. Wilson, M. A. ElSohly, and E. A. Abourashed, "Towards a better understanding of the psychopharmacology of nutmeg: activities in the mouse tetrad assay," *Journal of Ethnopharmacology*, vol. 126, no. 2, pp. 280–286, 2009.



Published in final edited form as:

*ACS Chem Neurosci.* 2014 November 19; 5(11): 1097–1106. doi:10.1021/cn5000875.

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

David R. Janero<sup>†</sup> and Alexandros Makriyannis<sup>\*,†,‡</sup>

<sup>†</sup>Center for Drug Discovery and Departments of Chemistry and Chemical Biology and Pharmaceutical Sciences, Northeastern University, Boston, Massachusetts 02115-5000, United States

<sup>‡</sup>King Abdulaziz University, Jeddah, 22254, Saudi Arabia

### Abstract

Endocannabinoid-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 antinociceptive action of some synthetic cannabinoids (WIN55,212-2 and AM1241), vasorelaxation by the endocannabinoid *N*-arachidonyl ethanolamide (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.,  $\Delta^9$ -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 cannabinergic and TRP-channel signaling suggest the possibility of dual-acting ligands as drugs.

### Graphical abstract

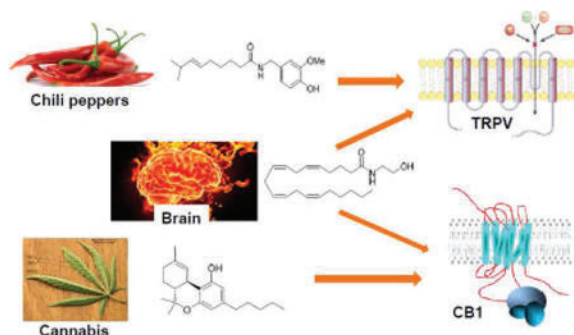
<sup>\*</sup>**Corresponding Author:** Mailing address: Northeastern University Center for Drug Discovery, 360 Huntington Avenue, 116 Mugar Hall, Boston, MA 02115-5000. Phone: 617-373-4200. Fax: 617-373-7493. a.makriyannis@neu.edu.

#### Author Contributions

Both authors contributed to the writing of the manuscript.

#### Notes

The authors declare no competing financial interest.



## Keywords

Drug discovery; endocannabinoid; 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.<sup>1</sup> 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 psychotropic component of marijuana,  $(-)\text{-}\Delta^9\text{-tetrahydrocannabinol}$  ( $\Delta^9\text{-THC}$ ), 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 immune-associated), whereas CB1 is a major GPCR in the central nervous system at presynaptic neurons and is also expressed in the periphery.<sup>2</sup> 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.<sup>3</sup> The best-studied endocannabinoids, 2-arachidonoylglycerol (2-AG) and N-arachidonyl ethanolamide (or anandamide) (AEA), are lipid mediators derived from diacylglycerol and N-acylphosphatidylcholine, respectively (Figure 2A). They originate from membrane phospholipids by distinct enzymatic pathways and possess specific functional and pharmacological properties.<sup>4</sup> In the central nervous system (CNS), the presynaptic serine hydrolase, monoacylglycerol lipase (MGL), is primarily responsible for 2-AG inactivation in vivo along with the  $\alpha,\beta$ -hydrolase domain-containing proteins 6 and 12 (ABHD6 and ABHD12), whereas AEA is inactivated by fatty acid amide hydrolase (FAAH) postsynaptically.<sup>5</sup> Aside from its canonical role in the CNS as

a key retrograde modulator of neurotransmitter release,<sup>6</sup> the endocannabinoid 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.<sup>7–10</sup>

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.<sup>11</sup> 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.<sup>12</sup> 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.<sup>13</sup> Reminiscent of the endocannabinoid system, TRP-channel signaling is involved in many (patho)physiological processes, for example, cell proliferation/differentiation, neurotransmitter release, chemical sensing, cell death, and inflammation.<sup>11,14,15</sup> Indeed, both endocannabinoid-system proteins<sup>8–10</sup> and TRP channels<sup>15–18</sup> 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.<sup>19</sup> 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,<sup>20</sup> also established physiological connectivity between endocannabinoid-system and TRP-channel-mediated cellular signaling, a relationship particularly relevant in the CNS where CB1 and TRPV1 activation provides a framework for  $\Delta^9$ -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.<sup>9,21,22</sup>

## PHYTOCANNABINOIDS AND LIGANDS BASED UPON A TERPENOID CHEMOTYPE

Among its approximately 500 endogenous phytochemicals,<sup>23</sup> the cannabis plant contains some 70 unique cannabinoids ("phytocannabinoids"), of which the most well-studied are shown in Figure 1A.  $\Delta^9$ -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.<sup>24</sup>  $\Delta^9$ -THC can engage and activate both CB1 and CB2 receptors with low nanomolar affinity, although the action of  $\Delta^9$ -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.  $\Delta^9$ -THC and its much less prominent natural isomer, (-)- $\Delta^9$ -tetrahydrocannabinol ( $\Delta^8$ -THC), are virtually equivalent as to CB-receptor affinity and pharmacological activity, although  $\Delta^8$ -THC is the more chemically stable isomer.<sup>25</sup> As compared to  $\Delta^9$ -THC, the phytocannabinoid (-)-cannabidiol (CBD) has significantly less affinity for CB receptors, modest CB2 selectivity, and negligible psychotropic activity.<sup>26,27</sup> Another classical terpenoid phytocannabinoid, (-)- $\Delta^9$ -tetrahydrocannabivarin ( $\Delta^9$ -THCV), is a shorter side-chain  $\Delta^9$ -THC homologue with a similar CB-receptor affinity and selectivity profile. As a "neutral" CB1 antagonist in some systems,  $\Delta^9$ -THCV at low doses can antagonize the effects of  $\Delta^9$ -THC in a manner distinct from that of typical CB1 antagonists/inverse agonists and may also have CB receptor-independent pharmacological activities.<sup>27,28</sup>

The classic terpenoid phytocannabinoids THC, CBD, and  $\Delta^9$ -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  $\Delta^9$ -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.<sup>2,7,8</sup> Other related xenocannabinoids include HU-210,<sup>29</sup> AM4054,<sup>30</sup> AM841,<sup>31</sup> and AM2389<sup>32</sup> (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.<sup>33</sup> 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.<sup>34</sup>

## 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.<sup>3,4</sup> As is the case for synthetic phytocannabinoid analogues, several lipid cannabinergic 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;<sup>35</sup> 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;<sup>36</sup> AMG313, the first AEA analogue with a chiral methyl arachidonoyl side chain;<sup>37</sup> 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.)<sup>38,39</sup> (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,<sup>40</sup> and the phytocannabinoid cannabidiol is both a CB1/CB2 agonist as well as an activator of TRPV1, TRPV2, and TRPV3<sup>41,42</sup> (Figure 3A). Other phytochemical TRP-channel activators include the four transient potential receptor channel ankyrin 1 (TRPA1) agonists cinnamaldehyde (found in cinnamon),<sup>43</sup> eugenol (found in cloves),<sup>44</sup> gingerol (found in ginger),<sup>45</sup> and umbellulone (found in California bay laurel, the “headache tree”)<sup>46</sup> and the two marine sphingoids leucettamol-A and leucettamol-B, which activate TRPA1 and inhibit transient receptor potential channel melastatin 8 (TRPM8)<sup>47</sup> (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.<sup>48</sup>

## ENDOGENOUS TRP-CHANNEL AND RELATED LIPID LIGANDS

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

include the FAAH inhibitor and TRPV1 antagonist *N*-arachidonoylserotonin<sup>53</sup> and the CB1 and TRPV1 agonist *N*-arachidonoyldopamine (NADA).<sup>54</sup> 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.<sup>55</sup> *N*-Acyl-amide lipids that act at TRP channels have inspired synthetic analogues including *N*-vanillylarachidonamide (“arvanil”), a TRPV1 agonist and CB1 partial agonist.<sup>56</sup> 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<sup>57–59</sup> (Figure 4B).

Lipids produced by the lipoxygenase-mediated oxygenation of polyunsaturated 20-carbon fatty acids (especially arachidonic acid), including the eicosanoids leukotriene B4 (LTB<sub>4</sub>) and 12-hydroperoxyeicosatetraenoic acid (12-HPETE), are potent, endogenous TRPV1 activators<sup>60,61</sup> (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-docosaehaenoic acid, is synthesized from docosaehaenoic acid lipoxygenation and acts as a TRPV1 antagonist<sup>62</sup> (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,<sup>63</sup> 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 TRPV1 and TRPV4<sup>64,65</sup> (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 antinociception and antihyperalgesia in acute pain models<sup>66</sup> and alleviate capsaicin-induced hyperalgesia/allodynia<sup>67,68</sup> 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,<sup>69</sup> whereas AM1241 is a CB2-selective agonist.<sup>70</sup> Cellular and in vivo animal data demonstrate that these CB agonists exert peripheral antinociceptive actions against the phytochemicals capsaicin and mustard oil by desensitizing TRPA1 and TRPV1 channels on sensory neurons.<sup>71</sup> 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 endocannabinoid-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.,<sup>72</sup> enzymatic hydrolysis of the endocannabinoid 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 endocannabinoid 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.<sup>59,73,74</sup> 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 endocannabinoid-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.<sup>57,59</sup> Thus, AM404's potent analgesic activity in vivo may reflect its pleiotropic activity profile and effects on multiple endocannabinoid-system and TRP-channel targets.

## INVOLVEMENT OF CANNABIBERGIC AND TRP-CHANNEL SIGNALING IN DRUG ABUSE

Potential 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  $\Delta^9$ -THC is generally accepted to be the basis for the negative cognitive effects of marijuana and its abuse liability,<sup>75</sup> inviting novel medicinal chemistry approaches (e.g., CB1 agonists with limited CNS penetration<sup>76</sup>) 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 endocannabinoid 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.<sup>77,78</sup> Thus, modulation of endocannabinoid-system activity has been of great therapeutic interest with respect to two general modalities: (a) regulating endocannabinoid-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  $\Delta^9$ -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<sup>79</sup> and CB1 (periphero-)neutral antagonists for cardiometabolic disease.<sup>80</sup> The latter modality would include FAAH inhibitors that enhance the CNS endocannabinoid elevation observed after brain injury to therapeutic levels.<sup>10</sup>

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 olfactory- and somatosensory-stimulating properties and interacts with TRPA1, an irritant-sensing TRP channel expressed by nociceptors in the lung and respiratory tract.<sup>81</sup> 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.<sup>82</sup> 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.<sup>83</sup> 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.<sup>84</sup> 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.<sup>85</sup> In the clinic, the CB1 antagonist/inverse agonist rimonabant has been shown to increase the likelihood that smokers will quit,<sup>86</sup> and inhaled CBD reduces cigarette consumption, perhaps by modulating the craving-related salience of smoking cues.<sup>87</sup> 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.<sup>88</sup> 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.<sup>89–91</sup> 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.<sup>91</sup> 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.<sup>92</sup> 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 phytochemicals 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)<sup>93</sup> 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.<sup>94</sup> 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.<sup>95</sup> 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.

## Acknowledgments

We thank Mr. Mitchel Ayer for expert bibliographic assistance.

## ABBREVIATIONS

<b>CB</b>	cannabinoid
<b>TRP</b>	transient receptor potential
<b>CB1</b>	cannabinoid 1 receptor
<b>(-)-<math>\Delta^9</math>-THC</b>	(-)- $\Delta^9$ -tetrahydrocannabinol
<b>CB2</b>	cannabinoid 2 receptor
<b>GPCR</b>	G-protein coupled receptor
<b>2-AG</b>	2-arachidonoylglycerol
<b>AEA</b>	anandamide
<b>CNS</b>	central nervous system
<b>MGL</b>	monoacylglycerol lipase
<b>ABHD6</b>	$\alpha,\beta$ -hydrolase domain-containing protein 6

<b>ABHD12</b>	$\alpha,\beta$ -hydrolase domain-containing protein 12
<b>FAAH</b>	fatty acid amide hydrolase
<b>TMS</b>	transmembrane segment
<b>TRPV</b>	transient receptor potential vanilloid
<b>(-)-<math>\Delta^8</math>-THC</b>	(-)- $\Delta^8$ -tetrahydrocannabinol
<b>CBD</b>	cannabidiol
<b><math>\Delta^9</math>-THCV</b>	$\Delta^9$ -tetrahydrocannabivarin
<b>ACPA</b>	arachidonoylcyclopropylamide
<b>TRPA1</b>	transient potential receptor channel ankyrin 1
<b>TRPM8</b>	transient receptor potential channel melastatin 8
<b>OEA</b>	oleoylethanolamide
<b>PPAR-<math>\alpha</math></b>	peroxisome proliferator-activated receptor alpha
<b>NADA</b>	<i>N</i> -arachidonoyl dopamine
<b>LTB<sub>4</sub></b>	leukotriene B <sub>4</sub>
<b>12-HPETE</b>	12-hydroperoxyeicosatrienoic acid
<b>EET</b>	epoxytrienoic acid
<b>5',6'-EET</b>	5',6'- epoxytrienoic acid

## REFERENCES

1. Machnicka B, Czogalla A, Hryniewicz-Jankowska A, Bogusławska DM, Grochowalaka R, Heger E, Sikorski AF. Spectrins: a structural platform for transporters and activation of membrane channels, receptors and transporters. *Biochim. Biophys. Acta.* 2014; 1838(2):620–634. [PubMed: 23673272]
2. Console-Bram L, Marcu J, Abood ME. Cannabinoid receptors: nomenclature and pharmacological principles. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* 2012; 38(1):4–15. [PubMed: 22421596]
3. Di Marzo V. The endocannabinoid system: its general strategy of action, tools for its pharmacological manipulation and potential therapeutic exploitation. *Pharmacol. Res.* 2009; 60(2): 77–84.
4. Luchicchi A, Pistis M. Anandamide and 2-arachidonoylglycerol: pharmacological properties, functional features, and emerging specificities of the two major endocannabinoids. *Mol. Neurobiol.* 2012; 46(2):374–392. [PubMed: 22801993]
5. Ueda N, Tsuboi K, Uyama T. Metabolism of endocannabinoids and related *N*-acylethanolamines: canonical and alternative pathways. *FEBS J.* 2013; 280(9):1874–1894. [PubMed: 23425575]
6. Ohno-Shosaku T, Tanimura A, Hashimoto-dani Y, Kano M. Endocannabinoids and retrograde modulation of synaptic transmission. *Neuroscientist.* 2012; 18(2):119–132. [PubMed: 21531987]
7. Yao B, Mackie K. Endocannabinoid receptor pharmacology. *Curr. Top. Behav. Neurosci.* 2009; 1:37–63. [PubMed: 21104379]
8. Pacher P, Kunos G. Modulating the endocannabinoid system in human health and disease- successes and failures. *FEBS J.* 2013; 280(9):1918–1943. [PubMed: 23551849]

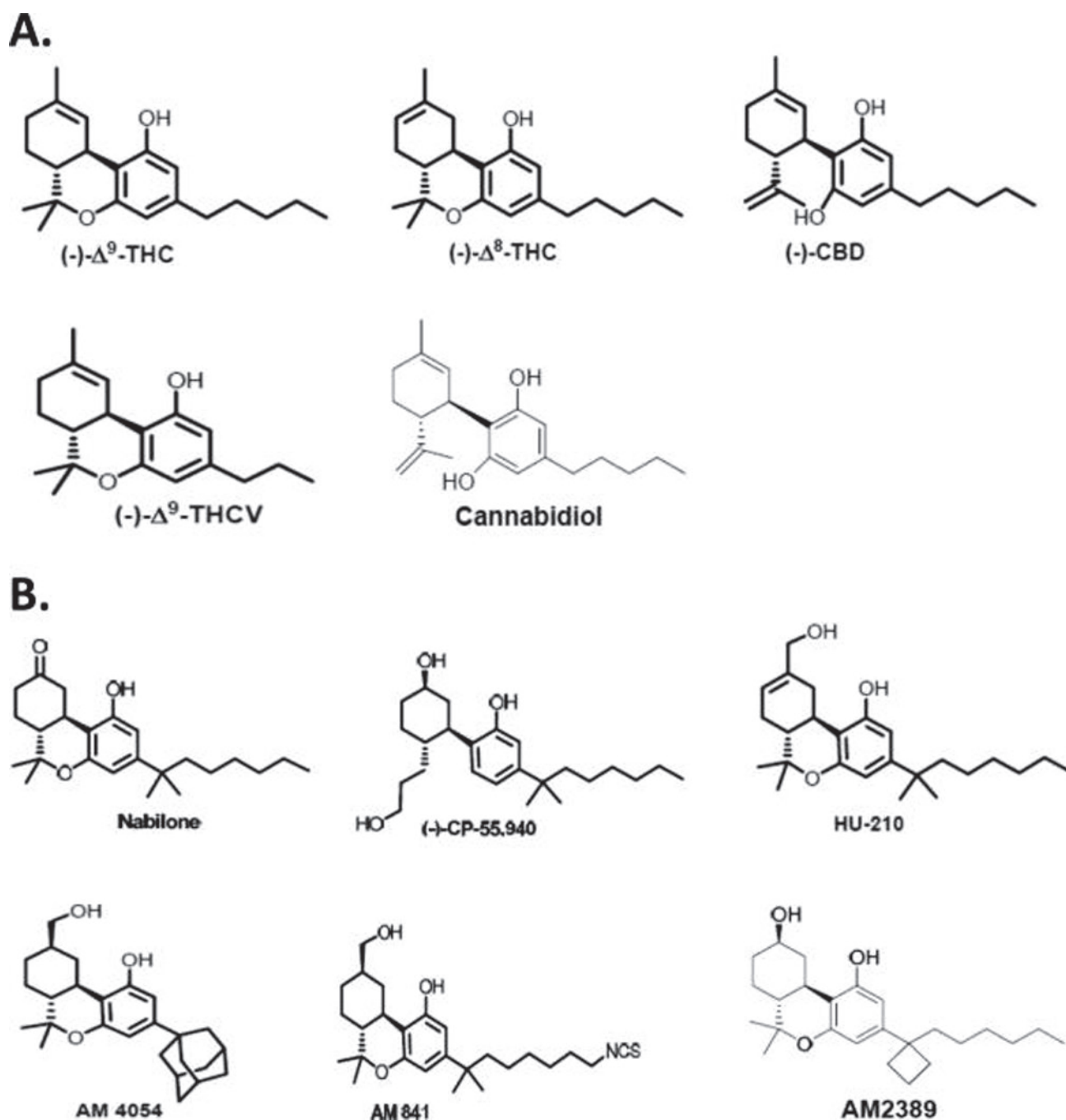
9. Janero DR, Lindsley L, Vemuri VK, Makriyannis A. Cannabinoid 1 G protein-coupled receptor (periphere-)neutral antagonists: emerging therapeutics for treating obesity-driven metabolic disease and reducing cardiovascular risk. *Expert Opin. Drug Discovery*. 2011; 6(10):995–1025.
10. Hwang J, Adamson C, Butler D, Janero DR, Makriyannis A, Bahr BA. Enhancement of endocannabinoid signaling by fatty acid amide hydrolase inhibition: a neuroprotective therapeutic modality. *Life Sci*. 2010; 86(15–16):615–623. [PubMed: 19527737]
11. Dadon D, Minke B. Cellular functions of transient receptor potential channels. *Int. J. Biochem. Cell Biol*. 2010; 42(9):1430–1445. [PubMed: 20399884]
12. Zheng J. Molecular mechanism of TRP channels. *Compr. Physiol*. 2013; 3(1):221–242. [PubMed: 23720286]
13. Vay L, Gu C, McNaughton PA. The thermo-TRP ion channel family: properties and therapeutic implications. *Br. J. Pharmacol*. 2012; 165(4):787–801. [PubMed: 21797839]
14. Nelson PL, Beck A, Cheng H. Transient receptor proteins illuminated: current views on TRPs and disease. *Vet. J*. 2011; 187(2):153–164. [PubMed: 20347603]
15. Wescott SA, Rauthan M, Xu XZ. When a TRP goes bad: transient receptor potential channels in addiction. *Life Sci*. 2013; 92(8–9):410–414. [PubMed: 22820171]
16. Moran MM, McAlexander MA, Biro T, Szallasi A. Transient receptor potential channels as therapeutic targets. *Nat. Rev. Drug Discovery*. 2011; 10(8):601–620. [PubMed: 21804597]
17. Quadid-Ahoidouch H, Dhennin-Duthille I, Gautoier M, Sevestere H, Ahidouch A. TRP channels: diagnostic markers and therapeutic targets for breast cancer? *Trends Mol. Med*. 2013; 19(2):117–124. [PubMed: 23253476]
18. Takeda Y, Numata T, Mori Y. Targeting TRPs in neurodegenerative disorders. *Curr. Top. Med. Chem*. 2013; 13(3):322–334. [PubMed: 23432063]
19. Di Marzo V, De Petrocellis L. Endocannabinoids as regulators of transient receptor potential (TRP) channels: A further opportunity to develop new endocannabinoid-based therapeutic drugs. *Curr. Med. Chem*. 2010; 17(14):1430–1439. [PubMed: 20166923]
20. Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sörgård M, Di Marzo V, Julius D, Högestätt ED. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature*. 1999; 400(6743):452–457. [PubMed: 10440374]
21. Pertwee RG. Receptors and channels targeted by synthetic cannabinoid receptor agonists and antagonists. *Curr. Med. Chem*. 2010; 17(14):1360–1381. [PubMed: 20166927]
22. Harteneck C, Klose C, Krautwurst D. Synthetic modulators of TRP channel activity. *Adv. Exp. Med. Biol*. 2011; 704:87–106. [PubMed: 21290290]
23. Pertwee RG. Cannabinoid pharmacology: the first 66 years. *Br. J. Pharmacol*. 2006; 147(Supplement 1):S163–S171. [PubMed: 16402100]
24. Gaoni Y, Mechoulam R. Isolation, structure, and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc*. 1964; 86(8):1646–1647.
25. Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids:  $\Delta^9$ -tetrahydrocannabinol, cannabidiol and  $\Delta^8$ -tetrahydrocannabivarin. *Br. J. Pharmacol*. 2008; 153(2):199–215. [PubMed: 17828291]
26. Mechoulam R, Parker LA, Gallily R. Cannabidiol: an overview of some pharmacological aspects. *J. Clin. Pharmacol*. 2002; 42(11 Supplement):11S–19S. [PubMed: 12412831]
27. Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R. Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol. Sci*. 2009; 30(10):515–527. [PubMed: 19729208]
28. Raffa RB, Ward SJ. CB1-independent mechanisms of  $\Delta^9$ -THCV, AM251 and SR141716. *J. Clin. Pharm. Ther*. 2012; 37(3):260–265. [PubMed: 21740450]
29. Ottani A, Giuliani D. HU 210: a potent tool for investigations of the cannabinoid system. *CNS Drug Rev*. 2001; 7(2):131–145. [PubMed: 11474421]
30. Thnakur GA, Bajaj S, Paronis C, Peng Y, Bowman AL, Barak LS, Caron MG, Parrish D, Deschamps JR, Makriyannis A. Novel adamantyl cannabinoids as CB1 receptor probes. *J. Med. Chem*. 2013; 56(10):3904–3921. [PubMed: 23621789]

31. Picone RP, Khanolkar AD, Ayotte LA, Thakur GA, Hurst DP, Abood ME, Reggio PH, Fournier DJ, Makriyannis A. (–)-7'-Isothiocyanato-11-hydroxy-1',1'-dime-thylheptylhexahydrocannabinol (AM841), a high-affinity electrophilic ligand, interacts covalently with a cysteine in helix six and activates the CB1 cannabinoid receptor. *Mol. Pharmacol.* 2005; 68(6):1623–1635. [PubMed: 16157695]
32. Nikas SP, Alapafuja SO, Papanastasiou I, Paronis CA, Shukla VG, Papahatjis DP, Bowman AL, Halikhedkar A, Han X, Makriyannis A. Novel 1',1'-chain substituted hexahydrocannabinols: 9 $\beta$ -hydroxy-3-(1-hexyl-cyclobut-1-yl)-hexahydrocannabinol (AM2389) a highly potent cannabinoid receptor 1 (CB1) agonist. *J. Med. Chem.* 2010; 53(19):6996–7010. [PubMed: 20925434]
33. Szymanski DW, Papanastasiou M, Melchior K, Zvonok N, Mercier RW, Janero DR, Thanur GA, Cha S, Wu B, Karger B, Makriyannis A. Mass spectrometry-based proteomics of human cannabinoid receptor 2: covalent cysteine 6.47(257)-ligand interaction affording megagonist receptor activation. *J. Proteome Res.* 2011; 10(10):4789–4798. [PubMed: 21861534]
34. Sharma R, Nikas SP, Paronis CA, Wood JT, Halikhedkar A, Guo JJ, Thakur GA, Kulkarni S, Benchama O, Raghav JG, Gifford RS, Järbe TU, Bergman J, Makriyannis A. Controlled-deactivation cannabinergic ligands. *J. Med. Chem.* 2013; 56(24):10142–10157. [PubMed: 24286207]
35. Hillard CJ, Manna S, Greenberg MJ, DiCamelli R, Ross RA, Stevenson LA, Murphy V, Pertwee RG, Campbell WB. Synthesis and characterization of potent and selective agonists of the neuronal cannabinoid receptor (CB1). *J. Pharmacol. Exp. Ther.* 1999; 289(3):1427–1433. [PubMed: 10336536]
36. Abadji V, Lin S, Taha G, Griffin G, Stevenson LA, Pertwee RG, Makriyannis A. (R)-methanandamide: a chiral novel anandamide possessing higher potency and metabolic stability. *J. Med. Chem.* 1994; 37(12):1889–1893. [PubMed: 8021930]
37. Papahatjis DP, Nahmias VR, Nikas SP, Schimpfen M, Makriyannis A. Design and synthesis of (13S)-methyl-substituted arachidonic acid analogues: templates for novel endocannabinoids. *Chemistry.* 2010; 16:4091–4099. [PubMed: 20187040]
38. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor—a heat-activated ion channel in the pain pathway. *Nature.* 1997; 389(6653):816–824. [PubMed: 9349813]
39. Barbero GF, Ruiz AG, Liazid A, Palma M, Vera JC, Barroso CG. Evolution of total and individual capsaicinoids in peppers during ripening of the Cayenne pepper plant (*Capsicum annuum* L.). *Food Chem.* 2014; 153:200–206. [PubMed: 24491721]
40. Szallasi A, Blumberg PM. Resiniferatoxin and its analogs provide novel insights into the pharmacology of the vanilloid (capsaicin) receptor. *Life Sci.* 1990; 47(16):1399–1408. [PubMed: 2174484]
41. De Petrocellis L, Ligresti A, Moriello AS, Allarà M, Bisogno T, Petrosino S, Stott CG, Di Marzo V. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. *Br. J. Pharmacol.* 2011; 163(7):1479–1494. [PubMed: 21175579]
42. De Petrocellis L, Orlando P, Moriello AS, Aviello G, Stott C, Izzo AA, Di Marzo V. Cannabinoid actions at TRPV channels: effects on TRPV3 and TRPV4 and their potential relevance to gastrointestinal inflammation. *Acta Physiol.* 2012; 204(2):255–266.
43. Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. *Neuron.* 2004; 41(6):849–857. [PubMed: 15046718]
44. Chung G, Im ST, Kim YH, Jung SJ, Rhyu MR, Oh SB. Activation of transient receptor potential ankyrin 1 by eugenol. *Neuroscience.* 2014; 261:153–160. [PubMed: 24384226]
45. Dedov VN, Tran VH, Duke CC, Connor M, Christie MJ, Mandadi S, Roufogalis BD. Gingerols: a novel class of vanilloid receptor (VR1) agonists. *Br. J. Pharmacol.* 2002; 137(6):793–798. [PubMed: 12411409]
46. Zhong J, Minassi A, Prenen J, Tagliabue S, Scafati O, Appendino G, Nilius B. Umbellulone modulates TRP channels. *Pfluegers Arch.* 2011; 462(6):861–870. [PubMed: 22038323]

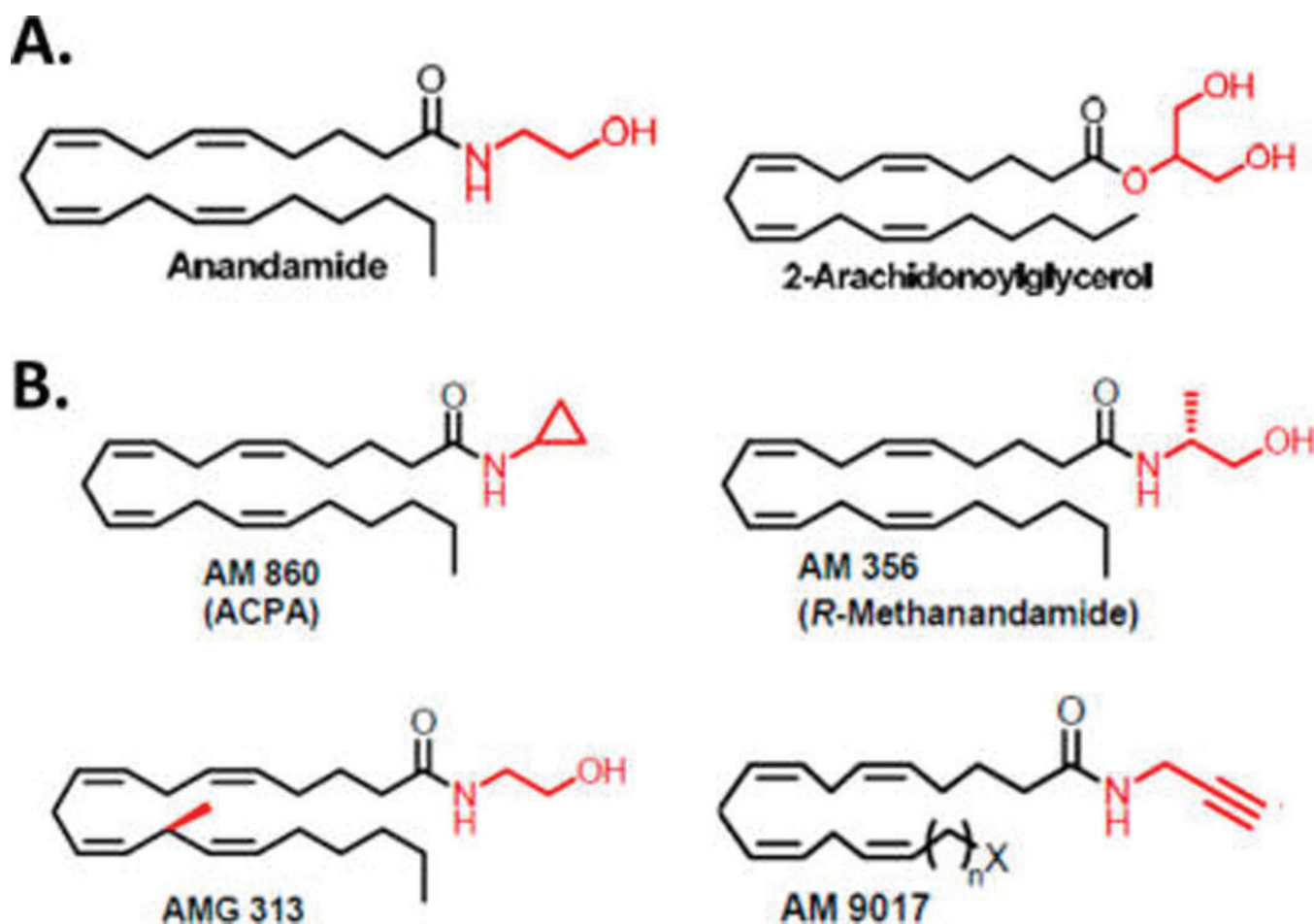
47. Chianese G, Fattorusso E, Putra MY, Calcinai B, Bavestrello G, Moriello AS, De Petrocellis L, Di Marzo V, Tagliatella-Scafati O. Leucettamols, bifunctionalized marine sphingoids, act as modulators of TRPA1 and TRPM8 channels. *Mar. Drugs*. 2012; 10(11):2435–2447. [PubMed: 23203269]
48. Morera E, De Petrocellis L, Morera L, Moriello AS, Nalli M, Di Marzo V, Ortar G. Synthesis and biological evaluation of [6]-gingerol analogues as transient receptor potential channel TRPV1 and TRPA1 modulators. *Bioorg. Med. Chem. Lett*. 2012; 22(4):1674–1677. [PubMed: 22257892]
49. Bang S, Yoo S, Oh U, Hwang SW. Endogenous lipid-derived ligands for sensory TRP ion channels and their pain modulation. *Arch. Pharm. Res*. 2010; 33(10):1509–1520. [PubMed: 21052930]
50. Palazzo E, Rossi F, de Novellis V, Maione S. Endogenous modulators of TRP channels. *Curr. Top. Med. Chem*. 2013; 13(3):398–407. [PubMed: 23432068]
51. Bradshaw HB, Raboune S, Hollis JL. Opportunistic activation of TRP receptors by endogenous lipids: exploiting lipidomics to understand TRP receptor cellular communication. *Life Sci*. 2013; 92(8–9):404–409. [PubMed: 23178153]
52. Syed SK, Bui HH, Beavers LS, Farb TB, Ficorilli J, Chesterfield AK, Kuo MS, Bokvist K, Barrett DG, Efanov AM. Regulation of GPR119 receptor activity with endocannabinoid-like lipids. *Am. J. Physiol. Endocrinol. Metab*. 2012; 303(12):E1469–E1478. [PubMed: 23074242]
53. Maione S, De Petrocellis L, de Novellis V, Moriello AS, Petrosino S, Palazzo E, Rossi FS, Woodward DF, Di Marzo V. Analgesic actions of N-arachidonoyl-serotonin, a fatty acid amide hydrolase inhibitor with antagonistic activity at vanilloid TRPV1 receptors. *Br. J. Pharmacol*. 2007; 150(6):766–781. [PubMed: 17279090]
54. Sagar DR, Smith PA, Millns PJ, Smart D, Kendall DA, Chapman V. TRPV1 and CB(1) receptor-mediated effects of the endovanilloid/endocannabinoid N-arachidonoyl-dopamine on primary afferent fibre and spinal cord neuronal responses in the rat. *Eur. J. Neurosci*. 2004; 20(1):175–184.
55. Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ, Krey JF, Chu CJ, Miller JD, Davies SN, Geppetti P, Walker JM, Di Marzo V. An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc. Natl. Acad. Sci. U.S.A.* 2002; 99(12):8400–8405. [PubMed: 12060783]
56. Di Marzo V, Griffin G, De Petrocellis L, Brandi I, Bisogno T, Williams W, Grier MC, Kulasegaram S, Mahadevan A, Razdan RK, Martin BR. A structure/activity relationship study on arvanil, an endocannabinoid and vanilloid hybrid. *J. Pharmacol. Exp. Ther*. 2002; 300(3):984–991. [PubMed: 11861807]
57. Beltramo M, Stella N, Calignano A, Lin SY, Makriyannis A, Piomelli D. Functional role of high-affinity anandamide transport, as revealed by selective inhibition. *science*. 1997; 277(5329):1094–1097. [PubMed: 9262477]
58. Rawls SM, Ding Z, Cowan A. Role of TRPV1 and cannabinoid CB1 receptors in AM 404-evoked hypothermia in rats. *Pharmacol. Biochem. Behav*. 2006; 83(4):508–516. [PubMed: 16647109]
59. Högestätt ED, Jönsson BA, Ermund A, Andersson DA, Björk H, Alexander JP, Cravatt BF, Basbaum AI, Zygmunt PM. Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. *J. Biol. Chem*. 2008; 280(36):31405–31412. [PubMed: 15987694]
60. Fernandes ES, Vong CT, Quek S, Cheong J, Awal S, Gentry C, Aubdool AA, Liang L, Bodkin JV, Bevan S, Heads R, Brain SD. Superoxide generation and leukocyte accumulation: key elements in the mediation of leukotriene B<sub>4</sub>-induced itch by transient receptor potential ankyrin 1 and transient receptor potential vanilloid 1. *FASEB J*. 2013; 27(4):1664–1673. [PubMed: 23271050]
61. Hwang SW, Cho H, Kwak J, Lee SY, Kang CJ, Jung J, Cho S, Min KH, Suh YG, Kim D, Oh U. Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. *Proc. Natl. Acad. Sci. U.S.A.* 2000; 97(11):6155–6160. [PubMed: 10823958]
62. Serhan CN, Dalli J, Karamnov S, Choi A, Park CK, Xu ZZ, Ji RR, Zhu M, Petasis NA. Macrophage proresolving mediator maresin 1 stimulates tissue regeneration and controls pain. *FASEB J*. 2012; 26(4):1755–1765. [PubMed: 22253477]
63. Trevisani M, Siemens J, Materazzi S, Bautista DM, Nassini R, Campi B, Imachi N, André E, Patacchini R, Cottrell GS, Gatti R, Basbaum AI, Bunnett NW, Julius D, Geppetti P. 4-Hydroxynonenal, an endogenous aldehyde, causes pain and neurogenic inflammation through

- activation of the irritant receptor TRPA1. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104(33):13519–13524. [PubMed: 17684094]
64. Sisignano M, Park CK, Angioni C, Zhang DD, von Hehn C, Cobos EJ, Ghasemlou N, Xu ZZ, Kumaran V, Lu R, Grant A, Fischer MJ, Schmidtke A, Reeh P, Ji RR, Woolf CJ, Geisslinger G, Scholich K, Brenneis C. 5,6-EET is released upon neuronal activity and induces mechanical pain hypersensitivity via TRPA1 on central afferent terminals. *J. Neurosci.* 2012; 32(18):6364–6372. [PubMed: 22553041]
65. Vriens J, Owsianik G, Fisslthaler B, Suzuki M, Janssens A, Voets T, Morisseau C, Hammock BD, Fleming I, Busse R, Nilius B. Modulation of the  $\text{Ca}^{2+}$  permeable cation channel TRPV4 by cytochrome P450 epoxigenases in vascular endothelium. *Circ. Res.* 2005; 97(9):908–915. [PubMed: 16179585]
66. Malan TP Jr, Ibrahim MM, Lai J, Vanderah TW, Makriyannis A, Porreca F. CB2 cannabinoid receptor agonists: pain relief without psychoactive effects? *Curr. Opin. Pharmacol.* 2003; 3(1):62–67. [PubMed: 12550743]
67. Li J, Daughters RS, Bullis C, Bengiamin R, Stucky MW, Brennan J, Simone DA. The cannabinoid receptor agonist WIN 55,212-2 mesylate blocks the development of hyperalgesia produced by capsaicin in rats. *Pain.* 1999; 81(1–2):25–33. [PubMed: 10353490]
68. Quartilho A, Mata HP, Ibrahim MM, Vanderah TW, Porreca F, Makriyannis A, Malan TP Jr. Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. *Anesthesiology.* 2003; 99(4):955–960. [PubMed: 14508331]
69. D'Ambra TE, Estep KG, Bell MR, Eissenstat MA, Josef KA, Ward SJ, Haycock DA, Baizman ER, Casiano FM, Beglin NC, Chippari SM, Grego JD, Kullnig RK, Daley GT. Conformationally restrained analogues of pravadoline: nanomolar potent, enantioselective, (aminoalkyl)indole agonists of the cannabinoid receptor. *J. Med. Chem.* 1992; 35(1):124–135. [PubMed: 1732519]
70. Malan TP Jr, Ibrahim MM, Deng H, Liu Q, Mata HP, Vanderah T, Porreca F, Makriyannis A. CB2 cannabinoid receptor-mediated peripheral antinociception. *Pain.* 2001; 93(3):239–245. [PubMed: 11514083]
71. Akopian AN, Ruparel NB, Patwardhan A, Hargreaves KM. Cannabinoids desensitize capsaicin and mustard oil responses in sensory neurons via TRPA1 activation. *J. Neurosci.* 2008; 28(5):1064–1075. [PubMed: 18234885]
72. Watanabe H, Vriens J, Prenen J, Droogmans G, Voets T, Nilius B. Anandamide and arachidonic acid use epoxyeicosatrienoic acids to activate TRPV4 channels. *Nature.* 2003; 424(6947):434–438. [PubMed: 12879072]
73. Zygmunt PM, Chuang H, Movahed P, Julius D, Högestätt ED. The anandamide transport inhibitor AM404 activates vanilloid receptors. *Eur. J. Pharmacol.* 2000; 396(1):39–42. [PubMed: 10822052]
74. Barrière DA, Mallet C, Blomgren A, Simonsen C, Daulhac L, Libert F, Chapuy E, Etienne M, Högestätt ED, Zygmunt PM, Eschalié A. Fatty acid amide hydrolase-dependent generation of antinociceptive drug metabolites acting on TRPV1 in the brain. *PLoS One.* 2013; 5(9):e12748. DOI: doi:10.1371/journal.pone.0012748. [PubMed: 20862299]
75. Maldonado R, Berrendero F, Ozaita A, Robledo P. Neurochemical basis of cannabis addiction. *Neuroscience.* 2011; 181:1–17. [PubMed: 21334423]
76. Pryce G, Visintin C, Ramagopalan SV, Al-Izki S, De Faveri LE, Nuamah RA, Mein CA, Montpetit A, Hardcastle AJ, Kooij G, de Vries HE, Amor S, Thomas SA, Ledent C, Marsicano G, Lutz B, Thompson AJ, Selwood DL, Giovannoni G, Baker D. Control of spasticity in a multiple sclerosis model using central nervous system-excluded CB1 cannabinoid receptor agonists. *FASEB J.* 2014; 28(1):117–130. [PubMed: 24121462]
77. Miller LK, Devi LA. The highs and lows of cannabinoid receptor expression in disease: mechanisms and their therapeutic implications. *Pharmacol. Rev.* 2011; 63(3):461–470. [PubMed: 21752875]
78. Rani Sagar D, Burston JJ, Woodhams SG, Chapman V. Dynamic changes to the endocannabinoid system in models of chronic pain. *Philos. Trans. R. Soc. London, Ser. B.* 2012; 367(1607):3300–3311. [PubMed: 23108548]
79. García-Merino A. Endocannabinoid system modulator use in everyday clinical practice in the UK and Spain. *Expert Rev. Neurother.* 2013; 13(Supplement 1):9–13. [PubMed: 23369054]

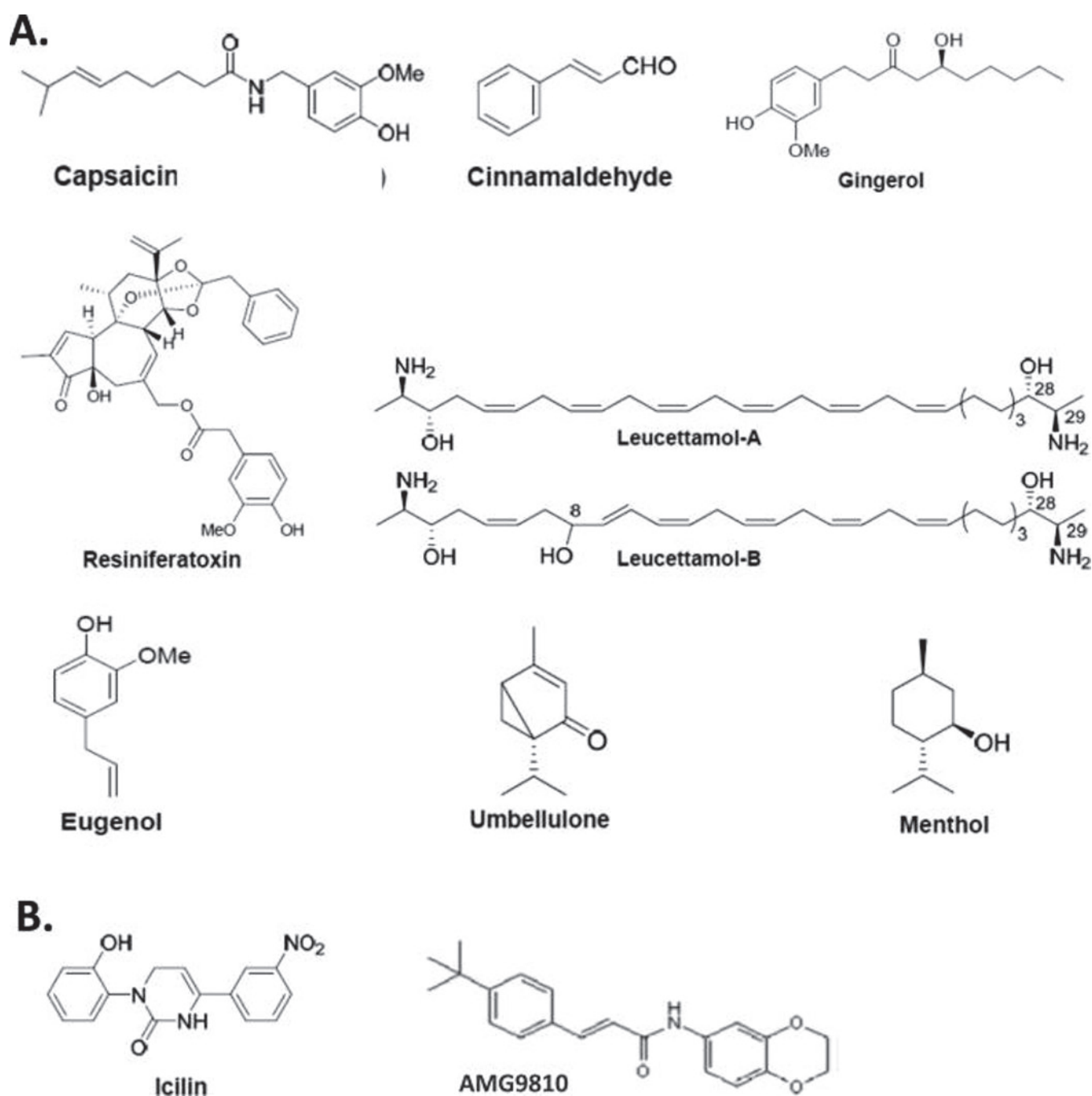
80. Janero DR, Lindsley L, Vemuri VK, Makriyannis A. Cannabinoid 1 G protein-coupled receptor (periphero-)neutral antagonists: emerging therapeutics for treating obesity-driven metabolic disease and reducing cardiovascular risk. *Expert Opin. Drug Discovery*. 2011; 6(10):995–1025.
81. Farco JA, Grundmann O. Menthol— pharmacology of an important naturally medicinal “cool”. *Mini Rev. Med. Chem*. 2013; 13:124–131. [PubMed: 23061635]
82. Uhl GR, Walther D, Behm FM, Rose JE. Menthol preference among smokers: association with TRPA1 variants. *Nicotine Tob. Res*. 2011; 13(12):1311–1315. [PubMed: 21719896]
83. Anderson SJ. Menthol cigarettes and smoking cessation behaviour: a review of tobacco industry documents. *Tob. Control*. 2011; 20(Supplement 2):49–56.
84. Willis DN, Liu B, Ha MA, Jordt SE, Morris JB. Menthol attenuates respiratory irritation responses to multiple cigarette smoke irritants. *FASEB J*. 2011; 25(12):4434–4444. [PubMed: 21903934]
85. Cahill K, Stevens S, Lancaster T. Pharmacological treatments for smoking cessation. *JAMA, J. Am. Med. Assoc*. 2014; 311(2):193–194.
86. Le Foll B, Forget B, Aubin HJ, Goldberg SR. Blocking cannabinoid CB1 receptors for the treatment of nicotine dependence: insights from pre-clinical and clinical studies. *Addict. Biol*. 2008; 13(2):239–252. [PubMed: 18482433]
87. Morgan CJ, Das RK, Joye A, Curran HV, Kamboj SK. Cannabidiol reduces cigarette consumption in tobacco smokers: preliminary findings. *Addict. Behav*. 2013; 38(9):2433–2436. [PubMed: 23685330]
88. RJ. Reynolds Tobacco Company Annual Performance Plan Review 1989 (890000). Available at: [http://tobaccodocuments.org/rjr/507930836-0893.html?start\\_page=1&end\\_page=58](http://tobaccodocuments.org/rjr/507930836-0893.html?start_page=1&end_page=58)
89. Peier AM, Moqrich A, Hergarden AC, Reeve AJ, Andersson DA, Story GM, Earley TJ, Dragoni I, McIntyre P, Bevan S, Patapoutian A. A TRP channel that senses cold stimuli and menthol. *Cell*. 2002; 108(5):705–715. [PubMed: 11893340]
90. Voets T, Droogmans G, Wissenbach U, Janssens A, Flockerzi V, Nilius B. The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. *Nature*. 2004; 430(7001):748–754. [PubMed: 15306801]
91. Chuang HH, Neuhausser WM, Julius D. The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. *Neuron*. 2004; 43(6):859–869. [PubMed: 15363396]
92. Macpherson LJ, Dubin AE, Evans MJ, Marr F, Schultz PG, Cravatt BF, Patapoutian A. Noxious compounds activate TRPA1 ion channels through covalent modification of cysteines. *Nature*. 2007; 445(7127):541–545. [PubMed: 17237762]
93. Cristino L, de Petrocellis L, Pryce G, Baker D, Guglielmotti V, Di Marzo V. Immunohistochemical localization of cannabinoid type 1 and vanilloid transient receptor potential vanilloid type 1 receptors in the mouse brain. *Neuroscience*. 2006; 139(4):1405–1415. [PubMed: 16603318]
94. Wilhelmssen K, Khakpour S, Tran A, Sheehan K, Schumacher M, Xu F, Hellman J. The endocannabinoid/endovanilloid *N*-arachidonoyl dopamine (NADA) and synthetic cannabinoid WIN55,212-2 abate the inflammatory activation of human endothelial cells. *J. Biol. Chem*. 2014; 289:13079–13100. [PubMed: 24644287]
95. Vilela LR, Medeiros DC, de Oliveira AC, Moraes MF, Moreira FA. Anticonvulsant Effects of *N*-arachidonoyl-serotonin, a dual fatty acid amide hydrolase enzyme and transient receptor potential vanilloid Type-1 (TRPV1) channel blocker, on experimental seizures: The roles of cannabinoid CB1 receptors and TRPV1 channels. *Basic Clin. Pharmacol. Toxicol*. 2014 DOI: 10.1111/bcpt.12232.



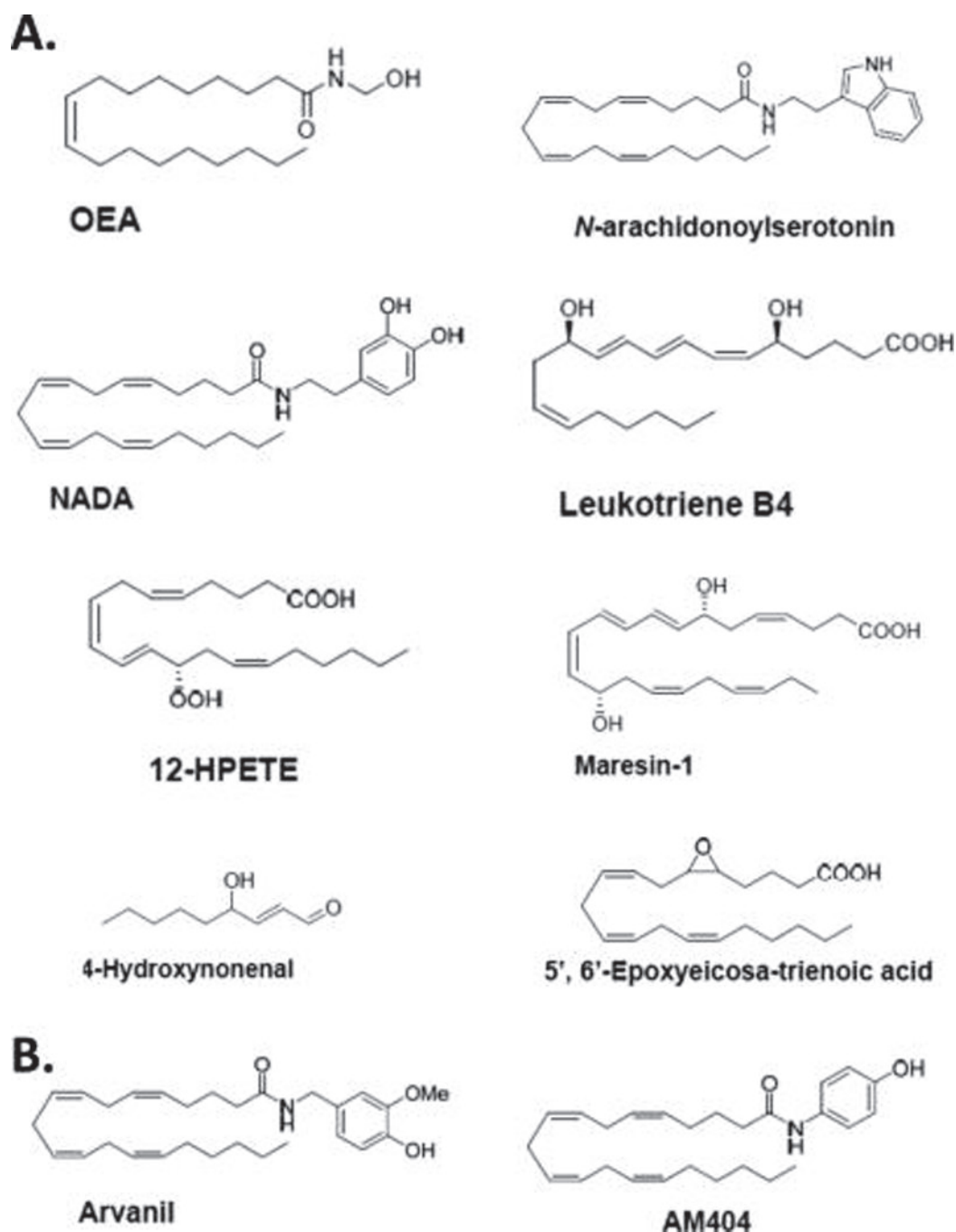
**Figure 1.**  
Chemical structures of select plant-derived terpenoid cannabinoids (phytocannabinoids) (A) and select synthetic terpenoid cannabinoids (B) discussed in the text.



