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# Molecular Pharmacology of Phytocannabinoids

**Sarah E. Turner, Claire M. Williams, Leslie Iversen, and Benjamin J. Whalley**

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S.E. Turner • C.M. Williams
School of Psychology and Clinical Language Sciences and School of Pharmacy, University of Reading, Earley Gate, Whiteknights, Reading RG6 6AL, UK
e-mail: s.glyn@pgr.reading.ac.uk; claire.williams@reading.ac.uk

L. Iversen (✉)
Department of Pharmacology, Oxford University, Mansfield Road, Oxford OX1 3QT, UK
e-mail: les.iversen@pharm.ox.ac.uk

B.J. Whalley
School of Pharmacy, University of Reading, Whiteknights, Reading RG6 6AP, UK
e-mail: b.j.whalley@reading.ac.uk



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# 1 Introduction

*Cannabis sativa* contains about 120 phytocannabinoids, which are the $C_{21}$ terpenophenolic constituents making up approximately 24% of the total natural products of the plant [1]. To date, eleven different chemical classes of phytocannabinoids have been identified (Table 1). The $\Delta^9$-tetrahydrocannabinol (**1**) type class represents the largest proportion, comprising 17.3% of the total phytocannabinoid content, closely followed by the cannabigerol (**6**) type (see [1] for a detailed review of these different classes). The proportion of each chemical class in the cannabis plant is, however, dependent on the growing conditions, geographical location, plant processing methods, and plant variety or chemotype. Thus, these factors influencing the relative proportions of each phytocannabinoid type will additionally influence the pharmacological effects of whole cannabis extracts, either through a polypharmacological effect of the phytocannabinoids themselves, or through modulation of phytocannabinoid effects by the non-cannabinoid content of the plant [2]. These variances are therefore important to take into account when assessing the effects of whole cannabis plant extracts. In this chapter, focus will be made on the seven individual phytocannabinoids that have been the most thoroughly studied.

**Table 1** Constituents of *Cannabis sativa* L. represented as a percentage of the total phytocannabinoid content. Adapted from [1]

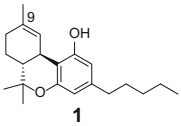
| Chemical class type | Percent of total phytocannabinoid content (%)[a] |
|---|---|
| **1** type | 17.3 |
| $\Delta^8$-THC type | 1.9 |
| **3** type | 9.6 |
| **4** type | 7.7 |
| **7** type | 7.7 |
| **6** type | 16.3 |
| CBND type | 1.9 |
| CBE type | 4.8 |
| CBL type | 2.9 |
| CBT type | 8.7 |
| Miscellaneous type | 21.2 |

[a]Total phytocannabinoid content = ca. 120

Phytocannabinoids have been of recreational, therapeutic, and other interest for thousands of years [3, 4]. Elucidation of the structure of the main phytocannabinoid obtained from cannabis, **1** [5], was reported in 1971. This discovery paved the way for further research that ultimately led to the discovery of the cannabinoid receptors, $CB_1$ [6], which predominates in the central nervous system, and the principally peripheral cannabinoid receptor, $CB_2$ [5]. The mammalian endocannabinoid system was then discovered [6], including the endogenous cannabinoid receptor ligands arachidonylethanolamide (AEA) and 2-arachidonylglycerol (2-AG) [7–9]. The psychotropic effect of **1**, mediated by its partial agonist activity at $CB_1$ receptors, has limited the extent of its use medicinally and it was removed from the *British Pharmacopeia* in 1971, and was declared of no medical benefit and placed under control in the Misuse of Drugs Act 1971 of the United Kingdom [10]. Despite this, patient-led self-medication campaigns claimed various therapeutic benefits, such as control of pain and emesis [11–15], control of seizures [16–21], and anti-inflammatory properties [17, 22], among others. This drove further investigation, leading to some licensed medications containing **1** being now available, such as Sativex®, which is used for the treatment of spasticity associated with multiple sclerosis. Although **1** also exerts some effects through non-CB receptor targets, the absence of psychotropic effects associated with the other phytocannabinoids present in cannabis has driven research into their discrete pharmacology and molecular targets that lie outside of the endocannabinoid system.

Over the years, a variety of molecular targets for plant cannabinoids outside the endocannabinoid system have been identified, such as ion channels, non-$CB_1$ or $CB_2$ G-protein coupled receptors, enzymes, and transporters. In this chapter, an overview of the molecular pharmacology of phytocannabinoids is presented, describing both targets within the endocannabinoid system and a wide range of other molecular targets. Since ca. 120 phytocannabinoids have now been identified and many have, as yet, poorly defined or unknown pharmacological profiles, particular focus is paid to phytocannabinoids that: (a) are reported to exert a behavioral effect in animal models or clinical reports, and (b) exert effects via specific molecular targets at sub-micromolar to low micromolar concentrations, which can realistically be achieved in vivo due to the lipophilic nature of these compounds [23].

## 2 $\Delta^9$-*trans*-Tetrahydrocannabinol




**1**

## 2.1 Activity at Cannabinoid Receptors

In 1986, Howlett and colleagues developed a biochemical model system that allowed the indirect identification of cannabimimetic drugs, i.e. those exhibiting properties like **1** (cAMP assay) [24]. This system provided an indication of cannabinoid receptor activation by monitoring the ability of a compound to inhibit forskolin-induced stimulation of cyclic adenosine monophosphate (cAMP) production. Along with the many synthetic $CB_1$ receptor agonists now developed [6, 25, 26], **1** can inhibit the activity of adenylate cyclase that synthesizes cyclic AMP. However, in this assay, **1** does not inhibit adenylate cyclase to the same extent as several other synthetic $CB_1$ receptor agonists, which led to its classification as a partial agonist at this receptor [27].

Two years later, in 1988, Devane and co-workers developed a radioligand displacement binding assay using the highly potent, synthetic $CB_1$ receptor agonist, CP-55940 [28]. In this assay, **1** effectively displaced radiolabeled CP-55940 and showed low micromolar affinity at the $CB_1$ receptor (Table 2). The properties of **1** as a $CB_1$ receptor partial agonist were further exemplified in binding assays assessing ligand-induced changes in GTPγS binding in cell membranes [27, 29, 30]. Here, the synthetic $CB_1$ receptor agonist, JWH-018, increased GTPγS binding in mouse brain membranes to a much greater extent than **1** [29].

Importantly, **1** not only activates $CB_1$ receptors in vitro but also in vivo as well. In vivo activity of **1** at $CB_1$ receptors was tested in a battery of animal behavior tasks known to produce outcomes associated with $CB_1$ receptor activation [31, 32]. The four simple behavioral tests in mice known as the "Billy Martin Tetrad" were reported, and these are: inhibition of locomotor activity; reduced sensitivity to pain; reduced body temperature; and immobility (catalepsy) [31]. At doses of 0.03–20 mg $kg^{-1}$ (i.v.), **1** was active in all of these tests, and the effects were blocked by the $CB_1$ receptor antagonist, rimonabant (10 mg $kg^{-1}$) [31–33]. However, it should be noted that rimonabant is not a specific ligand for the $CB_1$ receptor when employed at concentrations of $>1$ μ*M* [34, 35] and, therefore, at the concentrations reached in vivo.

With this dose, functional antagonism of these effects could also have been mediated by other targets of rimonabant such as agonism or antagonism of GPR55 receptors [36], antagonism of A1 adenosine receptors [37], and antagonism of TRPV1 channels [38].

In a feeding study in rats, **1** (0.5–4.0 mg $kg^{-1}$) stimulated hyperphagia. However, while rimonabant predictably inhibited hyperphagia at doses of $>0.67$ mg $kg^{-1}$, it also stimulated hyperphagia at lower doses. There was no significant difference in food intake between these two groups and this may be due to the differences in the feeding pattern being masked by effects on non-specific behavioral effects such as reduced motor co-ordination induced by **1** treatment [39].

There is also in vitro and in vivo evidence that **1** binds to, and activates the $CB_2$ receptor. The binding affinity of **1** at $CB_2$ receptors is, however, lower than that at $CB_1$ receptors, as shown in Table 2 [29, 40]. Evidence of a partial agonist effect of

**Table 2** Examples of $K_i$ values of $\Delta^9$-*trans*-tetrahydrocannibinol (**1**), $\Delta^9$-*trans*-tetrahydrocannabivarin (**2**), cannabinol (**3**), cannabidiol (**4**), cannabidivarin (**5**), cannabigerol (**6**), and cannabichromene (**7**) and half maximal responses where described

| | $K_i$/μ*M* | $EC_{50}$/ $IC_{50}$ | Assay | Cell type | Ref. |
|---|---|---|---|---|---|
| $\Delta^9$-Tetrahydrocannabinol (**1**) | | | | | |
| $CB_1$ | 0.0061 | ND | [$^3$H] CP55-940 binding assay | Whole brain/Rat | [28] |
| | 0.005 | ND | [$^3$H] CP55-940 binding assay | CHO cell membrane/ Human | [158] |
| | 0.008 | ND | [$^3$H] CP55-940 binding assay | CHO cell membrane/ Mouse | |
| | 0.013 | ND | [$^3$H] CP55-940 binding assay | CHO cell membrane/ Rat | |
| | 0.021 | ND | Filtration assay | Brain membranes/Rat | [86] |
| | 0.035 | ND | [$^3$H] CP55-940 binding assay | Brain synaptosomal membrane/Rat | [35] |
| | 0.0395 | 0.013 | [$^3$H] HU-243 binding assay | COS-7 cells/Rat | [40] |
| | 0.0477 | ND | [$^3$H] CP55-940 binding assay | Whole brain/Mouse | [90] |
| | 0.053 | 0.0165 | [$^3$H] CP55-940 binding assay | Fibroblast L cells/Rat | [87] |
| | 0.065 | ND | [$^3$H] HU-243 binding assay | Synaptosomal brain membrane/Rat | [85] |
| | 0.08 | ND | [$^3$H] HU-243 binding assay | COS-7 cells/Rat | |
| | 0.0356 | 0.087 | [$^3$H] CP55-940 binding assay | Sf9 cells/Human | [159] |
| $CB_2$ | 0.003 | ND | [$^3$H] CP55-940 binding assay | CHO cell membrane/ Human | [158] |
| | 0.0017 | ND | [$^3$H] CP55-940 binding assay | CHO cell membrane/ Mouse | |
| | 0.0068 | ND | [$^3$H] CP55-940 binding assay | CHO cell membrane/ Rat | |
| | 0.036 | ND | Filtration assay | Spleen membrane/Rat | [86] |
| | 0.0039 | ND | [$^3$H] CP55-940 binding assay | Spleen membrane/Rat | [35] |
| | 0.040 | ND | [$^3$H] HU-243 binding assay | CHO cell/Rat | [40] |
| | 0.075 | 0.0418 | [$^3$H] CP55-940 binding assay | CHO cell membrane/ Rat | [87] |
| | 0.032 | ND | [$^3$H] HU-243 binding assay | COS-7 cells/Rat | [85] |
| | 0.0084 | 0.061 | [$^3$H] CP55-940 binding assay | Sf9 cells/Human | [159] |
| $\Delta^9$-Tetrahydrocannabivarin (**2**) | | | | | |
| $CB_1$ | 0.075 | ND | [$^3$H] CP55-940 binding assay | Whole brain membranes/Mouse | [73] |
| | 0.047 | ND | [$^3$H] CP55-940 binding assay | Cortical brain membranes/Rat | [72] |
| | 0.286 | ND | [$^3$H] rimonabant binding assay | Cortical brain membranes/Rat | [160] |
| | 0.046 | ND | [$^3$H] CPP-940 binding assay | Whole brain/Mouse | [90] |