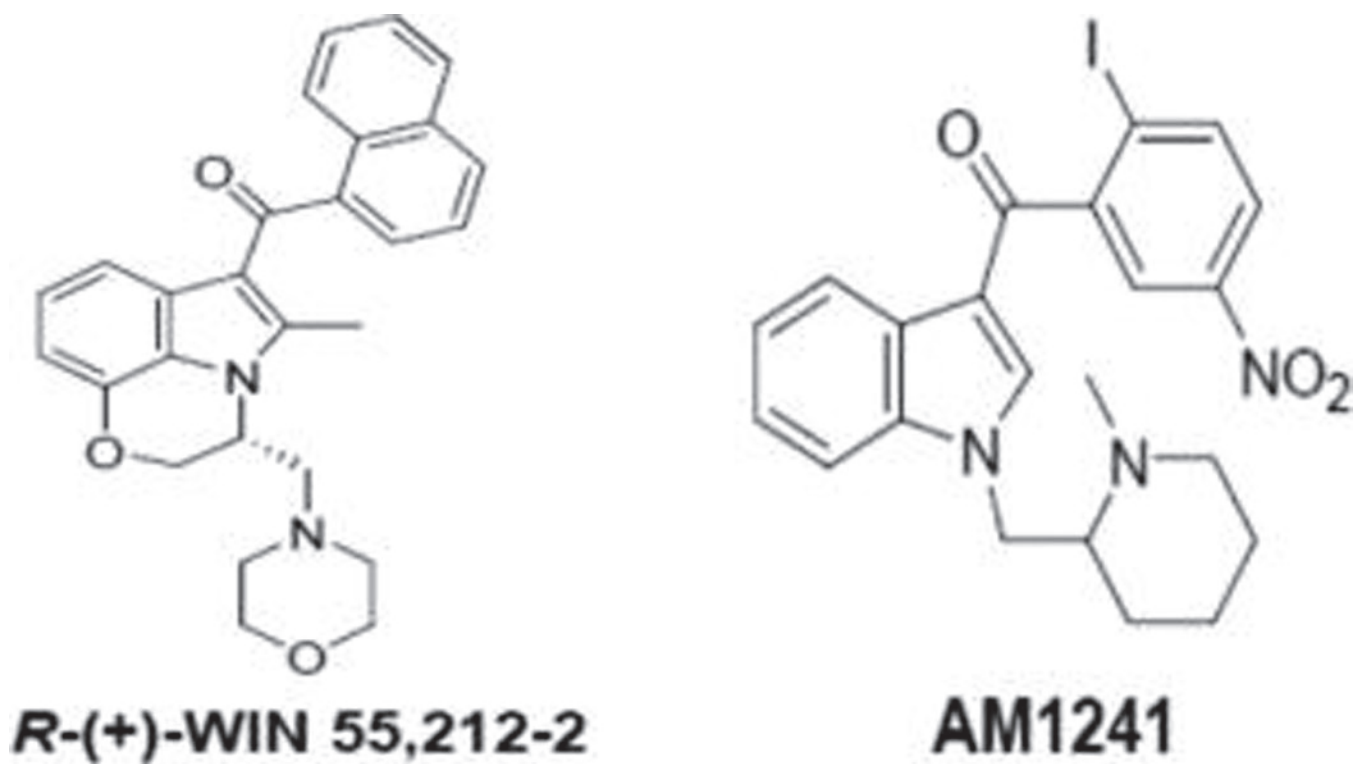
**Figure 2.**  
Chemical structures of select lipid endocannabinoids (A) and structurally related synthetic cannabinergic agents (B) discussed in the text.



**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.

Review

# Terpenoids, Cannabimimetic Ligands, beyond the *Cannabis* Plant

Elaine C. D. Gonçalves <sup>1,2</sup> , Gabriela M. Baldasso <sup>1,†</sup> , Máira A. Bicca <sup>3,†</sup> , Rodrigo S. Paes <sup>1</sup> , Raffaele Capasso <sup>4,\*</sup>  and Rafael C. Dutra <sup>1,2,\*</sup> 

<sup>1</sup> 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.)

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

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

<sup>4</sup> 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.

Academic Editor: Derek J. McPhee

Received: 29 February 2020; Accepted: 27 March 2020; Published: 29 March 2020



**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— $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -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 20<sup>th</sup> 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  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -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
$\Delta^9$ -THC types	23
$\Delta^8$ -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  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -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  $\Delta^9$ -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  $\Delta^9$ -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-arachidonylethanolamine molecule, named anandamide from porcine brain. Interestingly, anandamide interact to CBR and induces behavioral actions similar to the ones induced by  $\Delta^9$ -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].

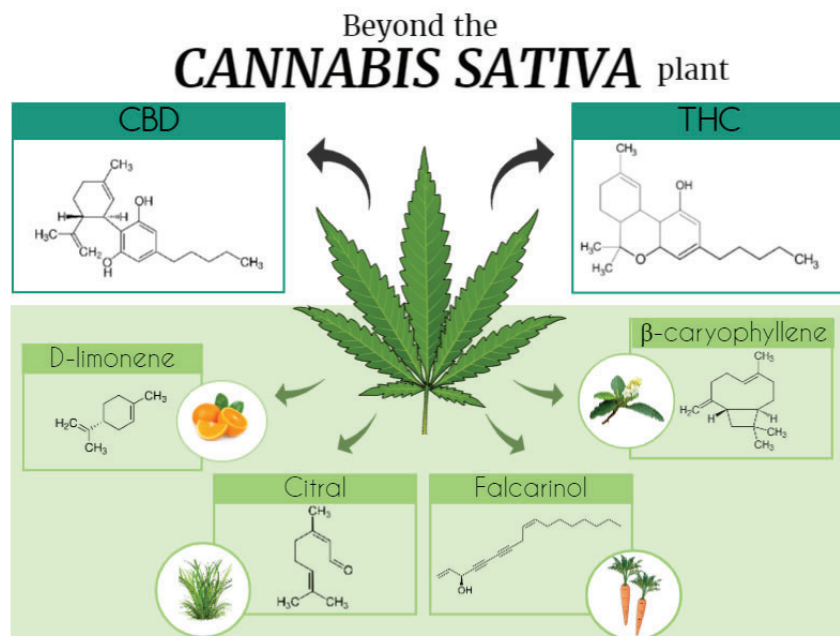
Importantly, the ECS (i.e., CBR, endogenous cannabinoids, and anabolic/catabolic enzymes) are present in the cardiovascular tissues (myocardium, smooth muscle, and vascular endothelial cells), as well as in the circulating blood cells [58]. CB1R are expressed in the peripheral nervous system, including 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 cannabinimimetic 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 cannabinimimetic 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 <i>tau</i> 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- $\kappa$ B and interferon- $\beta$ /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 <sub>1A</sub> -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- $\kappa$ B, nuclear factor kappa B; STAT, signal transducer and activator of transcription protein family; 5-HT<sub>1A</sub>, 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,  $\Delta^9$ -tetrahydrocannabinol (THC). However, many other natural compounds were also identified, totaling 565 substances among cannabinoids and non-cannabinoids constituents. This figure illustrates some of the *Cannabis sativa* compounds (D-limonene,  $\beta$ -caryophyllene, citral, and falcariinol) 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.

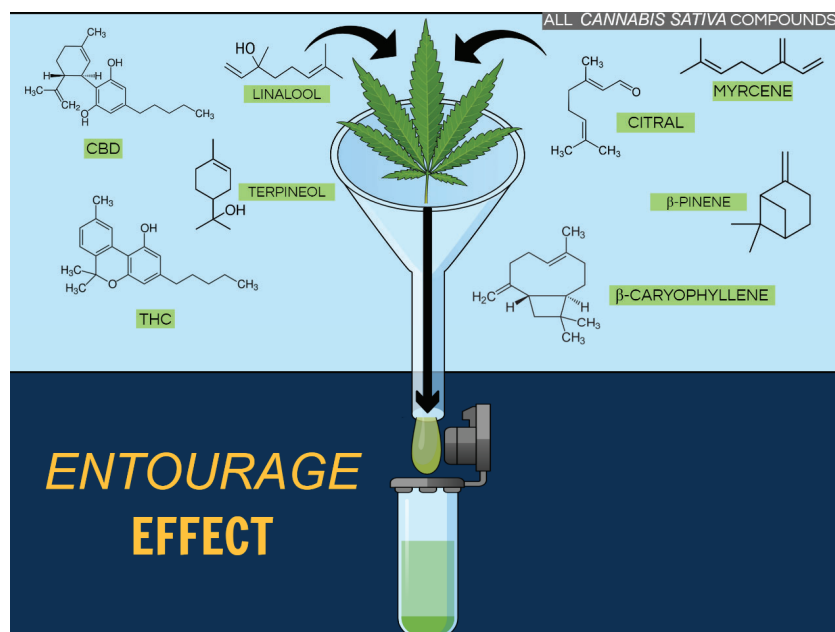
## 2. Cannabis Phytocannabinoids: Focus on Tetrahydrocannabinol and Cannabidiol

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,  $\Delta^8$ -e  $\Delta^9$ -THC, cannabigerol, and cannabivarin. The  $\Delta^9$ -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<sub>1</sub> 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  $\Delta^9$ -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  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -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  $\Delta^9$ -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 were 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,  $\beta$ -myrcene,  $\alpha$ -pinene,  $\alpha$ -terpineol,  $\beta$ -pinene,  $\beta$ -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 ( $\beta$ )- and $\alpha$ -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  $\beta$ -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  $\beta$ - and  $\alpha$ - 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-1 $\beta$  (IL-1 $\beta$ ) 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- $\gamma$ ) 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-1 $\beta$ , 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  $\beta$ -cyclodextrin 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 A $\beta$ 42-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- $\kappa$ B (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 A $\beta$ 1–40-induced cognitive impairment in mice, which it was suggested to be mediated by inhibition of apoptosis and oxidative stress induced by A $\beta$ -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  $\beta$ -cyclodextrin ( $\beta$ CD) (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,  $\alpha$ -terpineol- $\beta$ CD 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-HT<sub>3</sub> 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 its 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  $\alpha$ -terpineol were found to suppress the production of inflammatory mediators (e.g., NF- $\kappa$ B, 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  $\alpha$ -terpineol loaded chitosan nanoemulsion ( $\alpha$ -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 algacide [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 through modulation of NF- $\kappa$ B signaling pathway [135]. Thus, it is possible to 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  $\gamma$ -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  $\gamma$ -terpinene on pro- and anti-inflammatory macrophage production of cytokines in an animal model. The authors reported that  $\gamma$ -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%),  $\gamma$ -terpinene (20.15%), and  $\alpha$ -terpinene (9.85%), on MCF-7 breast cancer cells [147,148]. Authors reported  $\gamma$ -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  $\gamma$ -terpinene could serve as natural immunomodulatory agent with antioxidant, antimicrobial, and anticancer properties that could be useful therapeutically.

### 3.6. Alpha ( $\alpha$ )- and $\beta$ -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  $\alpha$ -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  $\alpha$ -pinene interacts with GABA<sub>A</sub>/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  $\alpha$ -pinene are also extended to convulsions [80,81], ischemic stroke [82], and schizophrenia [156]. Besides,  $\alpha$ -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  $\alpha$ -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  $\alpha$ -pinene appears to be devoid of anticonvulsant action, since only  $\beta$ -pinene affected the intensity of seizures and time of death of PTZ-treated mice [80]. Further, it was suggested that  $\alpha$ -pinene might serve as potential therapeutics for schizophrenia since it possibly suppresses neuronal activity. However, it has also been demonstrated that inhalation of  $\alpha$ -pinene inhibits dizocilpine (MK-801)-induced schizophrenia-like behavioral abnormalities in mice [156]. Lastly,  $\alpha$ -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  $\alpha$ -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,  $\alpha$ -pinene was able to prevent UVA-induced loss of mitochondrial membrane potential, lipid peroxidation, DNA damages, and ROS generation [158]. Likewise,  $\alpha$ -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/cm<sup>2</sup>/day, for 10 days [159]. In contrast,  $\alpha$ -pinene promoted cytotoxicity, 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  $\alpha$ -pinene was also able to inhibit human hepatoma tumor progression by inducing G2/M phase cell cycle arrest [162]. Regarding  $\alpha$ -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,  $\alpha$ -pinene exhibited significantly anti-inflammatory and analgesic effects through inhibition of COX-2. Moreover, the analgesic effect of  $\alpha$ -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  $\mu$ -opioid receptors [163].

Related to  $\alpha$ -pinene, another important monoterpene present in different *Cannabis sativa* L. varieties is  $\beta$ -pinene, which can also be found in many plants essential oils and obtained commercially by distillation or by  $\alpha$ -pinene conversion [165,166]. Literature describes  $\beta$ -pinene antimicrobial and antioxidant activity [167], as well as its derivatives have been associated to anticancer, anticoagulation, and antimalarial effects. Additionally,  $\beta$ -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  $\beta$ -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 Hisc12 (which encodes histamine-gated chloride channel subunit 2) [168]. However, according to Pajaro-Castro and colleagues,  $\beta$ -pinene showed little ability to dock on proteins associated with neurotransmission process in the *Tribolium castaneum* [168]. Even though the  $\beta$ -pinene-induced repellent effect still remains to be

fully addressed, it seems feasible to be considered that  $\beta$ -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  $\beta$ -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  $\beta$ -pinene, adult male ICR mice were pre-treated with (1S)-(-)- $\beta$ -pinene (100 mg/kg) and exposed to forced swimming test (FST). Results showed that  $\beta$ -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<sub>1A</sub> receptor antagonist prevented the antidepressant-like of  $\beta$ -pinene, demonstrating that this compound could interact with the serotonergic system. Likewise,  $\beta$ -pinene anti-immobility effects were also prevented by propranolol ( $\beta$ -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  $\beta$ -pinene as an antitumor, as well as antiviral and antifungal agent has also been explored. Regarding the former,  $\beta$ -pinene-based thiazole derivatives were investigated as antineoplastic agents in vitro. Twenty-four  $\beta$ -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,  $\beta$ -pinene reduced HSV-1 viral infectivity through interaction with free virus particles by 100% in a dose-dependent manner [174]. Similarly,  $\beta$ -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  $\beta$ -pinene and  $\alpha$ -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  $\alpha$ -pinene and  $\beta$ -pinene are able to play could be used to shed light on innovative approaches to treat a variety of neurological conditions.

### 3.7. $\beta$ -Elemene

$\beta$ -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,  $\beta$ -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  $\beta$ -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,  $\beta$ -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  $\beta$ -elemene

significantly inhibited growth of adenocarcinoma human alveolar basal epithelial cells A549 cells in a dose-dependent manner, when compared to  $\beta$ -elemene treatment alone [182]. Mechanistically, the exposition of A549 cells to hyperthermia plus  $\beta$ -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  $\beta$ -elemene co-loaded with chemotherapy drugs: i) cisplatin in co-loaded liposomes [193]; ii) doxorubicin (DOX) in pH-sensitive nanostructured lipid carriers (DOX/ $\beta$ -elemene Hyd NLCs) [194]; iii) cabazitaxel in complex liposome [195]. In summary, these reports described that  $\beta$ -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,  $\beta$ -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-NF $\kappa$ B-HIF-1 $\alpha$  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  $\beta$ -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  $\beta$ -elemene. Li and co-authors, for instance, provided evidence of  $\beta$ -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  $\beta$ -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,  $\beta$ -elemene exerted retino-protective effect by downregulation of hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ), VEGF, iNOS, and pro-inflammatory mediators during diabetes progression in a streptozotocin (STZ)-induced rat model [199]. Finally, the potential application of  $\beta$ -elemene in an EAE animal model was tested, in which mice were treated from day one after induction with  $\beta$ -elemene (20 mg/kg, i.p.) until the end of experiment. Beta-elemene reduced IFN- $\gamma$  and IL-17 levels and completely blocked EAE onset and the severity of clinical symptoms. Furthermore,  $\beta$ -elemene inhibited IL-17, IFN- $\gamma$ , ROR- $\gamma$ T, 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,  $\beta$ -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. $\beta$ -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, (+)- $\alpha$ -pinene, (–)-limonene, (+)- $\beta$ -pinene, terpinolene, and (E)- $\beta$ -ocimene [85]. Farré-Armengol and colleagues demonstrated that the emissions of  $\beta$ -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  $\beta$ -ocimene and  $\alpha$ -terpinolene, while  $\alpha$ - and  $\beta$ -pinene accompanied by extremely high  $\beta$ -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  $\mu\text{g/mL}$ , 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  $> 250 \mu\text{g/mL}$  [209]. Although there are not as much reports about  $\beta$ -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 peruvicol, is a noncyclic sesquiterpene alkene alcohol common to citrus peels, *Piper clausenianum*, *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- $\kappa$ B 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-1 $\beta$ , and

NF- $\kappa$ B 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- $\kappa$ B 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 chemical 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 bivoanae* 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-1 $\beta$ , 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- $\kappa$ B [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- $\kappa$ B activation via PPAR- $\gamma$  [254,255]. In accordance, Shen and colleagues demonstrated that GW9662 PPAR- $\gamma$  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<sup>+</sup> 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  $\beta$ -cyclodextrin and hydroxypropyl- $\beta$ -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 giganteus* 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 p53 activation, 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-octahydronicene-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- $\kappa$ B 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-1 $\beta$ -induced PGE2 through inhibition of NF- $\kappa$ B 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- $\gamma$ ) in response to the disease-related antigens, *ii*) IL-6/IL-17-related transcription factor STAT3, *iii*) cyclic citrullinated- and Bhs65-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 known 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/EBP $\alpha$  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- $\kappa$ B 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 PKC $\delta$  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<sup>®</sup> (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  $\beta$ -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<sub>1A</sub> 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  $\alpha$ 1A,  $\alpha$ 1B,  $\alpha$ 1D 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 (20 $\alpha$ -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/ $\beta$ -catenin signaling via GSK3 $\beta$  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- $\kappa$ B signaling inhibition during the carcinogenic process [338,339]. Corroborating, this triterpenoid has also been shown to attenuated colitis-associated colon cancer by modulating NF- $\kappa$ B 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 -EGFR2 and its downstream signaling pathways, which represent a key mechanism involved in the proliferation of cancer malignant phenotypes [344,345].

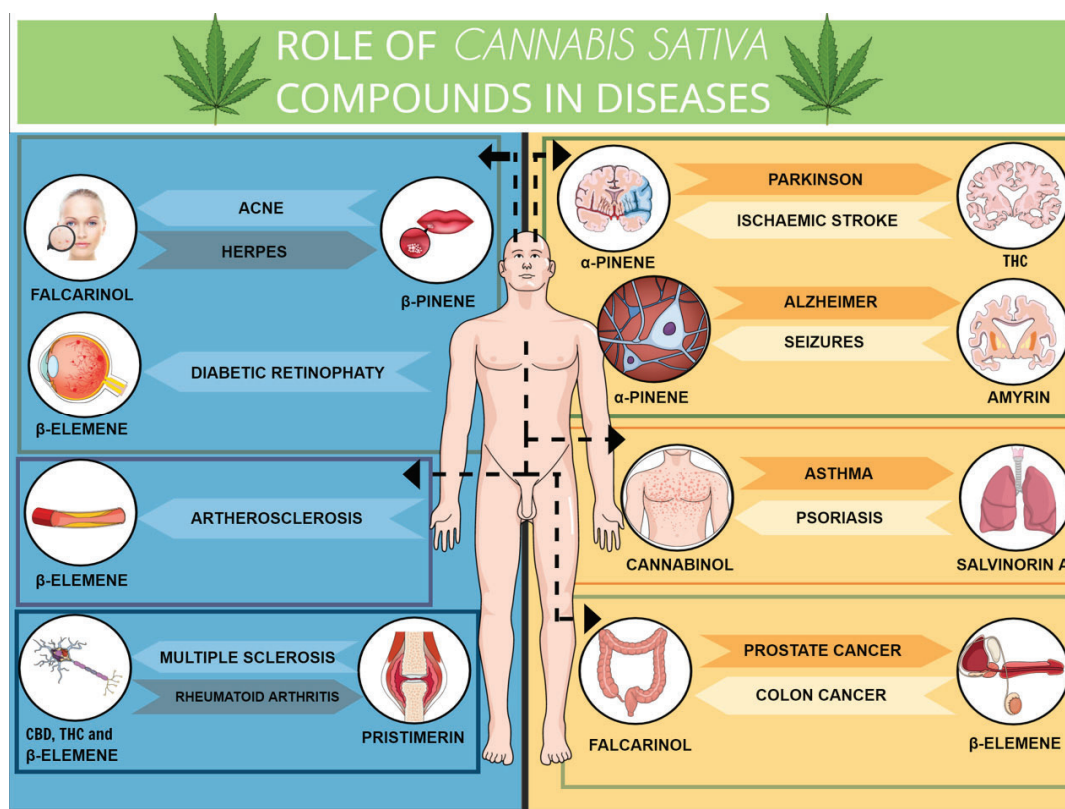
The antiproliferative activity of pristimerin goes beyond colon-related cancers, it is extended to breast [346–350], melanomas [351], osteosarcoma [352], pancreatic [353,354], and prostate cancers [355–358]. Herein, we describe a few examples focusing on articles that have demonstrated potential mechanisms 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- $\kappa$ B/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-1 $\alpha$  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- $\gamma$ t 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- $\kappa$ B 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  $\beta$ -caryophyllene, D-limonene, terpineol,  $\beta$ -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- $\kappa$ B, Nrf2, 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
$\beta$ - and $\alpha$ -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.
$\alpha$ -Pinene	Sedative, hypnotic, anti-seizure, anxiolytic, anticancer, and analgesic activities. Neuroprotective effects against memory loss.
$\beta$ -Pinene	Antiviral, antifungal, anticancer, antimalarial, antidepressant properties.
$\beta$ -Elemene	Anticancer and hypolipidemic compound. Potential treatment for demyelinating disease.
$\beta$ -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.

**Author Contributions:** Conceptualization, E.C.D.G., G.M.B., M.A.B., R.S.P., R.C.D.; Writing original draft preparation, E.C.D.G., G.M.B., M.A.B., R.S.P., R.C.D.; Review and Editing, M.A.B., E.C.D.G., G.M.B., R.C., R.C.D. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Acknowledgments:** The authors would like to thank the Laboratory of Autoimmunity and Immunopharmacology (LAIF) team member's—Universidade Federal de Santa Catarina—Graduate Program of Neuroscience, who have assisted with the literature analysis. R.C.D. is recipient of a research productivity fellowship supported by the Brazilian funding agency CNPq.

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

AC: Adenyl Cyclase; AChE, Acetylcholinesterase; AFB1, Aflatoxin B1; Ago2, Argonaute 2; ALS, Amyotrophic lateral sclerosis; AMPK, AMP-activated protein kinase; apoE, Apolipoprotein E; A $\beta$ , 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-3 $\beta$ , Glycogen synthase kinase-3 beta; HIF-1 $\alpha$ , 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- $\gamma$ , Interferon-gamma; IgE, Immunoglobulin E; IL-1 $\beta$ , Interleukin -1 $\beta$ ; 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 metalloproteinase – 9; MnSOD, Manganese superoxide dismutase; MPO, Myeloperoxidase; mTOR, Mammalian target of rapamycin; NF- $\kappa$ B, 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- $\gamma$ , Peroxisome proliferator-activated receptor gamma; pSTAT3, Phosphorylated STAT3; PTX, Paclitaxel; PTZ, Pentylentetrazole; RA, Rheumatoid arthritis; ROR- $\gamma$ t, Retinoid-related orphan receptor- $\gamma$ t; 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.

## References

- Di Marzo, V.; Bifulco, M.; De Petrocellis, L. The endocannabinoid system and its therapeutic exploitation. *Nat. Reviews. Drug Discov.* **2004**, *3*, 771–784. [[CrossRef](#)] [[PubMed](#)]
- Rubin, V. *Cannabis and Culture*; Mouton: The Hague, The Netherlands, 1975.
- Guy, G.W.; Whittle, B.A.; Robson, P. *The Medicinal Uses of Cannabis and Cannabinoids*; Pharmaceutical Press: London, UK, 2004.
- Pacher, P.; Batkai, S.; Kunos, G. The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.* **2006**, *58*, 389–462. [[CrossRef](#)] [[PubMed](#)]
- Russell, C.; Rueda, S.; Room, R.; Tyndall, M.; Fischer, B. Routes of administration for cannabis use—Basic prevalence and related health outcomes: A scoping review and synthesis. *Int. J. Drug Policy* **2018**, *52*, 87–96. [[CrossRef](#)] [[PubMed](#)]
- Azofeifa, A.; Mattson, M.E.; Schauer, G.; McAfee, T.; Grant, A.; Lyster, R. National Estimates of Marijuana Use and Related Indicators—National Survey on Drug Use and Health, United States, 2002–2014. *Morb. Mortal. Wkly. Rep. Surveill. Summ.* **2016**, *65*, 1–28. [[CrossRef](#)] [[PubMed](#)]
- Degenhardt, L.; Ferrari, A.J.; Calabria, B.; Hall, W.D.; Norman, R.E.; McGrath, J.; Flaxman, A.D.; Engell, R.E.; Freedman, G.D.; Whiteford, H.A.; et al. The global epidemiology and contribution of cannabis use and dependence to the global burden of disease: Results from the GBD 2010 study. *PLoS ONE* **2013**, *8*, e76635. [[CrossRef](#)]
- Hasin, D.S.; Saha, T.D.; Kerridge, B.T.; Goldstein, R.B.; Chou, S.P.; Zhang, H.; Jung, J.; Pickering, R.P.; Ruan, W.J.; Smith, S.M.; et al. Prevalence of Marijuana Use Disorders in the United States between 2001–2002 and 2012–2013. *JAMA Psychiatry* **2015**, *72*, 1235–1242. [[CrossRef](#)]
- Hasin, D.S.; Sarvet, A.L.; Cerda, M.; Keyes, K.M.; Stohl, M.; Galea, S.; Wall, M.M. US Adult Illicit Cannabis Use, Cannabis Use Disorder, and Medical Marijuana Laws: 1991–1992 to 2012–2013. *JAMA Psychiatry* **2017**, *74*, 579–588. [[CrossRef](#)]
- Han, B.; Compton, W.M.; Blanco, C.; Jones, C.M. Trends in and correlates of medical marijuana use among adults in the United States. *Drug Alcohol Depend.* **2018**, *186*, 120–129. [[CrossRef](#)]
- Han, B.; Compton, W.M.; Jones, C.M.; Blanco, C. Cannabis Use and Cannabis Use Disorders among Youth in the United States, 2002–2014. *J. Clin. Psychiatry* **2017**, *78*, 1404–1413. [[CrossRef](#)]
- Hill, K.P. Cannabis Use and Risk for Substance Use Disorders and Mood or Anxiety Disorders. *JAMA* **2017**, *317*, 1070–1071. [[CrossRef](#)]

13. Belackova, V.; Stefunkova, M. Interpreting the Czech drug decriminalization: The glass is half full—Response to Cervený, J., Chomynova, P., Mravčík, V., & van Ours, J.C. (2017). Cannabis decriminalization and the age of onset of cannabis use. *Int. J. Drug Policy* **2018**, *52*, 102–105. [[PubMed](#)]
14. Belackova, V.; Wilkins, C. Consumer agency in cannabis supply—Exploring auto-regulatory documents of the cannabis social clubs in Spain. *Int. J. Drug Policy* **2018**, *54*, 26–34. [[CrossRef](#)] [[PubMed](#)]
15. Room, R. Legalizing a market for cannabis for pleasure: Colorado, Washington, Uruguay and beyond. *Addiction* **2014**, *109*, 345–351. [[CrossRef](#)] [[PubMed](#)]
16. Room, R. Cannabis legalization and public health: Legal niceties, commercialization and countercultures. *Addiction* **2014**, *109*, 358–359. [[CrossRef](#)]
17. Wilkinson, S.T.; D’Souza, D.C. Problems with the medicalization of marijuana. *JAMA* **2014**, *311*, 2377–2378. [[CrossRef](#)]
18. Devane, W.A.; Dysarz, F.A., 3rd; Johnson, M.R.; Melvin, L.S.; Howlett, A.C. Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.* **1988**, *34*, 605–613.
19. Matsuda, L.A.; Lolait, S.J.; Brownstein, M.J.; Young, A.C.; Bonner, T.I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **1990**, *346*, 561–564. [[CrossRef](#)]
20. ElSohly, M.A.; Radwan, M.M.; Gul, W.; Chandra, S.; Galal, A. Phytochemistry of Cannabis sativa L. *Prog. Chem. Org. Nat. Prod.* **2017**, *103*, 1–36.
21. Russo, E.; Guy, G.W. A tale of two cannabinoids: The therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Med. Hypotheses* **2006**, *66*, 234–246. [[CrossRef](#)]
22. Esposito, G.; Scuderi, C.; Valenza, M.; Togna, G.I.; Latina, V.; De Filippis, D.; Cipriano, M.; Carratu, M.R.; Iuvone, T.; Steardo, L. Cannabidiol reduces Abeta-induced neuroinflammation and promotes hippocampal neurogenesis through PPARgamma involvement. *PLoS ONE* **2011**, *6*, e28668. [[CrossRef](#)]
23. Martin-Moreno, A.M.; Reigada, D.; Ramirez, B.G.; Mechoulam, R.; Innamorado, N.; Cuadrado, A.; de Ceballos, M.L. Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: Relevance to Alzheimer’s disease. *Mol. Pharmacol.* **2011**, *79*, 964–973. [[CrossRef](#)] [[PubMed](#)]
24. Mecha, M.; Torrao, A.S.; Mestre, L.; Carrillo-Salinas, F.J.; Mechoulam, R.; Guaza, C. Cannabidiol protects oligodendrocyte progenitor cells from inflammation-induced apoptosis by attenuating endoplasmic reticulum stress. *Cell Death Dis.* **2012**, *3*, e331. [[CrossRef](#)] [[PubMed](#)]
25. Shannon, S.; Opila-Lehman, J. Effectiveness of Cannabidiol Oil for Pediatric Anxiety and Insomnia as Part of Posttraumatic Stress Disorder: A Case Report. *Perm. J.* **2016**, *20*, 16–005. [[CrossRef](#)] [[PubMed](#)]
26. Marinho, A.L.; Vila-Verde, C.; Fogaca, M.V.; Guimaraes, F.S. Effects of intra-infralimbic prefrontal cortex injections of cannabidiol in the modulation of emotional behaviors in rats: Contribution of 5HT(1)A receptors and stressful experiences. *Behav. Brain Res.* **2015**, *286*, 49–56. [[CrossRef](#)] [[PubMed](#)]
27. Jadoon, K.A.; Ratcliffe, S.H.; Barrett, D.A.; Thomas, E.L.; Stott, C.; Bell, J.D.; O’Sullivan, S.E.; Tan, G.D. Efficacy and Safety of Cannabidiol and Tetrahydrocannabinol on Glycemic and Lipid Parameters in Patients With Type 2 Diabetes: A Randomized, Double-Blind, Placebo-Controlled, Parallel Group Pilot Study. *Diabetes Care* **2016**, *39*, 1777–1786. [[CrossRef](#)] [[PubMed](#)]
28. Dhital, S.; Stokes, J.V.; Park, N.; Seo, K.S.; Kaplan, B.L. Cannabidiol (CBD) induces functional Tregs in response to low-level T cell activation. *Cell. Immunol.* **2017**, *312*, 25–34. [[CrossRef](#)]
29. Krohn, R.M.; Parsons, S.A.; Fichna, J.; Patel, K.D.; Yates, R.M.; Sharkey, K.A.; Storr, M.A. Abnormal cannabidiol attenuates experimental colitis in mice, promotes wound healing and inhibits neutrophil recruitment. *J. Inflamm.* **2016**, *13*, 21. [[CrossRef](#)]
30. Norris, C.; Loureiro, M.; Kramar, C.; Zunder, J.; Renard, J.; Rushlow, W.; Laviolette, S.R. Cannabidiol Modulates Fear Memory Formation through Interactions with Serotonergic Transmission in the Mesolimbic System. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* **2016**, *41*, 2839–2850. [[CrossRef](#)]
31. Genaro, K.; Fabris, D.; Arantes, A.L.F.; Zuardi, A.W.; Crippa, J.A.S.; Prado, W.A. Cannabidiol Is a Potential Therapeutic for the Affective-Motivational Dimension of Incision Pain in Rats. *Front. Pharmacol.* **2017**, *8*, 391. [[CrossRef](#)]
32. Harris, H.M.; Sufka, K.J.; Gul, W.; ElSohly, M.A. Effects of Delta-9-Tetrahydrocannabinol and Cannabidiol on Cisplatin-Induced Neuropathy in Mice. *Planta Med.* **2016**, *82*, 1169–1172. [[CrossRef](#)]
33. Peres, F.F.; Levin, R.; Suíama, M.A.; Diana, M.C.; Gouvea, D.A.; Almeida, V.; Santos, C.M.; Lungato, L.; Zuardi, A.W.; Hallak, J.E.; et al. Cannabidiol Prevents Motor and Cognitive Impairments Induced by Reserpine in Rats. *Front. Pharmacol.* **2016**, *7*, 343. [[CrossRef](#)] [[PubMed](#)]

34. Peres, F.F.; Diana, M.C.; Suiama, M.A.; Justi, V.; Almeida, V.; Bressan, R.A.; Zuardi, A.W.; Hallak, J.E.; Crippa, J.A.; Abilio, V.C. Peripubertal treatment with cannabidiol prevents the emergence of psychosis in an animal model of schizophrenia. *Schizophr. Res.* **2016**, *172*, 220–221. [[CrossRef](#)] [[PubMed](#)]
35. Renard, J.; Loureiro, M.; Rosen, L.G.; Zunder, J.; de Oliveira, C.; Schmid, S.; Rushlow, W.J.; Laviolette, S.R. Cannabidiol Counteracts Amphetamine-Induced Neuronal and Behavioral Sensitization of the Mesolimbic Dopamine Pathway through a Novel mTOR/p70S6 Kinase Signaling Pathway. *J. Neurosci. Off. J. Soc. Neurosci.* **2016**, *36*, 5160–5169. [[CrossRef](#)] [[PubMed](#)]
36. Devinsky, O.; Cross, J.H.; Wright, S. Trial of Cannabidiol for Drug-Resistant Seizures in the Dravet Syndrome. *N. Engl. J. Med.* **2017**, *377*, 699–700. [[CrossRef](#)] [[PubMed](#)]
37. Hosseinzadeh, M.; Nikseresht, S.; Khodagholi, F.; Naderi, N.; Maghsoudi, N. Cannabidiol Post-Treatment Alleviates Rat Epileptic-Related Behaviors and Activates Hippocampal Cell Autophagy Pathway Along with Antioxidant Defense in Chronic Phase of Pilocarpine-Induced Seizure. *J. Mol. Neurosci. MN* **2016**, *58*, 432–440. [[CrossRef](#)] [[PubMed](#)]
38. Kaplan, E.H.; Offermann, E.A.; Sievers, J.W.; Comi, A.M. Cannabidiol Treatment for Refractory Seizures in Sturge-Weber Syndrome. *Pediatr. Neurol.* **2017**, *71*, 18–23 e2. [[CrossRef](#)] [[PubMed](#)]
39. Patel, R.R.; Barbosa, C.; Brustovetsky, T.; Brustovetsky, N.; Cummins, T.R. Aberrant epilepsy-associated mutant Nav1.6 sodium channel activity can be targeted with cannabidiol. *Brain J. Neurol.* **2016**, *139 Pt 8*, 2164–2181. [[CrossRef](#)]
40. Ibsen, M.S.; Connor, M.; Glass, M. Cannabinoid CB1 and CB2 Receptor Signaling and Bias. *Cannabis Cannabinoid Res.* **2017**, *2*, 48–60. [[CrossRef](#)]
41. McHugh, D.; Page, J.; Dunn, E.; Bradshaw, H.B. Delta(9) -Tetrahydrocannabinol and N-arachidonyl glycine are full agonists at GPR18 receptors and induce migration in human endometrial HEC-1B cells. *Br. J. Pharmacol.* **2012**, *165*, 2414–2424. [[CrossRef](#)]
42. Pertwee, R.G.; Howlett, A.C.; Abood, M.E.; Alexander, S.P.; Di Marzo, V.; Elphick, M.R.; Greasley, P.J.; Hansen, H.S.; Kunos, G.; Mackie, K.; et al. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: Beyond CB(1) and CB(2). *Pharmacol. Rev.* **2010**, *62*, 588–631. [[CrossRef](#)]
43. Davis, K.D.; Flor, H.; Greely, H.T.; Iannetti, G.D.; Mackey, S.; Ploner, M.; Pustilnik, A.; Tracey, I.; Treede, R.D.; Wager, T.D. Brain imaging tests for chronic pain: Medical, legal and ethical issues and recommendations. *Nat. Rev. Neurol.* **2017**, *13*, 624–638. [[CrossRef](#)] [[PubMed](#)]
44. Howlett, A.C.; Barth, F.; Bonner, T.I.; Cabral, G.; Casellas, P.; Devane, W.A.; Felder, C.C.; Herkenham, M.; Mackie, K.; Martin, B.R.; et al. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol. Rev.* **2002**, *54*, 161–202. [[CrossRef](#)] [[PubMed](#)]
45. Ashton, J.C.; Glass, M. The cannabinoid CB2 receptor as a target for inflammation-dependent neurodegeneration. *Curr. Neuropharmacol.* **2007**, *5*, 73–80. [[CrossRef](#)] [[PubMed](#)]
46. Rom, S.; Persidsky, Y. Cannabinoid receptor 2: Potential role in immunomodulation and neuroinflammation. *J. Neuroimmune Pharmacol. Off. J. Soc. Neuroimmune Pharmacol.* **2013**, *8*, 608–620. [[CrossRef](#)]
47. Pertwee, R.G. The pharmacology of cannabinoid receptors and their ligands: An overview. *Int. J. Obes.* **2006**, *30 (Suppl. 1)*, S13–S18. [[CrossRef](#)]
48. Pertwee, R.G. Pharmacological actions of cannabinoids. *Handb. Exp. Pharmacol.* **2005**, *168*, 1–51. [[CrossRef](#)]
49. Devane, W.A.; Hanus, L.; Breuer, A.; Pertwee, R.G.; Stevenson, L.A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* **1992**, *258*, 1946–1949. [[CrossRef](#)]
50. Mechoulam, R.; Ben-Shabat, S.; Hanus, L.; Ligumsky, M.; Kaminski, N.E.; Schatz, A.R.; Gopher, A.; Almog, S.; Martin, B.R.; Compton, D.R.; et al. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* **1995**, *50*, 83–90. [[CrossRef](#)]
51. Sugiura, T.; Kondo, S.; Sukagawa, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. 2-Arachidonoylglycerol: A possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* **1995**, *215*, 89–97. [[CrossRef](#)]
52. Pertwee, R.G. Receptors and channels targeted by synthetic cannabinoid receptor agonists and antagonists. *Curr. Med. Chem.* **2010**, *17*, 1360–1381. [[CrossRef](#)]
53. Goncalves, E.D.; Dutra, R.C. Cannabinoid receptors as therapeutic targets for autoimmune diseases: Where do we stand? *Drug Discov. Today* **2019**, *24*, 1845–1853. [[CrossRef](#)] [[PubMed](#)]

54. Di Iorio, G.; Lupi, M.; Sarchione, F.; Matarazzo, I.; Santacroce, R.; Petrucci, F.; Martinotti, G.; Di Giannantonio, M. The endocannabinoid system: A putative role in neurodegenerative diseases. *Int. J. High Risk Behav. Addict.* **2013**, *2*, 100–106. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Wolf, S.A.; Tauber, S.; Ullrich, O. CNS immune surveillance and neuroinflammation: Endocannabinoids keep control. *Curr. Pharm. Des.* **2008**, *14*, 2266–2278. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Cabral, G.A.; Ferreira, G.A.; Jamerson, M.J. Endocannabinoids and the Immune System in Health and Disease. *Handb. Exp. Pharmacol.* **2015**, *231*, 185–211. [\[PubMed\]](#)
57. De Laurentiis, A.; Araujo, H.A.; Rettori, V. Role of the endocannabinoid system in the neuroendocrine responses to inflammation. *Curr. Pharm. Des.* **2014**, *20*, 4697–4706. [\[CrossRef\]](#)
58. Pacher, P.; Steffens, S.; Hasko, G.; Schindler, T.H.; Kunos, G. Cardiovascular effects of marijuana and synthetic cannabinoids: The good, the bad, and the ugly. *Nat. Rev. Cardiol.* **2018**, *15*, 151–166. [\[CrossRef\]](#)
59. Ashton, J.C.; Smith, P.F. Cannabinoids and cardiovascular disease: The outlook for clinical treatments. *Curr. Vasc. Pharmacol.* **2007**, *5*, 175–185. [\[CrossRef\]](#)
60. Ho, W.S.V.; Kelly, M.E.M. Cannabinoids in the Cardiovascular System. *Adv. Pharmacol.* **2017**, *80*, 329–366.
61. Wolff, V.; Jouanous, E. Strokes are possible complications of cannabinoids use. *Epilepsy Behav. EB* **2017**, *70 Pt B*, 355–363. [\[CrossRef\]](#)
62. Singh, A.; Saluja, S.; Kumar, A.; Agrawal, S.; Thind, M.; Nanda, S.; Shirani, J. Cardiovascular Complications of Marijuana and Related Substances: A Review. *Cardiol. Ther.* **2018**, *7*, 45–59. [\[CrossRef\]](#)
63. Lu, Y.; Anderson, H.D. Cannabinoid signaling in health and disease. *Can. J. Physiol. Pharmacol.* **2017**, *95*, 311–327. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Huestis, M.A.; Boyd, S.J.; Heishman, S.J.; Preston, K.L.; Bonnet, D.; Le Fur, G.; Gorelick, D.A. Single and multiple doses of rimonabant antagonize acute effects of smoked cannabis in male cannabis users. *Psychopharmacology* **2007**, *194*, 505–515. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Kumar, A.; Premoli, M.; Aria, F.; Bonini, S.A.; Maccarinelli, G.; Gianoncelli, A.; Memo, M.; Mastinu, A. Cannabimimetic plants: Are they new cannabinoidergic modulators? *Planta* **2019**, *249*, 1681–1694. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Solymosi, K.; Kofalvi, A. Cannabis: A Treasure Trove or Pandora's Box? *Mini Rev. Med. Chem.* **2017**, *17*, 1223–1291. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Morales, P.; Hurst, D.P.; Reggio, P.H. Molecular Targets of the Phytocannabinoids: A Complex Picture. *Prog. Chem. Org. Nat. Prod.* **2017**, *103*, 103–131.
68. Campos, A.C.; Fogaca, M.V.; Sonego, A.B.; Guimaraes, F.S. Cannabidiol, neuroprotection and neuropsychiatric disorders. *Pharmacol. Res.* **2016**, *112*, 119–127. [\[CrossRef\]](#)
69. Crivelaro do Nascimento, G.; Ferrari, D.P.; Guimaraes, F.S.; Del Bel, E.A.; Bortolanza, M.; Ferreira-Junior, N.C. Cannabidiol increases the nociceptive threshold in a preclinical model of Parkinson's disease. *Neuropharmacology* **2020**, *163*, 107808. [\[CrossRef\]](#)
70. Mammana, S.; Cavalli, E.; Gugliandolo, A.; Silvestro, S.; Pollastro, F.; Bramanti, P.; Mazzon, E. Could the Combination of Two Non-Psychotropic Cannabinoids Counteract Neuroinflammation? Effectiveness of Cannabidiol Associated with Cannabigerol. *Medicina* **2019**, *55*, 747. [\[CrossRef\]](#)
71. Russo, E.B.; Marcu, J. Cannabis Pharmacology: The Usual Suspects and a Few Promising Leads. *Adv. Pharmacol.* **2017**, *80*, 67–134.
72. Borrelli, F.; Pagano, E.; Romano, B.; Panzera, S.; Maiello, F.; Coppola, D.; De Petrocellis, L.; Buono, L.; Orlando, P.; Izzo, A.A. Colon carcinogenesis is inhibited by the TRPM8 antagonist cannabigerol, a Cannabis-derived non-psychotropic cannabinoid. *Carcinogenesis* **2014**, *35*, 2787–2797. [\[CrossRef\]](#)
73. Udoh, M.; Santiago, M.; Devenish, S.; McGregor, I.S.; Connor, M. Cannabichromene is a cannabinoid CB2 receptor agonist. *Br. J. Pharmacol.* **2019**, *176*, 4537–4547. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Wilkinson, J.D.; Williamson, E.M. Cannabinoids inhibit human keratinocyte proliferation through a non-CB1/CB2 mechanism and have a potential therapeutic value in the treatment of psoriasis. *J. Dermatol. Sci.* **2007**, *45*, 87–92. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Al-Ghezi, Z.Z.; Miranda, K.; Nagarkatti, M.; Nagarkatti, P.S. Combination of Cannabinoids, Delta9-Tetrahydrocannabinol and Cannabidiol, Ameliorates Experimental Multiple Sclerosis by Suppressing Neuroinflammation Through Regulation of miRNA-Mediated Signaling Pathways. *Front. Immunol.* **2019**, *10*, 1921. [\[CrossRef\]](#) [\[PubMed\]](#)

76. Haupts, M.; Vila, C.; Jonas, A.; Witte, K.; Alvarez-Ossorio, L. Influence of Previous Failed Antispasticity Therapy on the Efficacy and Tolerability of THC:CBD Oromucosal Spray for Multiple Sclerosis Spasticity. *Eur. Neurol.* **2016**, *75*, 236–243. [[CrossRef](#)] [[PubMed](#)]
77. Laprairie, R.B.; Bagher, A.M.; Kelly, M.E.; Denovan-Wright, E.M. Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. *Br. J. Pharmacol.* **2015**, *172*, 4790–4805. [[CrossRef](#)] [[PubMed](#)]
78. Blasco-Benito, S.; Seijo-Vila, M.; Caro-Villalobos, M.; Tundidor, I.; Andradas, C.; Garcia-Taboada, E.; Wade, J.; Smith, S.; Guzman, M.; Perez-Gomez, E.; et al. Appraising the “entourage effect”: Antitumor action of a pure cannabinoid versus a botanical drug preparation in preclinical models of breast cancer. *Biochem. Pharmacol.* **2018**, *157*, 285–293. [[CrossRef](#)] [[PubMed](#)]
79. Russo, E.B. The Case for the Entourage Effect and Conventional Breeding of Clinical Cannabis: No “Strain,” No Gain. *Front. Plant Sci.* **2018**, *9*, 1969. [[CrossRef](#)]
80. Felipe, C.F.B.; Albuquerque, A.M.S.; de Pontes, J.L.X.; de Melo, J.I.V.; Rodrigues, T.; de Sousa, A.M.P.; Monteiro, A.B.; Ribeiro, A.; Lopes, J.P.; de Menezes, I.R.A.; et al. Comparative study of alpha- and beta-pinene effect on PTZ-induced convulsions in mice. *Fundam. Clin. Pharmacol.* **2019**, *33*, 181–190. [[CrossRef](#)]
81. Zamyad, M.; Abbasnejad, M.; Esmaeili-Mahani, S.; Mostafavi, A.; Sheibani, V. The anticonvulsant effects of *Ducrosia anethifolia* (Boiss) essential oil are produced by its main component alpha-pinene in rats. *Arq. De Neuro-Psiquiatr.* **2019**, *77*, 106–114. [[CrossRef](#)]
82. Khoshnazar, M.; Bigdeli, M.R.; Parvardeh, S.; Pouriran, R. Attenuating effect of alpha-pinene on neurobehavioural deficit, oxidative damage and inflammatory response following focal ischaemic stroke in rat. *J. Pharm. Pharmacol.* **2019**, *71*, 1725–1733. [[CrossRef](#)]
83. Zhao, Y.; Chen, R.; Wang, Y.; Yang, Y. alpha-Pinene Inhibits Human Prostate Cancer Growth in a Mouse Xenograft Model. *Chemotherapy* **2018**, *63*, 1–7. [[CrossRef](#)] [[PubMed](#)]
84. Araujo-Filho, H.G.; Pereira, E.W.M.; Rezende, M.M.; Menezes, P.P.; Araujo, A.A.S.; Barreto, R.S.S.; Martins, A.; Albuquerque, T.R.; Silva, B.A.F.; Alcantara, I.S.; et al. D-limonene exhibits superior antihyperalgesic effects in a beta-cyclodextrin-complexed form in chronic musculoskeletal pain reducing Fos protein expression on spinal cord in mice. *Neuroscience* **2017**, *358*, 158–169. [[CrossRef](#)] [[PubMed](#)]
85. Booth, J.K.; Page, J.E.; Bohlmann, J. Terpene synthases from Cannabis sativa. *PLoS ONE* **2017**, *12*, e0173911. [[CrossRef](#)] [[PubMed](#)]
86. Naz, S.; Hanif, M.A.; Ansari, T.M.; Al-Sabahi, J.N. A Comparative Study on Hemp (Cannabis sativa) Essential Oil Extraction Using Traditional and Advanced Techniques. *Guang Pu Xue Yu Guang Pu Fen Xi = Guang Pu* **2017**, *37*, 306–311.
87. Fidyt, K.; Fiedorowicz, A.; Strzadala, L.; Szumny, A. beta-caryophyllene and beta-caryophyllene oxide-natural compounds of anticancer and analgesic properties. *Cancer Med.* **2016**, *5*, 3007–3017. [[CrossRef](#)]
88. Sabulal, B.; Dan, M.; J, A.J.; Kurup, R.; Pradeep, N.S.; Valsamma, R.K.; George, V. Caryophyllene-rich rhizome oil of *Zingiber nimmonii* from South India: Chemical characterization and antimicrobial activity. *Phytochemistry* **2006**, *67*, 2469–2473. [[CrossRef](#)]
89. Su, Y.C.; Ho, C.L. Composition of the Leaf Essential Oil of *Phoebe formosana* from Taiwan and its in vitro Cytotoxic, Antibacterial, and Antifungal Activities. *Nat. Prod. Commun.* **2016**, *11*, 845–848. [[CrossRef](#)]
90. Sarvmeili, N.; Jafarian-Dehkordi, A.; Zolfaghari, B. Cytotoxic effects of *Pinus eldarica* essential oil and extracts on HeLa and MCF-7 cell lines. *Res. Pharm. Sci.* **2016**, *11*, 476–483.
91. Memariani, T.; Hosseini, T.; Kamali, H.; Mohammadi, A.; Ghorbani, M.; Shakeri, A.; Spandidos, D.A.; Tsatsakis, A.M.; Shahsavand, S. Evaluation of the cytotoxic effects of *Cyperus longus* extract, fractions and its essential oil on the PC3 and MCF7 cancer cell lines. *Oncol. Lett.* **2016**, *11*, 1353–1360. [[CrossRef](#)]
92. Segat, G.C.; Manjavachi, M.N.; Matias, D.O.; Passos, G.F.; Freitas, C.S.; Costa, R.; Calixto, J.B. Antiallodynic effect of beta-caryophyllene on paclitaxel-induced peripheral neuropathy in mice. *Neuropharmacology* **2017**, *125*, 207–219. [[CrossRef](#)]
93. Bento, A.F.; Marcon, R.; Dutra, R.C.; Claudino, R.F.; Cola, M.; Leite, D.F.; Calixto, J.B. beta-Caryophyllene inhibits dextran sulfate sodium-induced colitis in mice through CB2 receptor activation and PPARgamma pathway. *Am. J. Pathol.* **2011**, *178*, 1153–1166. [[CrossRef](#)] [[PubMed](#)]
94. Gertsch, J.; Leonti, M.; Raduner, S.; Racz, I.; Chen, J.Z.; Xie, X.Q.; Altmann, K.H.; Karsak, M.; Zimmer, A. Beta-caryophyllene is a dietary cannabinoid. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 9099–9104. [[CrossRef](#)] [[PubMed](#)]