(continued)

**Table 2** (continued)

| | $K_i$/μ$M$ | $EC_{50}$/ $IC_{50}$ | Assay | Cell type | Ref. |
|---|---|---|---|---|---|
| $CB_2$ | 0.225 | 0.038 | [$^3$H] CP55-940 binding assay | CHO cell membrane/ Human | [70] |
| | 0.145 | 0.143 | [$^3$H] CP55-940 binding assay | CHO cell membrane/ Human | [41] |
| Cannabinol (**3**) | | | | | |
| $CB_1$ | 0.326 | ND | Filtration assay | Brain/Rat | [86] |
| | 0.129 | ND | [$^3$H] CPP-940 binding assay | Whole brain/Mouse | [90] |
| | 1.13 | >1 | [$^3$H] CPP-940 binding assay | Fibroblast L cells/Rat | [87] |
| | 0.392 | ND | [$^3$H] HU-243 binding assay | Synaptosomal brain membrane/Rat | [85] |
| | 0.211 | ND | [$^3$H] HU-243 binding assay | COS-7 cells/Rat | |
| | 3.2 | ND | [$^3$H] CP55-940 binding assay | Sectioned brain/Rat | [25] |
| | 0.25 | ND | [$^3$H] CP55-940 binding assay | Whole brain/Rat | [161] |
| | 0.74 | ND | [$^3$H] rimonabant binding assay | Whole brain/Rat | |
| | 0.012 | 0.017 | [$^3$H] CP55-940 binding assay | Sf9 cells/Human | [159] |
| | 0.069 | ND | [$^3$H] CP55-940 binding assay | CHO cell/Human | [88] |
| $CB_2$ | 0.096 | ND | Filtration assay | Spleen/Rat | [86] |
| | 0.301 | >1 | [$^3$H] CPP-940 binding assay | CHO cell membrane/ Rat | [87] |
| | 0.126 | ND | [$^3$H] HU-243 binding assay | COS-7 cells/Rat | [85] |
| | 0.016 | 0.055 | [$^3$H] CP55-940 binding assay | Sf9 cells/Human | [159] |
| | 0.07 | 0.062 | [$^3$H] CP55-940 binding assay | CHO cell/Human | [88] |
| Cannabidiol (**4**) | | | | | |
| $CB_1$ | >10 | ND | [$^3$H] CP55-940 binding assay | Whole brain/Mouse | [90] |
| | 0.073 | ND | [$^3$H] 5′-trimethylammonium-$\Delta^9$-THC binding assay | Whole brain/Rat | [162] |
| | >0.5 | ND | [$^3$H] CP55-940 binding assay | Whole brain/Rat | [28] |
| | 53 | ND | [$^3$H] CPP-940 binding assay | Sectioned brain/Rat | [25] |
| | 4.3 | ND | [$^3$H] CP55-940 binding assay | Cortical brain membranes/Rat | [163] |
| | 2.3 | ND | [$^3$H] CP55-940 binding assay | Whole brain/Rat | [161] |
| | 1.3 | ND | [$^3$H] rimonabant binding assay | Whole brain/Rat | |
| | >10 | ND | [$^3$H] HU-243 binding assay | Whole brain/Rat | [136] |
| | 4.9 | ND | [$^3$H] HU-243 binding assay | Whole brain/Mouse | [104] |
| | 1.8 | ND/ NE | [$^3$H] rimonabant binding assay | Brain cortical membranes/Rat | [164] |
| | 4.7 | ND | [$^3$H] CP55-940 binding assay | Whole brain membranes/Mouse | [118] |
| | 1.45 | 3.86 | [$^3$H] CP55-940 binding assay | Sf9 cells/Human | [159] |
| $CB_2$ | >10 | ND | [$^3$H] HU-243 binding assay | COS-7 cells/Rat | [136] |
| | 2.86 | ND | [$^3$H] CP55-940 binding assay | CHO cell/Human | [86] |
| | 4.2 | 0.503 | [$^3$H] CP55-940 binding assay | CHO cell/Human | [104] |

(continued)