95. Alberti, T.B.; Marcon, R.; Bicca, M.A.; Raposo, N.R.; Calixto, J.B.; Dutra, R.C. Essential oil from *Pterodon emarginatus* seeds ameliorates experimental autoimmune encephalomyelitis by modulating Th1/Treg cell balance. *J. Ethnopharmacol.* **2014**, *155*, 485–494. [\[CrossRef\]](#)
96. Alberti, T.B.; Barbosa, W.L.; Vieira, J.L.; Raposo, N.R.; Dutra, R.C. (-)-beta-Caryophyllene, a CB2 Receptor-Selective Phytocannabinoid, Suppresses Motor Paralysis and Neuroinflammation in a Murine Model of Multiple Sclerosis. *Int. J. Mol. Sci.* **2017**, *18*, 691. [\[CrossRef\]](#) [\[PubMed\]](#)
97. Fernandes, E.S.; Passos, G.F.; Medeiros, R.; da Cunha, F.M.; Ferreira, J.; Campos, M.M.; Pianowski, L.F.; Calixto, J.B. Anti-inflammatory effects of compounds alpha-humulene and (-)-trans-caryophyllene isolated from the essential oil of *Cordia verbenacea*. *Eur. J. Pharmacol.* **2007**, *569*, 228–236. [\[CrossRef\]](#)
98. Ojha, S.; Javed, H.; Azimullah, S.; Haque, M.E. beta-Caryophyllene, a phytocannabinoid attenuates oxidative stress, neuroinflammation, glial activation, and salvages dopaminergic neurons in a rat model of Parkinson disease. *Mol. Cell. Biochem.* **2016**, *418*, 59–70. [\[CrossRef\]](#)
99. Sut, S.; Maggi, F.; Nicoletti, M.; Baldan, V.; Dall'Acqua, S. New Drugs from Old Natural Compounds: Scarcely Investigated Sesquiterpenes as New Possible Therapeutic Agents. *Curr. Med. Chem.* **2018**, *25*, 1241–1258. [\[CrossRef\]](#)
100. Santiago, M.; Sachdev, S.; Arnold, J.C.; McGregor, I.S.; Connor, M. Absence of Entourage: Terpenoids Commonly Found in *Cannabis sativa* Do Not Modulate the Functional Activity of Delta(9)-THC at Human CB1 and CB2 Receptors. *Cannabis Cannabinoid Res.* **2019**, *4*, 165–176. [\[CrossRef\]](#)
101. Sun, J. D-Limonene: Safety and clinical applications. *Altern. Med. Rev. J. Clin. Ther.* **2007**, *12*, 259–264.
102. Shah, B.B.; Baksi, R.; Chaudagar, K.K.; Nivsarkar, M.; Mehta, A.A. Anti-leukemic and anti-angiogenic effects of d-Limonene on K562-implanted C57BL/6 mice and the chick chorioallantoic membrane model. *Anim. Models Exp. Med.* **2018**, *1*, 328–333. [\[CrossRef\]](#)
103. d'Alessio, P.A.; Ostan, R.; Bisson, J.F.; Schulzke, J.D.; Ursini, M.V.; Bene, M.C. Oral administration of d-limonene controls inflammation in rat colitis and displays anti-inflammatory properties as diet supplementation in humans. *Life Sci.* **2013**, *92*, 1151–1156. [\[CrossRef\]](#) [\[PubMed\]](#)
104. Shin, M.; Liu, Q.F.; Choi, B.; Shin, C.; Lee, B.; Yuan, C.; Song, Y.J.; Yun, H.S.; Lee, I.S.; Koo, B.S.; et al. Neuroprotective effects of limonene (+) against Abeta42-induced neurotoxicity in a *Drosophila* model of Alzheimer's disease. *Biol. Pharm. Bull.* **2019**. [\[CrossRef\]](#)
105. de Almeida, A.A.; Silva, R.O.; Nicolau, L.A.; de Brito, T.V.; de Sousa, D.P.; Barbosa, A.L.; de Freitas, R.M.; Lopes, L.D.; Medeiros, J.R.; Ferreira, P.M. Physio-pharmacological Investigations About the Anti-inflammatory and Antinociceptive Efficacy of (+)-Limonene Epoxide. *Inflammation* **2017**, *40*, 511–522. [\[CrossRef\]](#) [\[PubMed\]](#)
106. Rozza, A.L.; Moraes Tde, M.; Kushima, H.; Tanimoto, A.; Marques, M.O.; Bauab, T.M.; Hiruma-Lima, C.A.; Pellizzon, C.H. Gastroprotective mechanisms of Citrus lemon (Rutaceae) essential oil and its majority compounds limonene and beta-pinene: Involvement of heat-shock protein-70, vasoactive intestinal peptide, glutathione, sulfhydryl compounds, nitric oxide and prostaglandin E(2). *Chem. Biol. Interact.* **2011**, *189*, 82–89.
107. de Souza, M.C.; Vieira, A.J.; Beserra, F.P.; Pellizzon, C.H.; Nobrega, R.H.; Rozza, A.L. Gastroprotective effect of limonene in rats: Influence on oxidative stress, inflammation and gene expression. *Phytomed. Int. J. Phytother. Phytopharm.* **2019**, *53*, 37–42. [\[CrossRef\]](#)
108. Wang, L.; Zhang, Y.; Fan, G.; Ren, J.N.; Zhang, L.L.; Pan, S.Y. Effects of orange essential oil on intestinal microflora in mice. *J. Sci. Food Agric.* **2019**, *99*, 4019–4028. [\[CrossRef\]](#)
109. do Amaral, J.F.; Silva, M.I.; Neto, M.R.; Neto, P.F.; Moura, B.A.; de Melo, C.T.; de Araujo, F.L.; de Sousa, D.P.; de Vasconcelos, P.F.; de Vasconcelos, S.M.; et al. Antinociceptive effect of the monoterpene R-(+)-limonene in mice. *Biol. Pharm. Bull.* **2007**, *30*, 1217–1220. [\[CrossRef\]](#)
110. Piccinelli, A.C.; Santos, J.A.; Konkiewitz, E.C.; Oesterreich, S.A.; Formagio, A.S.; Croda, J.; Ziff, E.B.; Kassuya, C.A. Antihyperalgesic and antidepressive actions of (R)-(+)-limonene, alpha-phellandrene, and essential oil from *Schinus terebinthifolius* fruits in a neuropathic pain model. *Nutr. Neurosci.* **2015**, *18*, 217–224. [\[CrossRef\]](#)
111. Smeriglio, A.; Alloisio, S.; Raimondo, F.M.; Denaro, M.; Xiao, J.; Cornara, L.; Trombetta, D. Essential oil of *Citrus lumia* Risso: Phytochemical profile, antioxidant properties and activity on the central nervous system. *Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* **2018**, *119*, 407–416. [\[CrossRef\]](#)

112. Zhang, Y.; Wang, J.; Cao, X.; Liu, W.; Yu, H.; Ye, L. High-level production of linalool by engineered *Saccharomyces cerevisiae* harboring dual mevalonate pathways in mitochondria and cytoplasm. *Enzym. Microb. Technol.* **2020**, *134*, 109462. [\[CrossRef\]](#)
113. Kim, M.G.; Kim, S.M.; Min, J.H.; Kwon, O.K.; Park, M.H.; Park, J.W.; Ahn, H.I.; Hwang, J.Y.; Oh, S.R.; Lee, J.W.; et al. Anti-inflammatory effects of linalool on ovalbumin-induced pulmonary inflammation. *Int. Immunopharmacol.* **2019**, *74*, 105706. [\[CrossRef\]](#) [\[PubMed\]](#)
114. Sabogal-Guaqueta, A.M.; Hobbie, F.; Keerthi, A.; Oun, A.; Kortholt, A.; Boddeke, E.; Dolga, A. Linalool attenuates oxidative stress and mitochondrial dysfunction mediated by glutamate and NMDA toxicity. *Biomed. Pharmacother.* **2019**, *118*, 109295. [\[CrossRef\]](#) [\[PubMed\]](#)
115. Harada, H.; Kashiwadani, H.; Kanmura, Y.; Kuwaki, T. Linalool Odor-Induced Anxiolytic Effects in Mice. *Front. Behav. Neurosci.* **2018**, *12*, 241. [\[CrossRef\]](#) [\[PubMed\]](#)
116. Xu, P.; Wang, K.; Lu, C.; Dong, L.; Gao, L.; Yan, M.; Aibai, S.; Yang, Y.; Liu, X. Protective effects of linalool against amyloid beta-induced cognitive deficits and damages in mice. *Life Sci.* **2017**, *174*, 21–27. [\[CrossRef\]](#) [\[PubMed\]](#)
117. Iwasaki, K.; Zheng, Y.W.; Murata, S.; Ito, H.; Nakayama, K.; Kurokawa, T.; Sano, N.; Nowatari, T.; Villareal, M.O.; Nagano, Y.N.; et al. Anticancer effect of linalool via cancer-specific hydroxyl radical generation in human colon cancer. *World J. Gastroenterol.* **2016**, *22*, 9765–9774. [\[CrossRef\]](#)
118. Gunaseelan, S.; Balupillai, A.; Govindasamy, K.; Muthusamy, G.; Ramasamy, K.; Shanmugam, M.; Prasad, N.R. The preventive effect of linalool on acute and chronic UVB-mediated skin carcinogenesis in Swiss albino mice. *Photochem. Photobiol. Sci. Off. J. Eur. Photochem. Assoc. Eur. Soc. Photobiol.* **2016**, *15*, 851–860. [\[CrossRef\]](#)
119. Katsuyama, S.; Kuwahata, H.; Yagi, T.; Kishikawa, Y.; Komatsu, T.; Sakurada, T.; Nakamura, H. Intraplantar injection of linalool reduces paclitaxel-induced acute pain in mice. *Biomed. Res.* **2012**, *33*, 175–181. [\[CrossRef\]](#)
120. Ibrahim, E.A.; Wang, M.; Radwan, M.M.; Wanas, A.S.; Majumdar, C.G.; Avula, B.; Wang, Y.H.; Khan, I.A.; Chandra, S.; Lata, H.; et al. Analysis of Terpenes in Cannabis sativa L. Using GC/MS: Method Development, Validation, and Application. *Planta Med.* **2019**, *85*, 431–438. [\[CrossRef\]](#)
121. Oliveira, M.G.; Brito, R.G.; Santos, P.L.; Araujo-Filho, H.G.; Quintans, J.S.; Menezes, P.P.; Serafini, M.R.; Carvalho, Y.M.; Silva, J.C.; Almeida, J.R.; et al. alpha-Terpeneol, a monoterpene alcohol, complexed with beta-cyclodextrin exerts antihyperalgesic effect in animal model for fibromyalgia aided with docking study. *Chem. Biol. Interact.* **2016**, *254*, 54–62. [\[CrossRef\]](#)
122. Kumar Chaudhari, A.; Singh, A.; Kumar Singh, V.; Kumar Dwivedy, A.; Das, S.; Grace Ramsdam, M.; Dkhar, M.S.; Kayang, H.; Kishore Dubey, N. Assessment of chitosan biopolymer encapsulated alpha-Terpeneol against fungal, aflatoxin B1 (AFB1) and free radicals mediated deterioration of stored maize and possible mode of action. *Food Chem.* **2020**, *311*, 126010. [\[CrossRef\]](#)
123. de Oliveira, M.G.; Marques, R.B.; de Santana, M.F.; Santos, A.B.; Brito, F.A.; Barreto, E.O.; De Sousa, D.P.; Almeida, F.R.; Badaue-Passos, D., Jr.; Antonioli, A.R.; et al. alpha-terpeneol reduces mechanical hypernociception and inflammatory response. *Basic Clin. Pharmacol. Toxicol.* **2012**, *111*, 120–125. [\[PubMed\]](#)
124. Dos Santos Negreiros, P.; da Costa, D.S.; da Silva, V.G.; de Carvalho Lima, I.B.; Nunes, D.B.; de Melo Sousa, F.B.; de Souza Lopes Araujo, T.; Medeiros, J.V.R.; Dos Santos, R.F.; de Cassia Meneses Oliveira, R. Antidiarrheal activity of alpha-terpeneol in mice. *Biomed. Pharmacother.* **2019**, *110*, 631–640. [\[CrossRef\]](#) [\[PubMed\]](#)
125. Gouveia, D.N.; Costa, J.S.; Oliveira, M.A.; Rabelo, T.K.; Silva, A.; Carvalho, A.A.; Miguel-Dos-Santos, R.; Lauton-Santos, S.; Scotti, L.; Scotti, M.T.; et al. alpha-Terpeneol reduces cancer pain via modulation of oxidative stress and inhibition of iNOS. *Biomed. Pharmacother.* **2018**, *105*, 652–661. [\[CrossRef\]](#) [\[PubMed\]](#)
126. Parvardeh, S.; Moghimi, M.; Eslami, P.; Masoudi, A. alpha-Terpeneol attenuates morphine-induced physical dependence and tolerance in mice: Role of nitric oxide. *Iran. J. Basic Med Sci.* **2016**, *19*, 201–208. [\[PubMed\]](#)
127. Moghimi, M.; Parvardeh, S.; Zanjani, T.M.; Ghafghazi, S. Protective effect of alpha-terpeneol against impairment of hippocampal synaptic plasticity and spatial memory following transient cerebral ischemia in rats. *Iran. J. Basic Med Sci.* **2016**, *19*, 960–969. [\[PubMed\]](#)
128. Kim, K.; Bu, Y.; Jeong, S.; Lim, J.; Kwon, Y.; Cha, D.S.; Kim, J.; Jeon, S.; Eun, J.; Jeon, H. Memory-enhancing effect of a supercritical carbon dioxide fluid extract of the needles of *Abies koreana* on scopolamine-induced amnesia in mice. *Biosci. Biotechnol. Biochem.* **2006**, *70*, 1821–1826. [\[CrossRef\]](#)

129. Nogueira, M.N.; Aquino, S.G.; Rossa Junior, C.; Spolidorio, D.M. Terpinen-4-ol and alpha-terpineol (tea tree oil components) inhibit the production of IL-1beta, IL-6 and IL-10 on human macrophages. *Inflamm. Res.* **2014**, *63*, 769–778. [\[CrossRef\]](#)
130. Jing, G.X.; Tao, N.G.; Jia, L.; Zhou, H.E. Influence of alpha-terpineol on the growth and morphogenesis of *Penicillium digitatum*. *Bot. Stud.* **2015**, *56*, 35. [\[CrossRef\]](#)
131. Sharifi-Rad, J.; Salehi, B.; Varoni, E.M.; Sharopov, F.; Yousaf, Z.; Ayatollahi, S.A.; Kobarfard, F.; Sharifi-Rad, M.; Afdjei, M.H.; Sharifi-Rad, M.; et al. Plants of the *Melaleuca* Genus as Antimicrobial Agents: From Farm to Pharmacy. *Phytother. Res. PTR* **2017**, *31*, 1475–1494. [\[CrossRef\]](#)
132. Chen, Y.; Weng, Y.; Zhou, M.; Meng, Y.; Liu, J.; Yang, L.; Zuo, Z. Linalool- and alpha-terpineol-induced programmed cell death in *Chlamydomonas reinhardtii*. *Ecotoxicol. Environ. Saf.* **2019**, *167*, 435–440. [\[CrossRef\]](#)
133. Wua, Y.; Chenb, H.P.; Wei, J.Y.; Yang, K.; Tian, Z.F.; Li, X.L.; Wang, P.J.; Wang, C.F.; Du, S.S.; Cai, Q. Repellent Constituents of essential oil from *Citrus wilsonii* stem barks against *Tribolium castaneum*. *Nat. Prod. Commun.* **2014**, *9*, 1515–1518. [\[CrossRef\]](#) [\[PubMed\]](#)
134. Villa-Ruano, N.; Becerra-Martinez, E.; Cruz-Duran, R.; Zarate-Reyes, J.A.; Landeta-Cortes, G.; Romero-Arenas, O. Volatile Profiling, Insecticidal, Antibacterial and Antiproliferative Properties of the Essential Oils of *Bursera glabrifolia* Leaves. *Chem. Biodivers.* **2018**, *15*, e1800354. [\[CrossRef\]](#) [\[PubMed\]](#)
135. Hassan, S.B.; Gali-Muhtasib, H.; Goransson, H.; Larsson, R. Alpha terpineol: A potential anticancer agent which acts through suppressing NF- $\kappa$ B signalling. *Anticancer Res.* **2010**, *30*, 1911–1919. [\[PubMed\]](#)
136. Djenane, D.; Yanguela, J.; Amrouche, T.; Boubrit, S.; Boussad, N.; Roncales, P. Chemical composition and antimicrobial effects of essential oils of *Eucalyptus globulus*, *Myrtus communis* and *Satureja hortensis* against *Escherichia coli* O157:H7 and *Staphylococcus aureus* in minced beef. *Food Sci. Technol. Int. = Cienc. Tecnol. Aliment. Int.* **2011**, *17*, 505–515. [\[CrossRef\]](#) [\[PubMed\]](#)
137. Ramalho, T.R.; Pacheco de Oliveira, M.T.; Lima, A.L.; Bezerra-Santos, C.R.; Piuvezam, M.R. Erratum for: Gamma-Terpinene Modulates Acute Inflammatory Response in Mice. *Planta Med.* **2015**, *81*, E3. [\[CrossRef\]](#) [\[PubMed\]](#)
138. Su, Y.C.; Hsu, K.P.; Hua, K.F.; Ho, C.L. Composition, in vitro Anti-inflammatory, Antioxidant and Antimicrobial Activities of Essential Oils from Leaf and Twig Parts of *Cupressus cashmeriana*. *Nat. Prod. Commun.* **2015**, *10*, 1461–1464. [\[CrossRef\]](#)
139. Xavier, A.L.; Pita, J.C.; Brito, M.T.; Meireles, D.R.; Tavares, J.F.; Silva, M.S.; Maia, J.G.; Andrade, E.H.; Diniz, M.F.; Silva, T.G.; et al. Chemical composition, antitumor activity, and toxicity of essential oil from the leaves of *Lippia microphylla*. *Z. Fur Naturforschung. Cjournal Biosci.* **2015**, *70*, 129–137. [\[CrossRef\]](#)
140. Mekonnen, A.; Tesfaye, S.; Christos, S.G.; Dires, K.; Zenebe, T.; Zegeye, N.; Shiferaw, Y.; Lulekal, E. Evaluation of Skin Irritation and Acute and Subacute Oral Toxicity of *Lavandula angustifolia* Essential Oils in Rabbit and Mice. *J. Toxicol.* **2019**, *2019*, 5979546. [\[CrossRef\]](#)
141. Plastina, P.; Apriantini, A.; Meijerink, J.; Witkamp, R.; Gabriele, B.; Fazio, A. In Vitro Anti-Inflammatory and Radical Scavenging Properties of Chinotto (*Citrus myrtifolia* Raf.) Essential Oils. *Nutrients* **2018**, *10*, 783. [\[CrossRef\]](#)
142. Lima Mda, S.; Quintans-Junior, L.J.; de Santana, W.A.; Martins Kaneto, C.; Pereira Soares, M.B.; Villarreal, C.F. Anti-inflammatory effects of carvacrol: Evidence for a key role of interleukin-10. *Eur. J. Pharmacol.* **2013**, *699*, 112–117. [\[CrossRef\]](#)
143. Guimaraes, A.G.; Quintans, J.S.; Quintans, L.J., Jr. Monoterpenes with analgesic activity—a systematic review. *Phytother. Res. PTR* **2013**, *27*, 1–15. [\[CrossRef\]](#)
144. Siveen, K.S.; Kuttan, G. Thujone inhibits lung metastasis induced by B16F-10 melanoma cells in C57BL/6 mice. *Can. J. Physiol. Pharmacol.* **2011**, *89*, 691–703. [\[CrossRef\]](#) [\[PubMed\]](#)
145. Ramalho, T.R.; Pacheco de Oliveira, M.T.; Lima, A.L.; Bezerra-Santos, C.R.; Piuvezam, M.R. Gamma-Terpinene Modulates Acute Inflammatory Response in Mice. *Planta Med.* **2015**, *81*, 1248–1254. [\[CrossRef\]](#) [\[PubMed\]](#)
146. Ramalho, T.R.; Filgueiras, L.R.; Pacheco de Oliveira, M.T.; Lima, A.L.; Bezerra-Santos, C.R.; Jancar, S.; Piuvezam, M.R. Gamma-Terpinene Modulation of LPS-Stimulated Macrophages is Dependent on the PGE2/IL-10 Axis. *Planta Med.* **2016**, *82*, 1341–1345. [\[CrossRef\]](#) [\[PubMed\]](#)
147. Baldissera, M.D.; Grando, T.H.; Souza, C.F.; Gressler, L.T.; Stefani, L.M.; da Silva, A.S.; Monteiro, S.G. In vitro and in vivo action of terpinen-4-ol, gamma-terpinene, and alpha-terpinene against *Trypanosoma evansi*. *Exp. Parasitol.* **2016**, *162*, 43–48. [\[CrossRef\]](#) [\[PubMed\]](#)

148. Assmann, C.E.; Cadona, F.C.; Bonadiman, B.; Dornelles, E.B.; Trevisan, G.; Cruz, I. Tea tree oil presents in vitro antitumor activity on breast cancer cells without cytotoxic effects on fibroblasts and on peripheral blood mononuclear cells. *Biomed. Pharmacother.* **2018**, *103*, 1253–1261. [\[CrossRef\]](#)
149. Begum, A.; Sandhya, S.; Shaffath Ali, S.; Vinod, K.R.; Reddy, S.; Banji, D. An in-depth review on the medicinal flora *Rosmarinus officinalis* (Lamiaceae). *Acta Sci. Polonorum. Technol. Aliment.* **2013**, *12*, 61–73.
150. Nissen, L.; Zatta, A.; Stefanini, I.; Grandi, S.; Sgorbati, B.; Biavati, B.; Monti, A. Characterization and antimicrobial activity of essential oils of industrial hemp varieties (*Cannabis sativa* L.). *Fitoterapia* **2010**, *81*, 413–419. [\[CrossRef\]](#)
151. Yang, H.; Woo, J.; Pae, A.N.; Um, M.Y.; Cho, N.C.; Park, K.D.; Yoon, M.; Kim, J.; Lee, C.J.; Cho, S. alpha-Pinene, a Major Constituent of Pine Tree Oils, Enhances Non-Rapid Eye Movement Sleep in Mice through GABAA-benzodiazepine Receptors. *Mol. Pharmacol.* **2016**, *90*, 530–539. [\[CrossRef\]](#)
152. Satou, T.; Kasuya, H.; Maeda, K.; Koike, K. Daily inhalation of alpha-pinene in mice: Effects on behavior and organ accumulation. *Phytother. Res. PTR* **2014**, *28*, 1284–1287. [\[CrossRef\]](#)
153. Mercier, B.; Prost, J.; Prost, M. The essential oil of turpentine and its major volatile fraction (alpha- and beta-pinenes): A review. *Int. J. Occup. Med. Environ. Health* **2009**, *22*, 331–342. [\[CrossRef\]](#) [\[PubMed\]](#)
154. Nuutinen, T. Medicinal properties of terpenes found in *Cannabis sativa* and *Humulus lupulus*. *Eur. J. Med. Chem.* **2018**, *157*, 198–228. [\[CrossRef\]](#) [\[PubMed\]](#)
155. Kasuya, H.; Iida, S.; Ono, K.; Satou, T.; Koike, K. Intracerebral Distribution of alpha-Pinene and the Anxiolytic-like Effect in Mice Following Inhaled Administration of Essential Oil from *Chamaecyparis obtusa*. *Nat. Prod. Commun.* **2015**, *10*, 1479–1482. [\[CrossRef\]](#) [\[PubMed\]](#)
156. Ueno, H.; Shimada, A.; Suemitsu, S.; Murakami, S.; Kitamura, N.; Wani, K.; Matsumoto, Y.; Okamoto, M.; Ishihara, T. Attenuation Effects of Alpha-Pinene Inhalation on Mice with Dizocilpine-Induced Psychiatric-Like Behaviour. *Evid. Based Complement. Altern. Med. ECAM* **2019**, *2019*, 2745453. [\[CrossRef\]](#)
157. Lee, G.Y.; Lee, C.; Park, G.H.; Jang, J.H. Amelioration of Scopolamine-Induced Learning and Memory Impairment by alpha-Pinene in C57BL/6 Mice. *Evid. Based Complement. Altern. Med. Ecam* **2017**, *2017*, 4926815. [\[CrossRef\]](#)
158. Karthikeyan, R.; Kanimozhi, G.; Prasad, N.R.; Agilan, B.; Ganesan, M.; Srithar, G. Alpha pinene modulates UVA-induced oxidative stress, DNA damage and apoptosis in human skin epidermal keratinocytes. *Life Sci.* **2018**, *212*, 150–158. [\[CrossRef\]](#)
159. Karthikeyan, R.; Kanimozhi, G.; Madhavan, N.R.; Agilan, B.; Ganesan, M.; Prasad, N.R.; Rathinaraj, P. Alpha-pinene attenuates UVA-induced photoaging through inhibition of matrix metalloproteinases expression in mouse skin. *Life Sci.* **2019**, *217*, 110–118. [\[CrossRef\]](#)
160. Bouzenna, H.; Hfaiedh, N.; Giroux-Metges, M.A.; Elfeki, A.; Talarmin, H. Potential protective effects of alpha-pinene against cytotoxicity caused by aspirin in the IEC-6 cells. *Biomed. Pharmacother.* **2017**, *93*, 961–968. [\[CrossRef\]](#)
161. Hou, J.; Zhang, Y.; Zhu, Y.; Zhou, B.; Ren, C.; Liang, S.; Guo, Y. alpha-Pinene Induces Apoptotic Cell Death via Caspase Activation in Human Ovarian Cancer Cells. *Med Sci. Monit.: Int. Med J. Exp. Clin. Res.* **2019**, *25*, 6631–6638. [\[CrossRef\]](#)
162. Xu, Q.; Li, M.; Yang, M.; Yang, J.; Xie, J.; Lu, X.; Wang, F.; Chen, W. alpha-pinene regulates miR-221 and induces G2/M phase cell cycle arrest in human hepatocellular carcinoma cells. *Biosci. Rep.* **2018**, *38*. [\[CrossRef\]](#)
163. Rahbar, I.; Abbasnejad, M.; Haghani, J.; Raoof, M.; Kooshki, R.; Esmaeili-Mahani, S. The effect of central administration of alpha-pinene on capsaicin-induced dental pulp nociception. *Int. Endod. J.* **2019**, *52*, 307–317. [\[CrossRef\]](#) [\[PubMed\]](#)
164. Li, X.J.; Yang, Y.J.; Li, Y.S.; Zhang, W.K.; Tang, H.B. alpha-Pinene, linalool, and 1-octanol contribute to the topical anti-inflammatory and analgesic activities of frankincense by inhibiting COX-2. *J. Ethnopharmacol.* **2016**, *179*, 22–26. [\[CrossRef\]](#) [\[PubMed\]](#)
165. Iseppi, R.; Brighenti, V.; Licata, M.; Lambertini, A.; Sabia, C.; Messi, P.; Pellati, F.; Benvenuti, S. Chemical Characterization and Evaluation of the Antibacterial Activity of Essential Oils from Fibre-Type *Cannabis sativa* L. (Hemp). *Molecules* **2019**, *24*, 2302. [\[CrossRef\]](#) [\[PubMed\]](#)
166. Pertwee, R.G.E. *Handbook of Cannabis*, 1st ed.; Oxford University Press: London, UK, 2016.
167. Mahajan, P.; Singh, H.P.; Kaur, S.; Batish, D.R.; Kohli, R.K. beta-Pinene moderates Cr(VI) phytotoxicity by quenching reactive oxygen species and altering antioxidant machinery in maize. *Environ. Sci. Pollut. Res. Int.* **2019**, *26*, 456–463. [\[CrossRef\]](#) [\[PubMed\]](#)

168. Pajaro-Castro, N.; Caballero-Gallardo, K.; Olivero-Verbel, J. Neurotoxic Effects of Linalool and beta-Pinene on *Tribolium castaneum* Herbst. *Molecules* **2017**, *22*, 2052. [[CrossRef](#)]
169. Guzman-Gutierrez, S.L.; Gomez-Cansino, R.; Garcia-Zebadua, J.C.; Jimenez-Perez, N.C.; Reyes-Chilpa, R. Antidepressant activity of *Litsea glaucescens* essential oil: Identification of beta-pinene and linalool as active principles. *J. Ethnopharmacol.* **2012**, *143*, 673–679. [[CrossRef](#)]
170. Guzman-Gutierrez, S.L.; Bonilla-Jaime, H.; Gomez-Cansino, R.; Reyes-Chilpa, R. Linalool and beta-pinene exert their antidepressant-like activity through the monoaminergic pathway. *Life Sci.* **2015**, *128*, 24–29. [[CrossRef](#)]
171. Wang, Y.; Wu, C.; Zhang, Q.; Shan, Y.; Gu, W.; Wang, S. Design, synthesis and biological evaluation of novel beta-pinene-based thiazole derivatives as potential anticancer agents via mitochondrial-mediated apoptosis pathway. *Bioorg. Chem.* **2019**, *84*, 468–477. [[CrossRef](#)]
172. Astani, A.; Reichling, J.; Schnitzler, P. Comparative study on the antiviral activity of selected monoterpenes derived from essential oils. *Phytother. Res. PTR* **2010**, *24*, 673–679. [[CrossRef](#)]
173. Zhou, L.; Zhang, Z.; Wei, M.; Xie, Y.; He, S.; Shi, H.; Lin, Z. Evaluation of the antifungal activity of individual and combined monoterpenes against *Rhizopus stolonifer* and *Absidia coerulea*. *Environ. Sci. Pollut. Res. Int.* **2019**, *26*, 7804–7809. [[CrossRef](#)]
174. Astani, A.; Schnitzler, P. Antiviral activity of monoterpenes beta-pinene and limonene against herpes simplex virus in vitro. *Iran. J. Microbiol.* **2014**, *6*, 149–155. [[PubMed](#)]
175. de Macedo Andrade, A.C.; Rosalen, P.L.; Freires, I.A.; Scotti, L.; Scotti, M.T.; Aquino, S.G.; de Castro, R.D. Antifungal Activity, Mode of Action, Docking Prediction and Anti-biofilm Effects of (+)-beta-pinene Enantiomers against *Candida* spp. *Curr. Top. Med. Chem.* **2018**, *18*, 2481–2490. [[CrossRef](#)] [[PubMed](#)]
176. Jia, N.; Arthington-Skaggs, B.; Lee, W.; Pierson, C.A.; Lees, N.D.; Eckstein, J.; Barbuch, R.; Bard, M. *Candida albicans* sterol C-14 reductase, encoded by the ERG24 gene, as a potential antifungal target site. *Antimicrob. Agents Chemother.* **2002**, *46*, 947–957. [[CrossRef](#)] [[PubMed](#)]
177. Rivas da Silva, A.C.; Lopes, P.M.; Barros de Azevedo, M.M.; Costa, D.C.; Alviano, C.S.; Alviano, D.S. Biological activities of alpha-pinene and beta-pinene enantiomers. *Molecules* **2012**, *17*, 6305–6316. [[CrossRef](#)] [[PubMed](#)]
178. Marchini, M.; Charvoz, C.; Dujourdy, L.; Baldovini, N.; Filippi, J.J. Multidimensional analysis of cannabis volatile constituents: Identification of 5,5-dimethyl-1-vinylbicyclo[2.1.1]hexane as a volatile marker of hashish, the resin of *Cannabis sativa* L. *J. Chromatography. A* **2014**, *1370*, 200–215. [[CrossRef](#)] [[PubMed](#)]
179. Booth, J.K.; Bohlmann, J. Terpenes in *Cannabis sativa*—From plant genome to humans. *Plant Sci. Int. J. Exp. Plant Biol.* **2019**, *284*, 67–72. [[CrossRef](#)] [[PubMed](#)]
180. Zhai, B.; Zhang, N.; Han, X.; Li, Q.; Zhang, M.; Chen, X.; Li, G.; Zhang, R.; Chen, P.; Wang, W.; et al. Molecular targets of beta-elemene, a herbal extract used in traditional Chinese medicine, and its potential role in cancer therapy: A review. *Biomed. Pharmacother.* **2019**, *114*, 108812. [[CrossRef](#)]
181. Deng, M.; Zhang, Y.; Liu, B.; Chen, Y.; Song, H.; Yu, R.; Che, X.; Qu, X.; Liu, Y.; Hu, X.; et al. beta-Elemene inhibits peritoneal metastasis of gastric cancer cells by modulating FAK/Claudin-1 signaling. *Phytother. Res. PTR* **2019**, *33*, 2448–2456. [[CrossRef](#)]
182. Wu, Z.; Wang, T.; Zhang, Y.; Zheng, Z.; Yu, S.; Jing, S.; Chen, S.; Jiang, H.; Ma, S. Anticancer effects of beta-elemene with hyperthermia in lung cancer cells. *Exp. Ther. Med.* **2017**, *13*, 3153–3157. [[CrossRef](#)]
183. Fang, M.; Mei, X.; Yao, H.; Zhang, T.; Zhang, T.; Lu, N.; Liu, Y.; Xu, W.; Wan, C. beta-elemene enhances anticancer and anti-metastatic effects of osteosarcoma of ligustrazine in vitro and in vivo. *Oncol. Lett.* **2018**, *15*, 3957–3964.
184. Cai, B.; Ma, L.; Nong, S.; Wu, Y.; Guo, X.; Pu, J. beta-elemene induced anticancer effect in bladder cancer through upregulation of PTEN and suppression of AKT phosphorylation. *Oncol. Lett.* **2018**, *16*, 6019–6025. [[PubMed](#)]
185. Liu, J.S.; He, S.C.; Zhang, Z.L.; Chen, R.; Fan, L.; Qiu, G.L.; Chang, S.; Li, L.; Che, X.M. Anticancer effects of beta-elemene in gastric cancer cells and its potential underlying proteins: A proteomic study. *Oncol. Rep.* **2014**, *32*, 2635–2647. [[CrossRef](#)] [[PubMed](#)]
186. Li, Q.Q.; Lee, R.X.; Liang, H.; Zhong, Y. Anticancer activity of beta-Elemene and its synthetic analogs in human malignant brain tumor cells. *Anticancer Res.* **2013**, *33*, 65–76. [[PubMed](#)]
187. Wei, Y.; Pu, X.; Zhao, L. Preclinical studies for the combination of paclitaxel and curcumin in cancer therapy. *Oncol. Rep.* **2017**, *37*, 3159–3166. [[CrossRef](#)] [[PubMed](#)]

188. Li, X.; Gao, C.; Wu, Y.; Cheng, C.Y.; Xia, W.; Zhang, Z. Combination delivery of Adjudin and Doxorubicin via integrating drug conjugation and nanocarrier approaches for the treatment of drug-resistant cancer cells. *J. Mater. Chem. B* **2015**, *3*, 1556–1564. [[CrossRef](#)] [[PubMed](#)]
189. Yao, Y.Q.; Ding, X.; Jia, Y.C.; Huang, C.X.; Wang, Y.Z.; Xu, Y.H. Anti-tumor effect of beta-elemene in glioblastoma cells depends on p38 MAPK activation. *Cancer Lett.* **2008**, *264*, 127–134. [[CrossRef](#)]
190. Jiang, Z.; Jacob, J.A.; Loganathachetti, D.S.; Nainangu, P.; Chen, B. beta-Elemene: Mechanistic Studies on Cancer Cell Interaction and Its Chemosensitization Effect. *Front. Pharmacol.* **2017**, *8*, 105. [[CrossRef](#)]
191. Li, Q.Q.; Wang, G.; Zhang, M.; Cuff, C.F.; Huang, L.; Reed, E. beta-Elemene, a novel plant-derived antineoplastic agent, increases cisplatin chemosensitivity of lung tumor cells by triggering apoptosis. *Oncol. Rep.* **2009**, *22*, 161–170. [[CrossRef](#)]
192. Eloy, J.O.; Claro de Souza, M.; Petrilli, R.; Barcellos, J.P.; Lee, R.J.; Marchetti, J.M. Liposomes as carriers of hydrophilic small molecule drugs: Strategies to enhance encapsulation and delivery. *Colloids Surf. Biointerfaces* **2014**, *123*, 345–363. [[CrossRef](#)]
193. Cao, M.; Long, M.; Chen, Q.; Lu, Y.; Luo, Q.; Zhao, Y.; Lu, A.; Ge, C.; Zhu, L.; Chen, Z. Development of beta-elemene and Cisplatin Co-Loaded Liposomes for Effective Lung Cancer Therapy and Evaluation in Patient-Derived Tumor Xenografts. *Pharm. Res.* **2019**, *36*, 121. [[CrossRef](#)]
194. Cao, C.; Wang, Q.; Liu, Y. Lung cancer combination therapy: Doxorubicin and beta-elemene co-loaded, pH-sensitive nanostructured lipid carriers. *Drug Des. Dev. Ther.* **2019**, *13*, 1087–1098. [[CrossRef](#)] [[PubMed](#)]
195. Zeng, Y.Y.; Zeng, Y.J.; Zhang, N.N.; Li, C.X.; Xie, T.; Zeng, Z.W. The Preparation, Determination of a Flexible Complex Liposome Co-Loaded with Cabazitaxel and beta-Elemene, and Animal Pharmacodynamics on Paclitaxel-Resistant Lung Adenocarcinoma. *Molecules* **2019**, *24*, 1697. [[CrossRef](#)] [[PubMed](#)]
196. Yoshida, T.; Hashimura, M.; Mastumoto, T.; Tazo, Y.; Inoue, H.; Kuwata, T.; Saegusa, M. Transcriptional upregulation of HIF-1alpha by NF- $\kappa$ B/p65 and its associations with beta-catenin/p300 complexes in endometrial carcinoma cells. *Lab. Investig. J. Tech. Methods Pathol.* **2013**, *93*, 1184–1193. [[CrossRef](#)] [[PubMed](#)]
197. Yu, X.; Li, Z.; Zhang, Y.; Xu, M.; Che, Y.; Tian, X.; Wang, R.; Zou, K.; Zou, L. beta-elemene inhibits radiation and hypoxia-induced macrophages infiltration via Prx-1/NF- $\kappa$ B/HIF-1alpha signaling pathway. *Oncotargets Ther.* **2019**, *12*, 4203–4211. [[CrossRef](#)] [[PubMed](#)]
198. Liu, M.; Chen, X.; Ma, J.; Hassan, W.; Wu, H.; Ling, J.; Shang, J. beta-Elemene attenuates atherosclerosis in apolipoprotein E-deficient mice via restoring NO levels and alleviating oxidative stress. *Biomed. Pharmacother.* **2017**, *95*, 1789–1798. [[CrossRef](#)] [[PubMed](#)]
199. Zhou, Y.; Liu, Y.; Chen, J.; Sun, Y.Z.; Li, L.H.; Chen, L. Inhibition of beta-elemene on the expressions of HIF-1alpha, VEGF and iNOS in diabetic rats model. *Int. J. Ophthalmol.* **2019**, *12*, 1693–1698. [[CrossRef](#)]
200. Zhang, R.; Tian, A.; Shi, X.; Yu, H.; Chen, L. Downregulation of IL-17 and IFN-gamma in the optic nerve by beta-elemene in experimental autoimmune encephalomyelitis. *Int. Immunopharmacol.* **2010**, *10*, 738–743. [[CrossRef](#)]
201. Farre-Armengol, G.; Filella, I.; Llusia, J.; Penuelas, J. beta-Ocimene, a Key Floral and Foliar Volatile Involved in Multiple Interactions between Plants and Other Organisms. *Molecules* **2017**, *22*, 1148. [[CrossRef](#)]
202. Pavlovic, R.; Panseri, S.; Giupponi, L.; Leoni, V.; Citti, C.; Cattaneo, C.; Cavaletto, M.; Giorgi, A. Phytochemical and Ecological Analysis of Two Varieties of Hemp (*Cannabis sativa* L.) Grown in a Mountain Environment of Italian Alps. *Front. Plant Sci.* **2019**, *10*, 1265. [[CrossRef](#)]
203. Bomfim, L.M.; Menezes, L.R.; Rodrigues, A.C.; Dias, R.B.; Rocha, C.A.; Soares, M.B.; Neto, A.F.; Nascimento, M.P.; Campos, A.F.; Silva, L.C.; et al. Antitumour Activity of the Microencapsulation of *Annona vepretorum* Essential Oil. *Basic Clin. Pharmacol. Toxicol.* **2016**, *118*, 208–213. [[CrossRef](#)]
204. Sayyah, M.; Nadjafnia, L.; Kamalinejad, M. Anticonvulsant activity and chemical composition of *Artemisia dracunculus* L. essential oil. *J. Ethnopharmacol.* **2004**, *94*, 283–287. [[CrossRef](#)]
205. Basler, K.H.C.; Buchbauer, G. *Handbook of Essential Oils: Science, Technology, and Applications*; CRC Press/Taylor & Francis: Boca Raton, FL, USA, 2010.
206. Feng, Y.X.; Wang, Y.; Chen, Z.Y.; Guo, S.S.; You, C.X.; Du, S.S. Efficacy of bornyl acetate and camphene from *Valeriana officinalis* essential oil against two storage insects. *Environ. Sci. Pollut. Res. Int.* **2019**, *26*, 16157–16165. [[CrossRef](#)] [[PubMed](#)]

207. Benelli, G.; Govindarajan, M.; Rajeswary, M.; Vaseeharan, B.; Alyahya, S.A.; Alharbi, N.S.; Kadaikunnan, S.; Khaled, J.M.; Maggi, F. Insecticidal activity of camphene, zerumbone and alpha-humulene from *Cheilocostus speciosus* rhizome essential oil against the Old-World bollworm, *Helicoverpa armigera*. *Ecotoxicol. Environ. Saf.* **2018**, *148*, 781–786. [[CrossRef](#)] [[PubMed](#)]
208. Benelli, G.; Govindarajan, M.; AlSalhi, M.S.; Devanesan, S.; Maggi, F. High toxicity of camphene and gamma-elemene from *Wedelia prostrata* essential oil against larvae of *Spodoptera litura* (Lepidoptera: Noctuidae). *Environ. Sci. Pollut. Res. Int.* **2018**, *25*, 10383–10391. [[CrossRef](#)] [[PubMed](#)]
209. Souza, M.R.P.; Coelho, N.P.; Baldin, V.P.; Scodro, R.B.L.; Cardoso, R.F.; da Silva, C.C.; Vandresen, F. Synthesis of novel (–)-Camphene-based thiosemicarbazones and evaluation of anti-*Mycobacterium tuberculosis* activity. *Nat. Prod. Res.* **2019**, *33*, 3372–3377. [[CrossRef](#)] [[PubMed](#)]
210. Baldissera, M.D.; Souza, C.F.; Rieffel, R.C.; Velho, M.C.; Ramos, A.P.; Nascimento, K.; Sagrillo, M.R.; Ourique, A.F.; da Silva, A.S.; Stefani, L.M.; et al. Protective effect of nerolidol-loaded in nanospheres against cerebral damage caused by *Trypanosoma evansi*. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2018**, *391*, 753–759. [[CrossRef](#)]
211. Ceole, L.F.; Cardoso, M.D.G.; Soares, M.J. Nerolidol, the main constituent of *Piper aduncum* essential oil, has anti-*Leishmania braziliensis* activity. *Parasitology* **2017**, *144*, 1179–1190. [[CrossRef](#)]
212. Alonso, L.; Fernandes, K.S.; Mendanha, S.A.; Goncalves, P.J.; Gomes, R.S.; Dorta, M.L.; Alonso, A. In vitro antileishmanial and cytotoxic activities of nerolidol are associated with changes in plasma membrane dynamics. *Biochim. Biophys. Acta Biomembr.* **2019**, *1861*, 1049–1056. [[CrossRef](#)]
213. Silva, M.P.; de Oliveira, R.N.; Mengarda, A.C.; Roquini, D.B.; Allegretti, S.M.; Salvadori, M.C.; Teixeira, F.S.; de Sousa, D.P.; Pinto, P.L.S.; da Silva Filho, A.A.; et al. Antiparasitic activity of nerolidol in a mouse model of schistosomiasis. *Int. J. Antimicrob. Agents* **2017**, *50*, 467–472. [[CrossRef](#)]
214. Zhang, L.; Sun, D.; Bao, Y.; Shi, Y.; Cui, Y.; Guo, M. Nerolidol Protects Against LPS-induced Acute Kidney Injury via Inhibiting TLR4/NF- $\kappa$ B Signaling. *Phytother. Res. PTR* **2017**, *31*, 459–465. [[CrossRef](#)]
215. Barros Silva Soares de Souza, E.P.; Trindade, G.; Lins Dantas Gomes, M.V.; Santos Silva, L.A.; Grespan, R.; Quintans Junior, L.J.; Cavalcanti de Albuquerque Junior, R.L.; Shanmugan, S.; Antunes de Souza Araujo, A. Anti-inflammatory effect of nano-encapsulated nerolidol on zymosan-induced arthritis in mice. *Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* **2019**, 110958. [[CrossRef](#)] [[PubMed](#)]
216. Hanusova, V.; Caltova, K.; Svobodova, H.; Ambroz, M.; Skarka, A.; Murinova, N.; Kralova, V.; Tomsik, P.; Skalova, L. The effects of beta-caryophyllene oxide and trans-nerolidol on the efficacy of doxorubicin in breast cancer cells and breast tumor-bearing mice. *Biomed. Pharmacother.* **2017**, *95*, 828–836. [[CrossRef](#)] [[PubMed](#)]
217. Iqbal, A.; Sharma, S.; Najmi, A.K.; Syed, M.A.; Ali, J.; Alam, M.M.; Haque, S.E. Nerolidol ameliorates cyclophosphamide-induced oxidative stress, neuroinflammation and cognitive dysfunction: Plausible role of Nrf2 and NF- $\kappa$ B. *Life Sci.* **2019**, *236*, 116867. [[CrossRef](#)] [[PubMed](#)]
218. Iqbal, A.; Sharma, S.; Ansari, M.A.; Najmi, A.K.; Syed, M.A.; Ali, J.; Alam, M.M.; Ahmad, S.; Haque, S.E. Nerolidol attenuates cyclophosphamide-induced cardiac inflammation, apoptosis and fibrosis in Swiss Albino mice. *Eur. J. Pharmacol.* **2019**, *863*, 172666. [[CrossRef](#)]
219. Asaikumar, L.; Vennila, L.; Akila, P.; Sivasangari, S.; Kanimozhi, K.; Premalatha, V.; Sindhu, G. Preventive effect of nerolidol on isoproterenol induced myocardial damage in Wistar rats: Evidences from biochemical and histopathological studies. *Drug Dev. Res.* **2019**, *80*, 814–823. [[CrossRef](#)]
220. Pellati, F.; Borgonetti, V.; Brighenti, V.; Biagi, M.; Benvenuti, S.; Corsi, L. Cannabis sativa L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer. *Biomed Res. Int.* **2018**, *2018*, 1691428. [[CrossRef](#)]
221. Betancur-Galvis, L.A.; Morales, G.E.; Forero, J.E.; Roldan, J. Cytotoxic and antiviral activities of Colombian medicinal plant extracts of the *Euphorbia* genus. *Mem. Inst. Oswaldo Cruz* **2002**, *97*, 541–546. [[CrossRef](#)]
222. Nes, W.D.; Wong, R.Y.; Benson, M.; Landrey, J.R.; Nes, W.R. Rotational isomerism about the 17(20)-bond of steroids and euphoids as shown by the crystal structures of euphol and tirucallol. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 5896–5900. [[CrossRef](#)]
223. Xie, X.; Li, Y.; Gao, D.; Zhang, Y.; Ren, Y. Quantitative determination of euphol in rat plasma by LC-MS/MS and its application to a pharmacokinetic study. *Biomed. Chromatogr. BMC* **2014**, *28*, 1229–1234. [[CrossRef](#)]

224. de Souza, L.S.; Puziol, L.C.; Tosta, C.L.; Bittencourt, M.L.F.; Ardisson, J.S.; Kitagawa, R.R.; Filgueiras, P.R.; Kuster, R.M. Analytical methods to access the chemical composition of an *Euphorbia tirucalli* anticancer latex from traditional Brazilian medicine. *J. Ethnopharmacol.* **2019**, *237*, 255–265. [[CrossRef](#)]
225. Prinsloo, G.; Marokane, C.K.; Street, R.A. Anti-HIV activity of southern African plants: Current developments, phytochemistry and future research. *J. Ethnopharmacol.* **2018**, *210*, 133–155. [[CrossRef](#)] [[PubMed](#)]
226. Mazoir, N.; Benharref, A.; Bailen, M.; Reina, M.; Gonzalez-Coloma, A.; Martinez-Diaz, R.A. Antileishmanial and antitrypanosomal activity of triterpene derivatives from latex of two *Euphorbia* species. *Z. Fur Naturforschung. Cjournal Biosci.* **2011**, *66*, 360–366. [[CrossRef](#)] [[PubMed](#)]
227. Ibrahim, E.H.; Kilany, M.; Mostafa, O.M.S.; Shaker, K.H.; Alshehri, M.; Alsyaad, K.M.; Alshehri, A.; Khan, K.A.; Qasim, M.; Kotb, N.; et al. TH1/TH2 chemokines/cytokines profile in rats treated with tetanus toxoid and *Euphorbia tirucalli*. *Saudi J. Biol. Sci.* **2019**, *26*, 1716–1723. [[CrossRef](#)] [[PubMed](#)]
228. Silva, V.A.O.; Rosa, M.N.; Tansini, A.; Oliveira, R.J.S.; Martinho, O.; Lima, J.P.; Pianowski, L.F.; Reis, R.M. In vitro screening of cytotoxic activity of euphol from *Euphorbia tirucalli* on a large panel of human cancer-derived cell lines. *Exp. Ther. Med.* **2018**, *16*, 557–566. [[CrossRef](#)]
229. Cruz, L.S.; de Oliveira, T.L.; Kanunfre, C.C.; Paludo, K.S.; Minozzo, B.R.; Prestes, A.P.; Wang, M.; Fernandes, D.; Santos, F.A.D.; Manda, V.K.; et al. Pharmacokinetics and cytotoxic study of euphol from *Euphorbia umbellata* (Bruyns) Pax latex. *Phytomedicine: Int. J. Phytother. Phytopharm.* **2018**, *47*, 105–112. [[CrossRef](#)]
230. Wang, L.; Wang, G.; Yang, D.; Guo, X.; Xu, Y.; Feng, B.; Kang, J. Euphol arrests breast cancer cells at the G1 phase through the modulation of cyclin D1, p21 and p27 expression. *Mol. Med. Rep.* **2013**, *8*, 1279–1285. [[CrossRef](#)]
231. Silva, V.A.O.; Rosa, M.N.; Miranda-Goncalves, V.; Costa, A.M.; Tansini, A.; Evangelista, A.F.; Martinho, O.; Carloni, A.C.; Jones, C.; Lima, J.P.; et al. Euphol, a tetracyclic triterpene, from *Euphorbia tirucalli* induces autophagy and sensitizes temozolomide cytotoxicity on glioblastoma cells. *Investig. New Drugs* **2019**, *37*, 223–237. [[CrossRef](#)]
232. Athmouni, K.; El Feki, A.; Ayadi, H. Hepatotoxic effects of Euphol-rich fractions from *Euphorbia bivonae*-Relevance to cytotoxic and anti-tumor activities. *Pathophysiol. Off. J. Int. Soc. Pathophysiol.* **2019**, *26*, 69–76. [[CrossRef](#)]
233. Dutra, R.C.; Claudino, R.F.; Bento, A.F.; Marcon, R.; Schmidt, E.C.; Bouzon, Z.L.; Pianowski, L.F.; Calixto, J.B. Preventive and therapeutic euphol treatment attenuates experimental colitis in mice. *PLoS ONE* **2011**, *6*, e27122. [[CrossRef](#)]
234. Dutra, R.C.; de Souza, P.R.; Bento, A.F.; Marcon, R.; Bicca, M.A.; Pianowski, L.F.; Calixto, J.B. Euphol prevents experimental autoimmune encephalomyelitis in mice: Evidence for the underlying mechanisms. *Biochem. Pharmacol.* **2012**, *83*, 531–542. [[CrossRef](#)]
235. Yasukawa, K.; Akihisa, T.; Yoshida, Z.Y.; Takido, M. Inhibitory effect of euphol, a triterpene alcohol from the roots of *Euphorbia kansui*, on tumour promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. *J. Pharm. Pharmacol.* **2000**, *52*, 119–124. [[CrossRef](#)] [[PubMed](#)]
236. Passos, G.F.; Medeiros, R.; Marcon, R.; Nascimento, A.F.; Calixto, J.B.; Pianowski, L.F. The role of PKC/ERK1/2 signaling in the anti-inflammatory effect of tetracyclic triterpene euphol on TPA-induced skin inflammation in mice. *Eur. J. Pharmacol.* **2013**, *698*, 413–420. [[CrossRef](#)] [[PubMed](#)]
237. Dutra, R.C.; Simao da Silva, K.A.; Bento, A.F.; Marcon, R.; Paszcuk, A.F.; Meotti, F.C.; Pianowski, L.F.; Calixto, J.B. Euphol, a tetracyclic triterpene produces antinociceptive effects in inflammatory and neuropathic pain: The involvement of cannabinoid system. *Neuropharmacology* **2012**, *63*, 593–605. [[CrossRef](#)] [[PubMed](#)]
238. Dutra, R.C.; Bicca, M.A.; Segat, G.C.; Silva, K.A.; Motta, E.M.; Pianowski, L.F.; Costa, R.; Calixto, J.B. The antinociceptive effects of the tetracyclic triterpene euphol in inflammatory and neuropathic pain models: The potential role of PKCepsilon. *Neuroscience* **2015**, *303*, 126–137. [[CrossRef](#)] [[PubMed](#)]
239. Yang, C.; Kennes, Y.M.; Lepp, D.; Yin, X.; Wang, Q.; Yu, H.; Yang, C.; Gong, J.; Diarra, M.S. Effects of encapsulated cinnamaldehyde and citral on the performance and cecal microbiota of broilers vaccinated or not vaccinated against coccidiosis. *Poult. Sci.* **2020**, *99*, 936–948. [[CrossRef](#)]
240. Shi, C.; Song, K.; Zhang, X.; Sun, Y.; Sui, Y.; Chen, Y.; Jia, Z.; Sun, H.; Sun, Z.; Xia, X. Antimicrobial Activity and Possible Mechanism of Action of Citral against *Cronobacter sakazakii*. *PLoS ONE* **2016**, *11*, e0159006. [[CrossRef](#)]

241. Parra-Flores, J.; Cerda-Leal, F.; Contreras, A.; Valenzuela-Riffo, N.; Rodriguez, A.; Aguirre, J. *Cronobacter sakazakii* and Microbiological Parameters in Dairy Formulas Associated With a Food Alert in Chile. *Front. Microbiol.* **2018**, *9*, 1708. [\[CrossRef\]](#)
242. Gupta, P.; Patel, D.K.; Gupta, V.K.; Pal, A.; Tandon, S.; Darokar, M.P. Citral, a monoterpenoid aldehyde interacts synergistically with norfloxacin against methicillin resistant *Staphylococcus aureus*. *Phytomed. Int. J. Phytother. Phytopharm.* **2017**, *34*, 85–96. [\[CrossRef\]](#)
243. Leite, M.C.; Bezerra, A.P.; de Sousa, J.P.; Guerra, F.Q.; Lima Ede, O. Evaluation of Antifungal Activity and Mechanism of Action of Citral against *Candida albicans*. *Evid. Based Complement. Altern. Med. ECAM* **2014**, *2014*, 378280. [\[CrossRef\]](#)
244. Qian, W.; Liu, M.; Fu, Y.; Wang, T.; Zhang, J.; Yang, M.; Sun, Z.; Li, X.; Li, Y. Antimicrobial and Antibiofilm Activities of Citral against Carbapenem-Resistant *Enterobacter cloacae*. *Foodborne Pathog. Dis.* **2020**. [\[CrossRef\]](#)
245. Fancello, F.; Petretto, G.L.; Marceddu, S.; Venditti, T.; Pintore, G.; Zara, G.; Mannazzu, I.; Budroni, M.; Zara, S. Antimicrobial activity of gaseous Citrus limon var pompia leaf essential oil against *Listeria monocytogenes* on ricotta salata cheese. *Food Microbiol.* **2020**, *87*, 103386. [\[CrossRef\]](#) [\[PubMed\]](#)
246. de Souza, R.C.; da Costa, M.M.; Baldisserotto, B.; Heinzmann, B.M.; Schmidt, D.; Caron, B.O.; Copatti, C.E. Antimicrobial and synergistic activity of essential oils of *Aloysia triphylla* and *Lippia alba* against *Aeromonas* spp. *Microb. Pathog.* **2017**, *113*, 29–33. [\[CrossRef\]](#) [\[PubMed\]](#)
247. Adil, M.; Baig, M.H.; Rupasinghe, H.P.V. Impact of Citral and Phloretin, Alone and in Combination, on Major Virulence Traits of *Streptococcus pyogenes*. *Molecules* **2019**, *24*, 4237. [\[CrossRef\]](#) [\[PubMed\]](#)
248. Lulekal, E.; Tesfaye, S.; Gebrechristos, S.; Dires, K.; Zenebe, T.; Zegeye, N.; Feleke, G.; Kassahun, A.; Shiferaw, Y.; Mekonnen, A. Phytochemical analysis and evaluation of skin irritation, acute and sub-acute toxicity of *Cymbopogon citratus* essential oil in mice and rabbits. *Toxicol. Rep.* **2019**, *6*, 1289–1294. [\[CrossRef\]](#)
249. Sharma, S.; Gupta, J.; Habib, S.; Sahu, D. Chemical properties and therapeutic potential of citral, a monoterpene isolated from lemongrass. *Med. Chem.* **2019**. [\[CrossRef\]](#)
250. Shen, Y.; Sun, Z.; Guo, X. Citral inhibits lipopolysaccharide-induced acute lung injury by activating PPAR-gamma. *Eur. J. Pharmacol.* **2015**, *747*, 45–51. [\[CrossRef\]](#)
251. Boukhatem, M.N.; Ferhat, M.A.; Kameli, A.; Saidi, F.; Kebir, H.T. Lemon grass (*Cymbopogon citratus*) essential oil as a potent anti-inflammatory and antifungal drugs. *Libyan J. Med.* **2014**, *9*, 25431. [\[CrossRef\]](#)
252. Yang, S.M.; Hua, K.F.; Lin, Y.C.; Chen, A.; Chang, J.M.; Kuoping Chao, L.; Ho, C.L.; Ka, S.M. Citral is renoprotective for focal segmental glomerulosclerosis by inhibiting oxidative stress and apoptosis and activating Nrf2 pathway in mice. *PLoS ONE* **2013**, *8*, e74871. [\[CrossRef\]](#)
253. Campos, C.A.; Lima, B.S.; Trindade, G.G.G.; Souza, E.; Mota, D.S.A.; Heimfarth, L.; Quintans, J.S.S.; Quintans-Junior, L.J.; Sussuchi, E.M.; Sarmiento, V.H.V.; et al. Anti-hyperalgesic and anti-inflammatory effects of citral with beta-cyclodextrin and hydroxypropyl-beta-cyclodextrin inclusion complexes in animal models. *Life Sci.* **2019**, *229*, 139–148. [\[CrossRef\]](#)
254. Song, Y.; Zhao, H.; Liu, J.; Fang, C.; Miao, R. Effects of Citral on Lipopolysaccharide-Induced Inflammation in Human Umbilical Vein Endothelial Cells. *Inflammation* **2016**, *39*, 663–671. [\[CrossRef\]](#)
255. Katsukawa, M.; Nakata, R.; Takizawa, Y.; Hori, K.; Takahashi, S.; Inoue, H. Citral, a component of lemongrass oil, activates PPARalpha and gamma and suppresses COX-2 expression. *Biochim. Biophys. Acta* **2010**, *1801*, 1214–1220. [\[CrossRef\]](#)
256. Goncalves, E.C.D.; Assis, P.M.; Junqueira, L.A.; Cola, M.; Santos, A.R.S.; Raposo, N.R.B.; Dutra, R.C. Citral Inhibits the Inflammatory Response and Hyperalgesia in Mice: The Role of TLR4, TLR2/Dectin-1, and CB2 Cannabinoid Receptor/ATP-Sensitive K(+) Channel Pathways. *J. Nat. Prod.* **2020**. [\[CrossRef\]](#) [\[PubMed\]](#)
257. Bouzenna, H.; Hfaiedh, N.; Giroux-Metges, M.A.; Elfeki, A.; Talarmin, H. Biological properties of citral and its potential protective effects against cytotoxicity caused by aspirin in the IEC-6 cells. *Biomed. Pharmacother.* **2017**, *87*, 653–660. [\[CrossRef\]](#) [\[PubMed\]](#)
258. Ortiz, M.I.; Ramirez-Montiel, M.L.; Gonzalez-Garcia, M.P.; Ponce-Monter, H.A.; Castaneda-Hernandez, G.; Carino-Cortes, R. The combination of naproxen and citral reduces nociception and gastric damage in rats. *Arch. Pharmacol. Res.* **2010**, *33*, 1691–1697. [\[CrossRef\]](#) [\[PubMed\]](#)

259. Sanches, L.J.; Marinello, P.C.; Panis, C.; Fagundes, T.R.; Morgado-Diaz, J.A.; de-Freitas-Junior, J.C.; Cecchini, R.; Cecchini, A.L.; Luiz, R.C. Cytotoxicity of citral against melanoma cells: The involvement of oxidative stress generation and cell growth protein reduction. *Tumour Biol. J. Int. Soc. Oncodev. Biol. Med.* **2017**, *39*. [[CrossRef](#)] [[PubMed](#)]
260. Sheikh, B.Y.; Sarker, M.M.R.; Kamarudin, M.N.A.; Mohan, G. Antiproliferative and apoptosis inducing effects of citral via p53 and ROS-induced mitochondrial-mediated apoptosis in human colorectal HCT116 and HT29 cell lines. *Biomed. Pharmacother.* **2017**, *96*, 834–846. [[CrossRef](#)] [[PubMed](#)]
261. Nordin, N.; Yeap, S.K.; Rahman, H.S.; Zamberi, N.R.; Abu, N.; Mohamad, N.E.; How, C.W.; Masarudin, M.J.; Abdullah, R.; Alitheen, N.B. In vitro cytotoxicity and anticancer effects of citral nanostructured lipid carrier on MDA MBA-231 human breast cancer cells. *Sci. Rep.* **2019**, *9*, 1614. [[CrossRef](#)]
262. Bayala, B.; Bassole, I.H.N.; Maqdasy, S.; Baron, S.; Simpore, J.; Lobaccaro, J.A. *Cymbopogon citratus* and *Cymbopogon giganteus* essential oils have cytotoxic effects on tumor cell cultures. Identification of citral as a new putative anti-proliferative molecule. *Biochimie* **2018**, *153*, 162–170. [[CrossRef](#)]
263. Zielinska, A.; Martins-Gomes, C.; Ferreira, N.R.; Silva, A.M.; Nowak, I.; Souto, E.B. Anti-inflammatory and anti-cancer activity of citral: Optimization of citral-loaded solid lipid nanoparticles (SLN) using experimental factorial design and LUMiSizer(R). *Int. J. Pharm.* **2018**, *553*, 428–440. [[CrossRef](#)]
264. Naz, F.; Khan, F.I.; Mohammad, T.; Khan, P.; Manzoor, S.; Hasan, G.M.; Lobb, K.A.; Luqman, S.; Islam, A.; Ahmad, F.; et al. Investigation of molecular mechanism of recognition between citral and MARK4: A newer therapeutic approach to attenuate cancer cell progression. *Int. J. Biol. Macromol.* **2018**, *107 Pt B*, 2580–2589. [[CrossRef](#)]
265. Voura, M.; Khan, P.; Thysiadis, S.; Katsamakos, S.; Queen, A.; Hasan, G.M.; Ali, S.; Sarli, V.; Hassan, M.I. Probing the Inhibition of Microtubule Affinity Regulating Kinase 4 by N-Substituted Acridones. *Sci. Rep.* **2019**, *9*, 1676. [[CrossRef](#)] [[PubMed](#)]
266. Nigjeh, S.E.; Yeap, S.K.; Nordin, N.; Rahman, H.; Rosli, R. In Vivo Anti-Tumor Effects of Citral on 4T1 Breast Cancer Cells via Induction of Apoptosis and Downregulation of Aldehyde Dehydrogenase Activity. *Molecules* **2019**, *24*, 3241. [[CrossRef](#)] [[PubMed](#)]
267. Dinavahi, S.S.; Bazewicz, C.G.; Gowda, R.; Robertson, G.P. Aldehyde Dehydrogenase Inhibitors for Cancer Therapeutics. *Trends Pharmacol. Sci.* **2019**, *40*, 774–789. [[CrossRef](#)] [[PubMed](#)]
268. Thomas, M.L.; de Antueno, R.; Coyle, K.M.; Sultan, M.; Cruickshank, B.M.; Giacomantonio, M.A.; Giacomantonio, C.A.; Duncan, R.; Marcato, P. Citral reduces breast tumor growth by inhibiting the cancer stem cell marker ALDH1A3. *Mol. Oncol.* **2016**, *10*, 1485–1496. [[CrossRef](#)] [[PubMed](#)]
269. Nordin, N.; Yeap, S.K.; Zamberi, N.R.; Abu, N.; Mohamad, N.E.; Rahman, H.S.; How, C.W.; Masarudin, M.J.; Abdullah, R.; Alitheen, N.B. Characterization and toxicity of citral incorporated with nanostructured lipid carrier. *PeerJ* **2018**, *6*, e3916. [[CrossRef](#)]
270. Maruoka, T.; Kitanaka, A.; Kubota, Y.; Yamaoka, G.; Kameda, T.; Imataki, O.; Dobashi, H.; Bandoh, S.; Kadowaki, N.; Tanaka, T. Lemongrass essential oil and citral inhibit Src/Stat3 activity and suppress the proliferation/survival of small-cell lung cancer cells, alone or in combination with chemotherapeutic agents. *Int. J. Oncol.* **2018**, *52*, 1738–1748. [[CrossRef](#)]
271. Fang, Z.; Wang, Y.; Li, H.; Yu, S.; Liu, Z.; Fan, Z.; Chen, X.; Wu, Y.; Pan, X.; Li, X.; et al. Combination Treatment of Citral Potentiates the Efficacy of Hyperthermic Intraperitoneal Chemoperfusion with Pirarubicin for Colorectal Cancer. *Mol. Pharm.* **2017**, *14*, 3588–3597. [[CrossRef](#)]
272. Kremer, J.L.; Melo, G.P.; Marinello, P.C.; Bordini, H.P.; Rossaneis, A.C.; Sabio, L.R.; Cecchini, R.; Cecchini, A.L.; Verri, W.A., Jr.; Luiz, R.C. Citral prevents UVB-induced skin carcinogenesis in hairless mice. *J. Photochem. Photobiol. Biol.* **2019**, *198*, 111565. [[CrossRef](#)]
273. Venkatesha, S.H.; Moudgil, K.D. Celastrol and Its Role in Controlling Chronic Diseases. *Adv. Exp. Med. Biol.* **2016**, *928*, 267–289.
274. Law, S.; Leung, A.W.; Xu, C. Folic acid-modified celastrol nanoparticles: Synthesis, characterization, anticancer activity in 2D and 3D breast cancer models. *Artif. Cells Nanomed. Biotechnol.* **2020**, *48*, 542–559. [[CrossRef](#)]
275. Zhu, B.; Wei, Y. Antitumor activity of celastrol by inhibition of proliferation, invasion, and migration in cholangiocarcinoma via PTEN/PI3K/Akt pathway. *Cancer Med.* **2020**, *9*, 783–796. [[CrossRef](#)] [[PubMed](#)]

276. Allison, A.C.; Cacabelos, R.; Lombardi, V.R.; Alvarez, X.A.; Vigo, C. Celastrol, a potent antioxidant and anti-inflammatory drug, as a possible treatment for Alzheimer's disease. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2001**, *25*, 1341–1357. [\[CrossRef\]](#)
277. Wang, H.; Ahn, K.S.; Alharbi, S.A.; Shair, O.H.; Arfuso, F.; Sethi, G.; Chinnathambi, A.; Tang, F.R. Celastrol Alleviates Gamma Irradiation-Induced Damage by Modulating Diverse Inflammatory Mediators. *Int. J. Mol. Sci.* **2020**, *21*, 1084. [\[CrossRef\]](#) [\[PubMed\]](#)
278. Kim, D.H.; Shin, E.K.; Kim, Y.H.; Lee, B.W.; Jun, J.G.; Park, J.H.; Kim, J.K. Suppression of inflammatory responses by celastrol, a quinone methide triterpenoid isolated from *Celastrus regelii*. *Eur. J. Clin. Investig.* **2009**, *39*, 819–827. [\[CrossRef\]](#)
279. Venkatesha, S.H.; Yu, H.; Rajaiah, R.; Tong, L.; Moudgil, K.D. Celastrus-derived celastrol suppresses autoimmune arthritis by modulating antigen-induced cellular and humoral effector responses. *J. Biol. Chem.* **2011**, *286*, 15138–15146. [\[CrossRef\]](#)
280. Li, H.; Yuan, Y.; Zhang, Y.; He, Q.; Xu, R.; Ge, F.; Wu, C. Celastrol inhibits IL-1 $\beta$ -induced inflammation in orbital fibroblasts through the suppression of NF- $\kappa$ B activity. *Mol. Med. Rep.* **2016**, *14*, 2799–2806. [\[CrossRef\]](#)
281. Paris, D.; Ganey, N.J.; Laporte, V.; Patel, N.S.; Beaulieu-Abdelahad, D.; Bachmeier, C.; March, A.; Ait-Ghezala, G.; Mullan, M.J. Reduction of beta-amyloid pathology by celastrol in a transgenic mouse model of Alzheimer's disease. *J. Neuroinflamm.* **2010**, *7*, 17. [\[CrossRef\]](#)
282. Shrivastava, S.; Jeengar, M.K.; Reddy, V.S.; Reddy, G.B.; Naidu, V.G. Anticancer effect of celastrol on human triple negative breast cancer: Possible involvement of oxidative stress, mitochondrial dysfunction, apoptosis and PI3K/Akt pathways. *Exp. Mol. Pathol.* **2015**, *98*, 313–327. [\[CrossRef\]](#)
283. Nagase, M.; Oto, J.; Sugiyama, S.; Yube, K.; Takaishi, Y.; Sakato, N. Apoptosis induction in HL-60 cells and inhibition of topoisomerase II by triterpene celastrol. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 1883–1887. [\[CrossRef\]](#)
284. Yu, X.; Ruan, X.; Zhang, J.; Zhao, Q. Celastrol Induces Cell Apoptosis and Inhibits the Expression of the AML1-ETO/C-KIT Oncoprotein in t(8;21) Leukemia. *Molecules* **2016**, *21*, 574. [\[CrossRef\]](#)
285. Hsieh, M.J.; Wang, C.W.; Lin, J.T.; Chuang, Y.C.; Hsi, Y.T.; Lo, Y.S.; Lin, C.C.; Chen, M.K. Celastrol, a plant-derived triterpene, induces cisplatin-resistance nasopharyngeal carcinoma cancer cell apoptosis through ERK1/2 and p38 MAPK signaling pathway. *Phytomed. Int. J. Phytother. Phytopharm.* **2019**, *58*, 152805. [\[CrossRef\]](#) [\[PubMed\]](#)
286. Liu, Z.; Ma, L.; Wen, Z.S.; Hu, Z.; Wu, F.Q.; Li, W.; Liu, J.; Zhou, G.B. Cancerous inhibitor of PP2A is targeted by natural compound celastrol for degradation in non-small-cell lung cancer. *Carcinogenesis* **2014**, *35*, 905–914. [\[CrossRef\]](#) [\[PubMed\]](#)
287. Kannaiyan, R.; Manu, K.A.; Chen, L.; Li, F.; Rajendran, P.; Subramaniam, A.; Lam, P.; Kumar, A.P.; Sethi, G. Celastrol inhibits tumor cell proliferation and promotes apoptosis through the activation of c-Jun N-terminal kinase and suppression of PI3 K/Akt signaling pathways. *Apoptosis: Int. J. Program. Cell Death* **2011**, *16*, 1028–1041. [\[CrossRef\]](#) [\[PubMed\]](#)
288. Kiaei, M.; Kipiani, K.; Petri, S.; Chen, J.; Calingasan, N.Y.; Beal, M.F. Celastrol blocks neuronal cell death and extends life in transgenic mouse model of amyotrophic lateral sclerosis. *Neuro-Degener. Dis.* **2005**, *2*, 246–254. [\[CrossRef\]](#)
289. Chow, A.M.; Tang, D.W.; Hanif, A.; Brown, I.R. Localization of heat shock proteins in cerebral cortical cultures following induction by celastrol. *Cell Stress Chaperones* **2014**, *19*, 845–851. [\[CrossRef\]](#)
290. Hou, W.; Liu, B.; Xu, H. Celastrol: Progresses in structure-modifications, structure-activity relationships, pharmacology and toxicology. *Eur. J. Med. Chem.* **2020**, *189*, 112081. [\[CrossRef\]](#)
291. Leonti, M.; Casu, L.; Raduner, S.; Cottiglia, F.; Floris, C.; Altmann, K.H.; Gertsch, J. Falcarinol is a covalent cannabinoid CB1 receptor antagonist and induces pro-allergic effects in skin. *Biochem. Pharmacol.* **2010**, *79*, 1815–1826. [\[CrossRef\]](#)
292. Kobaek-Larsen, M.; Nielsen, D.S.; Kot, W.; Krych, L.; Christensen, L.P.; Baatrup, G. Effect of the dietary polyacetylenes falcarinol and falcarindiol on the gut microbiota composition in a rat model of colorectal cancer. *BMC Res. Notes* **2018**, *11*, 411. [\[CrossRef\]](#)
293. Stefanson, A.L.; Bakovic, M. Falcarinol Is a Potent Inducer of Heme Oxygenase-1 and Was More Effective than Sulforaphane in Attenuating Intestinal Inflammation at Diet-Achievable Doses. *Oxidative Med. Cell. Longev.* **2018**, *2018*, 3153527. [\[CrossRef\]](#)

294. Yamauchi, T.; Kadowaki, T. Physiological and pathophysiological roles of adiponectin and adiponectin receptors in the integrated regulation of metabolic and cardiovascular diseases. *Int. J. Obes.* **2008**, *32* (Suppl. 7), S13–S18. [\[CrossRef\]](#)
295. Takagi, M.; Kimura, K.; Nakashima, K.I.; Inoue, M. Ameliorative effect of panaxynol on the reduction in high-molecular-weight adiponectin secretion from 3T3-L1 adipocytes treated with palmitic acids. *Eur. J. Pharmacol.* **2018**, *820*, 138–145. [\[CrossRef\]](#) [\[PubMed\]](#)
296. Yu, L.C. Microbiota dysbiosis and barrier dysfunction in inflammatory bowel disease and colorectal cancers: Exploring a common ground hypothesis. *J. Biomed. Sci.* **2018**, *25*, 79. [\[CrossRef\]](#) [\[PubMed\]](#)
297. Kho, Z.Y.; Lal, S.K. The Human Gut Microbiome—A Potential Controller of Wellness and Disease. *Front. Microbiol.* **2018**, *9*, 1835. [\[CrossRef\]](#) [\[PubMed\]](#)
298. Kobaek-Larsen, M.; El-Houri, R.B.; Christensen, L.P.; Al-Najami, I.; Frette, X.; Baatrup, G. Dietary polyacetylenes, falcarinol and falcariindiol, isolated from carrots prevents the formation of neoplastic lesions in the colon of azoxymethane-induced rats. *Food Funct.* **2017**, *8*, 964–974. [\[CrossRef\]](#)
299. Kobaek-Larsen, M.; Baatrup, G.; KhataeiNotabi, M.; El-Houri, R.B.; Pipo-Olle, E.; Christensen Arnspang, E.; Christensen, L.P. Dietary Polyacetylenic Oxy lipins Falcarinol and Falcariindiol Prevent Inflammation and Colorectal Neoplastic Transformation: A Mechanistic and Dose-Response Study in A Rat Model. *Nutrients* **2019**, *11*, 2223. [\[CrossRef\]](#)
300. Moharil, R.B.; Dive, A.; Khandekar, S.; Bodhade, A. Cancer stem cells: An insight. *J. Oral Maxillofac. Pathol. JOMFP* **2017**, *21*, 463. [\[CrossRef\]](#)
301. Zappa, C.; Mousa, S.A. Non-small cell lung cancer: Current treatment and future advances. *Transl. Lung Cancer Res.* **2016**, *5*, 288–300. [\[CrossRef\]](#)
302. Trepel, J.; Mollapour, M.; Giaccone, G.; Neckers, L. Targeting the dynamic HSP90 complex in cancer. *Nat. Rev. Cancer* **2010**, *10*, 537–549. [\[CrossRef\]](#)
303. Yan, Z.; Yang, R.; Jiang, Y.; Yang, Z.; Yang, J.; Zhao, Q.; Lu, Y. Induction of apoptosis in human promyelocytic leukemia HL60 cells by panaxynol and panaxydol. *Molecules* **2011**, *16*, 5561–5573. [\[CrossRef\]](#)
304. Henderson, S.K.; Cohen, H. Nalbuphine augmentation of analgesia and reversal of side effects following epidural hydromorphone. *Anesthesiology* **1986**, *65*, 216–218. [\[CrossRef\]](#)
305. Wang, Z.J.; Song, L.; Guo, L.C.; Yin, M.; Sun, Y.N. Induction of differentiation by panaxydol in human hepatocarcinoma SMMC-7721 cells via cAMP and MAP kinase dependent mechanism. *Yakugaku Zasshi: J. Pharm. Soc. Jpn.* **2011**, *131*, 993–1000. [\[CrossRef\]](#) [\[PubMed\]](#)
306. Sohn, J.; Lee, C.H.; Chung, D.J.; Park, S.H.; Kim, I.; Hwang, W.I. Effect of petroleum ether extract of Panax ginseng roots on proliferation and cell cycle progression of human renal cell carcinoma cells. *Exp. Mol. Med.* **1998**, *30*, 47–51. [\[CrossRef\]](#) [\[PubMed\]](#)
307. Hai, J.; Lin, Q.; Lu, Y.; Yi, J.; Zhang, H. Growth inhibition and induction of differentiation by panaxydol in rat C6 glioma cells. *Neurol. Res.* **2008**, *30*, 99–105. [\[CrossRef\]](#) [\[PubMed\]](#)
308. Machado, S.; Silva, E.; Massa, A. Occupational allergic contact dermatitis from falcarinol. *Contact Dermat.* **2002**, *47*, 113–114. [\[CrossRef\]](#)
309. Qu, C.; Li, B.; Lai, Y.; Li, H.; Windust, A.; Hofseth, L.J.; Nagarkatti, M.; Nagarkatti, P.; Wang, X.L.; Tang, D.; et al. Identifying panaxynol, a natural activator of nuclear factor erythroid-2 related factor 2 (Nrf2) from American ginseng as a suppressor of inflamed macrophage-induced cardiomyocyte hypertrophy. *J. Ethnopharmacol.* **2015**, *168*, 326–336. [\[CrossRef\]](#)
310. Phinney, S.D.; Tang, A.B.; Waggoner, C.R.; Tezanos-Pinto, R.G.; Davis, P.A. The transient hypercholesterolemia of major weight loss. *Am. J. Clin. Nutr.* **1991**, *53*, 1404–1410. [\[CrossRef\]](#)
311. MacLean, K.A.; Johnson, M.W.; Reissig, C.J.; Prinszano, T.E.; Griffiths, R.R. Dose-related effects of salvinorin A in humans: Dissociative, hallucinogenic, and memory effects. *Psychopharmacology* **2013**, *226*, 381–392. [\[CrossRef\]](#)
312. Roach, J.J.; Shenvi, R.A. A review of salvinorin analogs and their kappa-opioid receptor activity. *Bioorganic Med. Chem. Lett.* **2018**, *28*, 1436–1445. [\[CrossRef\]](#)
313. Walentiny, D.M.; Vann, R.E.; Warner, J.A.; King, L.S.; Seltzman, H.H.; Navarro, H.A.; Twine, C.E., Jr.; Thomas, B.F.; Gilliam, A.F.; Gilmour, B.P.; et al. Kappa opioid mediation of cannabinoid effects of the potent hallucinogen, salvinorin A, in rodents. *Psychopharmacology* **2010**, *210*, 275–284. [\[CrossRef\]](#)

314. Coffeen, U.; Canseco-Alba, A.; Simon-Arceo, K.; Almanza, A.; Mercado, F.; Leon-Olea, M.; Pellicer, F. Salvinorin A reduces neuropathic nociception in the insular cortex of the rat. *Eur. J. Pain* **2018**, *22*, 311–318. [\[CrossRef\]](#)
315. Guida, F.; Luongo, L.; Aviello, G.; Palazzo, E.; De Chiaro, M.; Gatta, L.; Boccella, S.; Marabese, I.; Zjawiony, J.K.; Capasso, R.; et al. Salvinorin A reduces mechanical allodynia and spinal neuronal hyperexcitability induced by peripheral formalin injection. *Mol. Pain* **2012**, *8*, 60. [\[CrossRef\]](#) [\[PubMed\]](#)
316. Butelman, E.R.; Prisinzano, T.E.; Deng, H.; Rus, S.; Kreek, M.J. Unconditioned behavioral effects of the powerful kappa-opioid hallucinogen salvinorin A in nonhuman primates: Fast onset and entry into cerebrospinal fluid. *J. Pharmacol. Exp. Ther.* **2009**, *328*, 588–597. [\[CrossRef\]](#) [\[PubMed\]](#)
317. Prisinzano, T.E. Psychopharmacology of the hallucinogenic sage *Salvia divinorum*. *Life Sci.* **2005**, *78*, 527–531. [\[CrossRef\]](#) [\[PubMed\]](#)
318. Ranganathan, M.; Schnakenberg, A.; Skosnik, P.D.; Cohen, B.M.; Pittman, B.; Sewell, R.A.; D'Souza, D.C. Dose-related behavioral, subjective, endocrine, and psychophysiological effects of the kappa opioid agonist Salvinorin A in humans. *Biol. Psychiatry* **2012**, *72*, 871–879. [\[CrossRef\]](#) [\[PubMed\]](#)
319. Paton, K.F.; Kumar, N.; Crowley, R.S.; Harper, J.L.; Prisinzano, T.E.; Kivell, B.M. The analgesic and anti-inflammatory effects of Salvinorin A analogue beta-tetrahydropyran Salvinorin B in mice. *Eur. J. Pain* **2017**, *21*, 1039–1050. [\[CrossRef\]](#)
320. Kivell, B.M.; Paton, K.F.; Kumar, N.; Morani, A.S.; Culverhouse, A.; Shepherd, A.; Welsh, S.A.; Biggerstaff, A.; Crowley, R.S.; Prisinzano, T.E. Kappa Opioid Receptor Agonist Mesyl Sal B Attenuates Behavioral Sensitization to Cocaine with Fewer Aversive Side-Effects than Salvinorin A in Rodents. *Molecules* **2018**, *23*, 2602. [\[CrossRef\]](#)
321. Zhou, Y.; Crowley, R.; Prisinzano, T.; Kreek, M.J. Effects of mesyl salvinorin B alone and in combination with naltrexone on alcohol deprivation effect in male and female mice. *Neurosci. Lett.* **2018**, *673*, 19–23. [\[CrossRef\]](#)
322. Salaga, M.; Polepally, P.R.; Zakrzewski, P.K.; Cygankiewicz, A.; Sobczak, M.; Kordek, R.; Zjawiony, J.K.; Krajewska, W.M.; Fichna, J. Novel orally available salvinorin A analog PR-38 protects against experimental colitis and reduces abdominal pain in mice by interaction with opioid and cannabinoid receptors. *Biochem. Pharmacol.* **2014**, *92*, 618–626. [\[CrossRef\]](#)
323. Wang, Z.; Ma, N.; Riley, J.; Armstead, W.M.; Liu, R. Salvinorin A administration after global cerebral hypoxia/ischemia preserves cerebrovascular autoregulation via kappa opioid receptor in piglets. *PLoS ONE* **2012**, *7*, e41724. [\[CrossRef\]](#)
324. Su, D.; Riley, J.; Armstead, W.M.; Liu, R. Salvinorin A pretreatment preserves cerebrovascular autoregulation after brain hypoxic/ischemic injury via extracellular signal-regulated kinase/mitogen-activated protein kinase in piglets. *Anesth. Analg.* **2012**, *114*, 200–204. [\[CrossRef\]](#)
325. Xin, J.; Zhang, Y.; He, Z.; Wang, Z. Highly selective non-opioid kappa opioid receptor (KOR) agonist salvinorin A protects against forebrain ischemia-induced brain injury in rats. *Brain Res.* **2016**, *1637*, 168–176. [\[CrossRef\]](#) [\[PubMed\]](#)
326. Sun, J.; Zhang, Y.; Lu, J.; Zhang, W.; Yan, J.; Yang, L.; Zhou, C.; Liu, R.; Chen, C. Salvinorin A ameliorates cerebral vasospasm through activation of endothelial nitric oxide synthase in a rat model of subarachnoid hemorrhage. *Microcirculation* **2018**, *25*, e12442. [\[CrossRef\]](#) [\[PubMed\]](#)
327. Dong, H.; Zhou, W.; Xin, J.; Shi, H.; Yao, X.; He, Z.; Wang, Z. Salvinorin A moderates postischemic brain injury by preserving endothelial mitochondrial function via AMPK/Mfn2 activation. *Exp. Neurol.* **2019**, *322*, 113045. [\[CrossRef\]](#)
328. Tang, G.; Yang, G.Y. Aquaporin-4: A Potential Therapeutic Target for Cerebral Edema. *Int. J. Mol. Sci.* **2016**, *17*, 1413. [\[CrossRef\]](#)
329. Braidia, D.; Capurro, V.; Zani, A.; Rubino, T.; Vigano, D.; Parolaro, D.; Sala, M. Potential anxiolytic- and antidepressant-like effects of salvinorin A, the main active ingredient of *Salvia divinorum*, in rodents. *Br. J. Pharmacol.* **2009**, *157*, 844–853. [\[CrossRef\]](#) [\[PubMed\]](#)
330. Ebner, S.R.; Roitman, M.F.; Potter, D.N.; Rachlin, A.B.; Chartoff, E.H. Depressive-like effects of the kappa opioid receptor agonist salvinorin A are associated with decreased phasic dopamine release in the nucleus accumbens. *Psychopharmacology* **2010**, *210*, 241–252. [\[CrossRef\]](#)
331. Kivell, B.; Uzelac, Z.; Sundaramurthy, S.; Rajamanickam, J.; Ewald, A.; Chefer, V.; Jaligam, V.; Bolan, E.; Simonson, B.; Annamalai, B.; et al. Salvinorin A regulates dopamine transporter function via a kappa opioid receptor and ERK1/2-dependent mechanism. *Neuropharmacology* **2014**, *86*, 228–240. [\[CrossRef\]](#)

332. Keasling, A.W.; Pandey, P.; Doerksen, R.J.; Pedrino, G.R.; Costa, E.A.; da Cunha, L.C.; Zjawiony, J.K.; Fajemiroye, J.O. Salvindolin elicits opioid system-mediated antinociceptive and antidepressant-like activities. *J. Psychopharmacol.* **2019**, *33*, 865–881. [\[CrossRef\]](#)
333. Fajemiroye, J.O.; Prabhakar, P.R.; Cunha, L.C.; Costa, E.A.; Zjawiony, J.K. 22-azidosalvinorin A exhibits antidepressant-like effect in mice. *Eur. J. Pharmacol.* **2017**, *800*, 96–106. [\[CrossRef\]](#)
334. Rossi, A.; Caiazzo, E.; Bilancia, R.; Riemma, M.A.; Pagano, E.; Cicala, C.; Ialenti, A.; Zjawiony, J.K.; Izzo, A.A.; Capasso, R.; et al. Salvinorin A Inhibits Airway Hyperreactivity Induced by Ovalbumin Sensitization. *Front. Pharmacol.* **2016**, *7*, 525. [\[CrossRef\]](#)
335. Salaga, M.; Polepally, P.R.; Zielinska, M.; Marynowski, M.; Fabisiak, A.; Murawska, N.; Sobczak, K.; Sacharczuk, M.; Do Rego, J.C.; Roth, B.L.; et al. Salvinorin A analogues PR-37 and PR-38 attenuate compound 48/80-induced itch responses in mice. *Br. J. Pharmacol.* **2015**, *172*, 4331–4341. [\[CrossRef\]](#) [\[PubMed\]](#)
336. Gertsch, J.; Pertwee, R.G.; Di Marzo, V. Phytocannabinoids beyond the Cannabis plant—Do they exist? *Br. J. Pharmacol.* **2010**, *160*, 523–529. [\[CrossRef\]](#) [\[PubMed\]](#)
337. Zhao, Q.; Bi, Y.; Zhong, J.; Ren, Z.; Liu, Y.; Jia, J.; Yu, M.; Tan, Y.; Zhang, Q.; Yu, X. Pristimerin suppresses colorectal cancer through inhibiting inflammatory responses and Wnt/beta-catenin signaling. *Toxicol. Appl. Pharmacol.* **2020**, *386*, 114813. [\[CrossRef\]](#) [\[PubMed\]](#)
338. Yousef, B.A.; Hassan, H.M.; Zhang, L.Y.; Jiang, Z.Z. Pristimerin exhibits in vitro and in vivo anticancer activities through inhibition of nuclear factor-small ka, CyrillicB signaling pathway in colorectal cancer cells. *Phytomed. Int. J. Phytother. Phytopharm.* **2018**, *40*, 140–147.
339. Slattery, M.L.; Mullany, L.E.; Sakoda, L.; Samowitz, W.S.; Wolff, R.K.; Stevens, J.R.; Herrick, J.S. The NF- $\kappa$ B signalling pathway in colorectal cancer: Associations between dysregulated gene and miRNA expression. *J. Cancer Res. Clin. Oncol.* **2018**, *144*, 269–283. [\[CrossRef\]](#)
340. Park, J.H.; Kim, J.K. Pristimerin, a naturally occurring triterpenoid, attenuates tumorigenesis in experimental colitis-associated colon cancer. *Phytomed. Int. J. Phytother. Phytopharm.* **2018**, *42*, 164–171. [\[CrossRef\]](#)
341. Grossi, V.; Fasano, C.; Celestini, V.; Lepore Signorile, M.; Sanese, P.; Simone, C. Chasing the FOXO3: Insights into Its New Mitochondrial Lair in Colorectal Cancer Landscape. *Cancers* **2019**, *11*, 414. [\[CrossRef\]](#)
342. Liu, Y.; Ao, X.; Ding, W.; Ponnusamy, M.; Wu, W.; Hao, X.; Yu, W.; Wang, Y.; Li, P.; Wang, J. Critical role of FOXO3a in carcinogenesis. *Mol. Cancer* **2018**, *17*, 104. [\[CrossRef\]](#)
343. Yousef, B.A.; Hassan, H.M.; Guerram, M.; Hamdi, A.M.; Wang, B.; Zhang, L.Y.; Jiang, Z.Z. Pristimerin inhibits proliferation, migration and invasion, and induces apoptosis in HCT-116 colorectal cancer cells. *Biomed. Pharmacother.* **2016**, *79*, 112–119. [\[CrossRef\]](#)
344. Yousef, B.A.; Guerram, M.; Hassan, H.M.; Hamdi, A.M.; Zhang, L.Y.; Jiang, Z.Z. Pristimerin demonstrates anticancer potential in colorectal cancer cells by inducing G1 phase arrest and apoptosis and suppressing various pro-survival signaling proteins. *Oncol. Rep.* **2016**, *35*, 1091–1100. [\[CrossRef\]](#)
345. Adams, R.; Brown, E.; Brown, L.; Butler, R.; Falk, S.; Fisher, D.; Kaplan, R.; Quirke, P.; Richman, S.; Samuel, L.; et al. Inhibition of EGFR, HER2, and HER3 signalling in patients with colorectal cancer wild-type for BRAF, PIK3CA, KRAS, and NRAS (FOCUS4-D): A phase 2–3 randomised trial. *Lancet. Gastroenterol. Hepatol.* **2018**, *3*, 162–171. [\[CrossRef\]](#)
346. Zhao, Q.; Liu, Y.; Zhong, J.; Bi, Y.; Liu, Y.; Ren, Z.; Li, X.; Jia, J.; Yu, M.; Yu, X. Pristimerin induces apoptosis and autophagy via activation of ROS/ASK1/JNK pathway in human breast cancer in vitro and in vivo. *Cell Death Discov.* **2019**, *5*, 125. [\[CrossRef\]](#) [\[PubMed\]](#)
347. Lee, Y.; Na, J.; Lee, M.S.; Cha, E.Y.; Sul, J.Y.; Park, J.B.; Lee, J.S. Combination of pristimerin and paclitaxel additively induces autophagy in human breast cancer cells via ERK1/2 regulation. *Mol. Med. Rep.* **2018**, *18*, 4281–4288. [\[CrossRef\]](#) [\[PubMed\]](#)
348. Cevatemre, B.; Erkisa, M.; Aztopal, N.; Karakas, D.; Alper, P.; Tsimplouli, C.; Sereti, E.; Dimas, K.; Armutak, E.I.I.; Gurevin, E.G.; et al. A promising natural product, pristimerin, results in cytotoxicity against breast cancer stem cells in vitro and xenografts in vivo through apoptosis and an incomplete autophagy in breast cancer. *Pharmacol. Res.* **2018**, *129*, 500–514. [\[CrossRef\]](#) [\[PubMed\]](#)
349. Xie, G.; Yu, X.; Liang, H.; Chen, J.; Tang, X.; Wu, S.; Liao, C. Pristimerin overcomes adriamycin resistance in breast cancer cells through suppressing Akt signaling. *Oncol. Lett.* **2016**, *11*, 3111–3116. [\[CrossRef\]](#)
350. Lee, J.S.; Yoon, I.S.; Lee, M.S.; Cha, E.Y.; Thuong, P.T.; Diep, T.T.; Kim, J.R. Anticancer activity of pristimerin in epidermal growth factor receptor 2-positive SKBR3 human breast cancer cells. *Biol. Pharm. Bull.* **2013**, *36*, 316–325. [\[CrossRef\]](#)

351. Zhang, B.; Zhang, J.; Pan, J. Pristimerin effectively inhibits the malignant phenotypes of uveal melanoma cells by targeting NFkappaB pathway. *Int. J. Oncol.* **2017**, *51*, 887–898. [[CrossRef](#)]
352. Mori, Y.; Shirai, T.; Terauchi, R.; Tsuchida, S.; Mizoshiri, N.; Hayashi, D.; Arai, Y.; Kishida, T.; Mazda, O.; Kubo, T. Antitumor effects of pristimerin on human osteosarcoma cells in vitro and in vivo. *Oncotargets Ther.* **2017**, *10*, 5703–5710. [[CrossRef](#)]
353. Deeb, D.; Gao, X.; Liu, Y.B.; Pindolia, K.; Gautam, S.C. Pristimerin, a quinonemethide triterpenoid, induces apoptosis in pancreatic cancer cells through the inhibition of pro-survival Akt/NF- $\kappa$ B/mTOR signaling proteins and anti-apoptotic Bcl-2. *Int. J. Oncol.* **2014**, *44*, 1707–1715. [[CrossRef](#)]
354. Deeb, D.; Gao, X.; Liu, Y.; Pindolia, K.; Gautam, S.C. Inhibition of hTERT/telomerase contributes to the antitumor activity of pristimerin in pancreatic ductal adenocarcinoma cells. *Oncol. Rep.* **2015**, *34*, 518–524. [[CrossRef](#)]
355. Lee, S.O.; Kim, J.S.; Lee, M.S.; Lee, H.J. Anti-cancer effect of pristimerin by inhibition of HIF-1 $\alpha$  involves the SPHK-1 pathway in hypoxic prostate cancer cells. *BMC Cancer* **2016**, *16*, 701. [[CrossRef](#)] [[PubMed](#)]
356. Liu, Y.B.; Gao, X.; Deeb, D.; Pindolia, K.; Gautam, S.C. Role of telomerase in anticancer activity of pristimerin in prostate cancer cells. *J. Exp. Ther. Oncol.* **2015**, *11*, 41–49. [[PubMed](#)]
357. Liu, Y.B.; Gao, X.; Deeb, D.; Brigolin, C.; Zhang, Y.; Shaw, J.; Pindolia, K.; Gautam, S.C. Ubiquitin-proteasomal degradation of antiapoptotic survivin facilitates induction of apoptosis in prostate cancer cells by pristimerin. *Int. J. Oncol.* **2014**, *45*, 1735–1741. [[CrossRef](#)] [[PubMed](#)]
358. Liu, Y.B.; Gao, X.; Deeb, D.; Arbab, A.S.; Gautam, S.C. Pristimerin Induces Apoptosis in Prostate Cancer Cells by Down-regulating Bcl-2 through ROS-dependent Ubiquitin-proteasomal Degradation Pathway. *J. Carcinog. Mutagenesis* **2013**. [[CrossRef](#)]
359. Clark, A.R.; Tokar, A. Signalling specificity in the Akt pathway in breast cancer. *Biochem. Soc. Trans.* **2014**, *42*, 1349–1355. [[CrossRef](#)] [[PubMed](#)]
360. Jin, Y.; Huynh, D.T.N.; Kang, K.W.; Myung, C.S.; Heo, K.S. Inhibition of p90RSK activation sensitizes triple-negative breast cancer cells to cisplatin by inhibiting proliferation, migration and EMT. *BMB Rep.* **2019**, *52*, 706–711. [[CrossRef](#)]
361. Li, Z.; Hu, C.; Zhen, Y.; Pang, B.; Yi, H.; Chen, X. Pristimerin inhibits glioma progression by targeting AGO2 and PTPN1 expression via miR-542-5p. *Biosci. Rep.* **2019**, *39*. [[CrossRef](#)]
362. Ye, Z.; Jin, H.; Qian, Q. Argonaute 2: A Novel Rising Star in Cancer Research. *J. Cancer* **2015**, *6*, 877–882. [[CrossRef](#)]
363. Liu, J.; Luan, W.; Zhang, Y.; Gu, J.; Shi, Y.; Yang, Y.; Feng, Z.; Qi, F. HDAC6 interacts with PTPN1 to enhance melanoma cells progression. *Biochem. Biophys. Res. Commun.* **2018**, *495*, 2630–2636. [[CrossRef](#)]
364. Zhao, H.; Wang, C.; Lu, B.; Zhou, Z.; Jin, Y.; Wang, Z.; Zheng, L.; Liu, K.; Luo, T.; Zhu, D.; et al. Pristimerin triggers AIF-dependent programmed necrosis in glioma cells via activation of JNK. *Cancer Lett.* **2016**, *374*, 136–148. [[CrossRef](#)]
365. Yan, Y.Y.; Bai, J.P.; Xie, Y.; Yu, J.Z.; Ma, C.G. The triterpenoid pristimerin induces U87 glioma cell apoptosis through reactive oxygen species-mediated mitochondrial dysfunction. *Oncol. Lett.* **2013**, *5*, 242–248. [[CrossRef](#)] [[PubMed](#)]
366. Thomas, R.; Weihua, Z. Rethink of EGFR in Cancer with Its Kinase Independent Function on Board. *Front. Oncol.* **2019**, *9*, 800. [[CrossRef](#)] [[PubMed](#)]
367. Wu, H.; Li, L.; Ai, Z.; Yin, J.; Chen, L. Pristimerin induces apoptosis of oral squamous cell carcinoma cells via G1 phase arrest and MAPK/Erk1/2 and Akt signaling inhibition. *Oncol. Lett.* **2019**, *17*, 3017–3025. [[CrossRef](#)] [[PubMed](#)]
368. Gao, X.; Liu, Y.; Deeb, D.; Arbab, A.S.; Gautam, S.C. Anticancer activity of pristimerin in ovarian carcinoma cells is mediated through the inhibition of prosurvival Akt/NF- $\kappa$ B/mTOR signaling. *J. Exp. Ther. Oncol.* **2014**, *10*, 275–283.
369. Cuvillier, O.; Ader, I. Hypoxia-inducible factors and sphingosine 1-phosphate signaling. *Anti-Cancer Agents Med. Chem.* **2011**, *11*, 854–862. [[CrossRef](#)]
370. Yang, H.; Landis-Piwowar, K.R.; Lu, D.; Yuan, P.; Li, L.; Reddy, G.P.; Yuan, X.; Dou, Q.P. Pristimerin induces apoptosis by targeting the proteasome in prostate cancer cells. *J. Cell. Biochem.* **2008**, *103*, 234–244. [[CrossRef](#)]
371. Wei, X.; Zeng, W.; Xie, K.; Diao, P.; Tang, P. Potential use of chymotrypsin-like proteasomal activity as a biomarker for prostate cancer. *Oncol. Lett.* **2018**, *15*, 5149–5154. [[CrossRef](#)]

372. Tong, L.; Nanjundaiah, S.M.; Venkatesha, S.H.; Astry, B.; Yu, H.; Moudgil, K.D. Pristimerin, a naturally occurring triterpenoid, protects against autoimmune arthritis by modulating the cellular and soluble immune mediators of inflammation and tissue damage. *Clin. Immunol.* **2014**, *155*, 220–230. [[CrossRef](#)]
373. Kim, H.J.; Park, G.M.; Kim, J.K. Anti-inflammatory effect of pristimerin on lipopolysaccharide-induced inflammatory responses in murine macrophages. *Arch. Pharmacol Res.* **2013**, *36*, 495–500. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

## 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

### Keywords

cannabinoids; terpenoids;  
essential oils; THC; CBD;  
limonene; pinene; linalool;  
caryophyllene; phytotherapy

### Received

19 November 2010

### Revised

29 December 2010

### Accepted

12 January 2011

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,  $\alpha$ -pinene, linalool,  $\beta$ -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<sup>-1</sup>. 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

This article is part of a themed issue on Cannabinoids in Biology and Medicine. To view the other articles in this issue visit <http://dx.doi.org/10.1111/bph.2011.163.issue-7>

### Abbreviations

2-AG, 2-arachidonoylglycerol; 5-HT, 5-hydroxytryptamine (serotonin); AD, antidepressant; AEA, arachidonylethanolamide (anandamide); AI, anti-inflammatory; AMPA,  $\alpha$ -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate; Ca<sup>++</sup>, calcium ion; CB<sub>1</sub>/CB<sub>2</sub>, 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<sub>50</sub>, 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<sub>1/2</sub>, half-life; TCA, tricyclic antidepressant; THC, tetrahydrocannabinol; THCA, tetrahydrocannabinolic acid; THCV, tetrahydrocannabivarin; TNF- $\alpha$ , 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<sub>1</sub> (Devane *et al.*, 1988), and the endocannabinoids, anandamide (arachidonylethanolamide, 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



**Figure 1**

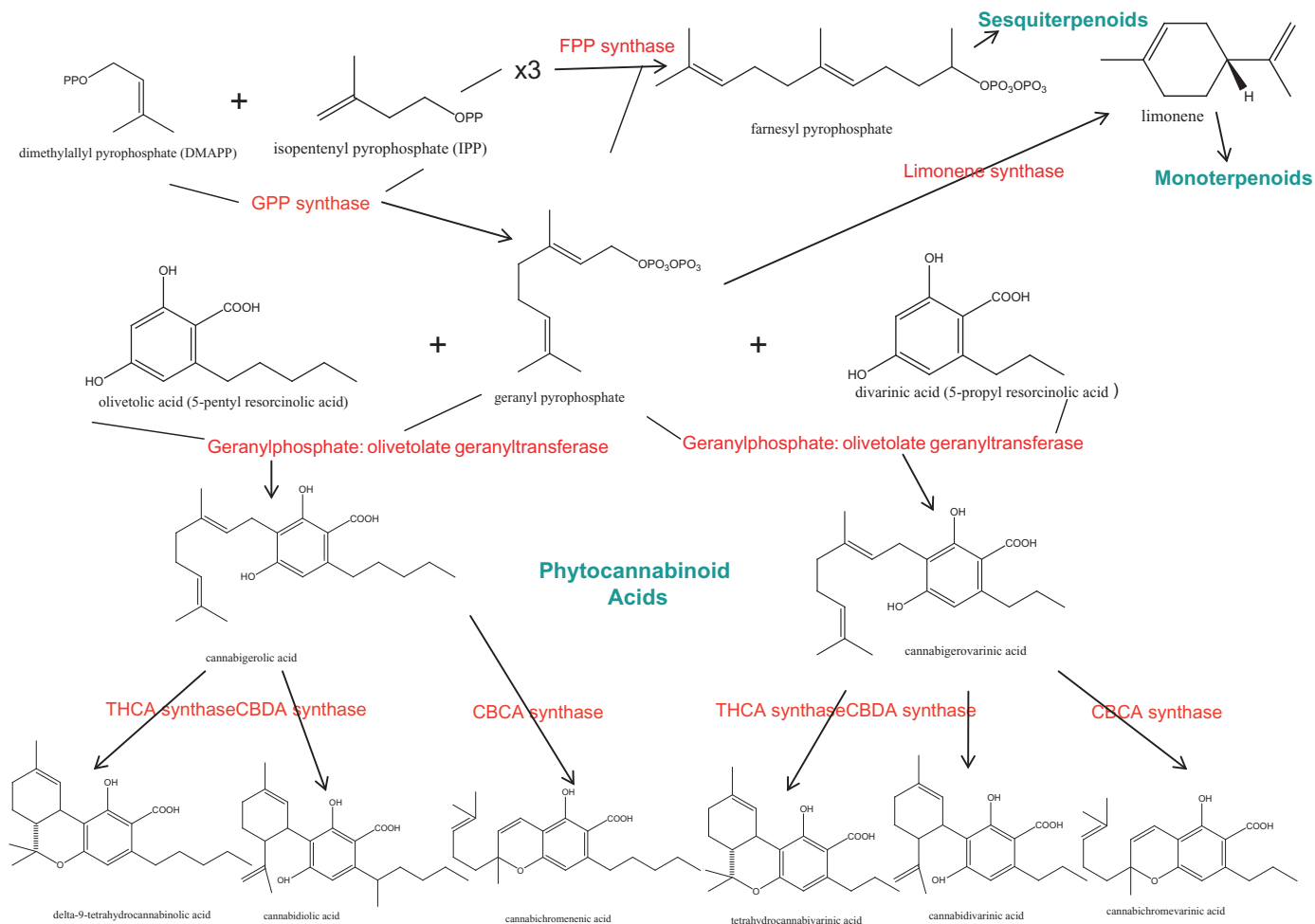
Cannabis capitata glandular (EBR by permission of Bedrocan BV, Netherlands).

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).