**Table 2** (continued)

| | $K_i$/μ$M$ | $EC_{50}$/ $IC_{50}$ | Assay | Cell type | Ref. |
|---|---|---|---|---|---|
| | 2.86 | ND | [$^3$H] CP55-940 binding assay | *E. coli* cell membranes/ Human | [118] |
| | 0.37 | 2.27 | [$^3$H] CP55-940 binding assay | Sf9 cells/ Human | [159] |
| Cannabichromene (**7**) | | | | | |
| $CB_1$ | >10 | ND | [$^3$H] CP55-940 binding assay | Whole brain/Mouse | [90] |
| | 0.71 | 1.68 | [$^3$H] CP55-940 binding assay | Sf9 cells/Human | [159] |
| $CB_2$ | 0.256 | 1.30 | [$^3$H] CP55-940 binding assay | Sf9 cells/Human | [159] |
| Cannabigerol (**6**) | | | | | |
| $CB_1$ | 275 | ND | [$^3$H] CP55-940 binding assay | Sectioned brain/Rat | [25] |
| | 0.896 | 1.12 | [$^3$H] CP55-940 binding assay | Sf9 cells/Human | [159] |
| $CB_2$ | 0.153 | 0.85 | [$^3$H] CP55-940 binding assay | Sf9 cells/Human | [159] |
| Cannabidivarin (**5**) | | | | | |
| $CB_1$ | 14.7 | 13.80 | [$^3$H] CP55-940 binding assay | Sf9 cells/Human | [159] |
| | 0.127* | ND | [$^3$H] CP55-940 binding assay | MF1 brain membranes/ Mouse | [2] |
| $CB_2$ | 0.57 | 3.45 | [$^3$H] CP55-940 binding assay | Sf9 cells/Human | [159] |

ND, not described; CHO, Chinese hamster ovary; COS, CV1 in origin with SV40 genes; Sf, *Spodoptera frugiperda*

**1** at $CB_2$ receptors came from a study where **1** antagonized the inhibition of adenylate cyclase in CHO cells transfected with human $CB_2$ receptors induced by the agonists HU-293a and HU-210 (Table 2) [40].

As is typical of a partial agonist, **1** has a mixed agonist–antagonist effect. This is likely dependent on the proportion of cannabinoid receptors that are in the "active" state in tissues, coupled to their effector mechanisms, or in the "inactive" state, uncoupled to their effector mechanisms [41]. Moreover, it would also depend on the presence of other synthetic or endogenous cannabinoid receptor agonists, and possibly species differences between studies. As a partial agonist, **1** can be expected to antagonize the actions of full agonists. In a mouse model of hypothermia, **1** alone acted as a partial agonist with less efficacy than the cannabinoid receptor full agonist, AM2389, but when co-administered with this compound, **1** antagonized AM2389's hypothermic effects [42].

In an in vitro study using the GTPγS binding assay in rat brain membranes from rats chronically treated with 10 mg $kg^{-1}$ **1** for 21 days, the stimulation of GTPγS binding by WIN 55212–2 was reduced by up to 70%, suggesting that chronic exposure to **1** led to a desensitization of cannabinoid-activated signal transduction.

In healthy human subjects, the intravenous administration of **1** caused acute psychotic reactions and a temporary decline in cognitive functioning [43].

## 2.2 Cannabinoid Receptor Independent Activity

The well-known psychotropic effect of **1** is mediated by its partial agonist activity at $CB_1$ receptors. However, **1** also exerts effects at molecular targets outside of the endocannabinoid system. Some of the physiological effects of **1** may be mediated by more than one target, as detailed below.

In this regard, **1** has been proposed to act in an allosteric manner on specific receptors outside of the endocannabinoid system. In vitro, **1** potently inhibited $5HT_{3A}$-induced currents in HEK293 cells transfected with $5HT_{3A}$ receptor cDNA [44], similar to the reported effect of the synthetic cannabinoid receptor agonist, WIN 55212–2,and also in cultured rat trigeminal ganglion neurons (Table 3) [44, 45]. Together with **1**, other cannabinoids such as WIN 55212–2, anandamide, JWH-015, and CP-55940, have been shown to stereoselectively inhibit currents at this receptor [44].

Cannabinoid receptors and $5HT_3$ receptors are both involved in control of pain and emesis [11–14]. The results above show that the activity of cannabinoid receptor agonists on the control of pain and emesis may be shared by their antagonistic effect on $5HT_3$ receptors [46–50]. This highlights the possibility of a ligand having a physiological effect that can be mediated by multiple targets. Therefore, an effect proven to be mediated through one target does not mean that other targets of the ligand mediating the same physiological effect can be ruled out.

At glycine receptors, low concentrations of **1** also acted through a possible allosteric mechanism by potentiating the amplitude of glycine-activated currents in rat isolated ventral tegmental area neurons via a cannabinoid receptor-independent mechanism (Table 3) [51]. Glycine receptor function was potentiated by **1** at physiologicallyrelevant concentrations. Glycine receptors are involved in pain transmission [52, 53] and dopamine release from ventral tegmental area neurons [54, 55], thus **1** may be important for analgesia and drug addiction. Analgesia is also produced through **1** activity at cannabinoid receptors [11, 12] but some of this analgesic effect may be mediated through glycine receptors as well. This again shows a physiological effect being mediated by more than one target of the same ligand.