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), anti-convulsant 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% cannabidiol (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

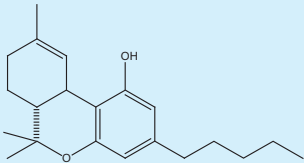
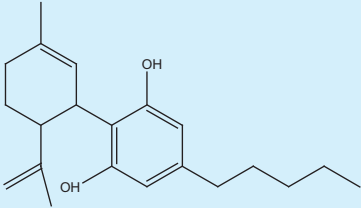
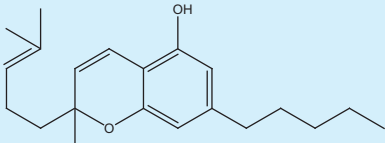
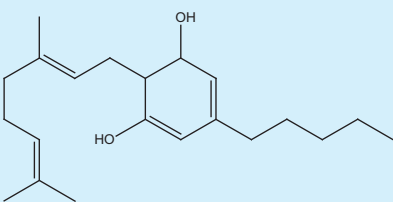
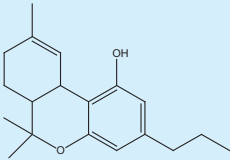
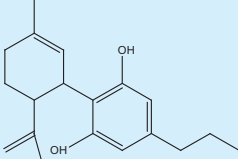
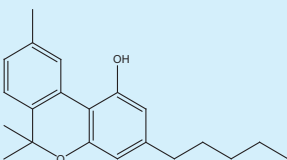
Phytocannabinoids are exclusively produced in cannabis (*vide infra* for exception), but their evolutionary and ecological *raison d'être* were obscure until recently. THC production is maximized with increased light energy (Potter, 2009). It has been known for some time that CBG and CBC are mildly antifungal (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
 <p>delta-9-tetrahydrocannabinol (THC)</p>	<p>Analgesic via CB<sub>1</sub> and CB<sub>2</sub> (Rahn and Hohmann, 2009)</p> <p>AI/antioxidant (Hampson <i>et al.</i>, 1998)</p> <p>Bronchodilatory (Williams <i>et al.</i>, 1976)</p> <p>↓ Sx. Alzheimer disease (Volicer <i>et al.</i>, 1997; Eubanks <i>et al.</i>, 2006)</p> <p>Benefit on duodenal ulcers (Douthwaite, 1947)</p> <p>Muscle relaxant (Kavia <i>et al.</i>, 2010)</p> <p>Antipruritic, cholestatic jaundice (Neff <i>et al.</i>, 2002)</p>	<p>Various</p> <p>Limonene <i>et al.</i></p> <p>Pinene</p> <p>Limonene, pinene, linalool</p> <p>Caryophyllene, limonene</p> <p>Linalool?</p> <p>Caryophyllene?</p>
 <p>cannabidiol</p>	<p>AI/antioxidant (Hampson <i>et al.</i>, 1998)</p> <p>Anti-anxiety via 5-HT<sub>1A</sub> (Russo <i>et al.</i>, 2005)</p> <p>Anticonvulsant (Jones <i>et al.</i>, 2010)</p> <p>Cytotoxic versus breast cancer (Ligresti <i>et al.</i>, 2006)</p> <p>↑ adenosine A<sub>2A</sub> signalling (Carrier <i>et al.</i>, 2006)</p> <p>Effective versus MRSA (Appendino <i>et al.</i>, 2008)</p> <p>Decreases sebum/sebocytes (Biro <i>et al.</i>, 2009)</p> <p>Treatment of addiction (see text)</p>	<p>Limonene <i>et al.</i></p> <p>Linalool, limonene</p> <p>Linalool</p> <p>Limonene</p> <p>Linalool</p> <p>Pinene</p> <p>Pinene, limonene, linalool</p> <p>Caryophyllene</p>
 <p>cannabichromene</p>	<p>Anti-inflammatory/analgesic (Davis and Hatoum, 1983)</p> <p>Antifungal (ElSohly <i>et al.</i>, 1982)</p> <p>AEA uptake inhibitor (De Petrocellis <i>et al.</i>, 2011)</p> <p>Antidepressant in rodent model (Deyo and Musty, 2003)</p>	<p>Various</p> <p>Caryophyllene oxide</p> <p>–</p> <p>Limonene</p>
 <p>cannabigerol</p>	<p>TRPM8 antagonist prostate cancer (De Petrocellis <i>et al.</i>, 2011)</p> <p>GABA uptake inhibitor (Banerjee <i>et al.</i>, 1975)</p> <p>Anti-fungal (ElSohly <i>et al.</i>, 1982)</p> <p>Antidepressant rodent model (Musty and Deyo, 2006); and via 5-HT<sub>1A</sub> antagonism (Cascio <i>et al.</i>, 2010)</p> <p>Analgesic, α-2 adrenergic blockade (Cascio <i>et al.</i>, 2010)</p> <p>↓ keratinocytes in psoriasis (Wilkinson and Williamson, 2007)</p> <p>Effective versus MRSA (Appendino <i>et al.</i>, 2008)</p>	<p>Various</p> <p>adjunctive role?</p> <p>Pinene</p>
 <p>tetrahydrocannabivarin</p>	<p>AI/anti-hyperalgesic (Bolognini <i>et al.</i>, 2010)</p> <p>Treatment of metabolic syndrome (Cawthorne <i>et al.</i>, 2007)</p> <p>Anticonvulsant (Hill <i>et al.</i>, 2010)</p>	<p>Caryophyllene <i>et al.</i> . . .</p> <p>–</p> <p>Linalool</p>
 <p>cannabidivarin</p>	<p>Inhibits diacylglycerol lipase (De Petrocellis <i>et al.</i>, 2011)</p> <p>Anticonvulsant in hippocampus (Hill <i>et al.</i>, 2010)</p>	<p>–</p> <p>Linalool</p>
 <p>cannabinol (CBN)</p>	<p>Sedative (Musty <i>et al.</i>, 1976)</p> <p>Effective versus MRSA (Appendino <i>et al.</i>, 2008)</p> <p>TRPV2 agonist for burns (Qin <i>et al.</i>, 2008)</p> <p>↓ keratinocytes in psoriasis (Wilkinson and Williamson, 2007)</p> <p>↓ breast cancer resistance protein (Holland <i>et al.</i>, 2008)</p>	<p>Nerolidol, myrcene</p> <p>Pinene</p> <p>Linalool</p> <p>adjunctive role?</p> <p>Limonene</p>

5-HT, 5-hydroxytryptamine (serotonin); AEA, arachidonylethanolamide (anandamide); AI, anti-inflammatory; CB<sub>1</sub>/CB<sub>2</sub>, 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<sub>1</sub> and cannabinoid receptor 2 (CB<sub>2</sub>) 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<sub>1</sub> 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- $\alpha$ ) in a rodent model of rheumatoid arthritis (Malfait *et al.*, 2000), enhances adenosine receptor A<sub>2A</sub> 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  $\mu\text{g}\cdot\text{mL}^{-1}$  (Appendino *et al.*, 2008). In 2005, it was demonstrated that CBD has agonistic activity at 5-hydroxytryptamine (5-HT)<sub>1A</sub> at 16  $\mu\text{M}$  (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  $\text{mg}\cdot\text{kg}^{-1}$  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<sub>1A</sub> 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  $\text{mg}\cdot\text{kg}^{-1}$ ) mediated by possible CB<sub>2</sub> 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<sub>2</sub> 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  $\text{mg}\cdot\text{kg}^{-1}$ , and at a fraction of THC activity, via a non-CB<sub>1</sub>, non-CB<sub>2</sub> 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<sup>2+</sup> 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<sub>1</sub> (K<sub>i</sub> 440 nM) and CB<sub>2</sub> (K<sub>i</sub> 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 lipoxygenase 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  $\alpha$ -2 adrenorecep-

tor agonist, supporting analgesic effects previously noted (Formukong *et al.*, 1988), and moderate 5-HT<sub>1A</sub> 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<sub>1</sub> antagonist at lower doses (Thomas *et al.*, 2005), but is a CB<sub>1</sub> 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<sub>2</sub>-based ability to suppress carageenan-induced hyperalgesia and inflammation, and both phases of formalin-induced pain behaviour via CB<sub>1</sub> and CB<sub>2</sub> 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<sub>50</sub>) 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<sub>1</sub> (K<sub>i</sub> 211.2 nM) and CB<sub>2</sub> (K<sub>i</sub> 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 µg·mL<sup>-1</sup>). 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<sub>50</sub> 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,  $\alpha$ -terpineol, terpineol-4-ol, p-cymene, borneol and  $\Delta$ -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<sup>-1</sup>, pinene 13.77% increase at trace concentration, terpineol 45% reduction at 4.7 ng·mL<sup>-1</sup>). 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<sup>-1</sup> 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 monoter-



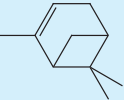

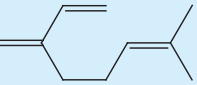

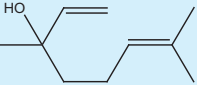

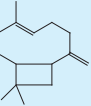
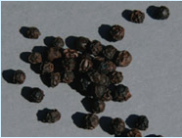
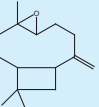

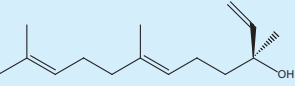

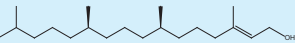

pene. Studies with varying methodology and dosing in citrus oils in mice suggest it to be a powerful anxiolytic agent (Carvalho-Freitas and Costa, 2002; Pultrini Ade *et al.*, 2006), with one EO increasing serotonin in the prefrontal cortex, and dopamine (DA) in hippocampus mediated via 5-HT<sub>1A</sub> (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, perillid 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<sup>-1</sup>) and non-sensitizing (Von Burg, 1995).

$\beta$ -Myrcene is another common monoterpene 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  $\alpha$ -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.

$\alpha$ -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).  $\alpha$ -Pinene forms the biosynthetic base for CB<sub>2</sub> 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<sub>50</sub> 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 <i>et al.</i> , 1995) Anxiolytic (Carvalho-Freitas and Costa, 2002; Pultrini Ade <i>et al.</i> , 2006) via 5-HT <sub>1A</sub> (Komiya <i>et al.</i> , 2006) Apoptosis of breast cancer cells (Vigushin <i>et al.</i> , 1998) Active against acne bacteria (Kim <i>et al.</i> , 2008) Dermatophytes (Sanguinetti <i>et al.</i> , 2007; Singh <i>et al.</i> , 2010) Gastro-oesophageal reflux (Harris, 2010)	CBD CBD CBD, CBG CBD CBG THC
$\alpha$ -Pinene		 Pine	Anti-inflammatory via PGE-1 (Gil <i>et al.</i> , 1989) Bronchodilatory in humans (Falk <i>et al.</i> , 1990) Acetylcholinesterase inhibitor, aiding memory (Perry <i>et al.</i> , 2000)	CBD THC THC?, CBD
$\beta$ -Myrcene		 Hops	Blocks inflammation via PGE-2 (Lorenzetti <i>et al.</i> , 1991) Analgesic, antagonized by naloxone (Rao <i>et al.</i> , 1990) Sedating, muscle relaxant, hypnotic (do Vale <i>et al.</i> , 2002) Blocks hepatic carcinogenesis by aflatoxin (de Oliveira <i>et al.</i> , 1997)	CBD CBD, THC THC CBD, CBG
Linalool		 Lavender	Anti-anxiety (Russo, 2001) Sedative on inhalation in mice (Buchbauer <i>et al.</i> , 1993) Local anesthetic (Re <i>et al.</i> , 2000) Analgesic via adenosine A <sub>2A</sub> (Peano <i>et al.</i> , 2006) Anticonvulsant/anti-glutamate (Elisabetsky <i>et al.</i> , 1995) Potent anti-leishmanial (do Socorro <i>et al.</i> , 2003)	CBD, CBG? THC THC CBD CBD, THCV, CBDV ?
$\beta$ -Caryophyllene		 Pepper	AI via PGE-1 comparable phenylbutazone (Basile <i>et al.</i> , 1988) Gastric cytoprotective (Tambe <i>et al.</i> , 1996) Anti-malarial (Campbell <i>et al.</i> , 1997) Selective CB <sub>2</sub> agonist (100 nM) (Gertsch <i>et al.</i> , 2008) Treatment of pruritus? (Karsak <i>et al.</i> , 2007) Treatment of addiction? (Xi <i>et al.</i> , 2010)	CBD THC ? THC THC CBD
Caryophyllene Oxide		 Lemon balm	Decreases platelet aggregation (Lin <i>et al.</i> , 2003) Antifungal in onychomycosis comparable to ciclopiroxolamine and sulconazole (Yang <i>et al.</i> , 1999) Insecticidal/anti-feedant (Bettarini <i>et al.</i> , 1993)	THC CBC,CBG THCA, CBGA
Nerolidol		 Orange	Sedative (Binet <i>et al.</i> , 1972) Skin penetrant (Cornwell and Barry, 1994) Potent antimalarial (Lopes <i>et al.</i> , 1999, Rodrigues Goulart <i>et al.</i> , 2004) Anti-leishmanial activity (Arruda <i>et al.</i> , 2005)	THC, CBN – ? ?
Phytol		 Green tea	Breakdown product of chlorophyll Prevents Vitamin A teratogenesis (Arnhold <i>et al.</i> , 2002) $\uparrow$ GABA via SSADH inhibition (Bang <i>et al.</i> , 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<sub>1</sub>/CB<sub>2</sub>, 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 anti-glutamatergic 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).

$\beta$ -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 anti-malarial 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<sub>2</sub> (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·kg<sup>-1</sup> in wild-type, but not CB<sub>2</sub> knockout mice (Gertsch, 2008). Given the lack of attributed psychoactivity of CB<sub>2</sub> 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  $\mu$ M 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  $\mu$ M) induced sebocyte apoptosis, which was augmented in the presence of AEA. The effect of CBD to increase

Ca<sup>++</sup> 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 µL·mL<sup>-1</sup>), more potently than triclosan (Kim *et al.*, 2008). Linalool alone demonstrated an MIC of 0.625 µL·mL<sup>-1</sup>. 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 µg·mL<sup>-1</sup>) (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 µg·mL<sup>-1</sup>). 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 anti-septic 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 (Rocheffort *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-HT<sub>1A</sub> 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·kg<sup>-1</sup> 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·kg<sup>-1</sup>·d<sup>-1</sup> 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  $\alpha$ -amino-3-hydroxyl-5-methyl-4-isoxazole-propionate glutamate and CB<sub>1</sub> 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<sub>2</sub> agonism and a newly discovered putative mechanism of action in addiction treatment.

CB<sub>2</sub> is expressed in dopaminergic neurones in the ventral tegmental area and nucleus accumbens, areas mediating addictive phenomena (Xi *et al.*, 2010). Activation of CB<sub>2</sub> 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<sub>2</sub> 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 physostigmine (Mukherjee *et al.*, 2007). Interestingly, the cannabis terpenoid,  $\alpha$ -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 first 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-Ukbary 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  $\beta$ -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 appreciably 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.

## Acknowledgements

The author offers appreciation to the following individuals, who provided materials and/or consultation: David Potter, Etienne de Meijer, John McPartland, David Watson, Rob Clarke, Indalecio Lozano, Tamas Biró, José Crippa, Roger

Pertwee, Colin Stott, Vincenzo Di Marzo, Luciano De Petrocellis, Patrick McGovern, John Riddle and Elisaldo Carlini. Most of all, I would like to thank Raphael Mechoulam for his example, guidance, friendship, a life of good works and for listening to many 'crazy ideas'.

## Conflict of Interest

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

## References

- Adams TB, Taylor SV (2010). Safety evaluation of essential oils: a constituent-based approach. In: Baser KHC, Buchbauer G (eds). *Handbook of Essential Oils: Science, Technology, and Applications*. CRC Press: Boca Raton, FL, pp. 185–208.
- Alexander SP, Mathie A, Peters JA (2009). Guide to Receptors and Channels (GRAC), 4th edition. *Br J Pharmacol* 158 (Suppl. 1): S1–254.
- Appendino G, Gibbons S, Giana A, Pagani A, Grassi G, Stavri M *et al.* (2008). Antibacterial cannabinoids from *Cannabis sativa*: a structure-activity study. *J Nat Prod* 71: 1427–1430.
- Arnhold T, Elmazar MM, Nau H (2002). Prevention of vitamin A teratogenesis by phytol or phytanic acid results from reduced metabolism of retinol to the teratogenic metabolite, all-trans-retinoic acid. *Toxicol Sci* 66: 274–282.
- Arruda DC, D’Alexandri FL, Katzin AM, Uliana SR (2005). Antileishmanial activity of the terpene nerolidol. *Antimicrob Agents Chemother* 49: 1679–1687.
- Baek SH, Kim YO, Kwag JS, Choi KE, Jung WY, Han DS (1998). Boron trifluoride etherate on silica-A modified Lewis acid reagent (VII). Antitumor activity of cannabigerol against human oral epitheloid carcinoma cells. *Arch Pharm Res* 21: 353–356.
- Bancroft EA (2007). Antimicrobial resistance: it’s not just for hospitals. *JAMA* 298: 1803–1804.
- Banerjee SP, Snyder SH, Mechoulam R (1975). Cannabinoids: influence on neurotransmitter uptake in rat brain synaptosomes. *J Pharmacol Exp Ther* 194: 74–81.
- Bang MH, Choi SY, Jang TO, Kim SK, Kwon OS, Kang TC *et al.* (2002). Phytol, SSADH inhibitory diterpenoid of *Lactuca sativa*. *Arch Pharm Res* 25: 643–646.
- Barnea G, O’Donnell S, Mancina F, Sun X, Nemes A, Mendelsohn M *et al.* (2004). Odorant receptors on axon termini in the brain. *Science* 304: 1468.
- Basile AC, Sertie JA, Freitas PC, Zanini AC (1988). Anti-inflammatory activity of oleoresin from Brazilian *Copaifera*. *J Ethnopharmacol* 22: 101–109.
- Batista PA, Werner MF, Oliveira EC, Burgos L, Pereira P, Brum LF *et al.* (2008). Evidence for the involvement of ionotropic glutamatergic receptors on the antinociceptive effect of (-)-linalool in mice. *Neurosci Lett* 440: 299–303.
- Ben-Shabat S, Fride E, Sheskin T, Tamiri T, Rhee MH, Vogel Z *et al.* (1998). An entourage effect: inactive endogenous fatty acid glycerol esters enhance 2-arachidonoyl-glycerol cannabinoid activity. *Eur J Pharmacol* 353: 23–31.

- Berenbaum MC (1989). What is synergy? *Pharmacol Rev* 41: 93–141.
- Bettarini F, Borgonovi GE, Fiorani T, Gagliardi I, Caprioli V, Massardo P *et al.* (1993). Antiparasitic compounds from East African plants: isolation and biological activity of anonaine, matricarinal, canthin-6-one, and caryophyllene oxide. *Insect Sci Appl* 14: 93–99.
- Bickers D, Calow P, Greim H, Hanifin JM, Rogers AE, Saurat JH *et al.* (2003). A toxicologic and dermatologic assessment of linalool and related esters when used as fragrance ingredients. *Food Chem Toxicol* 41: 919–942.
- Binet L, Binet P, Miocque M, Roux M, Bernier A (1972). Recherches sur les propriétés pharmacodynamiques (action sédative et action spasmolytique) de quelques alcools terpéniques aliphatiques. *Ann Pharm Fr* 30: 611–616.
- Biro T, Olah A, Toth BI, Czifra G, Zouboulis CC, Paus R (2009). Cannabidiol as a novel anti-acne agent? Cannabidiol inhibits lipid synthesis and induces cell death in human sebaceous gland-derived sebocytes. Proceedings 19th Annual Conference on the Cannabinoids. International Cannabinoid Research Society: Pheasant Run, St. Charles, IL, p. 28.
- Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I *et al.* (2001). Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br J Pharmacol* 134: 845–852.
- Bisset NG, Wichtl M (2004). *Herbal Drugs and Phytopharmaceuticals: A Handbook for Practice on a Scientific Basis*, 3rd edn. Medpharm Scientific Publishers: Stuttgart; CRC Press: Boca Raton, FL.
- Bloor RN, Wang TS, Spanel P, Smith D (2008). Ammonia release from heated ‘street’ cannabis leaf and its potential toxic effects on cannabis users. *Addiction* 103: 1671–1677.
- Bohlmann J, Meyer-Gauen G, Croteau R (1998). Plant terpenoid synthases: molecular biology and phylogenetic analysis. *Proc Natl Acad Sci USA* 95: 4126–4133.
- Bolognini D, Costa B, Maione S, Comelli F, Marini P, Di Marzo V *et al.* (2010). The plant cannabinoid Delta9-tetrahydrocannabinol can decrease signs of inflammation and inflammatory pain in mice. *Br J Pharmacol* 160: 677–687.
- Borgwardt SJ, Allen P, Bhattacharyya S, Fusar-Poli P, Crippa JA, Seal ML *et al.* (2008). Neural basis of Delta-9-tetrahydrocannabinol and cannabidiol: effects during response inhibition. *Biol Psychiatry* 64: 966–973.
- Bowles EJ (2003). *The Chemistry of Aromatherapeutic Oils*, 3rd edn. Allen & Unwin: Crow’s Nest, NSW.
- Bradshaw HB, Lee SH, McHugh D (2009). Orphan endogenous lipids and orphan GPCRs: a good match. *Prostaglandins Other Lipid Mediat* 89: 131–134.
- Brenneisen R (2007). Chemistry and analysis of phytocannabinoids and other *Cannabis* constituents. In: Elsohly M (ed.). *Marijuana and the Cannabinoids*. Humana Press: Totowa, NY, pp. 17–49.
- Buchbauer G (2010). Biological activities of essential oils. In: Baser KHC, Buchbauer G (eds). *Handbook of Essential Oils: Science, Technology, and Applications*. CRC Press: Boca Raton, FL, pp. 235–280.
- Buchbauer G, Jirovetz L, Jager W, Dietrich H, Plank C (1991). Aromatherapy: evidence for sedative effects of the essential oil of lavender after inhalation. *Z Naturforsch [C]* 46: 1067–1072.
- Buchbauer G, Jirovetz L, Jager W, Plank C, Dietrich H (1993). Fragrance compounds and essential oils with sedative effects upon inhalation. *J Pharm Sci* 82: 660–664.
- Calkins A (1871). *Opium and the Opium-Appetite: with Notices of Alcoholic Beverages, Cannabis Indica, Tobacco and Coca, and Tea and Coffee, in Their Hygienic Aspects and Pathologic Relationships*. J.B. Lippincott: Philadelphia, PA.
- Campbell WE, Gammon DW, Smith P, Abrahams M, Purves TD (1997). Composition and antimalarial activity in vitro of the essential oil of *Tetradenia riparia*. *Planta Med* 63: 270–272.
- Carlini EA, Cunha JM (1981). Hypnotic and antiepileptic effects of cannabidiol. *J Clin Pharmacol* 21 (Suppl.): 417S–427S.
- Carlini EA, Karniol IG, Renault PF, Schuster CR (1974). Effects of marihuana in laboratory animals and in man. *Br J Pharmacol* 50: 299–309.
- Carrier EJ, Auchampach JA, Hillard CJ (2006). Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proc Natl Acad Sci USA* 103: 7895–7900.
- Carvalho-Freitas MI, Costa M (2002). Anxiolytic and sedative effects of extracts and essential oil from *Citrus aurantium* L. *Biol Pharm Bull* 25: 1629–1633.
- Cascio MG, Gauson LA, Stevenson LA, Ross RA, Pertwee RG (2010). Evidence that the plant cannabinoid cannabigerol is a highly potent alpha2-adrenoceptor agonist and moderately potent 5HT1A receptor antagonist. *Br J Pharmacol* 159: 129–141.
- Cawthorne MA, Wargent E, Zaibi M, Stott C, Wright S (2007). The CB1 antagonist, delta-9-tetrahydrocannabinol (THCV) has anti-obesity activity in dietary-induced obese (DIO) mice. Proceedings 17th Annual Symposium on the Cannabinoids. International Cannabinoid Research Society: Saint-Sauveur, QC, p. 141.
- Choi HS, Song HS, Ukeda H, Sawamura M (2000). Radical-scavenging activities of citrus essential oils and their components: detection using 1,1-diphenyl-2-picrylhydrazyl. *J Agric Food Chem* 48: 4156–4161.
- Christison R (1848). *A Dispensary Or Commentary on the Pharmacopoeias of Great Britain and the United States*. Lea and Blanchard: Philadelphia, PA.
- Christison A (1851). On the natural history, action, and uses of Indian hemp. *Monthly J Med Sci Edinburgh, Scotland* 13: 26–45. 117–121.
- Clarke RC (2010). *Hashish!*, 2nd edn. Red Eye Press: Los Angeles, CA.
- Comelli F, Bettoni I, Colleoni M, Giagnoni G, Costa B (2009). Beneficial effects of a *Cannabis sativa* extract treatment on diabetes-induced neuropathy and oxidative stress. *Phytother Res* 23: 1678–1684.
- Cornwell PA, Barry BW (1994). Sesquiterpene components of volatile oils as skin penetration enhancers for the hydrophilic permeant 5-fluorouracil. *J Pharm Pharmacol* 46: 261–269.
- Costa B, Trovato AE, Comelli F, Giagnoni G, Colleoni M (2007). The non-psychoactive cannabis constituent cannabidiol is an orally effective therapeutic agent in rat chronic inflammatory and neuropathic pain. *Eur J Pharmacol* 556: 75–83.
- Couladis M, Ozcan M, Tzakou O, Akgul A (2003). Comparative essential oil composition of various parts of the turpentine tree (*Pistacia terebinthus*) growing wild in Turkey. *J Sci Food Agric* 83: 136–138.

- Crippa JA, Zuardi AW, Hallak JE (2010). [Therapeutical use of the cannabinoids in psychiatry]. *Rev Bras Psiquiatr* 32 (Suppl. 1): S56–S66.
- Crippa JA, Derenusson GN, Ferrari TB, Wichert-Ana L, Duran F, Marti NSRO *et al.* (2011). Neural basis of anxiolytic effects of cannabidiol (CBD) in generalized social anxiety disorder: a preliminary report. *J Psychopharmacol* 25: 121–130.
- Croteau R (1987). Biosynthesis and catabolism of monoterpenoids. *Chem Rev* 87: 929–954.
- De Oliveira AC, Ribeiro-Pinto LF, Paumgartten JR (1997). *In vitro* inhibition of CYP2B1 monooxygenase by beta-myrcene and other monoterpenoid compounds. *Toxicol Lett* 92: 39–46.
- D’Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu YT *et al.* (2004). The psychotomimetic effects of intravenous delta-9-tetrahydrocannabinol in healthy individuals: implications for psychosis. *Neuropsychopharmacology* 29: 1558–1572.
- Davalos SD, Fournier G, Boucher F, Paris M (1977). [Contribution to the study of Mexican marihuana. Preliminary studies: cannabinoids and essential oil (author’s transl)]. *J Pharm Belg* 32: 89–99.
- Davis WM, Hatoum NS (1983). Neurobehavioral actions of cannabichromene and interactions with delta 9-tetrahydrocannabinol. *Gen Pharmacol* 14: 247–252.
- De Oliveira AC, Ribeiro-Pinto LF, Paumgartten JR (1997). *In vitro* inhibition of CYP2B1 monooxygenase by beta-myrcene and other monoterpenoid compounds. *Toxicol Lett* 92: 39–46.
- De Petrocellis L, Di Marzo V (2010). Non-CB1, non-CB2 receptors for endocannabinoids, plant cannabinoids, and synthetic cannabimimetics: focus on G-protein-coupled receptors and transient receptor potential channels. *J Neuroimmune Pharmacol* 5: 103–121.
- De Petrocellis L, Vellani V, Schiano-Moriello A, Marini P, Magherini PC, Orlando P *et al.* (2008). Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. *J Pharmacol Exp Ther* 325: 1007–1015.
- De Petrocellis L, Ligresti A, Moriello AS, Allara M, Bisogno T, Petrosino S *et al.* (2011). Effects of cannabinoids and cannabinoid-enriched *Cannabis* extracts on TRP channels and endocannabinoid metabolic enzymes. *Br J Pharmacol* DOI:10.1111/j.1476-5381.2010.0166.x
- Delgado P, Moreno F (1999). Antidepressants and the brain. *Int Clin Psychopharmacol* 14 (Suppl. 1): S9–16.
- Delong GT, Wolf CE, Poklis A, Lichtman AH (2010). Pharmacological evaluation of the natural constituent of *Cannabis sativa*, cannabichromene and its modulation by Delta(9)-tetrahydrocannabinol. *Drug Alcohol Depend* 112: 126–133.
- Denson TF, Earleywine M (2006). Decreased depression in marijuana users. *Addict Behav* 31: 738–742.
- Devane WA, Dysarz FA 3rd, Johnson MR, Melvin LS, Howlett AC (1988). Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* 34: 605–613.
- Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G *et al.* (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258: 1946–1949.
- Deyo R, Musty R (2003). A cannabichromene (CBC) extract alters behavioral despair on the mouse tail suspension test of depression. *Proceedings 2003 Symposium on the Cannabinoids*. International Cannabinoid Research Society: Cornwall, ON, p. 146.
- Dirikoc S, Priola SA, Marella M, Zsurgner N, Chabry J (2007). Nonpsychoactive cannabidiol prevents prion accumulation and protects neurons against prion toxicity. *J Neurosci* 27: 9537–9544.
- Dobrosi N, Toth BI, Nagy G, Dozsa A, Geczy T, Nagy G *et al.* (2008). Endocannabinoids enhance lipid synthesis and apoptosis of human sebocytes via cannabinoid receptor-2-mediated signaling. *FASEB J* 22: 3685–3695.
- Douthwaite AH (1947). Choice of drugs in the treatment of duodenal ulcer. *Br Med J* 2: 43–47.
- Dreher M (2002). Crack heads and roots daughters: the therapeutic use of cannabis in Jamaica. *J Cannabis Therap* 2: 121–133.
- Duru ME, Cakir A, Kordali S, Zengin H, Harmandar M, Izumi S *et al.* (2003). Chemical composition and antifungal properties of essential oils of three *Pistacia* species. *Fitoterapia* 74: 170–176.
- Elisabetsky E, Marschner J, Souza DO (1995). Effects of Linalool on glutamatergic system in the rat cerebral cortex. *Neurochem Res* 20: 461–465.
- ElSohly HN, Turner CE, Clark AM, ElSohly MA (1982). Synthesis and antimicrobial activities of certain cannabichromene and cannabigerol related compounds. *J Pharm Sci* 71: 1319–1323.
- Esposito G, De Filippis D, Carnuccio R, Izzo AA, Iuvone T (2006a). The marijuana component cannabidiol inhibits beta-amyloid-induced tau protein hyperphosphorylation through Wnt/beta-catenin pathway rescue in PC12 cells. *J Mol Med* 84: 253–258.
- Esposito G, De Filippis D, Maiuri MC, De Stefano D, Carnuccio R, Iuvone T (2006b). Cannabidiol inhibits inducible nitric oxide synthase protein expression and nitric oxide production in beta-amyloid stimulated PC12 neurons through p38 MAP kinase and NF-kappaB involvement. *Neurosci Lett* 399: 91–95.
- Eubanks LM, Rogers CJ, Beuscher AE 4th, Koob GF, Olson AJ, Dickerson TJ *et al.* (2006). A molecular link between the active component of marijuana and Alzheimer’s disease pathology. *Mol Pharm* 3: 773–777.
- Evans FJ (1991). Cannabinoids: the separation of central from peripheral effects on a structural basis. *Planta Med* 57: S60–S67.
- Fairbairn JW, Pickens JT (1981). Activity of cannabis in relation to its delta’-trans-tetrahydro-cannabinol content. *Br J Pharmacol* 72: 401–409.
- Falk AA, Hagberg MT, Lof AE, Wigaeus-Hjelm EM, Wang ZP (1990). Uptake, distribution and elimination of alpha-pinene in man after exposure by inhalation. *Scand J Work Environ Health* 16: 372–378.
- Falk-Filipsson A, Lof A, Hagberg M, Hjelm EW, Wang Z (1993). d-limonene exposure to humans by inhalation: uptake, distribution, elimination, and effects on the pulmonary function. *J Toxicol Environ Health* 38: 77–88.
- Fan X, Gates RA (2001). Degradation of monoterpenes in orange juice by gamma radiation. *J Agric Food Chem* 49: 2422–2426.
- Fan X, Sokorai KJ (2002). Changes in volatile compounds of gamma-irradiated fresh cilantro leaves during cold storage. *J Agric Food Chem* 50: 7622–7626.
- Farag RS, Shalaby AS, El-Baroty GA, Ibrahim NA, Ali MA, Hassan EM (2004). Chemical and biological evaluation of the essential oils of different *Melaleuca* species. *Phytother Res* 18: 30–35.
- Favrat B, Menetrey A, Augsburg M, Rothuizen L, Appenzeller M, Buclin T *et al.* (2005). Two cases of ‘cannabis acute psychosis’ following the administration of oral cannabis. *BMC Psychiatry* 5: 17.

- Fellermeier M, Eisenreich W, Bacher A, Zenk MH (2001). Biosynthesis of cannabinoids. Incorporation experiments with (13)C-labeled glucoses. *Eur J Biochem* 268: 1596–1604.
- Fischedick JT, Hazekamp A, Erkelens T, Choi YH, Verpoorte R (2010). Metabolic fingerprinting of *Cannabis sativa* L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. *Phytochem* 71: 2058–2073.
- Formukong EA, Evans AT, Evans FJ (1988). Analgesic and antiinflammatory activity of constituents of *Cannabis sativa* L. *Inflammation* 12: 361–371.
- Franz C, Novak J (2010). Sources of essential oils. In: Baser KHC, Buchbauer G (eds). *Handbook of Essential Oils: Science, Technology, and Applications*. CRC Press: Boca Raton, FL, pp. 39–82.
- Fride E, Russo EB (2006). Neuropsychiatry: Schizophrenia, depression, and anxiety. In: Onaivi E, Sugiura T, Di Marzo V (eds). *Endocannabinoids: The Brain and Body's Marijuana and beyond*. Taylor & Francis: Boca Raton, FL, pp. 371–382.
- Friedrich RW (2004). Neurobiology: odorant receptors make scents. *Nature* 430: 511–512.
- Fukumoto S, Morishita A, Furutachi K, Terashima T, Nakayama T, Yokogoshi H (2008). Effect of flavour components in lemon essential oil on physical or psychological stress. *Stress Health* 24: 3–12.
- Fusar-Poli P, Allen P, Bhattacharyya S, Crippa JA, Mechelli A, Borgwardt S *et al.* (2010). Modulation of effective connectivity during emotional processing by Delta9-tetrahydrocannabinol and cannabidiol. *Int J Neuropsychopharmacol* 13: 421–432.
- Fusar-Poli P, Crippa JA, Bhattacharyya S, Borgwardt SJ, Allen P, Martin-Santos R *et al.* (2009). Distinct effects of {delta}9-tetrahydrocannabinol and cannabidiol on neural activation during emotional processing. *Archiv Gen Psychiatr* 66: 95–105.
- Gaoni Y, Mechoulam R (1964a). Isolation, structure and partial synthesis of an active constituent of hashish. *J Am Chem Soc* 86: 1646–1647.
- Gaoni Y, Mechoulam R (1964b). The structure and function of cannabigerol, a new hashish constituent. *Proc Chem Soc* 1: 82.
- Gaoni Y, Mechoulam R (1966). Cannabichromene, a new active principle in hashish. *Chem Commun* 1: 20–21.
- Gattefosse R-M (1993). *Gatefosse's Aromatherapy*. C.W. Daniel: Essex, MD.
- Gauson LA, Stevenson LA, Thomas A, Baillie GL, Ross RA, Pertwee RG (2007). Cannabigerol behaves as a partial agonist at both CB1 and CB2 receptors. *Proceedings 17th Annual Symposium on the Cannabinoids*. International Cannabinoid Research Society: Saint-Sauveur, QC, p. 206.
- Gerdeman GL, Lovinger DM (2003). Emerging roles for endocannabinoids in long-term synaptic plasticity. *Br J Pharmacol* 140: 781–789.
- Gershenzon J (1994). Metabolic costs of terpenoid accumulation in higher plants. *J Chem Ecol* 20: 1281–1328.
- Gershenzon J, Croteau R (1993). Terpenoid Biosynthesis: the basic pathway and formation of monoterpenes, sesquiterpenes, and diterpenes. In: Moore TS (ed.). *Lipid Metabolism in Plants*. CRC Press: Boca Raton, FL, pp. 339–388.
- Gertsch J (2008). Anti-inflammatory cannabinoids in diet: towards a better understanding of CB(2) receptor action? *Commun Integr Biol* 1: 26–28.
- Gertsch J, Leonti M, Raduner S, Racz I, Chen JZ, Xie XQ *et al.* (2008). Beta-caryophyllene is a dietary cannabinoid. *Proc Natl Acad Sci USA* 105: 9099–9104.
- Ghelardini C, Galeotti N, Salvatore G, Mazzanti G (1999). Local anaesthetic activity of the essential oil of *Lavandula angustifolia*. *Planta Med* 65: 700–703.
- Gil ML, Jimenez J, Ocete MA, Zarzuelo A, Cabo MM (1989). Comparative study of different essential oils of *Bupleurum gibraltarium* Lamarck. *Pharmazie* 44: 284–287.
- Gill EW, Paton WD, Pertwee RG (1970). Preliminary experiments on the chemistry and pharmacology of cannabis. *Nature* 228: 134–136.
- Giuffrida A, Leweke FM, Gerth CW, Schreiber D, Koethe D, Faulhaber J *et al.* (2004). Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacol* 29: 2108–2114.
- Gomes-Carneiro MR, Viana ME, Felzenszwalb I, Paumgarten FJ (2005). Evaluation of beta-myrcene, alpha-terpinene and (+)- and (-)-alpha-pinene in the Salmonella/microsome assay. *Food Chem Toxicol* 43: 247–252.
- Guy GW, Stott CG (2005). The development of Sativex- a natural cannabis-based medicine. In: Mechoulam R (ed.). *Cannabinoids As Therapeutics*. Birkhäuser Verlag: Basel, pp. 231–263.
- Hampson AJ, Grimaldi M, Axelrod J, Wink D (1998). Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci USA* 95: 8268–8273.
- Haney M, Hart CL, Vosburg SK, Nasser J, Bennett A, Zubarán C *et al.* (2004). Marijuana withdrawal in humans: effects of oral THC or divalproex. *Neuropsychopharmacol* 29: 158–170.
- Hanus L, Breuer A, Tchilibon S, Shiloah S, Goldenberg D, Horowitz M *et al.* (1999). HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. *Proc Natl Acad Sci USA* 96: 14228–14233.
- Harris B (2010). Phytotherapeutic uses of essential oils. In: Baser KHC, Buchbauer G (eds). *Handbook of Essential Oils: Science, Technology, and Applications*. CRC Press: Boca Raton, FL, pp. 315–352.
- Hart CL, Haney M, Ward AS, Fischman MW, Foltin RW (2002). Effects of oral THC maintenance on smoked marijuana self-administration. *Drug Alcohol Depend* 67: 301–309.
- Hatoum NS, Davis WM, Elsohly MA, Turner CE (1981). Cannabichromene and delta 9-tetrahydrocannabinol: interactions relative to lethality, hypothermia and hexobarbital hypnosis. *Gen Pharmacol* 12: 357–362.
- Hendriks H, Malingré TM, Batterman S, Bos R (1975). Mono- and sesqui-terpene hydrocarbons of the essential oil of *Cannabis sativa*. *Phytochem* 14: 814–815.
- Hendriks H, Malingré TM, Batterman S, Bos R (1977). Alkanes of the essential oil of *Cannabis sativa*. *Phytochem* 16: 719–721.
- Hill MN, Gorzalka BB (2005a). Is there a role for the endocannabinoid system in the etiology and treatment of melancholic depression? *Behav Pharmacol* 16: 333–352.
- Hill MN, Gorzalka BB (2005b). Pharmacological enhancement of cannabinoid CB1 receptor activity elicits an antidepressant-like response in the rat forced swim test. *Eur Neuropsychopharmacol* 15: 593–599.
- Hill AJ, Weston SE, Jones NA, Smith I, Bevan SA, Williamson EM *et al.* (2010). Delta-Tetrahydrocannabinol suppresses in vitro epileptiform and in vivo seizure activity in adult rats. *Epilepsia* 51: 1522–1532.

- Hohmann AG, Suplita RL, Bolton NM, Neely MH, Fegley D, Mangieri R *et al.* (2005). An endocannabinoid mechanism for stress-induced analgesia. *Nature* 435: 1108–1112.
- Holland ML, Allen JD, Arnold JC (2008). Interaction of plant cannabinoids with the multidrug transporter ABCC1 (MRP1). *Eur J Pharmacol* 591: 128–131.
- Hollister LE (1974). Structure-activity relationships in man of cannabis constituents, and homologs and metabolites of delta9-tetrahydrocannabinol. *Pharmacol* 11: 3–11.
- Hood LV, Dames ME, Barry GT (1973). Headspace volatiles of marijuana. *Nature* 242: 402–403.
- Howlett AC (1987). Cannabinoid inhibition of adenylate cyclase: relative activity of constituents and metabolites of marijuana. *Neuropharmacol* 26: 507–512.
- Huestis MA (2007). Human Cannabinoid Pharmacokinetics. *Chem Biodivers* 4: 1770–1804.
- ibn al-Baytar (1291) *Kitab al-Yami' li-mufradat al-adwiya wa-l-agdiya*. Bulaq: Egypt.
- ibn Sina (Avicenna) (1294). *Al-Qanun fi l-tibb (Canon of medicine)*. Bulaq: Egypt.
- Ignatowska-Jankowska B, Jankowski M, Glac W, Swiergel AH (2009). Cannabidiol-induced lymphopenia does not involve NKT and NK cells. *J Physiol Pharmacol* 60 (Suppl. 3): 99–103.
- Ilan AB, Gevins A, Coleman M, ElSohly MA, de Wit H (2005). Neurophysiological and subjective profile of marijuana with varying concentrations of cannabinoids. *Behav Pharmacol* 16: 487–496.
- Ismail M (2006). Central properties and chemical composition of *Ocimum basilicum* essential oil. *Pharm Biol* 44: 619–626.
- Iuvone T, Esposito G, Esposito R, Santamaria R, Di Rosa M, Izzo AA (2004). Neuroprotective effect of cannabidiol, a non-psychoactive component from *Cannabis sativa*, on beta-amyloid-induced toxicity in PC12 cells. *J Neurochem* 89: 134–141.
- Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R (2009). Non-psychoactive plant cannabinoids: new therapeutic opportunities from an ancient herb. *Trends Pharmacol Sci* 30: 515–527.
- Jäger W, Buchbauer G, Jirovetz L, Fritzer M (1992). Percutaneous absorption of lavender oil from a massage oil. *J Soc Cosmet Chem* 43 (Jan/Feb): 49–54.
- Jirovetz L, Buchbauer G, Jager W, Woidich A, Nikiforov A (1992). Analysis of fragrance compounds in blood samples of mice by gas chromatography, mass spectrometry, GC/FTIR and GC/AES after inhalation of sandalwood oil. *Biomed Chromatogr* 6: 133–134.
- Johnson JR, Burnell-Nugent M, Lossignol D, Ganae-Motan ED, Potts R, Fallon MT (2010). Multicenter, double-blind, randomized, placebo-controlled, parallel-group study of the efficacy, safety, and tolerability of THC:CBD extract and THC extract in patients with intractable cancer-related pain. *J Pain Symptom Manage* 39: 167–179.
- Jones NA, Hill AJ, Smith I, Bevan SA, Williams CM, Whalley BJ *et al.* (2010). Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. *J Pharmacol Exp Ther* 332: 569–577.
- Karsak M, Gaffal E, Date R, Wang-Eckhardt L, Rehnelt J, Petrosino S *et al.* (2007). Attenuation of allergic contact dermatitis through the endocannabinoid system. *Science* 316: 1494–1497.
- Kavia R, De Ridder D, Constantinescu C, Stott C, Fowler C (2010). Randomized controlled trial of Sativex to treat detrusor overactivity in multiple sclerosis. *Mult Scler* 16: 1349–1359.
- Kim JT, Ren CJ, Fielding GA, Pitti A, Kasumi T, Wajda M *et al.* (2007). Treatment with lavender aromatherapy in the post-anesthesia care unit reduces opioid requirements of morbidly obese patients undergoing laparoscopic adjustable gastric banding. *Obes Surg* 17: 920–925.
- Kim SS, Baik JS, Oh TH, Yoon WJ, Lee NH, Hyun CG (2008). Biological activities of Korean Citrus obovoides and Citrus natsudaoida essential oils against acne-inducing bacteria. *Biosci Biotechnol Biochem* 72: 2507–2513.
- King LA, Carpentier C, Griffiths P (2005). Cannabis potency in Europe. *Addiction* 100: 884–886.
- Komiya M, Takeuchi T, Harada E (2006). Lemon oil vapor causes an anti-stress effect via modulating the 5-HT and DA activities in mice. *Behav Brain Res* 172: 240–249.
- Komori T, Fujiwara R, Tanida M, Nomura J, Yokoyama MM (1995). Effects of citrus fragrance on immune function and depressive states. *Neuroimmunomodulation* 2: 174–180.
- Kose EO, Deniz IG, Sarikurkcu C, Aktas O, Yavuz M (2010). Chemical composition, antimicrobial and antioxidant activities of the essential oils of *Sideritis erythrantha* Boiss. and Heldr. (var. *erythrantha* and var. *cedretorum* P.H. Davis) endemic in Turkey. *Food Chem Toxicol* 48: 2960–2965.
- Labigalini E Jr, Rodrigues LR, Da Silveira DX (1999). Therapeutic use of cannabis by crack addicts in Brazil. *J Psychoactive Drugs* 31: 451–455.
- Lad V (1990). *Ayurveda: the Science of Self-Healing: A Practical Guide*. Lotus Light Publications: Milwaukee, WI.
- Langenheim JH (1994). Higher plant terpenoids: a phytocentric overview of their ecological roles. *J Chem Ecol* 20: 1223–1279.
- Lapczynski A, Bhatia SP, Letizia CS, Api AM (2008). Fragrance material review on nerolidol (isomer unspecified). *Food Chem Toxicol* 46 (Suppl. 11): S247–S250.
- Lawless J (1995). *The Illustrated Encyclopedia of Essential Oils : the Complete Guide to the Use of Oils in Aromatherapy and Herbalism*. Element: Shaftesbury, Dorset, [England]; Rockport, MA.
- Ligresti A, Moriello AS, Starowicz K, Matias I, Pisanti S, De Petrocellis L *et al.* (2006). Antitumor activity of plant cannabinoids with emphasis on the effect of cannabidiol on human breast carcinoma. *J Pharmacol Exp Ther* 318: 1375–1387.
- Lin WY, Kuo YH, Chang YL, Teng CM, Wang EC, Ishikawa T *et al.* (2003). Anti-platelet aggregation and chemical constituents from the rhizome of *Gynura japonica*. *Planta Med* 69: 757–764.
- Lis-Balchin M (2010). Aromatherapy with essential oils. In: Baser KHC, Buchbauer G (eds). *Handbook of Essential Oils: Science, Technology, and Applications*. CRC Press: Boca Raton, FL, pp. 549–584.
- Lopes NP, Kato MJ, Andrade EH, Maia JG, Yoshida M, Planchart AR *et al.* (1999). Antimalarial use of volatile oil from leaves of *Virola surinamensis* (Rol.) Warb. by Waiapi Amazon Indians. *J Ethnopharmacol* 67: 313–319.
- Lorenzetti BB, Souza GE, Sarti SJ, Santos Filho D, Ferreira SH (1991). Myrcene mimics the peripheral analgesic activity of lemongrass tea. *J Ethnopharmacol* 34: 43–48.
- Lozano I (1993). *Estudios Y Documentos Sobre La Historia Del Cáñamo Y Del Hachís En El Islam Medieval*. doctoral Dissertation. Universidad de Granada: Granada.

- Ludlow FH (1857). The Hasheesh Eater: Being Passages Form the Life of A Pythagorean. Harper: New York.
- McGinty D, Letizia CS, Api AM (2010). Fragrance material review on phytol. Food Chem Toxicol 48 (Suppl. 3): S59–S63.
- McGovern PE, Mirzoian A, Hall GR (2009). Ancient Egyptian herbal wines. Proc Natl Acad Sci USA 106: 7361–7366.
- McHugh D, Hu SS, Rimmerman N, Juknat A, Vogel Z, Walker JM *et al.* (2010). N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. BMC Neurosci 11: 44.
- McPartland J (1984). Pathogenicity of *Phomopsis ganjae* on *Cannabis sativa* and the fungistatic effect of cannabinoids produced by the host. Mycopathologia 87: 149–153.
- McPartland JM, Pruitt PL (1999). Side effects of pharmaceuticals not elicited by comparable herbal medicines: the case of tetrahydrocannabinol and marijuana. Altern Ther Health Med 5: 57–62.
- McPartland JM, Mediavilla V (2001a). Non-cannabinoids in cannabis. In: Grotenhermen F, Russo EB (eds). Cannabis and Cannabinoids. NY: Haworth Press: Binghamton, NY, pp. 401–409.
- McPartland JM, Russo EB (2001b). Cannabis and cannabis extracts: greater than the sum of their parts? J Cannabis Therap 1: 103–132.
- McPartland JM, Clarke RC, Watson DP (2000). Hemp Diseases and Pests: Management and Biological Control. CABI: Wallingford.
- McPartland JM, Blanchon DJ, Musty RE (2008). Cannabimimetic effects modulated by cholinergic compounds. Addict Biol 13: 411–415.
- Magen I, Avraham Y, Ackerman Z, Vorobiev L, Mechoulam R, Berry EM (2009). Cannabidiol ameliorates cognitive and motor impairments in mice with bile duct ligation. J Hepatol 51: 528–534.
- Malfait AM, Gallily R, Sumariwalla PF, Malik AS, Andreaskos E, Mechoulam R *et al.* (2000). The nonpsychoactive cannabis constituent cannabidiol is an oral anti-arthritic therapeutic in murine collagen-induced arthritis. Proc Natl Acad Sci USA 97: 9561–9566.
- Malingre T, Hendriks H, Batterman S, Bos R, Visser J (1975). The essential oil of *Cannabis sativa*. Planta Med 28: 56–61.
- Maor Y, Gallily R, Mechoulam R (2006). The relevance of the steric factor in the biological activity of CBD derivatives—a tool in identifying novel molecular target for cannabinoids. In: *Symposium on the Cannabinoids*. International Cannabinoid Research Society: Tihany, Hungary, p. 1.
- Marsicano G, Wotjak CT, Azad SC, Bisogno T, Rammes G, Cascio MG *et al.* (2002). The endogenous cannabinoid system controls extinction of aversive memories. Nature 418: 530–534.
- Matura M, Skold M, Borje A, Andersen KE, Bruze M, Frosch P *et al.* (2005). Selected oxidized fragrance terpenes are common contact allergens. Contact Dermatitis 52: 320–328.
- de Meijer E (2004). The breeding of cannabis cultivars for pharmaceutical end uses. In: Guy GW, Whittle BA, Robson P (eds). Medicinal Uses of Cannabis and Cannabinoids. Pharmaceutical Press: London, pp. 55–70.
- de Meijer EPM, Hammond KM (2005). The inheritance of chemical phenotype in *Cannabis sativa* L. (II): cannabigerol predominant plants. Euphytica 145: 189–198.
- de Meijer EP, Bagatta M, Carboni A, Crucitti P, Moliterni VM, Ranalli P *et al.* (2003). The inheritance of chemical phenotype in *Cannabis sativa* L. Genetics 163: 335–346.
- de Meijer EPM, Hammond KM, Micheler M (2009a). The inheritance of chemical phenotype in *Cannabis sativa* L. (III): variation in cannabichromene proportion. Euphytica 165: 293–311.
- de Meijer EPM, Hammond KM, Sutton A (2009b). The inheritance of chemical phenotype in *Cannabis sativa* L. (IV): cannabinoid-free plants. Euphytica 168: 95–112.
- Mechoulam R (1986). The pharmacohistory of *Cannabis sativa*. In: Mechoulam R (ed.). Cannabinoids As Therapeutic Agents. CRC Press: Boca Raton, FL, pp. 1–19.
- Mechoulam R (2005). Plant cannabinoids: a neglected pharmacological treasure trove. Br J Pharmacol 146: 913–915.
- Mechoulam R, Ben-Shabat S (1999). From gan-zi-gun-nu to anandamide and 2-arachidonoylglycerol: the ongoing story of cannabis. Nat Prod Rep 16: 131–143.
- Mechoulam R, Shvo Y (1963). Hashish-I. The structure of cannabidiol. Tetrahedron 19: 2073–2078.
- Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR *et al.* (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50: 83–90.
- Mechoulam R, Peters M, Murillo-Rodriguez E, Hanus LO (2007). Cannabidiol – recent advances. Chem Biodivers 4: 1678–1692.
- Mediavilla V, Steinemann S (1997). Essential oil of *Cannabis sativa* L. strains. J Intl Hemp Assoc 4: 82–84.
- Mehmedic Z, Chandra S, Slade D, Denham H, Foster S, Patel AS *et al.* (2010). Potency trends of delta(9)-THC and other cannabinoids in confiscated cannabis preparations from 1993 to 2008. J Forensic Sci 55: 1209–1217.
- Mello NK, Mendelson JH (1978). Marijuana, alcohol, and polydrug use: human self-administration studies. NIDA Res Monogr 20: 93–127.
- Merzouki A, Mesa JM (2002). Concerning kif, a Cannabis sativa L. preparation smoked in the Rif mountains of northern Morocco. J Ethnopharmacol 81: 403–406.
- Mishima K, Hayakawa K, Abe K, Ikeda T, Egashira N, Iwasaki K *et al.* (2005). Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine<sub>1A</sub> receptor-dependent mechanism. Stroke 36: 1077–1082.
- Miyazawa M, Yamafuji C (2005). Inhibition of acetylcholinesterase activity by bicyclic monoterpenoids. J Agric Food Chem 53: 1765–1768.
- Monti D, Chetoni P, Burgalassi S, Najarro M, Saettone MF, Boldrini E (2002). Effect of different terpene-containing essential oils on permeation of estradiol through hairless mouse skin. Int J Pharm 237: 209–214.
- Morgan CJ, Curran HV (2008). Effects of cannabidiol on schizophrenia-like symptoms in people who use cannabis. Br J Psychiatry 192: 306–307.
- Morgan CJ, Freeman TP, Schafer GL, Curran HV (2010a). Cannabidiol attenuates the appetitive effects of Delta 9-tetrahydrocannabinol in humans smoking their chosen cannabis. Neuropsychopharmacology 35: 1879–1885.
- Morgan CJ, Schafer G, Freeman TP, Curran HV (2010b). Impact of cannabidiol on the acute memory and psychotomimetic effects of smoked cannabis: naturalistic study. Br J Psychiatry 197: 285–290.
- Morimoto S, Tanaka Y, Sasaki K, Tanaka H, Fukamizu T, Shoyama Y *et al.* (2007). Identification and characterization of cannabinoids that induce cell death through mitochondrial permeability transition in Cannabis leaf cells. J Biol Chem 282: 20739–20751.