Compound **1** (0.1–10 μ*M*) is a peroxisome proliferator-activated receptor gamma (PPARγ) agonist. The studies below outline the relevance of the agonist effect at this nuclear receptor in the cardiovascular system and potentially in cancer treatment. Through agonism of the PPARγ receptor, **1** has time-dependent effects on vasorelaxation of the aorta and superior mesenteric arteries in a dose-dependent manner [56]. This relaxation effect of **1** was similar to the vascular relaxation effect of the PPARγ ligand rosiglitazone (46.7% and 69.7% respectively). Another study by the same group showed differences in the time-dependent effect of **1** on vasorelaxation in different vessel types; in resistance mesenteric arteries no time-dependent effect of **1** on PPARγ mediated vasorelaxation was noted [57]. These studies show that the effect of **1** on endothelium-dependent vasorelaxation is dependent on the predominant relaxing factor in a given artery. Agonism of

**Table 3** A comparison of select in vitro studies showing cannabinoid receptor independent activity of $\Delta^9$-*trans*-tetrahydrocannabinol (**1**) according to concentrations, assay types, and cell types used

| Target | Concentration/ μ*M* | $EC_{50}$/ μ*M* | $IC_{50}$/ μ*M* | Assay | Cell line | Ref. |
|---|---|---|---|---|---|---|
| GPR55 | <1 | | 0.008 | GTPγS binding assay | HEK293/Human | [67] |
| | 1–10 | | 5 | $[Ca^{2+}]$ mobilization assay | HEK293/Human | [165] |
| | <1 | | 0.64 | ERK1/2 MAPK phosphorylation | HEK293/Human | [69] |
| | 1 | | ND | ERK1/2 MAPK phosphorylation | HEK293/Human | |
| | | | ND | B-arrestin assay | HEK293/ND | [66] |
| GPR18 | <1 | 0.96 | | MAPK activation assay | HEK293/ Human | [62] |
| $5HT_{3A}$ | <1 | | 0.038 | Voltage clamp | HEK293/Human | [44] |
| Glycine ligand gated ion channels | | | | | | |
| α1 | <1 | 0.086 | | Whole cell patch clamp | *Xenopus laevis* oocytes/ Human | [51] |
| α1β1 | <1 | 0.073 | | Whole cell patch clamp | | |
| | | 0.115 | | | Ventral tegmental area neurons/ Rat | |
| PPARγ nuclear receptor | <1 | ND | | Contraction | HEK293/ND | [166] |
| TRP cation channels | | | | | | |
| TRPA1 | <1 | 0.23 | | $Ca^{2+}$ Fluorescence assay | HEK293/Rat | [80] |
| TRPV2 | | 0.65 | | | HEK293/Rat | |
| TRPM8 | | | 0.16 | | HEK293/Rat | |
| | | | 0.15 | | HEK293/Rat | [79] |
| TRPV3 | 1–10 | 9.5 | | | HEK293/Rat | [81] |
| TRPV4 | | 8.5 | | | HEK293/Rat | |
| CYP1A1 | 1–10 | | 0.53 | Fluorescence assay-FLUOSTAR OPTIMA | Recombinant/ Human | [167] |
| CYP1A2 | | | 4.59 | | | |
| CYP1B1 | | | 1.39 | | | |
| CYP2C9 | 1–10 | | 2.84 | HPLC | Recombinant/ Human | [168] |
| Adenosine uptake | <1 | | 0.27 | Scintillation counting [$^3$H]adenosine | EOC-20 microglia | [116] |
| | | | 0.334 | Scintillation counting [$^3$H]adenosine | RAW264.7 macrophages | |

GPR, G-protein-coupled receptor; 5HT, 5-hydroxytryptamine; PPAR, peroxisome proliferator-activated receptor; TRP, transient receptor potential; CYP, cytochrome P450; HEK, human embryonic kidney; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); $Ca^{2+}$, Calcium; ERK, extracellular signal-regulated kinases; MAPK, mitogen-activated protein kinase; also see footnote for Table 1

PPARγ by **1** leads to an increase in superoxide dismutase activity, thus leading to an increase in hydrogen peroxide ($H_2O_2$). In superior mesenteric arteries, $H_2O_2$ is the predominant relaxing factor and therefore **1** enhances endothelium-dependent vasorelaxation. In resistance mesenteric arteries, however, where endothelium-derived hyperpolarizing factor (EDHF) is the predominant relaxing factor, **1** inhibits EDHF production and therefore inhibits vasorelaxation in these arteries [57].

In vivo, **1** acts via the PPARγ mechanism to reduce tumor growth rate. In mice with induced tumour xenografts, **1** (15 mg $kg^{-1}$) showed antitumor properties by reducing tumor growth rate, which was prevented by co-administration with the PPARγ antagonist, GW9662 [58]. However, an antagonist-only treatment group was not included in this study and therefore the effect of **1** on tumor growth has not been validated as being mediated by PPARγ and so, as yet, can be considered a functional, rather than molecular antagonism.

Moreover, PPARγ is not only involved in the physiological roles outlined above. It is also involved in adipogenesis, where it is highly expressed, and in the treatment of type 2 diabetes [59, 60] and gastro-inflammatory disorders [61]. Compound **1** may therefore have as yet unproven effects on these disorders. There are other $G_{i/o}$ coupled receptors (GPCR) that are thought to be novel cannabinoid receptors. These are GPR18 and GPR55 [29, 30, 62, 63]. These receptors belong to the same class as $CB_1$ and $CB_2$ receptors but do not share many structural similarities [64], which would likely result in differing ligands and physiological effects at these receptors compared to $CB_1$ and $CB_2$ receptors.