- Morse K, Mamane D (2001). The Scent of Orange Blossoms : Sephardic Cuisine from Morocco. Ten Speed Press: Berkeley, CA.
- Mukerji G, Yiangou Y, Corcoran SL, Selmer IS, Smith GD, Benham CD *et al.* (2006). Cool and menthol receptor TRPM8 in human urinary bladder disorders and clinical correlations. BMC Urol 6: 6.
- Mukherjee PK, Kumar V, Mal M, Houghton PJ (2007). In vitro acetylcholinesterase inhibitory activity of the essential oil from *Acorus calamus* and its main constituents. Planta Med 73: 283–285.
- Murillo-Rodriguez E, Millan-Aldaco D, Palomero-Rivero M, Mechoulam R, Drucker-Colin R (2006). Cannabidiol, a constituent of *Cannabis sativa*, modulates sleep in rats. FEBS Lett 580: 4337–4345.
- Musty R, Deyo R (2006). A cannabigerol extract alters behavioral despair in an animal model of depression. Proceedings June 26; Symposium on the Cannabinoids. International Cannabinoid Research Society: Tihany, p. 32.
- Musty RE, Karniol IG, Shirikawa I, Takahashi RN, Knobel E (1976). Interactions of delta-9-tetrahydrocannabinol and cannabidiol in man. In: Braude MC, Szara S (eds). The Pharmacology of Marihuana, Vol. 2. Raven Press: New York, pp. 559–563.
- Naqvi NH, Bechara A (2009). The hidden island of addiction: the insula. Trends Neurosci 32: 56–67.
- Naqvi NH, Bechara A (2010). The insula and drug addiction: an interoceptive view of pleasure, urges, and decision-making. Brain Struct Funct 214: 435–450.
- Naqvi NH, Rudrauf D, Damasio H, Bechara A (2007). Damage to the insula disrupts addiction to cigarette smoking. Science 315: 531–534.
- Neff GW, O'Brien CB, Reddy KR, Bergasa NV, Regev A, Molina E *et al.* (2002). Preliminary observation with dronabinol in patients with intractable pruritus secondary to cholestatic liver disease. Am J Gastroenterol 97: 2117–2119.
- Nerio LS, Olivero-Verbel J, Stashenko E (2010). Repellent activity of essential oils: a review. Bioresour Technol 101: 372–378.
- Nicholson AN, Turner C, Stone BM, Robson PJ (2004). Effect of delta-9-tetrahydrocannabinol and cannabidiol on nocturnal sleep and early-morning behavior in young adults. J Clin Psychopharmacol 24: 305–313.
- Nissen L, Zatta A, Stefanini I, Grandi S, Sgorbati B, Biavati B *et al.* (2010). Characterization and antimicrobial activity of essential oils of industrial hemp varieties (*Cannabis sativa* L.). Fitoterapia 81: 413–419.
- Noma Y, Asakawa Y (2010). Biotransformation of monoterpenoids by microorganisms, insects, and mammals. In: Baser KHC, Buchbauer G (eds). Handbook of Essential Oils: Science, Technology, and Applications. CRC Press: Boca Raton, FL, pp. 585–736.
- Nunes DS, Linck VM, da Silva AL, Figueiro M, Elisabetsky E (2010). Psychopharmacology of essential oils. In: Baser KHC, Buchbauer G (eds). Handbook of Essential Oils: Science, Technology, and Applications. CRC Press: Boca Raton, FL, pp. 297–314.
- O'Shaughnessy WB (1843). Indian hemp. Prov Med J Retrospect Med Sci 5: 397–398.
- Oh DY, Yoon JM, Moon MJ, Hwang JI, Choe H, Lee JY *et al.* (2008). Identification of farnesyl pyrophosphate and N-arachidonylglycine as endogenous ligands for GPR92. J Biol Chem 283: 21054–21064.
- Opdyke DLJ (1983). Caryophyllene oxide. Food Chem Toxicol 21: 661–662.
- Ozek G, Demirci F, Ozek T, Tabanca N, Wedge DE, Khan SI *et al.* (2010). Gas chromatographic-mass spectrometric analysis of volatiles obtained by four different techniques from *Salvia rosifolia* Sm., and evaluation for biological activity. J Chromatog 1217: 741–748.
- Ozturk A, Ozbek H (2005). The anti-inflammatory activity of *Eugenia caryophyllata* essential oil: an animal model of anti-inflammatory activity. Eur J Gen Med 2: 159–163.
- Pacher P, Batkai S, Kunos G (2006). The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev 58: 389–462.
- Parker LA, Mechoulam R, Schlievert C (2002). Cannabidiol, a non-psychoactive component of cannabis and its synthetic dimethylheptyl homolog suppress nausea in an experimental model with rats. Neuroreport 13: 567–570.
- Parker LA, Burton P, Sorge RE, Yakiwchuk C, Mechoulam R (2004). Effect of low doses of Delta(9)-tetrahydrocannabinol and cannabidiol on the extinction of cocaine-induced and amphetamine-induced conditioned place preference learning in rats. Psychopharmacol (Berl) 175: 360–366.
- Parolaro D, Massi P (2008). Cannabinoids as potential new therapy for the treatment of gliomas. Expert Rev Neurother 8: 37–49.
- Pauli A, Schilcher H (2010). *In vitro* antimicrobial activities of essential oils monographed in the European Pharmacopoeia 6th Edition. In: Baser KHC, Buchbauer G (eds). Handbook of Essential Oils: Science, Technology, and Applications. CRC Press: Boca Raton, FL, pp. 353–548.
- Peana AT, Rubattu P, Piga GG, Fumagalli S, Boatto G, Pippia P *et al.* (2006). Involvement of adenosine A1 and A2A receptors in (-)-linalool-induced antinociception. Life Sci 78: 2471–2474.
- Perry NS, Houghton PJ, Theobald A, Jenner P, Perry EK (2000). In-vitro inhibition of human erythrocyte acetylcholinesterase by *salvia lavandulaefolia* essential oil and constituent terpenes. J Pharm Pharmacol 52: 895–902.
- Pertwee RG (2004). The pharmacology and therapeutic potential of cannabidiol. In: DiMarzo V (ed.). Cannabinoids. Kluwer Academic Publishers: Dordrecht, pp. 32–83.
- Pertwee RG, Thomas A, Stevenson LA, Ross RA, Varvel SA, Lichtman AH, Martin BR, Razdan RK (2007). The psychoactive plant cannabinoid, Delta9-tetrahydrocannabinol, is antagonized by Delta8- and Delta9-tetrahydrocannabivarin in mice *in vivo*. Br J Pharmacol 150: 586–594.
- Pertwee RG (2008). The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol 153: 199–215.
- Pliny (1980). Natural History, Books XXIV–XXVII., Vol. 7. Harvard University Press: Cambridge, MA.
- Potter D (2004). Growth and morphology of medicinal cannabis. In: Guy GW, Whittle BA, Robson P (eds). Medicinal Uses of Cannabis and Cannabinoids. Pharmaceutical Press: London, pp. 17–54.
- Potter DJ (2009). The propagation, characterisation and optimisation of *Cannabis sativa* L. as a phytopharmaceutical. PhD, King's College, London, 2009.
- Potter DJ, Clark P, Brown MB (2008). Potency of delta 9-THC and other cannabinoids in cannabis in England in 2005: implications for psychoactivity and pharmacology. J Forensic Sci 53: 90–94.

- Pultrini Ade M, Galindo LA, Costa M (2006). Effects of the essential oil from *Citrus aurantium* L. in experimental anxiety models in mice. *Life Sci* 78: 1720–1725.
- Qin N, Neeper MP, Liu Y, Hutchinson TL, Lubin ML, Flores CM (2008). TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. *J Neurosci* 28: 6231–6238.
- Rahn EJ, Hohmann AG (2009). Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. *Neurotherapeutics* 6: 713–737.
- Raman A, Weir U, Bloomfield SF (1995). Antimicrobial effects of tea-tree oil and its major components on *Staphylococcus aureus*, *Staph. epidermidis* and *Propionibacterium acnes*. *Lett Appl Microbiol* 21: 242–245.
- Rao VS, Menezes AM, Viana GS (1990). Effect of myrcene on nociception in mice. *J Pharm Pharmacol* 42: 877–878.
- Re L, Barocci S, Sonnino S, Mencarelli A, Vivani C, Paolucci G *et al.* (2000). Linalool modifies the nicotinic receptor-ion channel kinetics at the mouse neuromuscular junction. *Pharmacol Res* 42: 177–182.
- Ren Y, Whittard J, Higuera-Matas A, Morris CV, Hurd YL (2009). Cannabidiol, a nonpsychotropic component of cannabis, inhibits cue-induced heroin seeking and normalizes discrete mesolimbic neuronal disturbances. *J Neurosci* 29: 14764–14769.
- Resstel LB, Tavares RF, Lisboa SF, Joca SR, Correa FM, Guimaraes FS (2009). 5-HT<sub>1A</sub> receptors are involved in the cannabidiol-induced attenuation of behavioural and cardiovascular responses to acute restraint stress in rats. *Br J Pharmacol* 156: 181–188.
- Rhee MH, Vogel Z, Barg J, Bayewitch M, Levy R, Hanus L *et al.* (1997). Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylyl cyclase. *J Med Chem* 40: 3228–3233.
- Riedel G, Fadda P, McKillop-Smith S, Pertwee RG, Platt B, Robinson L (2009). Synthetic and plant-derived cannabinoid receptor antagonists show hypophagic properties in fasted and non-fasted mice. *Br J Pharmacol* 156: 1154–1166.
- Rocheffort C, Gheusi G, Vincent JD, Lledo PM (2002). Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. *J Neurosci* 22: 2679–2689.
- Rock EM, Limebeer CL, Mechoulam R, Parker LA (2009). Cannabidiol (the non-psychoactive component of cannabis) may act as a 5-HT<sub>1A</sub> auto-receptor agonist to reduce toxin-induced nausea and vomiting. *Proceedings 19th Annual Symposium on the Cannabinoids*. International Cannabinoid Research Society: St. Charles, IL, p. 29.
- Rodrigues Goulart H, Kimura EA, Peres VJ, Couto AS, Aquino Duarte FA, Katzin AM (2004). Terpenes arrest parasite development and inhibit biosynthesis of isoprenoids in *Plasmodium falciparum*. *Antimicrobial Agents Chemother* 48: 2502–2509.
- Rose JE, Behm FM (1994). Inhalation of vapor from black pepper extract reduces smoking withdrawal symptoms. *Drug Alcohol Depend* 34: 225–229.
- Ross SA, ElSohly MA (1996). The volatile oil composition of fresh and air-dried buds of *Cannabis sativa*. *J Nat Prod* 59: 49–51.
- Rothschild M, Bergstrom G, Wangberg S-A (2005). *Cannabis sativa*: volatile compounds from pollen and entire male and female plants of two variants, Northern Lights and Hawaiian Indica. *Bot J Linn Soc* 147: 387–397.
- Russo EB (2001). *Handbook of Psychotropic Herbs: A Scientific Analysis of Herbal Remedies for Psychiatric Conditions*. Haworth Press: Binghamton, NY.
- Russo EB (2006). The solution to the medicinal cannabis problem. In: Schatman ME (ed.). *Ethical Issues in Chronic Pain Management*. Taylor & Francis: Boca Raton, FL, pp. 165–194.
- Russo EB (2007). History of cannabis and its preparations in saga, science and sobriquet. *Chem Biodivers* 4: 2624–2648.
- Russo EB, Guy GW (2006). A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. *Med Hypotheses* 66: 234–246.
- Russo EB, McPartland JM (2003). Cannabis is more than simply Delta(9)-tetrahydrocannabinol. *Psychopharmacol (Berl)* 165: 431–432.
- Russo EB, Burnett A, Hall B, Parker KK (2005). Agonistic properties of cannabidiol at 5-HT<sub>1A</sub> receptors. *Neurochem Res* 30: 1037–1043.
- Russo EB, Guy GW, Robson PJ (2007). Cannabis, pain, and sleep: lessons from therapeutic clinical trials of Sativex, a cannabis-based medicine. *Chem Biodivers* 4: 1729–1743.
- Russo EB, Jiang HE, Li X, Sutton A, Carboni A, del Bianco F *et al.* (2008). Phytochemical and genetic analyses of ancient cannabis from Central Asia. *J Exp Bot* 59: 4171–4182.
- Ryan D, Drysdale AJ, Pertwee RG, Platt B (2006). Differential effects of cannabis extracts and pure plant cannabinoids on hippocampal neurones and glia. *Neurosci Lett* 408: 236–241.
- Salvadeo P, Boggia R, Evangelisti F, Zunin P (2007). Analysis of the volatile fraction of 'Pesto Genovese' by headspace sorptive extraction (HSSE). *Food Chem* 105: 1228–1235.
- Sanguinetti M, Posteraro B, Romano L, Battaglia F, Lopizzo T, De Carolis E *et al.* (2007). In vitro activity of *Citrus bergamia* (bergamot) oil against clinical isolates of dermatophytes. *J Antimicrob Chemother* 59: 305–308.
- Schmidt E (2010). Production of essential oils. In: Baser KHC, Buchbauer G (eds). *Handbook of Essential Oils: Science, Technology, and Applications*. CRC Press: Boca Raton, FL, pp. 83–120.
- Scutt A, Williamson EM (2007). Cannabinoids stimulate fibroblastic colony formation by bone marrow cells indirectly via CB<sub>2</sub> receptors. *Calcif Tissue Int* 80: 50–59.
- Shoyama Y, Sugawa C, Tanaka H, Morimoto S (2008). Cannabinoids act as necrosis-inducing factors in *Cannabis sativa*. *Plant Signal Behav* 3: 1111–1112.
- Silva Brum LF, Emanuelli T, Souza DO, Elisabetsky E (2001). Effects of linalool on glutamate release and uptake in mouse cortical synaptosomes. *Neurochem Res* 26: 191–194.
- Singh P, Shukla R, Prakash B, Kumar A, Singh S, Mishra PK *et al.* (2010). Chemical profile, antifungal, antiaflatoxinogenic and antioxidant activity of *Citrus maxima* Burm. and *Citrus sinensis* (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. *Food Chem Toxicol* 48: 1734–1740.
- Sirikantaramas S, Taura F, Tanaka Y, Ishikawa Y, Morimoto S, Shoyama Y (2005). Tetrahydrocannabinolic acid synthase, the enzyme controlling marijuana psychoactivity, is secreted into the storage cavity of the glandular trichomes. *Plant Cell Physiol* 46: 1578–1582.
- Skold M, Karlberg AT, Matura M, Borje A (2006). The fragrance chemical beta-caryophyllene-air oxidation and skin sensitization. *Food Chem Toxicol* 44: 538–545.

- Soares Vde P, Campos AC, Bortoli VC, Zangrossi H Jr, Guimaraes FS, Zuardi AW (2010). Intra-dorsal periaqueductal gray administration of cannabidiol blocks panic-like response by activating 5-HT1A receptors. *Behavioural Brain Res* 213: 225–229.
- do Socorro SRMS, Mendonca-Filho RR, Bizzo HR, de Almeida Rodrigues I, Soares RM, Souto-Padron T *et al.* (2003). Antileishmanial activity of a linalool-rich essential oil from *Croton cajucara*. *Antimicrob Agents Chemother* 47: 1895–1901.
- Stahl E, Kunde R (1973). Die Leitsubstanzen der Haschisch-Suchthunde. *Kriminalistik: Z Gesamte Kriminal Wiss Prax* 27: 385–389.
- Stott CG, Guy GW, Wright S, Whittle BA (2005). The effects of cannabis extracts Tetranabinex and Nabidiolex on human cytochrome P450-mediated metabolism. In: *Symposium on the Cannabinoids*, June 27. International Cannabinoid Research Association, Clearwater, FL, p. 163.
- Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K *et al.* (1995). 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem Biophys Res Commun* 215: 89–97.
- Tambe Y, Tsujiuchi H, Honda G, Ikeshiro Y, Tanaka S (1996). Gastric cytoprotection of the non-steroidal anti-inflammatory sesquiterpene, beta-caryophyllene. *Planta Med* 62: 469–470.
- Taylor B (1855). *The Lands of the Saracens*. G.P. Putnam & Sons: New York.
- Thomas A, Stevenson LA, Wease KN, Price MR, Baillie G, Ross RA *et al.* (2005). Evidence that the plant cannabinoid delta-9-tetrahydrocannabivarin is a cannabinoid CB1 and CB2 antagonist. *Br J Pharmacol* 146: 917–926.
- Thomas A, Baillie GL, Phillips AM, Razdan RK, Ross RA, Pertwee RG (2007). Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists in vitro. *Br J Pharmacol* 150: 613–623.
- Tisserand R, Balacs T (1995). *Essential Oil Safety: A Guide for Health Care Professionals*. Churchill Livingstone: Edinburgh.
- Trapp SC, Croteau RB (2001). Genomic organization of plant terpene synthases and molecular evolutionary implications. *Genet* 158: 811–832.
- Tsokou A, Georgopoulou K, Melliou E, Magiatis P, Tsitsa E (2007). Composition and enantiomeric analysis of the essential oil of the fruits and the leaves of *Pistacia vera* from Greece. *Molecules* 12: 1233–1239.
- Turner CE, Elsohly MA, Boeren EG (1980). Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. *J Nat Prod* 43: 169–234.
- Turner G, Gershenzon J, Nielson EE, Froehlich JE, Croteau R (1999). Limonene synthase, the enzyme responsible for monoterpene biosynthesis in peppermint, is localized to leucoplasts of oil gland secretory cells. *Plant Physiol* 120: 879–886.
- do Vale TG, Furtado EC, Santos JG Jr, Viana GS (2002). Central effects of citral, myrcene and limonene, constituents of essential oil chemotypes from *Lippia alba* (Mill.) n.e. Brown. *Phytomed* 9: 709–714.
- Varvel SA, Bridgen DT, Tao Q, Thomas BF, Martin BR, Lichtman AH (2005). Delta-9-tetrahydrocannabinol accounts for the antinociceptive, hypothermic, and cataleptic effects of marijuana in mice. *J Pharmacol Exp Ther* 314: 329–337.
- Vigushin DM, Poon GK, Boddy A, English J, Halbert GW, Pagonis C *et al.* (1998). Phase I and pharmacokinetic study of d-limonene in patients with advanced cancer. *Cancer Research Campaign Phase I/II Clinical Trials Committee. Cancer Chemother Pharmacol* 42: 111–117.
- Volicer L, Stelly M, Morris J, McLaughlin J, Volicer BJ (1997). Effects of dronabinol on anorexia and disturbed behavior in patients with Alzheimer's disease. *Int J Geriatr Psychiatry* 12: 913–919.
- Vollner L, Bieniek D, Korte F (1969). [Hashish. XX. Cannabidivarin, a new hashish constituent]. *Tetrahedron Lett* 3: 145–147.
- Von Burg R (1995). Toxicology update. Limonene. *J Appl Toxicol* 15: 495–499.
- Wachtel SR, ElSohly MA, Ross RA, Ambre J, de Wit H (2002). Comparison of the subjective effects of delta-9-tetrahydrocannabinol and marijuana in humans. *Psychopharmacol* 161: 331–339.
- Wagner H, Ulrich-Merzenich G (2009). Synergy research: approaching a new generation of phytopharmaceuticals. *Phytomed* 16: 97–110.
- Walton RP (1938). *Marihuana, America's New Drug Problem. A Sociologic Question with Its Basic Explanation Dependent on Biologic and Medical Principles*. J.B. Lippincott: Philadelphia, PA.
- Wattenberg LW (1991). Inhibition of azoxymethane-induced neoplasia of the large bowel by 3-hydroxy-3,7,11-trimethyl-1,6,10-dodecatriene (nerolidol). *Carcinogen* 12: 151–152.
- Wilkinson JD, Williamson EM (2007). Cannabinoids inhibit human keratinocyte proliferation through a non-CB1/CB2 mechanism and have a potential therapeutic value in the treatment of psoriasis. *J Dermatol Sci* 45: 87–92.
- Wilkinson JD, Whalley BJ, Baker D, Pryce G, Constanti A, Gibbons S *et al.* (2003). Medicinal cannabis: is delta-9-tetrahydrocannabinol necessary for all its effects? *J Pharm Pharmacol* 55: 1687–1694.
- Williams SJ, Hartley JP, Graham JD (1976). Bronchodilator effect of delta-1-tetrahydrocannabinol administered by aerosol of asthmatic patients. *Thorax* 31: 720–723.
- Williamson EM (2001). Synergy and other interactions in phytomedicines. *Phytomed* 8: 401–409.
- Wirth PW, Watson ES, ElSohly M, Turner CE, Murphy JC (1980). Anti-inflammatory properties of cannabichromene. *Life Sci* 26: 1991–1995.
- Xi Z-X, Peng X-Q, Li X, Zhang H, Li JG, Gardner EL (2010). Brain cannabinoid CB2 receptors inhibit cocaine self-administration and cocaine-enhanced extracellular dopamine in mice. *Proceedings 20th Annual Symposium on the Cannabinoids. International Cannabinoid Research Society: Lund*, p. 32.
- Yang D, Michel L, Chaumont JP, Millet-Clerc J (1999). Use of caryophyllene oxide as an antifungal agent in an in vitro experimental model of onychomycosis. *Mycopathologia* 148: 79–82.
- Zanelati TV, Biojone C, Moreira FA, Guimaraes FS, Joca SR (2010). Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. *Br J Pharmacol* 159: 122–128.
- Zuardi AW, Guimaraes FS (1997). Cannabidiol as an anxiolytic and antipsychotic. In: Mathre ML (ed.). *Cannabis in Medical Practice: A Legal, Historical and Pharmacological Overview of the Therapeutic Use of Marijuana*. McFarland: Jefferson, NC, pp. 133–141.
- Zuardi AW, Rodrigues JA, Cunha JM (1991). Effects of cannabidiol in animal models predictive of antipsychotic activity. *Psychopharmacol* 104: 260–264.
- Zuardi AW, Crippa JA, Hallak JE, Moreira FA, Guimaraes FS (2006). Cannabidiol, a *Cannabis sativa* constituent, as an antipsychotic drug. *Braz J Med Biol Res* 39: 421–429.

MINI-REVIEW

Open Access

# An Update on Non-CB<sub>1</sub>, Non-CB<sub>2</sub> 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<sub>1</sub>), which is the most abundant neuromodulatory receptor in the brain, and the cannabinoid receptor type 2 (CB<sub>2</sub>), 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<sub>1</sub>, non-CB<sub>2</sub> 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<sub>1</sub>) and cannabinoid receptor type 2 (CB<sub>2</sub>), 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.<sup>1</sup> 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 phys-

iological 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.<sup>2</sup>

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

© Paula Morales and Patricia H. Reggio 2017; Published by Mary Ann Liebert, Inc. This is an Open Access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



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.<sup>3,4</sup>

### 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.<sup>4</sup> Despite this fact, inconsistencies in pharmacological data in the literature are hampering their categorization.<sup>5,6</sup>

The cannabinoid-related class A GPCR GPR55 displays low sequence identity with CB<sub>1</sub> and CB<sub>2</sub> (~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.<sup>7–9</sup> This receptor displays G-protein coupling promiscuity associating with G<sub>α13</sub>,<sup>8,10</sup> G<sub>αq/11</sub>,<sup>11</sup> G<sub>α12</sub>,<sup>11</sup> or G<sub>α12/13</sub><sup>8,12</sup> depending on the cell line or tissue. GPR55 has been implicated in different physiopathological conditions such as cancer,<sup>13–15</sup> pain,<sup>11,16,17</sup> metabolic disorders,<sup>18,19</sup> vascular functions,<sup>20,21</sup> bone physiology,<sup>22</sup> and motor coordination.<sup>23</sup>

The phospholipid lysophosphatidylinositol (LPI) is considered the endogenous GPR55 ligand.<sup>8,24,25</sup> In fact, GPR55 has also been named the LPI1 receptor.<sup>26</sup> Numerous CB<sub>1</sub> and CB<sub>2</sub> ligands have also been reported to act as GPR55 modulators.<sup>6,27–29</sup> However, significant pharmacological discrepancies have been found depending on the tested functional outcome.<sup>6</sup> For instance, the well-known phytocannabinoid Δ<sup>9</sup>-tetrahydrocannabinol (Δ<sup>9</sup>-THC) displayed activation of GPR55 according to certain reports,<sup>10,11</sup> while it was unable to exert any effect in other functional assays.<sup>24,30</sup> Cannabinoid ligands reported to be recognized by GPR55 and their intriguing pharmacology have been recently reviewed elsewhere.<sup>31</sup>

Although its sequence presents low identity with CB<sub>1</sub> and CB<sub>2</sub> (~13% and 8%), GPR18 has also been tightly associated with the ECS.<sup>4,32</sup> 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.<sup>33,34</sup> Initially, GPR18 was found to couple to G<sub>αi/o</sub>; however, subsequent find-

ings suggested the participation of the G<sub>αq/11</sub> transduction pathway as well.<sup>34–36</sup> Different reports have shown the therapeutic potential of this target in the treatment of pathologies such as intraocular pressure,<sup>37</sup> cancer,<sup>38</sup> or metabolic disorders<sup>39</sup> among others.

N-arachidonoyl glycine (NAGly) has been suggested to be the endogenous GPR18 ligand by several research groups.<sup>32,34</sup> However, other researchers were not able to confirm these data.<sup>40</sup> 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.<sup>41</sup> In addition, and despite the pharmacological divergences observed among some reports, GPR18 has been shown to recognize an array of CB<sub>1</sub> and/or CB<sub>2</sub> ligands of endogenous, phytogenic, or synthetic nature (reviewed by others).<sup>39,42</sup>

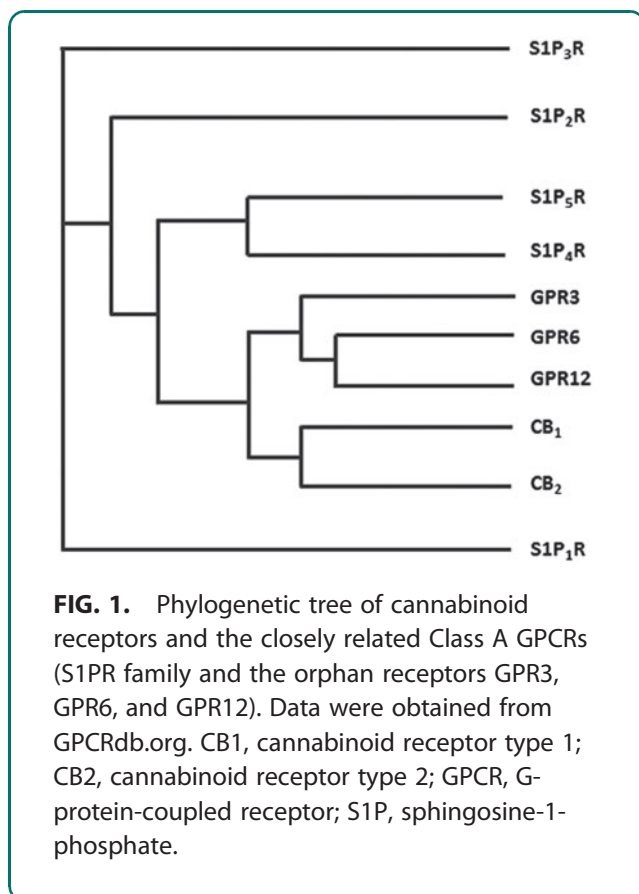
The pharmacological discrepancies on the appraisal of cannabinoids in these two receptors, as well as the lack of selective ligands targeting them, are delaying an insightful understanding of the relation of GPR55 and GPR18 with the ECS. These inconsistencies, which may rely on intrinsic properties of 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,<sup>43</sup> 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<sub>1</sub> and CB<sub>2</sub> (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).<sup>44,45</sup> Because of their phylogenetic proximity, these orphan receptors share common conserved residues and unique sequence motifs with CB<sub>1</sub> and CB<sub>2</sub>.<sup>46</sup> According to Fredriksson et al. these orphan receptors may share a common ancestor with the cannabinoid receptors since they share the same chromosomal positions.<sup>45</sup>

GPR3, GPR6, and GPR12, which share over 60% of sequence similarity, were first cloned in 1995.<sup>47,48</sup> These receptors constitutively activate adenylate cyclase by coupling to G<sub>zs</sub> proteins. In fact, different





groups have reported that when expressed in diverse cell lines, they can stimulate adenylate cyclase to levels similar in amplitude to agonist-activated GPCRs.<sup>47,49,50</sup> In addition to  $G_{\alpha_s}$ , GPR6 and GPR12 have also been suggested to couple to  $G_{\alpha_i/o}$ ,<sup>51,52</sup> 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.<sup>49</sup> This family of constitutively active GPCRs is involved in neuronal differentiation and growth, as well as in the formation of synaptic contacts.<sup>49</sup> Therefore, their role in different neurological processes such as neurite outgrowth,<sup>49</sup> Alzheimer's disease,<sup>53–57</sup> development of cerebellar granule neurons,<sup>58,59</sup> neuropathic pain,<sup>60</sup> early phases of cocaine reinforcement,<sup>61</sup> emotional-like responses,<sup>62</sup> instrumental learning,<sup>63</sup> or Parkinson's disease<sup>64,65</sup> has been studied. Other pathophysiological conditions such as oocyte maturation,<sup>66,67</sup> dyslipidemia,<sup>68</sup> and cell proliferation<sup>69</sup> may also be impacted by the modulation of some of these receptors.

The bioactive lipids, sphingosine-1-phosphate<sup>50,52</sup> and/or sphingosylphosphorylcholine,<sup>51</sup> have been pro-

posed 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.<sup>30,70,71</sup> 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,<sup>72</sup> acting as a  $\beta$ -arrestin2 inverse agonist of both receptors. This functionality is of high interest in the GPR3 field because  $\beta$ -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  $\gamma$ -secretase.<sup>56,57</sup> 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.<sup>30,72</sup>

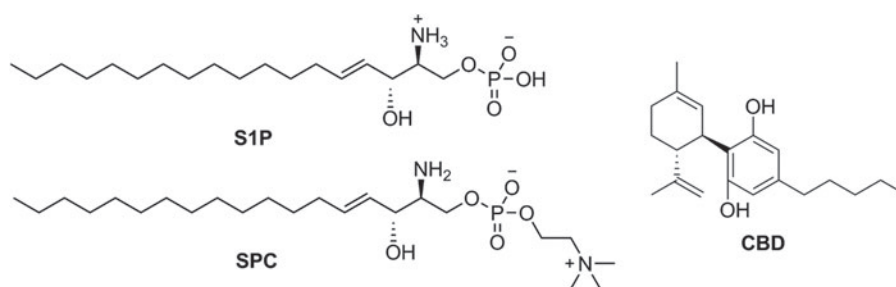
So, a relationship between the cannabinoids and the orphan receptors GPR3, GPR6, and GPR12 has been evidenced. Nonetheless, extensive research and more pharmacological tools are needed to extract significant conclusions about the association of these receptors with the ECS and its ligands.

### 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<sub>1</sub> and CB<sub>2</sub>.<sup>73–75</sup> This fact led to the identification of novel targets commonly referred to as the alkylindole (AI)-sensitive receptors.<sup>74,76,77</sup> These cannabinoid-related receptors are modulated by AI derivatives, but not by other classes of cannabinoid ligands.<sup>76</sup> Diverse evidence suggests that the AI-sensitive receptors are  $G_{\alpha_s}$ -protein coupled receptors that are mainly expressed in microglia and astrocytomas.<sup>76–79</sup> 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.<sup>77,78</sup> 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.<sup>78</sup> These compounds display affinities in the nanomolar range when competing with [<sup>3</sup>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<sub>1</sub> and CB<sub>2</sub> receptors.<sup>80</sup> Compound ST-11 stands out from this study because of its potency at AI-sensitive receptors, while not interacting with CB<sub>1</sub> and CB<sub>2</sub> 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.<sup>81</sup>

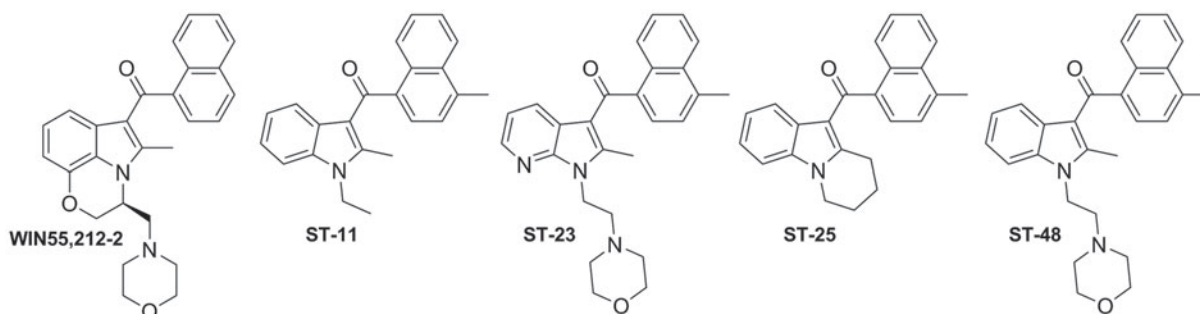
Even though extensive research is clearly needed to understand the pathophysiological function of these receptors, reported data suggest that AI-sensitive receptor agonists could represent a novel class of potential brain cancer antitumor drugs.

### Cannabinoid-Related Oligomers

Numerous studies have shown that GPCRs, cannabinoid receptors among them, can exist and function as dimers or complexes of higher order.<sup>82–85</sup> 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.

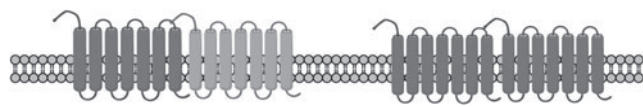
For the CB<sub>1</sub> receptor, heteromers have been suggested to exist under certain physiological conditions with serotonin,<sup>86</sup> angiotensin,<sup>87</sup> opioid,<sup>88–90</sup> GPR55,<sup>91</sup> somatostatin,<sup>92</sup> orexin,<sup>93,94</sup> dopamine,<sup>95–97</sup> and adenosine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



**FIG. 3.** Alkyndole 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 <sub>1</sub> –D2	95,97	CB <sub>1</sub> –CB <sub>1</sub>	104–106
CB <sub>1</sub> –A <sub>2A</sub>	98	CB <sub>2</sub> –CB <sub>2</sub>	107–109
CB <sub>1</sub> –5HT <sub>2A</sub>	86		
CB <sub>1</sub> –AT <sub>1</sub>	87		
CB <sub>1</sub> –GPR55	91		
CB <sub>1</sub> –SST5	92		
CB <sub>1</sub> –OX1	94		
CB <sub>1</sub> –OX2	93		
CB <sub>1</sub> –μOR	90		
CB <sub>1</sub> –δOR	88		
CB <sub>1</sub> –CB <sub>2</sub>	99		
CB <sub>2</sub> –GPR55	100,101		
CB <sub>2</sub> –5HT <sub>1A</sub>	102		
CB <sub>2</sub> –CXCR4	103		

CB<sub>1</sub>, cannabinoid receptor type 1; CB<sub>2</sub>, 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<sub>1</sub> homodimers than on their CB<sub>2</sub> counterparts. The presence of CB<sub>1</sub> receptor homodimers has been reported in different biological tissues,<sup>104–106</sup> but their functional role has not been determined. In contrast, CB<sub>2</sub> homodimers have been evidenced,<sup>107–109</sup> 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.<sup>110–113</sup> Despite their poor pharmacokinetic properties,<sup>114</sup> 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,<sup>115,116</sup> dopamine,<sup>117,118</sup> and histamine<sup>119</sup> are some of the receptors for which a bivalent compound provided higher activity than their monomer counterparts. CB<sub>1</sub> homobivalent<sup>120–122</sup> and heterobivalent<sup>123–125</sup> 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.<sup>126</sup> 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<sub>3</sub>,<sup>127</sup> the muscarinic acetylcholine receptors M1 and M4,<sup>128</sup> and the serotonin receptors 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub><sup>129</sup> among others. In addition, phytocannabinoids such as Δ<sup>9</sup>-THC and CBD have been shown to modulate the μ- and δ-opioid receptors,<sup>130</sup> while other plant-derived compounds such as CBG (cannabigerol) and Δ<sup>9</sup>-THCV (tetrahydrocannabinavarin) display activity at the 5-HT<sub>1A</sub> receptor.<sup>131,132</sup> Likewise, synthetic cannabinoids, such as the CB<sub>1</sub> inverse agonists taranabant (MK-0364) and rimonabant (SR141716), have also displayed activity in well-established targets. These include the adenosine A<sub>3</sub> and the tachykinin NK2 receptors.<sup>133</sup>

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<sub>1</sub> and/or CB<sub>2</sub> 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<sub>1</sub> and CB<sub>2</sub>, 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.<sup>4</sup> However, there is no available evidence since they do not meet some of the criteria established by the International Union of Pharmacology.<sup>4,70</sup>



## Conclusions

Two cannabinoid receptors, CB<sub>1</sub> and CB<sub>2</sub>, 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.<sup>1</sup> Great efforts are being made to structurally understand these receptors; in fact, CB<sub>1</sub> in its inactive<sup>134,135</sup> and active<sup>136</sup> 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<sub>3</sub> receptor has yet been confirmed.<sup>2</sup>

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<sub>1</sub>, non-CB<sub>2</sub> GPCRs in the modulation of the ECS.

## Acknowledgments

The authors acknowledge research support from NIH/NIDA grants R01 DA003934 and K05 DA021358 (P.H.R.).

## Author Disclosure Statement

No competing financial interests exist.

## References

1. Pertwee RG. Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities. *Philos Trans R Soc B* 2012;367:3353–3363.
2. IUPHAR BPS Guide to PHARMACOLOGY. Cannabinoid receptors. [www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=13](http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=13) (accessed June 7, 2017).
3. Morales P, Hurst DP, Reggio PH. In: Molecular targets of the phytocannabinoids: a complex picture. In: Kinghorn AD, Gibbons S, eds. *Phytocannabinoids: Unraveling the Complex Chemistry and Pharmacology of Cannabis sativa*. Cham, Switzerland: Springer International Publishing, 2017; Vol. 103, pp. 103–131.
4. Pertwee RG, Howlett AC, Abood ME, et al. International union of basic and clinical pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB<sub>1</sub> and CB<sub>2</sub>. *Pharmacol Rev* 2010;62:588–631.
5. IUPHAR BPS Guide to PHARMACOLOGY. GPR18, GPR55 and GPR119. [www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=114](http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=114) (accessed June 7, 2017).
6. Ross RA. The enigmatic pharmacology of GPR55. *Trends Pharmacol Sci* 2009;30:156–163.
7. Balenga NAB, Henstridge CM, Kargl J, et al. Pharmacology, signaling and physiological relevance of the G protein-coupled receptor 55. In: Neubig RR, ed. *Pharmacology of G Protein Coupled Receptors*. San Diego, CA: Advances in Pharmacology, Volume 62, Elsevier Inc., 2011, pp. 251–277.
8. Henstridge CM, Balenga NAB, Ford LA, et al. The GPR55 ligand L- $\alpha$ -lysophosphatidylinositol promotes RhoA-dependent Ca<sup>2+</sup> signaling and NFAT activation. *FASEB J* 2009;23:183–193.
9. Sawzdargo M, Nguyen T, Lee DK, et al. Identification and cloning of three novel human G protein-coupled receptor genes GPR52, GPR53 and GPR55: GPR55 is extensively expressed in human brain. *Brain research. Mol Brain Res* 1999;64:193–198.
10. Ryberg E, Larsson N, Sjögren S, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 2007;152:1092–1101.
11. Lauckner JE, Jensen JB, Chen H-Y, et al. GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc Natl Acad Sci U S A* 2008;105:2699–2704.
12. Henstridge CM, Balenga NA, Schröder R, et al. GPR55 ligands promote receptor coupling to multiple signalling pathways. *Br J Pharmacol* 2010;160:604–614.
13. Andradas C, Blasco-Benito S, Castillo-Lluis S, et al. Activation of the orphan receptor GPR55 by lysophosphatidylinositol promotes metastasis in triple-negative breast cancer. *Oncotarget* 2016;7:47565–47575.
14. Hofmann NA, Yang J, Trauger SA, et al. The GPR55 agonist, L- $\alpha$ -lysophosphatidylinositol, mediates ovarian carcinoma cell-induced angiogenesis. *Br J Pharmacol* 2015;172:4107–4118.
15. Kargl J, Andersen L, Hasenöhr C, et al. GPR55 promotes migration and adhesion of colon cancer cells indicating a role in metastasis. *Br J Pharmacol* 2016;173:142–154.
16. Carey LM, Gutierrez T, Deng L, et al. Inflammatory and neuropathic nociception is preserved in GPR55 knockout mice. *Sci Rep* 2017;7:944.
17. Staton PC, Hatcher JP, Walker DJ, et al. The putative cannabinoid receptor GPR55 plays a role in mechanical hyperalgesia associated with inflammatory and neuropathic pain. *Pain* 2008;139:225–236.
18. Tudurí E, Imbernon M, Hernández-Bautista RJ. GPR55: a new promising target for metabolism? *J Mol Endocrinol* 2017;58:R191–R202.
19. Tudurí E, López M, Diéguez C, et al. GPR55 and the regulation of glucose homeostasis. *Int J Biochem Cell Biol* 2017;88:204–207.
20. AlSuleimani YM, Hiley CR. The GPR55 agonist lysophosphatidylinositol relaxes rat mesenteric resistance artery and induces calcium release in rat mesenteric artery endothelial cells. *Br J Pharmacol* 2015;172:3043–3057.



21. Bondarenko AI, Montecucco F, Panasiuk O, et al. GPR55 agonist lysophosphatidylinositol and lysophosphatidylcholine inhibit endothelial cell hyperpolarization via GPR-independent suppression of Na<sup>+</sup>-Ca<sup>2+</sup> exchanger and endoplasmic reticulum Ca<sup>2+</sup> refilling. *Vascul Pharmacol* 2017;89:39–48.
22. Whyte LS, Ryberg E, Sims NA, et al. The putative cannabinoid receptor GPR55 affects osteoclast function in vitro and bone mass in vivo. *Proc Natl Acad Sci U S A* 2009;106:16511–16516.
23. Wu C-S, Chen H, Sun H, et al. GPR55, a G-protein coupled receptor for lysophosphatidylinositol, plays a role in motor coordination. *PLoS One* 2013;8:e60314.
24. Oka S, Nakajima K, Yamashita A, et al. Identification of GPR55 as a lysophosphatidylinositol receptor. *Biochem Biophys Res Commun* 2007;362:928–934.
25. Oka S, Toshida T, Maruyama K, et al. 2-Arachidonoyl-sn-glycero-3-phosphoinositol: a possible natural ligand for GPR55. *J Biochem* 2009;145:13–20.
26. Kihara Y, Maceyka M, Spiegel S, et al. Lysophospholipid receptor nomenclature review: IUPHAR review 8. *Br J Pharmacol* 2014;171:3575–3594.
27. Moriconi A, Cerbara I, Maccarrone M, et al. GPR55: current knowledge and future perspectives of a purported “Type-3” cannabinoid receptor. *Curr Med Chem* 2010;17:1411–1429.
28. Nevalainen T, Irving AJ. GPR55, a Lysophosphatidylinositol receptor with cannabinoid sensitivity? *Curr Top Med Chem* 2010;1:799–813.
29. Sharir H, Abood ME. Pharmacological characterization of GPR55, a putative cannabinoid receptor. *Pharmacol Ther* 2010;126:301–313.
30. Yin H, Chu A, Li W, et al. Lipid G protein-coupled receptor ligand identification using beta-arrestin PathHunter assay. *J Biol Chem* 2009;284:12328–12338.
31. Morales P, Jagerovic N. Advances towards the discovery of GPR55 ligands. *Curr Med Chem* 2016;23:2087–2100.
32. McHugh D, Hu SSJ, Rimmerman N, et al. N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. *BMC Neurosci* 2010;11:44.
33. Gantz I, Muraoka A, Yang YK, et al. Cloning and chromosomal localization of a gene (GPR18) encoding a novel seven transmembrane receptor highly expressed in spleen and testis. *Genomics* 1997;42:462–466.
34. Kohno M, Hasegawa H, Inoue A, et al. Identification of N-arachidonoylglycine as the endogenous ligand for orphan G-protein-coupled receptor GPR18. *Biochem Biophys Res Commun* 2006;347:827–832.
35. Console-Bram L, Brailoiu E, Brailoiu GC, et al. Activation of GPR18 by cannabinoid compounds: a tale of biased agonism. *Br J Pharmacol* 2014;171:3908–3917.
36. Takenouchi R, Inoue K, Kambe Y, et al. N-arachidonoyl glycine induces macrophage apoptosis via GPR18. *Biochem Biophys Res Commun* 2012;418:366–371.
37. Miller S, Leishman E, Oehler O, et al. Evidence for a GPR18 role in diurnal regulation of intraocular pressure. *Invest Ophthalmol Vis Sci* 2016;57:6419–6426.
38. Qin Y, Verdegaaal EME, Siderius M, et al. Quantitative expression profiling of G-protein-coupled receptors (GPCRs) in metastatic melanoma: the constitutively active orphan GPCR GPR18 as novel drug target. *Pigment Cell Melanoma Res* 2011;24:207–218.
39. Rajaraman G, Simcocks A, Hryciw DH, et al. G protein coupled receptor 18: a potential role for endocannabinoid signaling in metabolic dysfunction. *Mol Nutr Food Res* 2016;60:92–102.
40. Lu VB, Puhl HL, Ikeda SR. N-arachidonoyl glycine does not activate G protein-coupled receptor 18 signaling via canonical pathways. *Mol Pharmacol* 2013;83:267–282.
41. Chiang N, Dalli J, Colas RA, et al. Identification of resolvin D2 receptor mediating resolution of infections and organ protection. *J Exp Med* 2015;212:1203–1217.
42. McHugh D. GPR18 in microglia: implications for the CNS and endocannabinoid system signalling. *Br J Pharmacol* 2012;167:1575–1582.
43. Lingerfelt MA, Zhao P, Sharir HP, et al. Identification of crucial amino acid residues involved in agonist signaling at the GPR55 receptor. *Biochemistry* 2017;56:473–486.
44. Fredriksson R, Schio HB. The repertoire of G-protein-coupled receptors in fully. *Mol Pharmacol* 2005;67:1414–1425.
45. Fredriksson R, Lagerström MC, Lundin L-G, et al. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol* 2003;63:1256–1272.
46. Morales P, Hurst DP, Reggio PH. Methods for the development of in silico GPCR models. In: Reggio PH, ed. *Cannabinoids and Their Receptors*. London: Methods in Enzymology, Volume 593, Elsevier Inc., 2017, pp. 405–448.
47. Eggerickx D, Denef JF, Labbe O, et al. Molecular cloning of an orphan G-protein-coupled receptor that constitutively activates adenylate cyclase. *Biochem J* 1995;309:837–843.
48. Song Z-H, Modi W, Bonner TI. Molecular cloning and chromosomal localization of human genes encoding three closely related G protein-coupled receptors. *Genomics* 1995;28:347–349.
49. Tanaka S, Ishii K, Kasai K, et al. Neural expression of G protein-coupled receptors GPR3, GPR6, and GPR12 up-regulates cyclic AMP levels and promotes neurite outgrowth. *J Biol Chem* 2007;282:10506–10515.
50. Uhlenbrock K, Gassenhuber H, Kostenis E. Sphingosine 1-phosphate is a ligand of the human gpr3, gpr6 and gpr12 family of constitutively active G protein-coupled receptors. *Cell Signal* 2002;14:941–953.
51. Ignatov A, Lintzel J, Hermans-Borgmeyer I, et al. Role of the G-protein-coupled receptor GPR12 as high-affinity receptor for sphingosylphosphorylcholine and its expression and function in brain development. *J Neurosci* 2003;23:907–914.
52. Ignatov A, Lintzel J, Kreienkamp HJ, et al. Sphingosine-1-phosphate is a high-affinity ligand for the G protein-coupled receptor GPR6 from mouse and induces intracellular Ca<sup>2+</sup> release by activating the sphingosine-kinase pathway. *Biochem Biophys Res Commun* 2003;311:329–336.
53. Benoit ME, Hernandez MX, Dinh ML, et al. C1q-induced LRP1B and GPR6 proteins expressed early in Alzheimer disease mouse models, are essential for the C1q-mediated protection against amyloid neurotoxicity. *J Biol Chem* 2013;288:654–665.
54. Huang Y, Skwarek-Maruszewska A, Horré K, et al. Loss of GPR3 reduces the amyloid plaque burden and improves memory in Alzheimer’s disease mouse models. *Sci Transl Med* 2015;7:309ra164.
55. Nelson CD, Sheng M. Gpr3 stimulates Aβ production via interactions with APP and β-arrestin2. *PLoS One* 2013;8:e74680.
56. Thathiah A, Horré K, Snellinx A, et al. β-arrestin 2 regulates Aβ generation and γ-secretase activity in Alzheimer’s disease. *Nat Med* 2013;19:43–49.
57. Thathiah A, Spittaels K, Hoffmann M, et al. The orphan G protein-coupled receptor 3 modulates amyloid-beta peptide generation in neurons. *Science* 2009;323:946–951.
58. Tanaka S, Shaikh IM, Chiocca EA, et al. The Gs-linked receptor GPR3 inhibits the proliferation of cerebellar granule cells during postnatal development. *PLoS One* 2009;4:1–12.
59. Tanaka S, Miyagi T, Dohi E, et al. Developmental expression of GPR3 in rodent cerebellar granule neurons is associated with cell survival and protects neurons from various apoptotic stimuli. *Neurobiol Dis* 2014;68:215–227.
60. Ruiz-Medina J, Ledent C, Valverde O. GPR3 orphan receptor is involved in neuropathic pain after peripheral nerve injury and regulates morphine-induced antinociception. *Neuropharmacology* 2011;61:43–50.
61. Tourino C, Valjent E, Ruiz-Medina J, et al. The orphan receptor GPR3 modulates the early phases of cocaine reinforcement. *Br J Pharmacol* 2012;167:892–904.
62. Valverde O, Celerier E, Baranyi M, et al. GPR3 receptor, a novel actor in the emotional-like responses. *PLoS One* 2009;4:e4704.
63. Lobo MK, Cui Y, Ostlund SB, et al. Genetic control of instrumental conditioning by striatopallidal neuron-specific S1P receptor Gpr6. *Nat Neurosci* 2007;10:1395–1397.
64. Oeckl P, Fenger B, Ferger B. G-protein coupled receptor 6 deficiency alters striatal dopamine and cAMP concentrations and reduces dyskinesia in a mouse model of Parkinson’s disease. *Exp Neurol* 2014;257:1–9.
65. Oeckl P, Fenger B. Increased susceptibility of G-protein coupled receptor 6 deficient mice to MPTP neurotoxicity. *Neuroscience* 2016;337:218–223.
66. Hinckley M, Vaccari S, Horner K, et al. The G-protein-coupled receptors GPR3 and GPR12 are involved in cAMP signaling and maintenance of meiotic arrest in rodent oocytes. *Dev Biol* 2005;287:249–261.



67. Mehlmann LM, Saeki Y, Tanaka S, et al. The Gs-linked receptor GPR3 maintains meiotic arrest in mammalian oocytes. *Science* 2004;306: 1947–1950.
68. Bjursell M, Gerdin AK, Jönsson M, et al. G protein-coupled receptor 12 deficiency results in dyslipidemia and obesity in mice. *Biochem Biophys Res Commun* 2006;348:359–366.
69. Lu X, Zhang N, Meng B, et al. Involvement of GPR12 in the regulation of cell proliferation and survival. *Mol Cell Biochem* 2012;366:101–110.
70. Davenport AP, Alexander SPH, Sharman JL, et al. International Union of Basic and Clinical Pharmacology. LXXXVIII. G protein-coupled receptor list: recommendations for new pairings with cognate ligands. *Pharmacol Rev* 2013;65:967–986.
71. Southern C, Cook JM, Neetoo-Isseljee Z, et al. Screening beta-arrestin recruitment for the identification of natural ligands for orphan G-protein-coupled receptors. *J Biomol Screen* 2013;18:599–609.
72. Laun AS, Song Z-H. GPR3 and GPR6, novel molecular targets for cannabidiol. *Biochem Biophys Res Commun* 2017;490:17–21.
73. Abood ME, Sorensen RG, Stella N. Endocannabinoids: actions at non-CB1/CB2 cannabinoid receptors. Springer: New York, 2013.
74. Hájos N, Ledent C, Freund TF. Novel cannabinoid-sensitive receptor mediates inhibition of glutamatergic synaptic transmission in the hippocampus. *Neuroscience* 2001;106:1–4.
75. Monory K, Tzavara ET, Lexime J, et al. Novel, not adenylyl cyclase-coupled cannabinoid binding site in cerebellum of mice. *Biochem Biophys Res Commun* 2002;292:231–235.
76. Breivogel CS, Griffin G, Di Marzo V, et al. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Mol Pharmacol* 2001;60:155–163.
77. Fung S, Cherry AE, Xu C, et al. Alkylindole-sensitive receptors modulate microglial cell migration and proliferation. *Glia* 2015;63:1797–1808.
78. Fung S, Xu C, Hamel E, et al. Novel indole-based compounds that differentiate alkylindole-sensitive receptors from cannabinoid receptors and microtubules: characterization of their activity on glioma cell migration. *Pharmacol Res* 2017;115:233–241.
79. Stella N. Cannabinoid and cannabinoid-like receptors in microglia, astrocytes, and astrocytomas. *Glia* 2010;58:1017–1030.
80. Cudaback E, Marrs W, Moeller T, et al. The expression level of CB1 and CB2 receptors determines their efficacy at inducing apoptosis in astrocytomas. *PLoS One* 2010;5:e8702.
81. Cherry AE, Haas BR, Naydenov AV, et al. ST-11: a new brain-penetrant microtubule-destabilizing agent with therapeutic potential for glioblastoma multiforme. *Mol Cancer Ther* 2016;15:2018–2029.
82. Farran B. An Update on the physiological and therapeutic relevance of GPCR oligomers. *Pharmacol Res* 2017;117:303–327.
83. Franco R, Martínez-Pinilla E, Lanciego JL, et al. Basic pharmacological and structural evidence for class A G-protein-coupled receptor heteromerization. *Front Pharmacol* 2016;7:1–10.
84. Scarselli M, Annibale P, McCormick PJ, et al. Revealing G-protein-coupled receptor oligomerization at the single-molecule level through a nanoscopic lens: methods, dynamics and biological function. *FEBS J* 2016;283:1197–1217.
85. Vischer HF, Castro M, Pin J-P. G protein-coupled receptor multimers: a question still open despite the use of novel approaches. *Mol Pharmacol* 2015;88:561–571.
86. Viñals X, Moreno E, Lanfumey L, et al. Cognitive impairment induced by Delta9-tetrahydrocannabinol occurs through heteromers between cannabinoid CB1 and serotonin 5-HT2A receptors. *PLoS Biol* 2015;13:e1002194.
87. Rozenfeld R, Gupta A, Gagnidze K, et al. AT1R-CB1R heteromerization reveals a new mechanism for the pathogenic properties of angiotensin II. *EMBO J* 2011;30:2350–2363.
88. Bushlin I, Gupta A, Stockton SD, et al. Dimerization with cannabinoid receptors allosterically modulates delta opioid receptor activity during neuropathic pain. *PLoS One* 2012;7:e49789.
89. Fujita W, Gomes I, Devi LA. Revolution in GPCR signalling: opioid receptor heteromers as novel therapeutic targets: IUPHAR review 10. *Br J Pharmacol* 2014;171:4155–4176.
90. Hojo M, Sudo Y, Ando Y, et al. mu-Opioid receptor forms a functional heterodimer with cannabinoid CB1 receptor: electrophysiological and FRET assay analysis. *J Pharmacol Sci* 2008;108:308–319.
91. Martínez-Pinilla E, Reyes-Resina I, Oñatibia-Astibia A, et al. CB1 and GPR55 receptors are co-expressed and form heteromers in rat and monkey striatum. *Exp Neurol* 2014;261:44–52.
92. Zou S, Somvanshi RK, Kumar U. Somatostatin receptor 5 is a prominent regulator of signaling pathways in cells with coexpression of cannabinoid receptors 1. *Neuroscience* 2017;340:218–231.
93. Jäntti MH, Mandrika I, Kukkonen JP. Human orexin/hypocretin receptors form constitutive homo- and heteromeric complexes with each other and with human CB1 cannabinoid receptors. *Biochem Biophys Res Commun* 2014;445:486–490.
94. Ward RJ, Pediani JD, Milligan G. Heteromultimerization of cannabinoid CB(1) receptor and orexin OX(1) receptor generates a unique complex in which both protomers are regulated by orexin A. *J Biol Chem* 2011; 286:37414–37428.
95. Glass M, Felder CC. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *J Neurosci* 1997;17:5327–5333.
96. Kearn CS, Blake-Palmer K, Daniel E, et al. Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? *Mol Pharmacol* 2005;67:1697–1704.
97. Przybyla JA, Watts VJ. Ligand-induced regulation and localization of cannabinoid CB1 and dopamine D2L receptor heterodimers. *J Pharmacol Exp Ther* 2010;332:710–719.
98. Moreno E, Chiarlone A, Medrano M, et al. Singular location and signaling profile of adenosine A2A-cannabinoid CB1 receptor heteromers in the dorsal striatum. *Neuropsychopharmacology* 2017 [Epub ahead of print]; DOI: 10.1038/npp.2017.
99. Callén L, Moreno E, Barroso-Chinea P, et al. Cannabinoid receptors CB1 and CB2 form functional heteromers in brain. *J Biol Chem* 2012;287:20851–20865.
100. Balenga NA, Martínez-Pinilla E, Kargl J, et al. Heteromerization of GPR55 and cannabinoid CB2 receptors modulates signaling. *Br J Pharmacol* 2014;171:5387–5406.
101. Moreno E, Andradás C, Medrano M, et al. Targeting CB2-GPR55 receptor heteromers modulates cancer cell signaling. *J Biol Chem* 2014;289:21960–21972.
102. Pazos MR, Mohammed N, Lafuente H, et al. Mechanisms of cannabidiol neuroprotection in hypoxic-ischemic newborn pigs: role of 5HT(1A) and CB2 receptors. *Neuropharmacology* 2013;71:282–291.
103. Coke CJ, Scarlett KA, Chetram MA, et al. Simultaneous activation of induced heterodimerization between CXCR4 chemokine receptor and cannabinoid receptor 2 (CB2) reveals a mechanism for regulation of tumor progression. *J Biol Chem* 2016;291:9991–10005.
104. Mackie K. Cannabinoid receptor homo- and heterodimerization. *Life Sci* 2005;77:1667–1673.
105. Wager-Miller J, Westenbroek R, Mackie K. Dimerization of G protein-coupled receptors: CB1 cannabinoid receptors as an example. *Chem Phys Lipids* 2002;121:83–89.
106. Xu W, Filippula S, Mercier R, et al. Purification and mass spectroscopic analysis of human CB1 cannabinoid receptor functionally expressed using the baculovirus system. *J Pept Res* 2005;66:138–150.
107. Filippula S, Yaddanapudi S, Mercier R, et al. Purification and mass spectroscopic analysis of human CB2 cannabinoid receptor functionally expressed using the baculovirus system. *J Pept Res* 2004;64:225–236.
108. Singh J, Song Z-H, Reggio PH. Structure of a cannabinoid receptor subtype 2 homodimer determined by cysteine and homobifunctional crosslinking experiments combined with computational studies. *Biophys J* 2012;102:244a.
109. Zvonok N, Yaddanapudi S, Williams J, et al. Comprehensive proteomic mass spectrometric characterization of human cannabinoid CB2 receptor. *J Proteome Res* 2007;6:2068–2079.
110. Ferré S, Casadó V, Devi LA, et al. G protein-coupled receptor oligomerization revisited: functional and pharmacological perspectives. *Pharmacol Rev* 2014;66:413–434.
111. Franco R, Martínez-Pinilla E, Ricobaraza A, et al. Challenges in the development of heteromer-GPCR-based drugs. *Prog Mol Biol Transl Sci* 2013;117:143–162.
112. Hiller C, Kühhorn J, Gmeiner P. Class A G-protein-coupled receptor (GPCR) dimers and bivalent ligands. *J Med Chem* 2013;56:6542–6559.
113. Hübner H, Schellhorn T, Gienger M, et al. Structure-guided development of heterodimer-selective GPCR ligands. *Nat Commun* 2016;7:12298.
114. Shonberg J, Scammells PJ, Capuano B. Design strategies for bivalent ligands targeting GPCRs. *ChemMedChem* 2011;6:963–974.