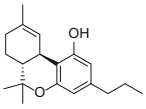
In HEK293 cells transfected with the novel $G_{i/0}$ coupled GPCR cannabinoid receptor, GPR18, **1** acts as a potent agonist (Table 3) [62]. Interestingly, the phytocannabinoid cannabidiol (**4**) can antagonize the effect of the agonists such as **1** at this receptor [62, 63].

There are conflicting reports on the activity of **1** at the GPR55 receptor in vitro. This receptor has been claimed, by many authors, to be a third cannabinoid receptor [29–31, 65]. Using two different assays in the same cell line (HEK293) transfected with human GPR55, **1** weakly activated GPR55 in a β-arrestin assay [66], but potently activated it in a GTPγS binding assay with a submicromolar half maximal response (Table 3) [67]. However, using the same cell line transfected with human GPR55 and the β-arrestin assay, Kapur and co-workers found no detectable activity of **1** at this receptor [68]. Moreover, again in the same cell line also transfected with human GPR55, **1** has been reported to exhibit differential effects in a concentration-dependent fashion. It was reported in the same study that **1** is an inhibitor of the proposed endogenous agonist of GPR55, lysophosphatidylinositol (LPI), at concentrations of 1 μ*M*, by inducing a rightward shift in the log concentration-response

curve of LPI as well as activating this receptor at micromolar concentrations [69]. These findings of agonism and inhibition suggest that there could be two distinct binding sites on GPR55 receptors. By itself, **1** may bind to either an orthosteric binding site or an allosteric binding site producing agonism of the receptor or, by binding to an allosteric site, produces a conformational change in the orthosteric binding site, thus reducing the effect of LPI [69]. The binding of **1** to a particular binding site may be dependent on the concentrations used.

Even though **1** has undesirable psychotropic effects, mediated by $CB_1$ receptors, it is important to remember that this phytocannabinoid has a range of important therapeutic benefits. These effects may be mediated both by cannabinoid receptors, either $CB_1$ and $CB_2$ receptors or novel GPCRs, and non-cannabinoid targets.

## 3 $\Delta^9$-Tetrahydrocannabivarin




**2**

$\Delta^9$-Tetrahydrocannabivarin (**2**) is included in the $\Delta^9$-THC chemical class which, as mentioned earlier, constitutes the majority of the phytocannabinoid content [1] of *C. sativa*. This phytocannabinoid is the *n*-propyl analog of **1**, with the slight structural change resulting in some different molecular targets and physiological effects when compared to **1**.

### *3.1 Activity at Cannabinoid Receptors*

In vitro, **2** is a $CB_2$ receptor partial agonist, as shown by its lower efficacy at $CB_2$ receptors than the agonist CP-55940 in both CHO cells transfected with human $CB_2$ receptors and in the GTPγS binding assay in membranes from these cells, as shown in Table 2 [70].

Importantly, there is also in vivo evidence of **2** as a $CB_2$ receptor partial agonist. Garcia and co-workers showed that **2** (2 mg $kg^{-1}$) can show signs of neuroprotection in a model of Parkinson's disease in mice that have received

intrastriatal injections of lipopolysaccharide (LPS), similar to the effects shown by the $CB_2$ selective agonist HU-308 [71]. $CB_2$ receptor-deficient mice were more vulnerable to LPS-induced lesions, which supports the effects of **2** being mediated, at least in part, by agonism at $CB_2$ receptors.

At low concentrations (0.1–5 μ*M*), **2** blocks $CB_1$ receptors both in vitro and in vivo, but interestingly at high doses acts as a $CB_1$ agonist in vivo but not in vitro. Two studies reported that **2** blocks the agonist effects of CP-55940- and (+)-(*R*)-WIN55212-induced stimulation of GTPγS binding to mouse whole membranes at a low concentration of 1 μ*M* (Table 2) [72, 73], while Dennis and co-workers showed, using the same assay, this antagonistic effect of **2** from the lower concentration of 0.1 μ*M* up to 5 μ*M* on (+)-(*R*)-WIN55212 in the mouse cerebellum and piriform cortex membranes [74]. The antagonist effect of **2** is the same as two established $CB_1$ receptor-selective antagonists, rimonabant and AM251 [72, 73, 75, 76]. Thus, antagonism of $CB_1$ receptors by **2** modulates inhibitory neurotransmission in the cerebellum [76].

In vivo, **2** acts as both an antagonist and agonist at low doses and high doses, respectively. This antagonist and agonist phenomenon results in opposing effects on antinociception and on locomotor activity depending on the concentration used. This disparity in pharmacological effect of **2**, dependent upon the concentration used, highlights the importance of knowing the concentration of each phytocannabinoid in whole cannabis plant material and extracts when this is being used for therapeutic use.

At low doses of 0.3 and 3 mg $kg^{-1}$, **2** blocks the antinociceptive effect of **1** in a mouse model of acute pain and hypothermia [72]. Using the same model, **2** also partially antagonized the $CB_1$ agonist effects of CP-55940 at a dose of 2 mg $kg^{-1}$ and also partially antagonized CP-55940-induced inhibition of rat locomotor activity in a model of Parkinson's disease [71]. There was no effect of **2** treatment alone on either of these parameters and therefore these studies support the molecular antagonism of **2** at $CB_1$ receptors.

At higher doses of 3, 10, 30, and 56 mg $kg^{-1}$, **2** acts as an agonist by producing antinociception in an acute model of pain and causes immobility in the ring test (a quantitative test for measuring catalepsy [65]) [72]. In this study, the $CB_1$ receptor antagonist rimonabant blocked the agonist effect of **2** on antinociception but not on immobility in the ring test. A rimonabant-only treatment group was not included in this study to rule out whether this antagonist worsens nociceptive pain. It is therefore not clear from this study whether the effect found is functional or molecular.