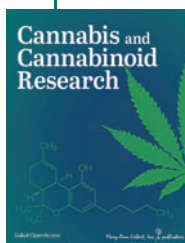
115. Fulton BS, Knapp BL, Bidlack JM, et al. Effect of linker substitution on the binding of butorphan univalent and bivalent ligands to opioid receptors. *Bioorg Med Chem Lett* 2010;20:1507–1509.
116. Zhang B, Zhang T, Sromek AW, et al. Synthesis and binding affinity of novel mono- and bivalent morphinan ligands for  $\kappa$ ,  $\mu$ , and  $\delta$  opioid receptors. *Bioorg Med Chem* 2011;19:2808–2816.
117. Gogoi S, Biswas S, Modi G, et al. Novel bivalent ligands for D2/D3 dopamine receptors: significant cooperative gain in D2 affinity and potency. *ACS Med Chem Lett* 2012;3:991–996.
118. Huber D, Löber S, Hübner H, et al. Bivalent molecular probes for dopamine D2-like receptors. *Bioorg Med Chem* 2012;20:455–466.
119. Birnkammer T, Spickenreither A, Brunskole I, et al. The bivalent ligand approach leads to highly potent and selective acylguanidine-type histamine H<sub>2</sub> receptor agonists. *J Med Chem* 2012;55:1147–1160.
120. Fernández-Fernández C, Decara J, Bermúdez-Silva FJ, et al. Description of a bivalent cannabinoid ligand with hypophagic properties. *Arch Pharm* 2013;346:171–179.
121. Huang G, Pemp D, Stadtmüller P, et al. Design, synthesis and in vitro evaluation of novel uni- and bivalent ligands for the cannabinoid receptor type 1 with variation of spacer length and structure. *Bioorg Med Chem Lett* 2014;24:4209–4214.
122. Zhang Y, Gilliam A, Maitra R, et al. Synthesis and biological evaluation of bivalent ligands for the cannabinoid 1 receptor. *J Med Chem* 2010;53:7048–7060.
123. Fernández-Fernández C, Callado LF, Girón R, et al. Combining rimonabant and fentanyl in a single entity: preparation and pharmacological results. *Drug Design Devel Ther* 2014;8:263–277.
124. Le Naour M, Akgün E, Yekkirala A, et al. Bivalent ligands that target  $\mu$  opioid (MOP) and cannabinoid1 (CB1) receptors are potent analgesics devoid of tolerance. *J Med Chem* 2013;56:5505–5513.
125. Perrey DA, Gilmour BP, Thomas BF, et al. Toward the development of bivalent ligand probes of cannabinoid CB1 and Orexin OX1 receptor heterodimers. *ACS Med Chem Lett* 2014;5:634–638.
126. Glass M, Govindpani K, Furkert DP, et al. One for the price of two...are bivalent ligands targeting cannabinoid receptor dimers capable of simultaneously binding to both receptors? *Trends Pharmacol Sci* 2016;xx:1–11.
127. Lane JR, Beukers MW, Mulder-Krieger T, et al. The endocannabinoid 2-arachidonylglycerol is a negative allosteric modulator of the human A3 adenosine receptor. *Biochem Pharmacol* 2010;79:48–56.
128. Christopoulos A, Wilson K. Interaction of anandamide with the M1 and M4 muscarinic acetylcholine receptors. *Brain Res* 2001;915:70–78.
129. Boger DL, Patterson JE, Jin Q. Structural requirements for 5-HT<sub>2A</sub> and 5-HT<sub>1A</sub> serotonin receptor potentiation by the biologically active lipid oleamide. *Proc Natl Acad Sci U S A* 1998;95:4102–4107.
130. Kathmann M, Flau K, Redmer A, et al. Cannabidiol is an allosteric modulator at  $\mu$ - and delta-opioid receptors. *Naunyn Schmiedebergs Arch Pharmacol* 2006;372:354–361.
131. Cascio MG, Gauson LA, Stevenson LA, et al. Evidence that the plant cannabinoid cannabigerol is a highly potent  $\alpha$ 2-adrenoceptor agonist and moderately potent 5HT<sub>1A</sub> receptor antagonist. *Br J Pharmacol* 2010;159:129–141.
132. Cascio MG, Zamberletti E, Marini P, et al. The phytocannabinoid,  $\Delta$ 9-tetrahydrocannabivarin, can act through 5-HT<sub>1A</sub> receptors to produce antipsychotic effects. *Br J Pharmacol* 2015;172:1305–1318.
133. Fong TM, Shearman LP, Stribling DS, et al. Pharmacological efficacy and safety profile of taranabant in preclinical species. *Drug Dev Res* 2009;70:349–362.
134. Hua T, Vemuri K, Pu M, et al. Crystal structure of the human cannabinoid CB<sub>1</sub>. *Cell* 2016;167:750–762.
135. Shao Z, Yin J, Chapman K, et al. High-resolution crystal structure of the human CB<sub>1</sub> cannabinoid receptor. *Nature* 2016;540:602–606.
136. Hua T, Vemuri K, Nikas SP, et al. Crystal structures of agonist-bound human cannabinoid receptor CB<sub>1</sub>. *Nature* 2017;547:468–471.

**Cite this article as:** Morales P, Reggio PH (2017) An update on non-CB<sub>1</sub>, non-CB<sub>2</sub> cannabinoid related G-protein-coupled receptors, *Cannabis and Cannabinoid Research* 2:1, 265–273, DOI: 10.1089/can.2017.0036.

#### Abbreviations Used

Al = alkylindole  
CB<sub>1</sub> = cannabinoid receptor type 1  
CB<sub>2</sub> = cannabinoid receptor type 2  
CBD = cannabidiol  
ECS = endocannabinoid system  
GPCR = G-protein-coupled receptor  
LPI = lysophosphatidylinositol  
THC = tetrahydrocannabinol

#### Publish in Cannabis and Cannabinoid Research



- Immediate, unrestricted online access
- Rigorous peer review
- Compliance with open access mandates
- Authors retain copyright
- Highly indexed
- Targeted email marketing

liebertpub.com/can





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

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

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

## OPEN ACCESS

### Edited by:

Luis F. Callado,  
Universidad del País Vasco, Spain

### Reviewed by:

Styliani Vlachou,  
Dublin City University, Ireland  
Thomas Heinbockel,  
Howard University, United States

### \*Correspondence:

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

### Specialty section:

This article was submitted to  
Neuropharmacology,  
a section of the journal  
Frontiers in Pharmacology

Received: 27 August 2018

Accepted: 07 December 2018

Published: 08 January 2019

### Citation:

Guerrero-Alba R, Barragán-Iglesias P, González-Hernández A, Valdez-Morales EE, Granados-Soto V, Condés-Lara M, Rodríguez MG and Marichal-Cancino BA (2019) Some Prospective Alternatives for Treating Pain: The Endocannabinoid System and Its Putative Receptors GPR18 and GPR55. *Front. Pharmacol.* 9:1496. doi: 10.3389/fphar.2018.01496

**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<sub>1</sub> and CB<sub>2</sub>) 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<sub>1</sub>/CB<sub>2</sub> 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<sub>1</sub> and CB<sub>2</sub> 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 ( $\sim 1$  m/s), sensing and transducing thermal, chemical and mechanical stimuli; and (2) thinly myelinated A $\delta$ -fibers that show fast conduction velocity ( $\sim 5$ – $30$  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 ( $\text{Na}^+$ -TTXr) voltage-gated sodium ( $\text{Na}^+$ ) channels, purinergic P $_2$ X receptors, serotonin (5-HT $_3$ ) channel receptor, and calcium ( $\text{Ca}^{2+}$ ) 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

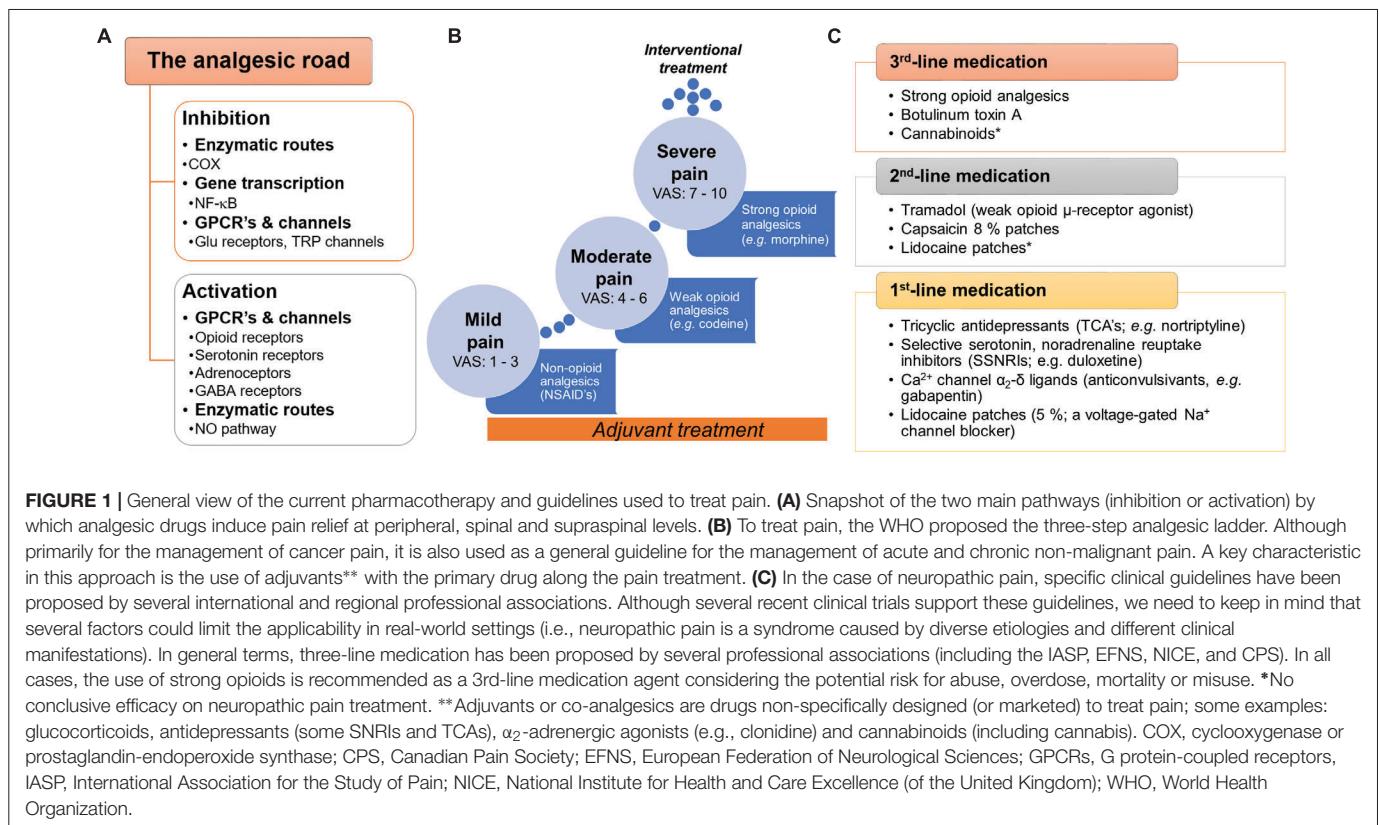
Pain treatment can be categorized as pharmacologic and non-pharmacologic. In the first case, there are a variety of druggable targets in both central and peripheral nervous system commonly used for pain treatment. Analgesics are classified as: (i) non-opioid analgesics; (ii) opioid analgesics; and (iii) 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 ( $\mu$ -,  $\delta$ -, and  $\kappa$ -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 ( $\text{Na}^+$ ) 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



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 United 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-hydroxytryptamine (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 ( $\text{Ca}^{2+}$ )-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<sub>1</sub>) and cannabinoid type-2 (CB<sub>2</sub>) 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.

## ENDOCANNABINOID SYSTEM 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  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) (Gaoni and Mechoulam, 1964), and the subsequent isolation of cannabinoid receptors (CB<sub>1</sub> and CB<sub>2</sub> receptors, both G-proteins-coupled receptors linked to G<sub>i/o</sub> 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),  $\Delta^9$ -THC or  $\Delta^9$ -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,  $\Delta^9$ -THC could have a narrow therapeutic index. Nevertheless, the above cannabimimetic effects seem to be mainly mediated by CB<sub>1</sub> 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

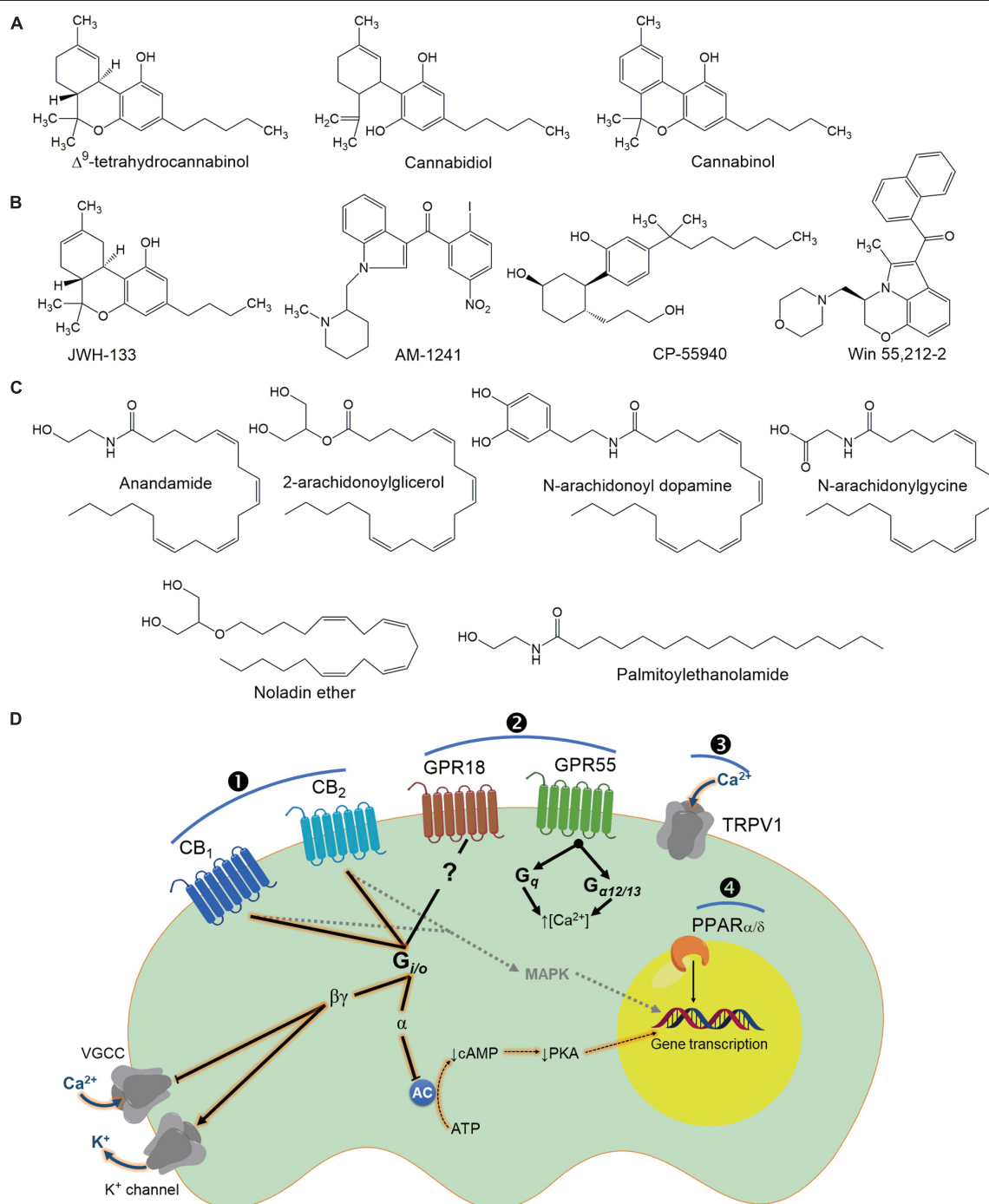
The first eCB isolated in the brain was *N*-arachidonoyl ethanolamide (AEA), or anandamide (a name taken from the Sanskrit word Ananda, which means “bliss, joy,” and amide) (Devane et al., 1992; **Figure 2**). AEA is a fatty acid neuromodulator derived from the non-oxidative metabolism of arachidonic 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  $\Delta^9$ -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<sub>1</sub> and CB<sub>2</sub> receptors. Certainly, anandamide shows preferential affinity for CB<sub>1</sub> ( $K_i$  = 89 nM) compared to CB<sub>2</sub> ( $K_i$  = 371 nM) receptors (Gauldie et al., 2001), whereas 2-AG is considered a full agonist at both CB<sub>1</sub> and CB<sub>2</sub> 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<sub>1</sub>-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  $\alpha$  (PPAR $\alpha$ ) (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<sub>1</sub> and CB<sub>2</sub> receptors. These receptors belong to the 7-transmembrane G-protein coupled receptors (GPCRs) primarily coupled to G<sub>i/o</sub> proteins that inhibit adenylyl cyclase (AC) and increase mitogen-activated protein kinase (MAPK) activity downstream of  $\beta$ -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 (protect ① and ②) or modulate other family receptors (protect ③ and ④), which contribute to the biological effect of these molecules (particularly for the endocannabinoids). In general terms, classic cannabinoid receptors (CB<sub>1</sub> and CB<sub>2</sub>) are GPCRs, which are canonically coupled to G<sub>i/o</sub> proteins. Consequently, under CB<sub>1/2</sub> receptors: (i) a decrease of adenylyl cyclase (AC) activity; (ii) an inactivation of Ca<sup>2+</sup> channels; and (iii) activation of inwardly rectifying K<sup>+</sup> 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<sup>2+</sup> channels (VDCCs) through the release of associated  $\beta\gamma$  subunits (apparently CB<sub>2</sub> receptors are ineffective, compared with CB<sub>1</sub>, for shifting ionic currents *via*  $\beta\gamma$  subunits). In addition to PKA inhibition, CB<sub>1/2</sub> 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<sub>i/o</sub> proteins, whereas GPR55 has been associated with an increase of intracellular Ca<sup>2+</sup> *via* G $\alpha_{12/13}$ . In the case of TRPV1 channels (a non-selective cation channel for Ca<sup>2+</sup>, Mg<sup>2+</sup>, and Na<sup>+</sup> 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<sub>1</sub> 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, prostate, 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<sub>2</sub> 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-HT<sub>3</sub>) receptor antagonist MDL72222, suggesting a cannabinoid-mediated inhibition of serotonin (5-HT<sub>3</sub>) 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-HT<sub>3</sub>) receptors on the terminals of cardiopulmonary afferent C-fibers (Godlewski et al., 2003) through an allosteric interaction at a (5-HT<sub>3</sub>) 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<sub>1</sub>-mediated inhibitory effect at nM concentration, while it exerts a TRPV1-mediated stimulatory effect at higher concentrations ( $\mu$ M) 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<sub>1</sub> 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<sub>1</sub> receptors inhibit CGRP and substance P (SP) release from trigeminal sensory nerves (Akerman et al., 2004; Oshita et al., 2005). Moreover, CB<sub>1</sub> receptor agonists reduce voltage-activated Ca<sup>2+</sup> 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<sub>2</sub> 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<sub>1</sub> receptors, nerve damage upregulates CB<sub>2</sub> 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<sub>2</sub> receptor in sensory neurons remains unclear, several functional studies in sensory neurons point to an antinociceptive role (Burstin and Woodhams, 2014). For instance, the putative CB<sub>2</sub> 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<sup>2+</sup> response in DRG neurons in neuropathic rats (Sagar et al., 2009), while GW818646X (other CB<sub>2</sub> receptor agonist) diminishes capsaicin-induced inward cation currents and elevation of cytoplasmic Ca<sup>2+</sup> (Anand et al., 2008). Another CB<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> knock-out mice and blocked by AM630, a CB<sub>2</sub> receptor inverse agonist. Local injection of the PEA analog *N*-(4-methoxy-2-nitrophenyl)hexadecanamide induces CB<sub>1</sub>- and CB<sub>2</sub>-dependent antinociception in rats (Roa-Coria et al., 2012). Similar results were observed with GW833972A, another putative CB<sub>2</sub> receptor agonist (Belvisi et al., 2008). Interestingly, repeated systemic administration of the CB<sub>2</sub> receptor selective agonist AM1710

suppresses paclitaxel-induced allodynia (Deng et al., 2015). Taken together, the data strongly suggest that CB<sub>1</sub> and CB<sub>2</sub> receptors have an antinociceptive role. Despite this evidence, there are few cannabinoid-based drugs currently available for clinical use (see below).

## CB<sub>1</sub> and CB<sub>2</sub>-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%  $\Delta^9$ -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  $\Delta^9$ -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  $\Delta^9$ -THC analog) on 41 patients with postoperative pain (Beaulieu, 2006). Another study evaluated cannabis extract capsules (20 mg of  $\Delta^9$ -THC) in 18 healthy female volunteers (Kraft et al., 2008). Treatment with  $\Delta^9$ -THC was not able to reduce pain induced by capsaicin, electrical stimulation or sunburn. Taken together, it seems that  $\Delta^9$ -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  $\Delta^9$ -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  $\Delta^9$ -THC analog, modestly reduced central pain in patients with multiple sclerosis (Svendsen et al., 2004). Nabilone, another synthetic  $\Delta^9$ -THC analog, diminished neuropathic pain in diabetic patients (Toth et al., 2012). Oral administration of  $\Delta^9$ -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 $\mu$ mol/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 $\mu$ g/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 tactile 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 $\mu$ mol/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; SNL, spinal nerve ligation; FCA, Freund'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<sub>1</sub> and CB<sub>2</sub> 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  $\Delta^9$ -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<sup>1</sup>/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<sub>1</sub> and CB<sub>2</sub>) with several other pharmacological receptors, including the cannabinoid putative receptors GPR18 and GPR55 (which have been even suggested as CB<sub>x</sub> and CB<sub>3</sub> receptors). It is likely that the contradictory effects observed in clinical trials using *Cannabis sp.*-based treatments (e.g.,  $\Delta^9$ -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<sub>1</sub>, 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)<sup>2</sup>(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

<sup>1</sup>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.

<sup>2</sup><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 (Kohnno 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- $\beta$ -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
	CID16020046	10 $\mu$ M	Intra-ACC	Attenuation of phase II response Reduction of p-ERK in the ACC Attenuation of spinal <i>c-fos</i> 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 <sup>-/-</sup> 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 <sup>-/-</sup> mice	GPR55 signaling	Staton et al., 2008
Capsaicin/mouse	N/T	N/T	N/T	GPR55 <sup>-/-</sup> 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 <sup>-/-</sup> mice GPR55 <sup>-/-</sup> 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 <sup>-/-</sup> 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 <sup>-/-</sup> mice: reduction of LPI-induced acute allodynia, attenuation of LPI-induced long-term mechanical hyperalgesia	GPR55, G $\alpha_{q/11}$ , and G $\alpha_{13}$ pathways, and their signaling via RhoA-ROCK as well as ERK1/2	Gangadharan et al., 2013
Hot plate test/rat	LPI	1 $\mu$ g	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 cingulate 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 cingulate 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  $\beta$ -arrestin trafficking, MAPK, ERK1/2 phosphorylation and activates the G-protein signaling by a  $PKC\beta II$ -independent mechanism (Oka et al., 2007; Kapur et al., 2009). Interestingly, the effects on  $\beta$ -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- $\kappa B$  (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- $\kappa B$  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.

## FUNDING

This work was supported by “Dirección General de Investigación y Posgrado” from Autonomous University of Aguascalientes. It was also supported by CONACyT through Postdoctoral Fellowship no. 274414 to PB-I.

## ACKNOWLEDGMENTS

We especially thank Prof. Dr. Theodore J. Price for revising the manuscript. We also thank to PRODEP's program for supporting this work.

## REFERENCES

- Agarwal, N., Pacher, P., Tegeder, I., Amaya, F., Constantin, C. E., Brenner, G. J., et al. (2007). Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors. *Nat. Neurosci.* 10, 870–879. doi: 10.1038/nn1916
- Agrawal, R. P., Goswami, J., Jain, S., and Kochar, D. K. (2009). Management of diabetic neuropathy by sodium valproate and glyceryl trinitrate spray: a prospective double-blind randomized placebo-controlled study. *Diabetes Res. Clin. Pract.* 83, 371–378. doi: 10.1016/j.diabres.2008.12.018
- Ahluwalia, J., Urban, L., Capogna, M., Bevan, S., and Nagy, I. (2000). Cannabinoid 1 receptors are expressed in nociceptive primary sensory neurons. *Neuroscience* 100, 685–688. doi: 10.1016/S0306-4522(00)00389-4
- Akerman, S., Kaube, H., and Goadsby, P. J. (2004). Anandamide is able to inhibit trigeminal neurons using an in vivo model of trigeminovascular-mediated nociception. *J. Pharmacol. Exp. Ther.* 309, 56–63. doi: 10.1124/jpet.103.059808
- Albe-Fessard, D., Berkley, K. J., Kruger, L., Ralston, H. J. III, and Willis, W. D. Jr. (1985). Diencephalic mechanisms of pain sensation. *Brain Res.* 356, 217–296. doi: 10.1016/0165-0173(85)90013-X
- Alexander, S. P. (2012). 2012 cannabinoid themed section. *Br. J. Pharmacol.* 167, 1573–1574. doi: 10.1111/j.1476-5381.2012.02238.x
- Alexander, S. P., Christopoulos, A., Davenport, A. P., Kelly, E., Marrion, N. V., Peters, J. A., et al. (2017). The concise guide to pharmacology 2017/18: G protein-coupled receptors. *Br. J. Pharmacol.* 174(Suppl. 1), S17–S129. doi: 10.1111/bph.13878
- Anand, U., Otto, W. R., Sanchez-Herrera, D., Facer, P., Yiangou, Y., Korchev, Y., et al. (2008). Cannabinoid receptor CB2 localisation and agonist-mediated inhibition of capsaicin responses in human sensory neurons. *Pain* 138, 667–680. doi: 10.1016/j.pain.2008.06.007
- Andersson, D. A., Nash, M., and Bevan, S. (2007). Modulation of the cold-activated channel TRPM8 by lysophospholipids and polyunsaturated fatty acids. *J. Neurosci.* 27, 3347–3355. doi: 10.1523/JNEUROSCI.4846-06.2007
- Attal, N., Brasseur, L., Guirimand, D., Clermond-Gnamien, S., Atlami, S., and Bouhassira, D. (2004). Are oral cannabinoids safe and effective in refractory neuropathic pain? *Eur. J. Pain* 8, 173–177.
- Ballantyne, J. C., and Shin, N. S. (2008). Efficacy of opioids for chronic pain: a review of the evidence. *Clin. J. Pain* 24, 469–478. doi: 10.1097/AJP.0b013e31816b2f26
- Barann, M., Molderings, G., Bruss, M., Bonisch, H., Urban, B. W., and Gothert, M. (2002). Direct inhibition by cannabinoids of human 5-HT3A receptors: probable involvement of an allosteric modulator site. *Br. J. Pharmacol.* 137, 589–596. doi: 10.1038/sj.bjp.0704829
- Basbaum, A. I., Bautista, D. M., Scherrer, G., and Julius, D. (2009). Cellular and molecular mechanisms of pain. *Cell* 139, 267–284. doi: 10.1016/j.cell.2009.09.028
- Basbaum, A. I., and Woolf, C. J. (1999). Pain. *Curr. Biol.* 9, R429–R431. doi: 10.1016/S0960-9822(99)80273-5
- Beaulieu, P. (2006). Effects of nabilone, a synthetic cannabinoid, on postoperative pain. *Can. J. Anaesth.* 53, 769–775. doi: 10.1007/BF03022793
- Beltramo, M., Bernardini, N., Bertorelli, R., Campanella, M., Nicolussi, E., Fredduzzi, S., et al. (2006). CB2 receptor-mediated antihyperalgesia: possible direct involvement of neural mechanisms. *Eur. J. Neurosci.* 23, 1530–1538. doi: 10.1111/j.1460-9568.2006.04684.x
- Belvisi, M. G., Patel, H. J., Freund-Michel, V., Hele, D. J., Crispino, N., and Birrell, M. A. (2008). Inhibitory activity of the novel CB2 receptor agonist, GW833972A, on guinea-pig and human sensory nerve function in the airways. *Br. J. Pharmacol.* 155, 547–557. doi: 10.1038/bjp.2008.298
- Berman, J. S., Symonds, C., and Birch, R. (2004). Efficacy of two cannabis based medicinal extracts for relief of central neuropathic pain from brachial plexus avulsion: results of a randomised controlled trial. *Pain* 112, 299–306. doi: 10.1016/j.pain.2004.09.013
- Bhosale, U. A., Quraishi, N., Yegnanarayan, R., and Devasthale, D. (2015). A comparative study to evaluate the cardiovascular risk of selective and nonselective cyclooxygenase inhibitors (COX-Is) in arthritic patients. *J. Basic Clin. Physiol. Pharmacol.* 26, 73–79. doi: 10.1515/jbcpp-2014-0005
- Bondarenko, A. I., Malli, R., and Graier, W. F. (2011a). The GPR55 agonist lysophosphatidylinositol acts as an intracellular messenger and bidirectionally modulates Ca<sup>2+</sup>-activated large-conductance K<sup>+</sup> channels in endothelial cells. *Pflugers Arch.* 461, 177–189. doi: 10.1007/s00424-010-0898-x
- Bondarenko, A. I., Malli, R., and Graier, W. F. (2011b). The GPR55 agonist lysophosphatidylinositol directly activates intermediate-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> channels. *Pflugers Arch.* 462, 245–255. doi: 10.1007/s00424-011-0977-7
- Booker, L., Kinsey, S. G., Abdullah, R. A., Blankman, J. L., Long, J. Z., Ezzili, C., et al. (2012). The fatty acid amide hydrolase (FAAH) inhibitor PF-3845 acts in the nervous system to reverse LPS-induced tactile allodynia in mice. *Br. J. Pharmacol.* 165, 2485–2496. doi: 10.1111/j.1476-5381.2011.01445.x
- Bouaboula, M., Hilairiet, S., Marchand, J., Fajas, L., Le Fur, G., and Casellas, P. (2005). Anandamide induced PPARgamma transcriptional activation and 3T3-L1 preadipocyte differentiation. *Eur. J. Pharmacol.* 517, 174–181. doi: 10.1016/j.ejphar.2005.05.032
- Breen, C., Brownjohn, P. W., and Ashton, J. C. (2012). The atypical cannabinoid O-1602 increases hind paw sensitisation in the chronic constriction injury model of neuropathic pain. *Neurosci. Lett.* 508, 119–122. doi: 10.1016/j.neulet.2011.12.039
- Breuer, B., Pappagallo, M., Knotkova, H., Guleyupoglu, N., Wallenstein, S., and Portenoy, R. K. (2007). A randomized, double-blind, placebo-controlled, two-period, crossover, pilot trial of lamotrigine in patients with central pain due to multiple sclerosis. *Clin. Ther.* 29, 2022–2030. doi: 10.1016/j.clinthera.2007.09.023
- Bridges, D., Rice, A. S., Egertova, M., Elphick, M. R., Winter, J., and Michael, G. J. (2003). Localisation of cannabinoid receptor 1 in rat dorsal root ganglion using in situ hybridisation and immunohistochemistry. *Neuroscience* 119, 803–812. doi: 10.1016/S0306-4522(03)00200-8
- Buffum, M., and Buffum, J. C. (2000). Nonsteroidal anti-inflammatory drugs in the elderly. *Pain Manag. Nurs.* 1, 40–50. doi: 10.1053/jpmn.2000.7779
- Burstein, S. H., McQuain, C. A., Ross, A. H., Salmonsens, R. A., and Zurier, R. E. (2011). Resolution of inflammation by N-arachidonoylglycine. *J. Cell. Biochem.* 112, 3227–3233. doi: 10.1002/jcb.23245
- Burston, J. J., and Woodhams, S. G. (2014). Endocannabinoid system and pain: an introduction. *Proc. Nutr. Soc.* 73, 106–117. doi: 10.1017/S0029665113003650
- Calabro, R. S., Gervasi, G., Marino, S., Mondo, P. N., and Bramanti, P. (2010). Misdiagnosed chronic pelvic pain: pudendal neuralgia responding to a novel use of palmitoylethanolamide. *Pain Med.* 11, 781–784. doi: 10.1111/j.1526-4637.2010.00823.x
- Calignano, A., La Rana, G., and Piomelli, D. (2001). Antinociceptive activity of the endogenous fatty acid amide, palmitoylethanolamide. *Eur. J. Pharmacol.* 419, 191–198. doi: 10.1016/S0014-2999(01)00988-8
- Callen, L., Moreno, E., Barroso-Chinea, P., Moreno-Delgado, D., Cortes, A., Mallol, J., et al. (2012). Cannabinoid receptors CB1 and CB2 form functional heteromers in brain. *J. Biol. Chem.* 287, 20851–20865. doi: 10.1074/jbc.M111.335273
- Carey, L. M., Gutierrez, T., Deng, L., Lee, W. H., Mackie, K., and Hohmann, A. G. (2017). Inflammatory and neuropathic nociception is preserved in GPR55 knockout mice. *Sci. Rep.* 7:944. doi: 10.1038/s41598-017-01062-2
- Celorio, M., Rojo-Bustamante, E., Fernandez-Suarez, D., Saez, E., Estella-Hermoso De Mendoza, A., Muller, C. E., et al. (2017). GPR55: a therapeutic target for Parkinson's disease? *Neuropharmacology* 125, 319–332. doi: 10.1016/j.neuropharm.2017.08.017
- Chen, J., Li, L., Chen, S. R., Chen, H., Xie, J. D., Sirrieh, R. E., et al. (2018). The alpha2delta-1-NMDA receptor complex is critically involved in neuropathic pain development and gabapentin therapeutic actions. *Cell Rep.* 22, 2307–2321. doi: 10.1016/j.celrep.2018.02.021
- Chiang, N., Dalli, J., Colas, R. A., and Serhan, C. N. (2015). Identification of resolvin D2 receptor mediating resolution of infections and organ protection. *J. Exp. Med.* 212, 1203–1217. doi: 10.1084/jem.20150225
- Chu, L. F., Angst, M. S., and Clark, D. (2008). Opioid-induced hyperalgesia in humans: molecular mechanisms and clinical considerations. *Clin. J. Pain* 24, 479–496. doi: 10.1097/AJP.0b013e31816b2f43
- Clapper, J. R., Moreno-Sanz, G., Russo, R., Guizarro, A., Vacondio, F., Duranti, A., et al. (2010). Anandamide suppresses pain initiation through a peripheral endocannabinoid mechanism. *Nat. Neurosci.* 13, 1265–1270. doi: 10.1038/nn.2632
- Clayton, N. M., Wilson, A. W., Collins, S. D., Giblin, G. M., Mitchell, B. L., Goldsmith, P., et al. (2004). Anti-hypersensitive and anti-inflammatory activity

- of the potent and selective CB2 agonist GW842166X. *Proc. Br. Pharmacol. Soc.* 21:050P. Available at: <http://www.pa2online.org/Vol2Issue4abst050P.html>
- Clermont-Gnamien, S., Atlani, S., Attal, N., Le Mercier, F., Guirimand, F., and Brasseur, L. (2002). [The therapeutic use of D9-tetrahydrocannabinol (dronabinol) in refractory neuropathic pain]. *Presse Med.* 31, 1840–1845.
- Condés-Lara, M., Martínez-Lorezana, G., Rubio-Beltrán, E., Rodríguez-Jiménez, J., Rojas-Piloni, G., and González-Hernández, A. (2015). Hypothalamic paraventricular nucleus stimulation enhances c-Fos expression in spinal and supraspinal structures related to pain modulation. *Neurosci. Res.* 98, 59–63. doi: 10.1016/j.neures.2015.04.004
- Conigliaro, R., Drago, V., Foster, P. S., Schievano, C., and Di Marzo, V. (2011). Use of palmitoylethanolamide in the entrapment neuropathy of the median in the wrist. *Minerva Med.* 102, 141–147.
- Console-Bram, L., Brailoiu, E., Brailoiu, G. C., Sharir, H., and Abood, M. E. (2014). Activation of GPR18 by cannabinoid compounds: a tale of biased agonism. *Br. J. Pharmacol.* 171, 3908–3917. doi: 10.1111/bph.12746
- Console-Bram, L., Ciuciu, S. M., Zhao, P., Zipkin, R. E., Brailoiu, E., and Abood, M. E. (2017). N-arachidonoyl glycine, another endogenous agonist of GPR55. *Biochem. Biophys. Res. Commun.* 490, 1389–1393. doi: 10.1016/j.bbrc.2017.07.038
- Corchero, J., Avila, M. A., Fuentes, J. A., and Manzanares, J. (1997a). delta-9-Tetrahydrocannabinol increases prodynorphin and proenkephalin gene expression in the spinal cord of the rat. *Life Sci.* 61, 39–43.
- Corchero, J., Fuentes, J. A., and Manzanares, J. (1997b). delta-9-Tetrahydrocannabinol increases proopiomelanocortin gene expression in the arcuate nucleus of the rat hypothalamus. *Eur. J. Pharmacol.* 323, 193–195. doi: 10.1016/S0014-2999(97)00144-1
- Corey-Bloom, J., Wolfson, T., Gamst, A., Jin, S., Marcotte, T. D., Bentley, H., et al. (2012). Smoked cannabis for spasticity in multiple sclerosis: a randomized, placebo-controlled trial. *CMAJ* 184, 1143–1150. doi: 10.1503/cmaj.110837
- Costa, B., Bettoni, I., Petrosini, S., Comelli, F., Giagnoni, G., and Di Marzo, V. (2010). The dual fatty acid amide hydrolase/TRPV1 blocker, N-arachidonoyl-serotonin, relieves carrageenan-induced inflammation and hyperalgesia in mice. *Pharmacol. Res.* 61, 537–546. doi: 10.1016/j.phrs.2010.02.001
- Crawley, J. N., Corwin, R. L., Robinson, J. K., Felder, C. C., Devane, W. A., and Axelrod, J. (1993). Anandamide, an endogenous ligand of the cannabinoid receptor, induces hypomotility and hypothermia in vivo in rodents. *Pharmacol. Biochem. Behav.* 46, 967–972. doi: 10.1016/0091-3057(93)90230-Q
- Crean, R. D., Tapert, S. F., Minassian, A., Macdonald, K., Crane, N. A., and Mason, B. J. (2011). Effects of chronic, heavy cannabis use on executive functions. *J. Addict. Med.* 5, 9–15. doi: 10.1097/ADM.0b013e31820cdd57
- D'Agostino, G., La Rana, G., Russo, R., Sasso, O., Iacono, A., Esposito, E., et al. (2007). Acute intracerebroventricular administration of palmitoylethanolamide, an endogenous peroxisome proliferator-activated receptor-alpha agonist, modulates carrageenan-induced paw edema in mice. *J. Pharmacol. Exp. Ther.* 322, 1137–1143. doi: 10.1124/jpet.107.123265
- Delieu, E., Sperow, M., Console-Bram, L., Carter, R. L., Tilley, D. G., Kalamirides, D. J., et al. (2015). The lysophosphatidylinositol receptor GPR55 modulates pain perception in the periaqueductal gray. *Mol. Pharmacol.* 88, 265–272. doi: 10.1124/mol.115.099333
- Danthi, S., Enyeart, J. A., and Enyeart, J. J. (2003). Modulation of native TREK-1 and Kv1.4 K<sup>+</sup> channels by polyunsaturated fatty acids and lysophospholipids. *J. Membr. Biol.* 195, 147–164. doi: 10.1007/s00232-003-0616-0
- Davis, M. P. (2014). Cannabinoids in pain management: CB1, CB2 and non-classic receptor ligands. *Expert Opin. Investig. Drugs* 23, 1123–1140. doi: 10.1517/13543784.2014.918603
- Demuth, D. G., and Molleman, A. (2006). Cannabinoid signalling. *Life Sci.* 78, 549–563. doi: 10.1016/j.lfs.2005.05.055
- Deng, L., Guindon, J., Cornett, B. L., Makriyannis, A., Mackie, K., and Hohmann, A. G. (2015). Chronic cannabinoid receptor 2 activation reverses palmitaxel neuropathy without tolerance or cannabinoid receptor 1-dependent withdrawal. *Biol. Psychiatry* 77, 475–487. doi: 10.1016/j.biopsych.2014.04.009
- Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., et al. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258, 1946–1949. doi: 10.1126/science.1470919
- Di Marzo, V., Bifulco, M., and De Petrocellis, L. (2004). The endocannabinoid system and its therapeutic exploitation. *Nat. Rev. Drug Discov.* 3, 771–784. doi: 10.1038/nrd1495
- Dowell, D., Haegerich, T. M., and Chou, R. (2016). CDC guideline for prescribing opioids for chronic pain—United States, 2016. *JAMA* 315, 1624–1645. doi: 10.1001/jama.2016.1464
- Drewes, A. M., Andreasen, A., and Poulsen, L. H. (1994). Valproate for treatment of chronic central pain after spinal cord injury. A double-blind cross-over study. *Paraplegia* 32, 565–569.
- Dumas, E. O., and Pollack, G. M. (2008). Opioid tolerance development: a pharmacokinetic/pharmacodynamic perspective. *AAPS J.* 10, 537–551. doi: 10.1208/s12248-008-9056-1
- Dunn, S. L., Wilkinson, J. M., Crawford, A., Bunning, R. A. D., and Le Maitre, C. L. (2016). Expression of cannabinoid receptors in human osteoarthritic cartilage: implications for future therapies. *Cannabis Cannabinoid Res.* 1, 3–15. doi: 10.1089/can.2015.0001
- Eisenberg, E., Lurie, Y., Braker, C., Daoud, D., and Ishay, A. (2001). Lamotrigine reduces painful diabetic neuropathy: a randomized, controlled study. *Neurology* 57, 505–509. doi: 10.1212/WNL.57.3.505
- Elmes, S. J., Jhaveri, M. D., Smart, D., Kendall, D. A., and Chapman, V. (2004). Cannabinoid CB2 receptor activation inhibits mechanically evoked responses of wide dynamic range dorsal horn neurons in naive rats and in rat models of inflammatory and neuropathic pain. *Eur. J. Neurosci.* 20, 2311–2320. doi: 10.1111/j.1460-9568.2004.03690.x
- Evans, R. M., Scott, R. H., and Ross, R. A. (2004). Multiple actions of anandamide on neonatal rat cultured sensory neurones. *Br. J. Pharmacol.* 141, 1223–1233. doi: 10.1038/sj.bjp.0705723
- Fabbro, F., and Crescentini, C. (2014). Facing the experience of pain: a neuropsychological perspective. *Phys. Life Rev.* 11, 540–552. doi: 10.1016/j.plrev.2013.12.010
- Fan, P. (1995). Cannabinoid agonists inhibit the activation of 5-HT3 receptors in rat nodose ganglion neurons. *J. Neurophysiol.* 73, 907–910. doi: 10.1152/jn.1995.73.2.907
- Felder, C. C., Joyce, K. E., Briley, E. M., Mansouri, J., Mackie, K., Blond, O., et al. (1995). Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. *Mol. Pharmacol.* 48, 443–450.
- Finnerup, N. B., Sindrup, S. H., and Jensen, T. S. (2010). The evidence for pharmacological treatment of neuropathic pain. *Pain* 150, 573–581. doi: 10.1016/j.pain.2010.06.019
- Fischbach, T., Greffrath, W., Nawrath, H., and Treede, R. D. (2007). Effects of anandamide and noxious heat on intracellular calcium concentration in nociceptive DRG neurons of rats. *J. Neurophysiol.* 98, 929–938. doi: 10.1152/jn.01096.2006
- Fox, A., Kesingland, A., Gentry, C., McNair, K., Patel, S., Urban, L., et al. (2001). The role of central and peripheral Cannabinoid1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. *Pain* 92, 91–100. doi: 10.1016/S0304-3959(00)00474-7
- Fu, J., Gaetani, S., Oveisi, F., Lo Verme, J., Serrano, A., Rodriguez De Fonseca, F., et al. (2003). Oleylethanolamide regulates feeding and body weight through activation of the nuclear receptor PPAR-alpha. *Nature* 425, 90–93. doi: 10.1038/nature01921
- Galiegue, S., Mary, S., Marchand, J., Dussossoy, D., Carriere, D., Carayon, P., et al. (1995). Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. *Eur. J. Biochem.* 232, 54–61. doi: 10.1111/j.1432-1033.1995.tb02780.x
- Gangadharan, V., Selvaraj, D., Kurejova, M., Njoo, C., Gritsch, S., Skoriceva, D., et al. (2013). A novel biological role for the phospholipid lysophosphatidylinositol in nociceptive sensitization via activation of diverse G-protein signalling pathways in sensory nerves in vivo. *Pain* 154, 2801–2812. doi: 10.1016/j.pain.2013.08.019
- Gantz, I., Muraoka, A., Yang, Y. K., Samuelson, L. C., Zimmerman, E. M., Cook, H., et al. (1997). Cloning and chromosomal localization of a gene (GPR18) encoding a novel seven transmembrane receptor highly expressed in spleen and testis. *Genomics* 42, 462–466. doi: 10.1006/geno.1997.4752
- Gaoni, Y., and Mechoulam, R. (1964). Isolation, structure, and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* 86, 1646–1647. doi: 10.1021/ja01062a046
- Gatti, A., Lazzari, M., Gianfelice, V., Di Paolo, A., Sabato, E., and Sabato, A. F. (2012). Palmitoylethanolamide in the treatment of chronic pain caused by different etiopathogenesis. *Pain Med.* 13, 1121–1130. doi: 10.1111/j.1526-4637.2012.01432.x