In other in vivo experiments, **2** (3, 10, 30 mg $kg^{-1}$) suppressed food consumption in non-fasted mice, similar to the $CB_1$-selective antagonist AM251 [77]. Signs of motor inhibition, induced by 6-hydroxydopamine, were reduced by **2** (2 mg $kg^{-1}$), similar to the effect of the $CB_1$ antagonist, rimonabant [71]. It is unclear without further investigation whether this effect of **2** is via inverse agonism of the $CB_1$ receptor, competitive inhibition with endogenous cannabinoids at $CB_1$ receptors or by activity at another target, since comparisons made were based on functional effects of the compounds without confirmation of the molecular targets [78].

## 3.2 Cannabinoid Receptor Independent Activity

There is little available evidence to suggest that **2** acts at $CB_1$ or $CB_2$ receptor-independent targets but it may have other targets within the cannabinoid system, such as the novel cannabinoid receptor GPR55 [69]. There is, however, only one study to date describing agonism of GPR55 receptors by **2** [69]. In this investigation, **2** was an agonist of GPR55 in HEK293 cells expressing human GPR55 with a similar potency to **1** (Table 4) and 1 μ*M* **2** was shown to inhibit LPI induced stimulation of GPR55 with 50% efficacy, higher than that of **1** [69].

The evidence of **2** acting at targets outside the cannabinoid system comes from the proven interaction between **2** and transient receptor potential (TRP) cation channels at higher concentrations than at which it acts at $CB_1$ or $CB_2$ receptors [79–81].

Despite there being limited known pharmacological targets for **2**, its activity at TRP channels may have wide-reaching physiological effects. These TRP channels are present in the plasma membrane of a broad range of cell types in many tissues and act as ligand-gated, non-selective cation channels permeable to sodium, calcium and magnesium ions, thereby being powerful regulators of many cell functions [82].

De Petrocellis and co-workers studied the efficacy and potency of numerous phytocannabinoids at various TRP channels [79–81]. At TRPA1 and TRPV1 cation channels, **2** is an agonist with the same high potency and at TRPV2 with a slightly

**Table 4** A comparison of selected in vitro studies showing cannabinoid receptor independent activity of $\Delta^9$-*trans*-tetrahydrocannabivarin (**2**) according to concentrations, assay types, and cell types used

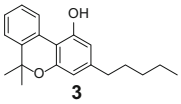
| Target | Concentration/ μ*M* | $EC_{50}$/ μ*M* | $IC_{50}$/ μ*M* | Assay | Cell type | Ref. |
|---|---|---|---|---|---|---|
| GPR55 | >1 | 0.88 | | ERK1/2 MAPK phosphorylation | HEK293/Human | [69] |
| | 1 | | ND | ERK1/2 MAPK phosphorylation | HEK293/ Human | |
| $5HT_{1A}$ | <1 | 5.4 | | GTPγS binding assay 8-OH-DPAT | Brainstem membranes/Rat | [169] |
| | | 28.3 | | GTPγS binding assay 8-OH-DPAT | CHO cells/ Human | |
| TRP cation channels | | | | | | |
| TRPA1 | 1–10 | 1.5 | | $Ca^{2+}$ fluorescence assay | HEK293/Rat | [80] |
| TRPM8 | <1 | | 0.87 | | HEK293/Rat | |
| TRPV1 | 1–10 | 1.5 | | | HEK293/ Human | |
| TRPV2 | | 4.1 | | | HEK293/Rat | |
| TRPV3 | | 3.8 | | | HEK293/Rat | [81] |
| TRPV4 | | 6.4 | | | HEK293/Rat | |

See Tables 1 and 2

lower potency. The TRPM8 cation channels are blocked by **2** with relatively high potency (Table 4) [80].

In summary, **2** is known to be an antagonist at $CB_1$ receptors at low concentrations both in vitro and in vivo but at high concentrations it shows agonistic effects at $CB_1$ receptors only in vivo. This antagonistic effect at $CB_1$ receptors has been shown to have adverse effects in the clinic, with removal of the $CB_1$ receptor antagonist, rimonabant, from the market due to adverse psychological effects [83]. In vivo and in vitro evidence supports partial agonism activity at $CB_2$ receptors and at higher concentrations than at which it activates cannabinoid receptors it has activity at TRP cation channels, which may have benefits for regulating a variety of cell functions.

## 4 Cannabinol




**3**

Cannabinol (**3**) is an oxidation product of **1** and is found in large quantities in dried and aged cannabis material [84]. The acid form of **3** is also found in large quantities in the cannabis plant but upon heating this acid is decarboxylated to **3** [84]. This is important to take into account when considering how cannabis that is being used for medicinal or recreational purposes is processed, and stored, and how it is administered.