- Gauldie, S. D., McQueen, D. S., Pertwee, R., and Chessell, I. P. (2001). Anandamide activates peripheral nociceptors in normal and arthritic rat knee joints. *Br. J. Pharmacol.* 132, 617–621. doi: 10.1038/sj.bjp.0703890
- Gee, N. S., Brown, J. P., Dissanayake, V. U., Offord, J., Thurlow, R., and Woodruff, G. N. (1996). The novel anticonvulsant drug, gabapentin (Neurontin), binds to the  $\alpha$ 2delta subunit of a calcium channel. *J. Biol. Chem.* 271, 5768–5776. doi: 10.1074/jbc.271.10.5768
- Giblin, G. M. P., O'shaughnessy, C. T., Naylor, A., Mitchell, W. L., Eatherton, A. J., Slingsby, B. P., et al. (2007). Discovery of 2-[(2,4-dichlorophenyl)amino]-N-[(tetrahydro-2H-pyran-4-yl)methyl]-4-(trifluoromethyl)-5-pyrimidinecarboxamide, a selective CB2 receptor agonist for the treatment of inflammatory pain. *J. Med. Chem.* 50, 2597–2600. doi: 10.1021/jm061195+
- Glass, M., Dragunow, M., and Faull, R. L. (1997). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* 77, 299–318. doi: 10.1016/S0306-4522(96)00428-9
- Godlewski, G., Gothert, M., and Malinowska, B. (2003). Cannabinoid receptor-independent inhibition by cannabinoid agonists of the peripheral 5-HT<sub>3</sub> receptor-mediated von Bezold-Jarisch reflex. *Br. J. Pharmacol.* 138, 767–774. doi: 10.1038/sj.bjp.0705114
- Gong, J. P., Onaivi, E. S., Ishiguro, H., Liu, Q. R., Tagliaferro, P. A., Brusco, A., et al. (2006). Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res.* 1071, 10–23. doi: 10.1016/j.brainres.2005.11.035
- González-Hernández, A., Martínez-Lorenzana, G., Rodríguez-Jiménez, J., Rojas-Piloni, G., and Condés-Lara, M. (2015). Intracisternal injection of palmitoylethanolamide inhibits the peripheral nociceptive evoked responses of dorsal horn wide dynamic range neurons. *J. Neural Transm.* 122, 369–374. doi: 10.1007/s00702-014-1255-6
- Griffin, M. R., Piper, J. M., Daugherty, J. R., Snowden, M., and Ray, W. A. (1991). Nonsteroidal anti-inflammatory drug use and increased risk for peptic ulcer disease in elderly persons. *Ann. Intern. Med.* 114, 257–263. doi: 10.7326/0003-4819-114-4-257
- Guy, A. T., Nagatsuka, Y., Ooashi, N., Inoue, M., Nakata, A., Greimel, P., et al. (2015). Glycerophospholipid regulation of modality-specific sensory axon guidance in the spinal cord. *Science* 349, 974–977. doi: 10.1126/science.aab3516
- Hanus, L., Abu-Lafi, S., Frider, E., Breuer, A., Vogel, Z., Shalev, D. E., et al. (2001). 2-arachidonyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc. Natl. Acad. Sci. U.S.A.* 98, 3662–3665. doi: 10.1073/pnas.061029898
- Hanus, L., Breuer, A., Tchilibon, S., Shiloah, S., Goldenberg, D., Horowitz, M., et al. (1999). HU-308: a specific agonist for CB2, a peripheral cannabinoid receptor. *Proc. Natl. Acad. Sci. U.S.A.* 96, 14228–14233. doi: 10.1073/pnas.96.25.14228
- Harada, K., Kitaguchi, T., Kamiya, T., Aung, K. H., Nakamura, K., Ohta, K., et al. (2017). Lysophosphatidylinositol-induced activation of the cation channel TRPV2 triggers glucagon-like peptide-1 secretion in enteroendocrine L cells. *J. Biol. Chem.* 292, 10855–10864. doi: 10.1074/jbc.M117.788653
- Heinricher, M. M. (2016). Pain modulation and the transition from acute to chronic pain. *Adv. Exp. Med. Biol.* 904, 105–115. doi: 10.1007/978-94-017-7537-3\_8
- Henstridge, C. M., Balenga, N. A., Ford, L. A., Ross, R. A., Waldhoer, M., and Irving, A. J. (2009). The GPR55 ligand L- $\alpha$ -lysophosphatidylinositol promotes RhoA-dependent Ca<sup>2+</sup> signaling and NFAT activation. *FASEB J.* 23, 183–193. doi: 10.1096/fj.08-108670
- Henstridge, C. M., Balenga, N. A., Schroder, R., Kargl, J. K., Platzer, W., Martini, L., et al. (2010). GPR55 ligands promote receptor coupling to multiple signalling pathways. *Br. J. Pharmacol.* 160, 604–614. doi: 10.1111/j.1476-5381.2009.00625.x
- Herzberg, U., Eliav, E., Bennett, G. J., and Kopin, I. J. (1997). The analgesic effects of R(+)-WIN 55,212-2 mesylate, a high affinity cannabinoid agonist, in a rat model of neuropathic pain. *Neurosci. Lett.* 221, 157–160. doi: 10.1016/S0304-3940(96)13308-5
- Hillsley, K., Mccaul, C., Aerssens, J., Peeters, P. J., Gijsen, H., Moechars, D., et al. (2007). Activation of the cannabinoid 2 (CB2) receptor inhibits murine mesenteric afferent nerve activity. *Neurogastroenterol. Motil.* 19, 769–777. doi: 10.1111/j.1365-2982.2007.00950.x
- Hohmann, A. G., Briley, E. M., and Herkenham, M. (1999). Pre- and postsynaptic distribution of cannabinoid and mu opioid receptors in rat spinal cord. *Brain Res.* 822, 17–25. doi: 10.1016/S0006-8993(98)01321-3
- Hohmann, A. G., and Herkenham, M. (1999). Localization of central cannabinoid CB1 receptor messenger RNA in neuronal subpopulations of rat dorsal root ganglia: a double-label in situ hybridization study. *Neuroscience* 90, 923–931. doi: 10.1016/S0306-4522(98)00524-7
- Horl, W. H. (2010). Nonsteroidal anti-inflammatory drugs and the kidney. *Pharmaceuticals* 3, 2291–2321. doi: 10.3390/ph3072291
- Howlett, A. C. (2002). The cannabinoid receptors. *Prostaglandins Other Lipid Mediat.* 68–69, 619–631. doi: 10.1016/S0090-6980(02)00060-6
- Hsieh, G. C., Pai, M., Chandran, P., Hooker, B. A., Zhu, C. Z., Salyers, A. K., et al. (2011). Central and peripheral sites of action for CB2 receptor mediated analgesic activity in chronic inflammatory and neuropathic pain models in rats. *Br. J. Pharmacol.* 162, 428–440. doi: 10.1111/j.1476-5381.2010.01046.x
- Huang, S. M., Bisogno, T., Petros, T. J., Chang, S. Y., Zavitsanos, P. A., Zipkin, R. E., et al. (2001). Identification of a new class of molecules, the arachidonyl amino acids, and characterization of one member that inhibits pain. *J. Biol. Chem.* 276, 42639–42644. doi: 10.1074/jbc.M107351200
- Ibrahim, M. M., Deng, H., Zvonok, A., Cockayne, D. A., Kwan, J., Mata, H. P., et al. (2003). Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc. Natl. Acad. Sci. U.S.A.* 100, 10529–10533. doi: 10.1073/pnas.1834309100
- Ishac, E. J., Jiang, L., Lake, K. D., Varga, K., Abood, M. E., and Kunos, G. (1996). Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB1 receptors on peripheral sympathetic nerves. *Br. J. Pharmacol.* 118, 2023–2028. doi: 10.1111/j.1476-5381.1996.tb15639.x
- Jaggat, S. I., Hasnie, F. S., Sellaturay, S., and Rice, A. S. (1998). The anti-hyperalgesic actions of the cannabinoid anandamide and the putative CB2 receptor agonist palmitoylethanolamide in visceral and somatic inflammatory pain. *Pain* 76, 189–199. doi: 10.1016/S0304-3959(98)00041-4
- Jensen, B., Chen, J., Furnish, T., and Wallace, M. (2015). Medical marijuana and chronic pain: a review of basic science and clinical evidence. *Curr. Pain Headache Rep.* 19:50. doi: 10.1007/s11916-015-0524-x
- Jeong, H. J., Vandenberg, R. J., and Vaughan, C. W. (2010). N-arachidonyl-glycine modulates synaptic transmission in superficial dorsal horn. *Br. J. Pharmacol.* 161, 925–935. doi: 10.1111/j.1476-5381.2010.00935.x
- Ji, R. R., Gereau, R. W. T., Malcangio, M., and Strichartz, G. R. (2009). MAP kinase and pain. *Brain Res. Rev.* 60, 135–148. doi: 10.1016/j.brainresrev.2008.12.011
- Johanek, L. M., Heitmiller, D. R., Turner, M., Nader, N., Hodges, J., and Simone, D. A. (2001). Cannabinoids attenuate capsaicin-evoked hyperalgesia through spinal and peripheral mechanisms. *Pain* 93, 303–315. doi: 10.1016/S0304-3959(01)00336-0
- Johanek, L. M., and Simone, D. A. (2004). Activation of peripheral cannabinoid receptors attenuates cutaneous hyperalgesia produced by a heat injury. *Pain* 109, 432–442. doi: 10.1016/j.pain.2004.02.020
- Julius, D., and Basbaum, A. I. (2001). Molecular mechanisms of nociception. *Nature* 413, 203–210. doi: 10.1038/35093019
- Kalliomäki, J., Segerdahl, M., Webster, L., Reimfeldt, A., Huizar, K., Annas, P., et al. (2013). Evaluation of the analgesic efficacy of AZD1940, a novel cannabinoid agonist, on post-operative pain after lower third molar surgical removal. *Scand. J. Pain* 4, 17–22. doi: 10.1016/j.sjpain.2012.08.004
- Kaplan, J. S., Stella, N., Catterall, W. A., and Westenbroek, R. E. (2017). Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. *Proc. Natl. Acad. Sci. U.S.A.* 114, 11229–11234. doi: 10.1073/pnas.1711351114
- Kapur, A., Zhao, P., Sharir, H., Bai, Y., Caron, M. G., Barak, L. S., et al. (2009). Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. *J. Biol. Chem.* 284, 29817–29827. doi: 10.1074/jbc.M109.050187
- Keppel Hesselink, J. M., Kopsky, D. J., and Sajben, N. L. (2014). Vulvodynia and proctodynia treated with topical baclofen 5 % and palmitoylethanolamide. *Arch. Gynecol. Obstet.* 290, 389–393. doi: 10.1007/s00404-014-3218-4
- Kinsey, S. G., Naidu, P. S., Cravatt, B. F., Dudley, D. T., and Lichtman, A. H. (2011). Fatty acid amide hydrolase blockade attenuates the development of collagen-induced arthritis and related thermal hyperalgesia in mice. *Pharmacol. Biochem. Behav.* 99, 718–725. doi: 10.1016/j.pbb.2011.06.022
- Knerlich-Lukoschus, F., Noack, M., Von Der Ropp-Brenner, B., Lucius, R., Mehdorn, H. M., and Held-Feindt, J. (2011). Spinal cord injuries induce changes in CB1 cannabinoid receptor and C-C chemokine expression in brain

- areas underlying circuitry of chronic pain conditions. *J. Neurotrauma* 28, 619–634. doi: 10.1089/neu.2010.1652
- Ko, M. C., and Woods, J. H. (1999). Local administration of delta9-tetrahydrocannabinol attenuates capsaicin-induced thermal nociception in rhesus monkeys: a peripheral cannabinoid action. *Psychopharmacology* 143, 322–326. doi: 10.1007/s002130050955
- Kohno, M., Hasegawa, H., Inoue, A., Muraoka, M., Miyazaki, T., Oka, K., et al. (2006). Identification of N-arachidonylglycine as the endogenous ligand for orphan G-protein-coupled receptor GPR18. *Biochem. Biophys. Res. Commun.* 347, 827–832. doi: 10.1016/j.bbrc.2006.06.175
- Kraft, B., Frickey, N. A., Kaufmann, R. M., Reif, M., Frey, R., Gustorff, B., et al. (2008). Lack of analgesia by oral standardized cannabis extract on acute inflammatory pain and hyperalgesia in volunteers. *Anesthesiology* 109, 101–110. doi: 10.1097/ALN.0b013e31817881e1
- LaBuda, C. J., Kobilsh, M., and Little, P. J. (2005). Cannabinoid CB2 receptor agonist activity in the hindpaw incision model of postoperative pain. *Eur. J. Pharmacol.* 527, 172–174. doi: 10.1016/j.ejphar.2005.10.020
- Langford, R. M., Mares, J., Novotna, A., Vachova, M., Novakova, I., Notcutt, W., et al. (2013). A double-blind, randomized, placebo-controlled, parallel-group study of THC/CBD oromucosal spray in combination with the existing treatment regimen, in the relief of central neuropathic pain in patients with multiple sclerosis. *J. Neurol.* 260, 984–997. doi: 10.1007/s00415-012-6739-4
- Laprairie, R. B., Kelly, M. E., and Denovan-Wright, E. M. (2012). The dynamic nature of type 1 cannabinoid receptor (CB1) gene transcription. *Br. J. Pharmacol.* 167, 1583–1595. doi: 10.1111/j.1476-5381.2012.02175.x
- Lauckner, J. E., Jensen, J. B., Chen, H. Y., Lu, H. C., Hille, B., and Mackie, K. (2008). GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc. Natl. Acad. Sci. U.S.A.* 105, 2699–2704. doi: 10.1073/pnas.0711278105
- Li, H.-L. (1974). An archaeological and historical account of cannabis in China. *Econ. Bot.* 28, 437–448. doi: 10.1007/BF02862859
- Li, J., Daughters, R. S., Bullis, C., Bengiamin, R., Stucky, M. W., Brennan, J., et al. (1999). The cannabinoid receptor agonist WIN 55,212-2 mesylate blocks the development of hyperalgesia produced by capsaicin in rats. *Pain* 81, 25–33. doi: 10.1016/S0304-3959(98)00263-2
- Lim, G., Sung, B., Ji, R. R., and Mao, J. (2003). Upregulation of spinal cannabinoid-1-receptors following nerve injury enhances the effects of Win 55,212-2 on neuropathic pain behaviors in rats. *Pain* 105, 275–283. doi: 10.1016/S0304-3959(03)00242-2
- Llorca-Torralba, M., Borges, G., Neto, F., Mico, J. A., and Berrocoso, E. (2016). Noradrenergic *Locus coeruleus* pathways in pain modulation. *Neuroscience* 338, 93–113. doi: 10.1016/j.neuroscience.2016.05.057
- Lo Verme, J., Fu, J., Astarita, G., La Rana, G., Russo, R., Calignano, A., et al. (2005). The nuclear receptor peroxisome proliferator-activated receptor- $\alpha$  mediates the anti-inflammatory actions of palmitoylethanolamide. *Mol. Pharmacol.* 67, 15–19. doi: 10.1124/mol.104.006353
- Loyd, D. R., and Murphy, A. Z. (2009). The role of the periaqueductal gray in the modulation of pain in males and females: are the anatomy and physiology really that different? *Neural Plast.* 2009:462879. doi: 10.1155/2009/462879
- Lu, V. B., Puhl, H. L. III, and Ikeda, S. R. (2013). N-Arachidonyl glycine does not activate G protein-coupled receptor 18 signaling via canonical pathways. *Mol. Pharmacol.* 83, 267–282. doi: 10.1124/mol.112.081182
- Luchicchi, A., and Pistis, M. (2012). Anandamide and 2-arachidonoylglycerol: pharmacological properties, functional features, and emerging specificities of the two major endocannabinoids. *Mol. Neurobiol.* 46, 374–392. doi: 10.1007/s12035-012-8299-0
- Lynch, M. E., Cesar-Rittenberg, P., and Hohmann, A. G. (2014). A double-blind, placebo-controlled, crossover pilot trial with extension using an oral mucosal cannabinoid extract for treatment of chemotherapy-induced neuropathic pain. *J. Pain Symptom Manage.* 47, 166–173. doi: 10.1016/j.jpainsymman.2013.02.018
- Ma, H., Chen, S. R., Chen, H., Li, L., Li, D. P., Zhou, J. J., et al. (2018). Alpha2delta-1 is essential for sympathetic output and NMDA receptor activity potentiated by angiotensin II in the hypothalamus. *J. Neurosci.* 38, 6388–6398. doi: 10.1523/JNEUROSCI.0447-18.2018
- Mackie, K. (2005). Cannabinoid receptor homo- and heterodimerization. *Life Sci.* 77, 1667–1673. doi: 10.1016/j.lfs.2005.05.011
- Mackie, K. (2008a). Cannabinoid receptors: where they are and what they do. *J. Neuroendocrinol.* 20(Suppl. 1), 10–14. doi: 10.1111/j.1365-2826.2008.01671.x
- Mackie, K. (2008b). Signaling via CNS cannabinoid receptors. *Mol. Cell. Endocrinol.* 286, S60–S65. doi: 10.1016/j.mce.2008.01.022
- Maingret, F., Patel, A. J., Lesage, F., Lazdunski, M., and Honore, E. (2000). Lysophospholipids open the two-pore domain mechano-gated K<sup>+</sup> channels TREK-1 and TRAAK. *J. Biol. Chem.* 275, 10128–10133. doi: 10.1074/jbc.275.14.10128
- Maione, S., Costa, B., Piscitelli, F., Morera, E., De Chiaro, M., Comelli, F., et al. (2013). Piperazinyl carbamate fatty acid amide hydrolase inhibitors and transient receptor potential channel modulators as “dual-target” analgesics. *Pharmacol. Res.* 76, 98–105. doi: 10.1016/j.phrs.2013.07.003
- Maione, S., De Petrocellis, L., De Novellis, V., Moriello, A. S., Petrosino, S., Palazzo, E., et al. (2007). Analgesic actions of N-arachidonoyl-serotonin, a fatty acid amide hydrolase inhibitor with antagonistic activity at vanilloid TRPV1 receptors. *Br. J. Pharmacol.* 150, 766–781. doi: 10.1038/sj.bjp.070.7145
- Malan, T. P. Jr., Ibrahim, M. M., Vanderah, T. W., Makriyannis, A., and Porreca, F. (2002). Inhibition of pain responses by activation of CB2 cannabinoid receptors. *Chem. Phys. Lipids* 121, 191–200. doi: 10.1016/S0009-3084(02)00155-X
- Malan, T. P. Jr., Ibrahim, M. M., Deng, H., Liu, Q., Mata, H. P., Vanderah, T., et al. (2001). CB2 cannabinoid receptor-mediated peripheral antinociception. *Pain* 93, 239–245. doi: 10.1016/S0304-3959(01)00321-9
- Malek, N., Kostrzewa, M., Makuch, W., Pajak, A., Kucharczyk, M., Piscitelli, F., et al. (2016). The multiplicity of spinal AA-5-HT anti-nociceptive action in a rat model of neuropathic pain. *Pharmacol. Res.* 111, 251–263. doi: 10.1016/j.phrs.2016.06.012
- Malek, N., Mrugala, M., Makuch, W., Kolosowska, N., Przewlocka, B., Binkowski, M., et al. (2015). A multi-target approach for pain treatment: dual inhibition of fatty acid amide hydrolase and TRPV1 in a rat model of osteoarthritis. *Pain* 156, 890–903. doi: 10.1097/j.pain.0000000000000132
- Manzanares, J., Corchero, J., Romero, J., Fernandez-Ruiz, J. J., Ramos, J. A., and Fuentes, J. A. (1999). Pharmacological and biochemical interactions between opioids and cannabinoids. *Trends Pharmacol. Sci.* 20, 287–294. doi: 10.1016/S0165-6147(99)01339-5
- Marichal-Cancino, B. A., Fajardo-Valdez, A., Ruiz-Contreras, A. E., Méndez-Díaz, M., and Prospero-García, O. (2017). Advances in the physiology of GPR55 in the central nervous system. *Curr. Neuropharmacol.* 15, 771–778. doi: 10.2174/1570159X14666160729155441
- Marichal-Cancino, B. A., Fajardo-Valdez, A., Ruiz-Contreras, A. E., Méndez-Díaz, M., and Prospero-García, O. (2018). Possible role of hippocampal GPR55 in spatial learning and memory in rats. *Acta Neurobiol. Exp.* 78, 41–50. doi: 10.21307/ane-2018-001
- Marichal-Cancino, B. A., Manrique-Maldonado, G., Altamirano-Espinoza, A. H., Ruiz-Salinas, I., González-Hernández, A., Maassen-vandenbrink, A., et al. (2013). Analysis of anandamide- and lysophosphatidylinositol-induced inhibition of the vasopressor responses produced by sympathetic stimulation or noradrenaline in pithed rats. *Eur. J. Pharmacol.* 721, 168–177. doi: 10.1016/j.ejphar.2013.09.039
- Marichal-Cancino, B. A., Sánchez-Fuentes, A., Méndez-Díaz, M., Ruiz-Contreras, A. E., and Prospero-García, O. (2016). Blockade of GPR55 in the dorsolateral striatum impairs performance of rats in a T-maze paradigm. *Behav. Pharmacol.* 27, 393–396. doi: 10.1097/FBP.0000000000000185
- Martell, B. A., O'Connor, P. G., Kerns, R. D., Becker, W. C., Morales, K. H., Kosten, T. R., et al. (2007). Systematic review: opioid treatment for chronic back pain: prevalence, efficacy, and association with addiction. *Ann. Intern. Med.* 146, 116–127. doi: 10.7326/0003-4819-146-2-200701160-00006
- Martin-Sanchez, E., Furukawa, T. A., Taylor, J., and Martin, J. L. (2009). Systematic review and meta-analysis of cannabis treatment for chronic pain. *Pain Med.* 10, 1353–1368. doi: 10.1111/j.1526-4637.2009.00703.x
- Mason, P. (2001). Contributions of the medullary raphe and ventromedial reticular region to pain modulation and other homeostatic functions. *Annu. Rev. Neurosci.* 24, 737–777. doi: 10.1146/annurev.neuro.24.1.737
- Massi, P., Vaccani, A., Rubino, T., and Parolaro, D. (2003). Cannabinoids and opioids share cAMP pathway in rat splenocytes. *J. Neuroimmunol.* 145, 46–54. doi: 10.1016/j.jneuroim.2003.09.006
- McGaraughty, S., Chu, K. L., Dart, M. J., Yao, B. B., and Meyer, M. D. (2009). A CB2 receptor agonist, A-836339, modulates wide dynamic range neuronal activity in neuropathic rats: contributions of spinal and peripheral CB2 receptors. *Neuroscience* 158, 1652–1661. doi: 10.1016/j.neuroscience.2008.11.015

- McHugh, D., Hu, S. S., Rimmerman, N., Juknat, A., Vogel, Z., Walker, J. M., et al. (2010). N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. *BMC Neurosci.* 11:44. doi: 10.1186/1471-2202-11-44
- Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., et al. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* 50, 83–90. doi: 10.1016/0006-2952(95)00109-D
- Meng, I. D., Manning, B. H., Martin, W. J., and Fields, H. L. (1998). An analgesia circuit activated by cannabinoids. *Nature* 395, 381–383. doi: 10.1038/26481
- Merriam, F. V., Wang, Z. Y., Guerios, S. D., and Bjorling, D. E. (2008). Cannabinoid receptor 2 is increased in acutely and chronically inflamed bladder of rats. *Neurosci. Lett.* 445, 130–134. doi: 10.1016/j.neulet.2008.08.076
- Mico, J. A., Ardid, D., Berrocoso, E., and Eschaliere, A. (2006). Antidepressants and pain. *Trends Pharmacol. Sci.* 27, 348–354. doi: 10.1016/j.tips.2006.05.004
- Mika, J., Zychowska, M., Makuch, W., Rojewska, E., and Przewlocka, B. (2013). Neuronal and immunological basis of action of antidepressants in chronic pain - clinical and experimental studies. *Pharmacol. Rep.* 65, 1611–1621. doi: 10.1016/S1734-1140(13)71522-6
- Miller, S., Leishman, E., Oehler, O., Daily, L., Murataeva, N., Wager-Miller, J., et al. (2016). Evidence for a GPR18 role in diurnal Regulation of intraocular pressure. *Invest. Ophthalmol. Vis. Sci.* 57, 6419–6426. doi: 10.1167/iov.16-19437
- Millns, P. J., Chapman, V., and Kendall, D. A. (2001). Cannabinoid inhibition of the capsaicin-induced calcium response in rat dorsal root ganglion neurones. *Br. J. Pharmacol.* 132, 969–971. doi: 10.1038/sj.bjp.0703919
- Moffat, R., and Rae, C. P. (2011). Anatomy, physiology and pharmacology of pain. *Anaesth. Intensive Care Med.* 12, 12–15. doi: 10.1016/j.mpaic.2010.10.011
- Monet, M., Gkika, D., Lehen'kyi, V., Pourtier, A., Vanden Abeele, F., Bidaux, G., et al. (2009). Lysophospholipids stimulate prostate cancer cell migration via TRPV2 channel activation. *Biochim. Biophys. Acta* 1793, 528–539. doi: 10.1016/j.bbamcr.2009.01.003
- Morera, E., De Petrocellis, L., Morera, L., Moriello, A. S., Ligresti, A., Nalli, M., et al. (2009). Synthesis and biological evaluation of piperazinyl carbamates and ureas as fatty acid amide hydrolase (FAAH) and transient receptor potential (TRP) channel dual ligands. *Bioorg. Med. Chem. Lett.* 19, 6806–6809. doi: 10.1016/j.bmcl.2009.09.033
- Munro, S., Thomas, K. L., and Abu-Shaar, M. (1993). Molecular characterization of a peripheral receptor for cannabinoids. *Nature* 365, 61–65. doi: 10.1038/365061a0
- Narang, S., Gibson, D., Wasan, A. D., Ross, E. L., Michna, E., Nedeljkovic, S. S., et al. (2008). Efficacy of dronabinol as an adjuvant treatment for chronic pain patients on opioid therapy. *J. Pain* 9, 254–264. doi: 10.1016/j.jpain.2007.10.018
- Niederberger, E., and Geisslinger, G. (2008). The IKK-NF- $\kappa$ B pathway: a source for novel molecular drug targets in pain therapy? *FASEB J.* 22, 3432–3442. doi: 10.1096/fj.08-109355
- Nurmikko, T. J., Serpell, M. G., Hoggart, B., Toomey, P. J., Morlion, B. J., and Haines, D. (2007). Sativex successfully treats neuropathic pain characterised by allodynia: a randomised, double-blind, placebo-controlled clinical trial. *Pain* 133, 210–220. doi: 10.1016/j.pain.2007.08.028
- Oka, S., Kimura, S., Toshida, T., Ota, R., Yamashita, A., and Sugiura, T. (2010). Lysophosphatidylinositol induces rapid phosphorylation of p38 mitogen-activated protein kinase and activating transcription factor 2 in HEK293 cells expressing GPR55 and IM-9 lymphoblastoid cells. *J. Biochem.* 147, 671–678. doi: 10.1093/jb/mvp208
- Oka, S., Nakajima, K., Yamashita, A., Kishimoto, S., and Sugiura, T. (2007). Identification of GPR55 as a lysophosphatidylinositol receptor. *Biochem. Biophys. Res. Commun.* 362, 928–934. doi: 10.1016/j.bbrc.2007.08.078
- Okamoto, Y., Wang, J., Morishita, J., and Ueda, N. (2007). Biosynthetic pathways of the endocannabinoid anandamide. *Chem. Biodivers.* 4, 1842–1857. doi: 10.1002/cbdv.200790155
- Okine, B. N., Madasu, M. K., McGowan, F., Prendergast, C., Gaspar, J. C., Harhen, B., et al. (2016). N-palmitoylethanolamide in the anterior cingulate cortex attenuates inflammatory pain behaviour indirectly via a CB1 receptor-mediated mechanism. *Pain* 157, 2687–2696. doi: 10.1097/j.pain.0000000000000687
- Onaivi, E. S., Ishiguro, H., Gong, J. P., Patel, S., Perchuk, A., Meozzi, P. A., et al. (2006). Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. *Ann. N. Y. Acad. Sci.* 1074, 514–536. doi: 10.1196/annals.1369.052
- Oshita, K., Inoue, A., Tang, H. B., Nakata, Y., Kawamoto, M., and Yuge, O. (2005). CB(1) cannabinoid receptor stimulation modulates transient receptor potential vanilloid receptor 1 activities in calcium influx and substance P Release in cultured rat dorsal root ganglion cells. *J. Pharmacol. Sci.* 97, 377–385. doi: 10.1254/jphs.FP0040872
- Otto, M., Bach, F. W., Jensen, T. S., and Sindrup, S. H. (2004). Valproic acid has no effect on pain in polyneuropathy: a randomized, controlled trial. *Neurology* 62, 285–288. doi: 10.1212/WNL.62.2.285
- Park, C. K., Xu, Z. Z., Liu, T., Lu, N., Serhan, C. N., and Ji, R. R. (2011). Resolvin D2 is a potent endogenous inhibitor for transient receptor potential subtype V1/A1, inflammatory pain, and spinal cord synaptic plasticity in mice: distinct roles of resolvin D1, D2, and E1. *J. Neurosci.* 31, 18433–18438. doi: 10.1523/JNEUROSCI.4192-11.2011
- Patel, H. J., Birrell, M. A., Crispino, N., Hele, D. J., Venkatesan, P., Barnes, P. J., et al. (2003). Inhibition of guinea-pig and human sensory nerve activity and the cough reflex in guinea-pigs by cannabinoid (CB2) receptor activation. *Br. J. Pharmacol.* 140, 261–268. doi: 10.1038/sj.bjp.0705435
- Pertwee, R. G. (2009). Emerging strategies for exploiting cannabinoid receptor agonists as medicines. *Br. J. Pharmacol.* 156, 397–411. doi: 10.1111/j.1476-5381.2008.00048.x
- Pertwee, R. G., Howlett, A. C., Abood, M. E., Alexander, S. P. H., Di Marzo, V., Elphick, M. R., et al. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB<sub>1</sub> and CB<sub>2</sub>. *Pharmacol. Rev.* 62, 588–631. doi: 10.1124/pr.110.003004
- Pineiro, R., Maffucci, T., and Falasca, M. (2011). The putative cannabinoid receptor GPR55 defines a novel autocrine loop in cancer cell proliferation. *Oncogene* 30, 142–152. doi: 10.1038/ncr.2010.417
- Pini, L. A., Guerzoni, S., Cainazzo, M. M., Ferrari, A., Sarchielli, P., Tiraferri, I., et al. (2012). Nabilone for the treatment of medication overuse headache: results of a preliminary double-blind, active-controlled, randomized trial. *J. Headache Pain* 13, 677–684. doi: 10.1007/s10194-012-0490-1
- Piomelli, D. (2003). The molecular logic of endocannabinoid signalling. *Nat. Rev. Neurosci.* 4, 873–884. doi: 10.1038/nrn1247
- Porter, A. C., Sauer, J. M., Knierman, M. D., Becker, G. W., Berna, M. J., Bao, J., et al. (2002). Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J. Pharmacol. Exp. Ther.* 301, 1020–1024. doi: 10.1124/jpet.301.3.1020
- Price, T. J., Helesic, G., Parghi, D., Hargreaves, K. M., and Flores, C. M. (2003). The neuronal distribution of cannabinoid receptor type 1 in the trigeminal ganglion of the rat. *Neuroscience* 120, 155–162. doi: 10.1016/S0306-4522(03)00333-6
- Pugh, G. Jr., Smith, P. B., Dombrowski, D. S., and Welch, S. P. (1996). The role of endogenous opioids in enhancing the antinociception produced by the combination of delta 9-tetrahydrocannabinol and morphine in the spinal cord. *J. Pharmacol. Exp. Ther.* 279, 608–616.
- Quartilho, A., Mata, H. P., Ibrahim, M. M., Vanderah, T. W., Porreca, F., Makriyannis, A., et al. (2003). Inhibition of inflammatory hyperalgesia by activation of peripheral CB2 cannabinoid receptors. *Anesthesiology* 99, 955–960. doi: 10.1097/0000542-200310000-00031
- Quintero, G. C. (2017). Review about gabapentin misuse, interactions, contraindications and side effects. *J. Exp. Pharmacol.* 9, 13–21. doi: 10.2147/JEP.S124391
- Rahn, E. J., and Hohmann, A. G. (2009). Cannabinoids as pharmacotherapies for neuropathic pain: from the bench to the bedside. *Neurotherapeutics* 6, 713–737. doi: 10.1016/j.nurt.2009.08.002
- Rahn, E. J., Thakur, G. A., Wood, J. A., Zvonok, A. M., Makriyannis, A., and Hohmann, A. G. (2011). Pharmacological characterization of AM1710, a putative cannabinoid CB2 agonist from the cannabillactone class: antinociception without central nervous system side-effects. *Pharmacol. Biochem. Behav.* 98, 493–502. doi: 10.1016/j.pbb.2011.02.024
- Rajapakse, C. S., Lisai, M., Deregnaucourt, C., Sinou, V., Latour, C., Roy, D., et al. (2015). Synthesis of new 4-aminoquinolines and evaluation of their in vitro activity against chloroquine-sensitive and chloroquine-resistant plasmodium falciparum. *PLoS One* 10:e0140878. doi: 10.1371/journal.pone.0140878
- Ranganathan, M., and D'Souza, D. C. (2006). The acute effects of cannabinoids on memory in humans: a review. *Psychopharmacology* 188, 425–444. doi: 10.1007/s00213-006-0508-y

- Ray, P., Torck, A., Quigley, L., Wangzhou, A., Neiman, M., Rao, C., et al. (2018). Comparative transcriptome profiling of the human and mouse dorsal root ganglia: an RNA-seq-based resource for pain and sensory neuroscience research. *Pain* 159, 1325–1345. doi: 10.1097/j.pain.0000000000001217
- Ray, W. A., Meredith, S., Thapa, P. B., Hall, K., and Murray, K. T. (2004). Cyclic antidepressants and the risk of sudden cardiac death. *Clin. Pharmacol. Ther.* 75, 234–241. doi: 10.1016/j.clpt.2003.09.019
- Rice, W., Shannon, J. M., Burton, F., and Fiedeldey, D. (1997). Expression of a brain-type cannabinoid receptor (CB1) in alveolar Type II cells in the lung: regulation by hydrocortisone. *Eur. J. Pharmacol.* 327, 227–232. doi: 10.1016/S0014-2999(97)89665-3
- Roa-Coria, J. E., Navarrete-Vázquez, G., Fowler, C. J., Flores-Murrieta, F. J., Déciga-Campos, M., and Granados-Soto, V. (2012). N-(4-Methoxy-2-nitrophenyl)hexadecanamide, a palmitoylethanolamide analogue, reduces formalin-induced nociception. *Life Sci.* 91, 1288–1294. doi: 10.1016/j.lfs.2012.09.024
- Roberts, J. D., Gennings, C., and Shih, M. (2006). Synergistic affective analgesic interaction between delta-9-tetrahydrocannabinol and morphine. *Eur. J. Pharmacol.* 530, 54–58. doi: 10.1016/j.ejphar.2005.11.036
- Roberts, L. A., Christie, M. J., and Connor, M. (2002). Anandamide is a partial agonist at native vanilloid receptors in acutely isolated mouse trigeminal sensory neurons. *Br. J. Pharmacol.* 137, 421–428. doi: 10.1038/sj.bjp.0704904
- Rog, D. J., Nurmikko, T. J., Friede, T., and Young, C. A. (2005). Randomized, controlled trial of cannabis-based medicine in central pain in multiple sclerosis. *Neurology* 65, 812–819. doi: 10.1212/01.wnl.0000176753.45410.8b
- Rosenkilde, M. M., Benned-Jensen, T., Andersen, H., Holst, P. J., Kledal, T. N., Luttichau, H. R., et al. (2006). Molecular pharmacological phenotyping of EBI2. An orphan seven-transmembrane receptor with constitutive activity. *J. Biol. Chem.* 281, 13199–13208. doi: 10.1074/jbc.M602245200
- Ross, R. A. (2003). Anandamide and vanilloid TRPV1 receptors. *Br. J. Pharmacol.* 140, 790–801. doi: 10.1038/sj.bjp.0705467
- Ross, R. A., Coutts, A. A., McFarlane, S. M., Anavi-Goffer, S., Irving, A. J., Pertwee, R. G., et al. (2001). Actions of cannabinoid receptor ligands on rat cultured sensory neurones: implications for antinociception. *Neuropharmacology* 40, 221–232. doi: 10.1016/S0028-3908(00)00135-0
- Ryberg, E., Larsson, N., Sjogren, S., Hjorth, S., Hermansson, N. O., Leonova, J., et al. (2007). The orphan receptor GPR55 is a novel cannabinoid receptor. *Br. J. Pharmacol.* 152, 1092–1101. doi: 10.1038/sj.bjp.0707460
- Sagar, D. R., Jhaveri, M., and Chapman, V. (2009). Targeting the cannabinoid system to produce analgesia. *Curr. Top. Behav. Neurosci.* 1, 275–287. doi: 10.1007/978-3-540-88955-7\_11
- Salio, C., Fischer, J., Franzoni, M. F., and Conrath, M. (2002). Pre- and postsynaptic localizations of the CB1 cannabinoid receptor in the dorsal horn of the rat spinal cord. *Neuroscience* 110, 755–764. doi: 10.1016/S0306-4522(01)00584-X
- Santha, P., Jenes, A., Somogyi, C., and Nagy, I. (2010). The endogenous cannabinoid anandamide inhibits transient receptor potential vanilloid type 1 receptor-mediated currents in rat cultured primary sensory neurons. *Acta Physiol. Hung.* 97, 149–158. doi: 10.1556/APhysiol.97.2010.2.1
- Sanudo-Pena, M. C., Strangman, N. M., Mackie, K., Walker, J. M., and Tsou, K. (1999). CB1 receptor localization in rat spinal cord and roots, dorsal root ganglion, and peripheral nerve. *Zhongguo Yao Li Xue Bao* 20, 1115–1120.
- Scavone, J. L., Sterling, R. C., and Van Bockstaele, E. J. (2013). Cannabinoid and opioid interactions: implications for opiate dependence and withdrawal. *Neuroscience* 248, 637–654. doi: 10.1016/j.neuroscience.2013.04.034
- Schicho, R., Bashashati, M., Bawa, M., Mchugh, D., Saur, D., Hu, H. M., et al. (2011). The atypical cannabinoid O-1602 protects against experimental colitis and inhibits neutrophil recruitment. *Inflamm. Bowel Dis.* 17, 1651–1664. doi: 10.1002/ibd.21538
- Schuelert, N., and McDougall, J. J. (2011). The abnormal cannabidiol analogue O-1602 reduces nociception in a rat model of acute arthritis via the putative cannabinoid receptor GPR55. *Neurosci. Lett.* 500, 72–76. doi: 10.1016/j.neulet.2011.06.004
- Schuelert, N., Zhang, C., Mogg, A. J., Broad, L. M., Hepburn, D. L., Nisenbaum, E. S., et al. (2010). Paradoxical effects of the cannabinoid CB2 receptor agonist GW405833 on rat osteoarthritic knee joint pain. *Osteoarthritis Cartilage* 18, 1536–1543. doi: 10.1016/j.joca.2010.09.005
- Serpell, M., Ratcliffe, S., Hovorka, J., Schofield, M., Taylor, L., Lauder, H., et al. (2014). A double-blind, randomized, placebo-controlled, parallel group study of THC/CBD spray in peripheral neuropathic pain treatment. *Eur. J. Pain* 18, 999–1012. doi: 10.1002/j.1532-2149.2013.00445.x
- Shiue, S. J., Peng, H. Y., Lin, C. R., Wang, S. W., Rau, R. H., and Cheng, J. K. (2017). Continuous intrathecal infusion of cannabinoid receptor agonists attenuates nerve ligation-induced pain in rats. *Reg. Anesth. Pain Med.* 42, 499–506. doi: 10.1097/AAP.0000000000000601
- Silver, M., Blum, D., Grainger, J., Hammer, A. E., and Quessy, S. (2007). Double-blind, placebo-controlled trial of lamotrigine in combination with other medications for neuropathic pain. *J. Pain Symptom Manage.* 34, 446–454. doi: 10.1016/j.jpainsymman.2006.12.015
- Simon, G. M., and Cravatt, B. F. (2006). Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. *J. Biol. Chem.* 281, 26465–26472. doi: 10.1074/jbc.M604660200
- Sinha, S., Schreiner, A. J., Biernaskie, J., Nickerson, D., and Gabriel, V. A. (2017). Treating pain on skin graft donor sites: review and clinical recommendations. *J. Trauma Acute Care Surg.* 83, 954–964. doi: 10.1097/TA.0000000000001615
- Skrabek, R. Q., Galimova, L., Ethans, K., and Perry, D. (2008). Nabilone for the treatment of pain in fibromyalgia. *J. Pain* 9, 164–173. doi: 10.1016/j.jpain.2007.09.002
- Smart, D., Gunthorpe, M. J., Jerman, J. C., Nasir, S., Gray, J., Muir, A. I., et al. (2000). The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). *Br. J. Pharmacol.* 129, 227–230. doi: 10.1038/sj.bjp.0703050
- Smith, F. L., Fujimori, K., Lowe, J., and Welch, S. P. (1998). Characterization of delta9-tetrahydrocannabinol and anandamide antinociception in nonarthritic and arthritic rats. *Pharmacol. Biochem. Behav.* 60, 183–191. doi: 10.1016/S0091-3057(97)00583-2
- Snider, W. D., and McMahon, S. B. (1998). Tackling pain at the source: new ideas about nociceptors. *Neuron* 20, 629–632. doi: 10.1016/S0896-6273(00)81003-X
- Sousa-Valente, J., Varga, A., Ananthan, K., Khajuria, A., and Nagy, I. (2014). Anandamide in primary sensory neurons: too much of a good thing? *Eur. J. Neurosci.* 39, 409–418. doi: 10.1111/ejn.12467
- Stahl, S. M., Grady, M. M., Moret, C., and Briley, M. (2005). SNRIs: their pharmacology, clinical efficacy, and tolerability in comparison with other classes of antidepressants. *CNS Spectr.* 10, 732–747. doi: 10.1017/S1092852900019726
- Staton, P. C., Hatcher, J. P., Walker, D. J., Morrison, A. D., Shapland, E. M., Hughes, J. P., et al. (2008). The putative cannabinoid receptor GPR55 plays a role in mechanical hyperalgesia associated with inflammatory and neuropathic pain. *Pain* 139, 225–236. doi: 10.1016/j.pain.2008.04.006
- Steeds, C. E. (2009). The anatomy and physiology of pain. *Surgery* 27, 507–511.
- Stein, A. T., Ufret-Vincenty, C. A., Hua, L., Santana, L. F., and Gordon, S. E. (2006). Phosphoinositide 3-kinase binds to TRPV1 and mediates NGF-stimulated TRPV1 trafficking to the plasma membrane. *J. Gen. Physiol.* 128, 509–522. doi: 10.1085/jgp.200609576
- Stella, N., Schweitzer, P., and Piomelli, D. (1997). A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 388, 773–778. doi: 10.1038/42015
- Stevens, A. J., and Higgins, M. D. (2017). A systematic review of the analgesic efficacy of cannabinoid medications in the management of acute pain. *Acta Anaesthesiol. Scand.* 61, 268–280. doi: 10.1111/aas.12851
- Straiker, A., Wager-Miller, J., Hutchens, J., and Mackie, K. (2012). Differential signalling in human cannabinoid CB1 receptors and their splice variants in autaptic hippocampal neurones. *Br. J. Pharmacol.* 165, 2660–2671. doi: 10.1111/j.1476-5381.2011.01744.x
- Succar, R., Mitchell, V. A., and Vaughan, C. W. (2007). Actions of N-arachidonyl-glycine in a rat inflammatory pain model. *Mol. Pain* 3:24. doi: 10.1186/1744-8069-3-24
- Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., et al. (1995). 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* 215, 89–97. doi: 10.1006/bbrc.1995.2437
- Sugiura, T., and Waku, K. (2000). 2-arachidonoylglycerol: a possible multifunctional lipid mediator in the nervous and immune systems. *Ann. N. Y. Acad. Sci.* 905, 344–346. doi: 10.1111/j.1749-6632.2000.tb06575.x

- Svensden, K. B., Jensen, T. S., and Bach, F. W. (2004). Does the cannabinoid dronabinol reduce central pain in multiple sclerosis? Randomised double blind placebo controlled crossover trial. *BMJ* 329:253. doi: 10.1136/bmj.38149.566979.AE
- Takenouchi, R., Inoue, K., Kambe, Y., and Miyata, A. (2012). N-arachidonoyl glycine induces macrophage apoptosis via GPR18. *Biochem. Biophys. Res. Commun.* 418, 366–371. doi: 10.1016/j.bbrc.2012.01.027
- Tognetto, M., Amadesi, S., Harrison, S., Creminon, C., Trevisani, M., Carreras, M., et al. (2001). Anandamide excites central terminals of dorsal root ganglion neurons via vanilloid receptor-1 activation. *J. Neurosci.* 21, 1104–1109. doi: 10.1523/JNEUROSCI.21-04-01104.2001
- Toth, C., Mawani, S., Brady, S., Chan, C., Liu, C., Mehina, E., et al. (2012). An enriched-enrolment, randomized withdrawal, flexible-dose, double-blind, placebo-controlled, parallel assignment efficacy study of nabilone as adjuvant in the treatment of diabetic peripheral neuropathic pain. *Pain* 153, 2073–2082. doi: 10.1016/j.pain.2012.06.024
- Touw, M. (1981). The religious and medicinal uses of Cannabis in China, India and Tibet. *J. Psychoactive Drugs* 13, 23–34. doi: 10.1080/02791072.1981.10471447
- Turcotte, D., Le Dorze, J. A., Esfahani, F., Frost, E., Gomori, A., and Namaka, M. (2010). Examining the roles of cannabinoids in pain and other therapeutic indications: a review. *Expert Opin. Pharmacother.* 11, 17–31. doi: 10.1517/14656560903413534
- Ueda, N., Tsuboi, K., Uyama, T., and Ohnishi, T. (2011). Biosynthesis and degradation of the endocannabinoid 2-arachidonoylglycerol. *Biofactors* 37, 1–7. doi: 10.1002/biof.131
- Valenzano, K. J., Tafesse, L., Lee, G., Harrison, J. E., Boulet, J. M., Gottshall, S. L., et al. (2005). Pharmacological and pharmacokinetic characterization of the cannabinoid receptor 2 agonist, GW405833, utilizing rodent models of acute and chronic pain, anxiety, ataxia and catalepsy. *Neuropharmacology* 48, 658–672. doi: 10.1016/j.neuropharm.2004.12.008
- Valverde, O., Noble, F., Beslot, F., Dauge, V., Fournie-Zaluski, M. C., and Roques, B. P. (2001). Delta9-tetrahydrocannabinol releases and facilitates the effects of endogenous enkephalins: reduction in morphine withdrawal syndrome without change in rewarding effect. *Eur. J. Neurosci.* 13, 1816–1824. doi: 10.1046/j.0953-816x.2001.01558.x
- van Amerongen, G., Kanhai, K., Baakman, A. C., Heuberger, J., Klaassen, E., Beumer, T. L., et al. (2017). Effects on spasticity and neuropathic pain of an oral formulation of delta9-tetrahydrocannabinol in patients with progressive multiple sclerosis. *Clin. Ther.* 40, 1467–1482. doi: 10.1016/j.clinthera.2017.01.016
- Vanden Abele, F., Zholos, A., Bidaux, G., Shuba, Y., Thebault, S., Beck, B., et al. (2006). Ca<sup>2+</sup>-independent phospholipase A2-dependent gating of TRPM8 by lysophospholipids. *J. Biol. Chem.* 281, 40174–40182. doi: 10.1074/jbc.M605779200
- Vanegas, H., and Schaible, H. G. (2004). Descending control of persistent pain: inhibitory or facilitatory? *Brain Res. Brain Res. Rev.* 46, 295–309. doi: 10.1016/j.brainresrev.2004.07.004
- Vasileiou, I., Fotopoulou, G., Matzourani, M., Patsouris, E., and Theocharis, S. (2013). Evidence for the involvement of cannabinoid receptors' polymorphisms in the pathophysiology of human diseases. *Expert Opin. Ther. Targets* 17, 363–377. doi: 10.1517/14728222.2013.754426
- Vassilatis, D. K., Hohmann, J. G., Zeng, H., Li, F., Ranchalis, J. E., Mortrud, M. T., et al. (2003). The G protein-coupled receptor repertoires of human and mouse. *Proc. Natl. Acad. Sci. U.S.A.* 100, 4903–4908. doi: 10.1073/pnas.0230374100
- Veress, G., Meszar, Z., Muszil, D., Avelino, A., Matesz, K., Mackie, K., et al. (2013). Characterisation of cannabinoid 1 receptor expression in the perikarya, and peripheral and spinal processes of primary sensory neurons. *Brain Struct. Funct.* 218, 733–750. doi: 10.1007/s00429-012-0425-2
- Vestergaard, K., Andersen, G., Gottrup, H., Kristensen, B. T., and Jensen, T. S. (2001). Lamotrigine for central poststroke pain: a randomized controlled trial. *Neurology* 56, 184–190. doi: 10.1212/WNL.56.2.184
- Vizi, E. S., Katona, I., and Freund, T. F. (2001). Evidence for presynaptic cannabinoid CB1 receptor-mediated inhibition of noradrenaline release in the guinea pig lung. *Eur. J. Pharmacol.* 431, 237–244. doi: 10.1016/S0014-2999(01)01413-3
- Vuong, L. A., Mitchell, V. A., and Vaughan, C. W. (2008). Actions of N-arachidonoyl-glycine in a rat neuropathic pain model. *Neuropharmacology* 54, 189–193. doi: 10.1016/j.neuropharm.2007.05.004
- Waldeck-Weiermair, M., Zoratti, C., Osibow, K., Balenga, N., Goessnitzer, E., Waldhoer, M., et al. (2008). Integrin clustering enables anandamide-induced Ca<sup>2+</sup> signaling in endothelial cells via GPR55 by protection against CB1-receptor-triggered repression. *J. Cell Sci.* 121, 1704–1717. doi: 10.1242/jcs.020958
- Walker, J. M., and Hohmann, A. G. (2005). Cannabinoid mechanisms of pain suppression. *Handb. Exp. Pharmacol.* 168, 509–554. doi: 10.1007/3-540-26573-2\_17
- Walker, J. M., and Huang, S. M. (2002). Cannabinoid analgesia. *Pharmacol. Ther.* 95, 127–135. doi: 10.1016/S0163-7258(02)00252-8
- Wallace, M., Schulteis, G., Atkinson, J. H., Wolfson, T., Lazzaretto, D., Bentley, H., et al. (2007). Dose-dependent effects of smoked cannabis on capsaicin-induced pain and hyperalgesia in healthy volunteers. *Anesthesiology* 107, 785–796. doi: 10.1097/01.anes.0000286986.92475.b7
- Wallace, M. S., Marcotte, T. D., Umlauf, A., Gouaux, B., and Atkinson, J. H. (2015). Efficacy of inhaled cannabis on painful diabetic neuropathy. *J. Pain* 16, 616–627. doi: 10.1016/j.jpain.2015.03.008
- Wang, S., Lim, G., Mao, J., Sung, B., Yang, L., and Mao, J. (2007). Central glucocorticoid receptors regulate the upregulation of spinal cannabinoid-1 receptors after peripheral nerve injury in rats. *Pain* 131, 96–105. doi: 10.1016/j.pain.2006.12.019
- Wang, Z. Y., McDowell, T., Wang, P., Alvarez, R., Gomez, T., and Bjorling, D. E. (2014). Activation of CB1 inhibits NGF-induced sensitization of TRPV1 in adult mouse afferent neurons. *Neuroscience* 277, 679–689. doi: 10.1016/j.neuroscience.2014.07.041
- Whiting, P. F., Wolff, R. F., Deshpande, S., Di Nisio, M., Duffy, S., Hernandez, A. V., et al. (2015). Cannabinoids for medical use: a systematic review and meta-analysis. *JAMA* 313, 2456–2473. doi: 10.1001/jama.2015.6358
- Wiles, A. L., Pearlman, R. J., Rosvall, M., Aubrey, K. R., and Vandenberg, R. J. (2006). N-Arachidonoyl-glycine inhibits the glycine transporter, GLYT2a. *J. Neurochem.* 99, 781–786. doi: 10.1111/j.1471-4159.2006.04107.x
- Wilsey, B., Marcotte, T., Deutsch, R., Gouaux, B., Sakai, S., and Donaghe, H. (2013). Low-dose vaporized cannabis significantly improves neuropathic pain. *J. Pain* 14, 136–148. doi: 10.1016/j.jpain.2012.10.009
- Wotherspoon, G., Fox, A., McIntyre, P., Colley, S., Bevan, S., and Winter, J. (2005). Peripheral nerve injury induces cannabinoid receptor 2 protein expression in rat sensory neurons. *Neuroscience* 135, 235–245. doi: 10.1016/j.neuroscience.2005.06.009
- Yamamoto, W., Mikami, T., and Iwamura, H. (2008). Involvement of central cannabinoid CB2 receptor in reducing mechanical allodynia in a mouse model of neuropathic pain. *Eur. J. Pharmacol.* 583, 56–61. doi: 10.1016/j.ejphar.2008.01.010
- Yao, B. B., Hsieh, G. C., Frost, J. M., Fan, Y., Garrison, T. R., Daza, A. V., et al. (2008). In vitro and in vivo characterization of A-796260: a selective cannabinoid CB2 receptor agonist exhibiting analgesic activity in rodent pain models. *Br. J. Pharmacol.* 153, 390–401. doi: 10.1038/sj.bjp.0707568
- Yin, H., Chu, A., Li, W., Wang, B., Shelton, F., Otero, F., et al. (2009). Lipid G protein-coupled receptor ligand identification using beta-arrestin PathHunter assay. *J. Biol. Chem.* 284, 12328–12338. doi: 10.1074/jbc.M806516200
- Yrjola, S., Kallioikoski, T., Laitinen, T., Poso, A., Parkkari, T., and Nevalainen, T. (2013). Discovery of novel cannabinoid receptor ligands by a virtual screening approach: further development of 2,4,6-trisubstituted 1,3,5-triazines as CB2 agonists. *Eur. J. Pharm. Sci.* 48, 9–20. doi: 10.1016/j.ejps.2012.10.020
- Yu, X. H., Cao, C. Q., Martino, G., Puma, C., Morinville, A., St-Onge, S., et al. (2010). A peripherally restricted cannabinoid receptor agonist produces robust anti-nociceptive effects in rodent models of inflammatory and neuropathic pain. *Pain* 151, 337–344. doi: 10.1016/j.pain.2010.07.019
- Zhang, M. J., Sansbury, B. E., Hellmann, J., Baker, J. F., Guo, L., Parmer, C. M., et al. (2016). Resolvin D2 enhances postischemic revascularization while resolving inflammation. *Circulation* 134, 666–680. doi: 10.1161/CIRCULATIONAHA.116.021894
- Zhu, W., and Oxford, G. S. (2007). Phosphoinositide-3-kinase and mitogen activated protein kinase signaling pathways mediate acute NGF sensitization of TRPV1. *Mol. Cell. Neurosci.* 34, 689–700. doi: 10.1016/j.mcn.2007.01.005
- Zhuang, Z. Y., Xu, H., Clapham, D. E., and Ji, R. R. (2004). Phosphatidylinositol 3-kinase activates ERK in primary sensory neurons and mediates inflammatory heat hyperalgesia through TRPV1 sensitization. *J. Neurosci.* 24, 8300–8309. doi: 10.1523/JNEUROSCI.2893-04.2004

Zygmunt, P. M., Petersson, J., Andersson, D. A., Chuang, H., Sorgard, M., Di Marzo, V., et al. (1999). Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. *Nature* 400, 452–457. doi: 10.1038/22761

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Guerrero-Alba, Barragán-Iglesias, González-Hernández, Valdez-Morales, Granados-Soto, Condés-Lara, Rodríguez and Marichal-Cancino. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

## Quick guide

# Cannabinoid receptors

Jane M. Sullivan

**What are they?** Cannabinoid receptors are G protein-coupled receptors with 7 trans-membrane 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?**  $\Delta^9$ -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

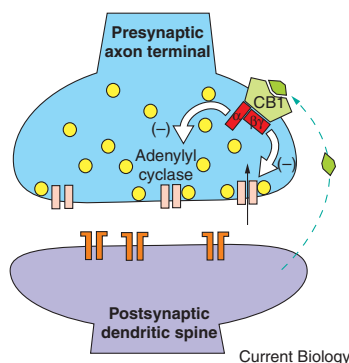


Figure 1. Depolarization of a postsynaptic neuron releases endocannabinoids. Binding of ligand to the CB1 receptor causes dissociation of the  $\alpha$  and  $\beta\gamma$  subunits (red) of the G protein that is coupled to the receptor. The  $\alpha$  subunit inhibits adenylyl cyclase while the  $\beta\gamma$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

these compounds are physiologically relevant cannabinoid receptor ligands is still an open question.

**Events downstream of cannabinoid receptors...** Like other G protein-coupled receptors, binding of ligand to cannabinoid receptors causes dissociation of the  $\alpha$  and  $\beta\gamma$  G protein subunits from the cannabinoid receptor and from each other. Release of the  $\alpha$  subunit leads to inhibition of adenylyl cyclase, reducing cAMP levels in the cell. In neurons dissociated  $\beta\gamma$  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?

- Ameri, A. (1999). The effects of cannabinoids on the brain. *Prog. Neurobiol.* 58, 315–348.
- Piomelli, D. et al. (2000). The endocannabinoid system as a target for therapeutic drugs. *Trends Pharmacol. Sci.* 21, 218–224.
- Schlicker, E. and Kathmann, M. (2001). Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol. Sci.* 22, 565–572.
- Sullivan, J. (2000). Cellular and molecular mechanisms underlying learning and memory impairments produced by cannabinoids. *Learn. Mem.* 7, 132–139.
- Wilson, R.I. and Nicoll, R.A. (2002). Endocannabinoid signaling in the brain. *Science* 296, 678–682.

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