### *4.1 Activity at Cannabinoid Receptors*

Cannabinol (**3**) like **1**, acts at both $CB_1$ and $CB_2$ receptors but with higher affinity for $CB_2$ than $CB_1$ receptors, as shown in Table 2 [85–87]. It is an agonist at $CB_1$ receptors [29], but there are conflicting reports about its activity at $CB_2$ receptors. In COS-7 cells transfected with rat $CB_2$ receptors, **3** acted as a $CB_2$ receptor agonist in the cyclic AMP assay at 1 μM [85] but in another study performed in CHO cells transfected with human $CB_2$ receptors, **3** acted as an inverse agonist in the GTPγS binding assay at submicromolar concentrations [88]. These discrepancies may be due to the differences in concentrations of **3** used between the studies and could also depend on the conformational state of the receptors in the tissues. Receptors can either be in the active conformational state, where G-proteins are activated and

elicit a physiological response, or the inactive conformational state, where there is no activation of G-proteins. The amount of receptors in either state can differ in different tissues and under different conditions. If a ligand has a greater affinity for a specific conformational state (active or inactive), then the presence of the ligand will cause a redistribution of the concentrations of each conformational state. Thus, the concentration of ligand present will dictate the distribution of the receptor conformational state and either induce or inhibit a physiological response [89]. Further investigations are warranted to determine the activity of **3** at $CB_2$ receptors.

In vivo, **3** (50 mg $kg^{-1}$) has been shown to be a $CB_1$ receptor agonist by suppressing acetic acid-induced abdominal stretching behavior in mice, which was blocked by the $CB_1$ antagonist, rimonabant. The administration of rimonabant alone did not significantly affect abdominal stretching, indicating that this effect of **3** is likely to be a molecular one [90]. Moreover, in this study, the effect of **3** on locomotor suppression was also investigated. This was performed to determine whether the effect of **3** on hypomotility could be excluded from the observed effect of **3** on abdominal stretching behavior. The dose of **3** used (50 mg $kg^{-1}$) did not elicit locomotor suppression thereby indicating the suppression of abdominal stretching was not due to motor dysfunction [90].

Additionally, **3** (0.26–26.0 mg $kg^{-1}$ p.o.) exerts $CB_1$ receptor-dependent effects on rat feeding behavior by decreasing latency to feed and increasing food consumption over the whole test period with these effects being abolished in the presence of rimonabant [91]. However, a rimonabant-only treatment group was not included in this study and therefore it is not clear whether this effect of **3** is via functional mechanisms or molecular mechanisms. In numerous other feeding studies rimonabant decreases food consumption [92–94], but there is speculation as to whether this is due to suppressive effects of rimonabant on spontaneous locomotion [95, 96] and stimulation of emesis and nausea [97–99]. Together with these studies it is unclear whether the effects of **3** and rimonabant on feeding are mediated via molecular mechanisms.

For further information on binding affinities of **3** at $CB_1$ and $CB_2$ receptors, see Table 2.

## *4.2 Cannabinoid Receptor Independent Activity*

Cannabinol also acts at targets outside of the endocannabinoid system. It is a potent agonist of TRPA1 cation channels, potently blocks TRPM8 cation channels, and also desensitizes TRPA1 cation channels to activation by the agonist allyl isothiocyanate (Table 5) [80].

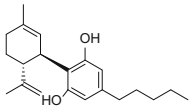
There is little recent literature on the pharmacology of **3** and thus further investigations need to be conducted to determine whether this compound has other therapeutic or recreational effects and how it modulates or enhances the physiological effects of whole cannabis-derived preparations.

**Table 5** A comparison of selected in vitro studies showing cannabinoid receptor independent activity of cannabinol (**3**) according to concentrations, assay types, and cell types used

| Target | Concentration/ μ*M* | $EC_{50}$/ μ*M* | $IC_{50}$/ μ*M* | Assay | Cell type | Ref. |
|---|---|---|---|---|---|---|
| TRP cation channels | | | | | | |
| TRPA1 | <1 | 0.18 | 0.4 | $Ca^{2+}$ fluorescence assay | HEK293/Rat | [80] |
| TRPM8 | | | 0.21 | | HEK293/Rat | |
| TRPV1 | 1–10 | 6.2 | | | HEK293/ Human | |
| TRPV2 | >10 | 19.0 | | | | |
| TRPV3 | 1–10 | 5.3 | | | HEK293/Rat | [81] |
| TRPV4 | >10 | 16.1 | | | HEK293/Rat | |
| CYP1A1 | 1–10 | | 0.685 | fluorescence assay—FLUOSTAR OPTIMA | Recombinant/ Human | [167] |
| CYP1A2 | | | 3.92 | | | |
| CYP1B1 | | | 1.50 | | | |
| CYP2C9 | 1–10 | | 2.86 | HPLC | Recombinant/ Human | [168] |

See Tables 1 and 2

## 5 Cannabidiol




**4**

Cannabidiol (**4**) is a non-psychotropic phytocannabinoid and the **4** chemical class type of phytocannabinoids is currently the third most abundant chemical class type in cannabis, after **1** and **6** [1]. Another phytocannabinoid in this class, cannabimovone, was isolated in 2010 [100], thereby increasing the number of phytocannabinoids of this type from seven in 2005 [101] to eight [1]. This class now makes up 7.7% of phytocannabinoid content (Table 1).

### *5.1 Activity at Cannabinoid Receptors*

Cannabidiol (**4**) has been investigated in a number of studies to determine its activity at cannabinoid receptors and shows very low affinity at these receptors (Table 2) [102, 103]. There has been a single report where **4** was shown to act as an antagonist of both $CB_1$ and $CB_2$ receptors at submicromolar concentrations [104]. However, a meta-analysis examining interspecies differences in ligand-binding affinity and receptor distribution identified eight methodological covariates

that could explain the discrepancies between results from various studies on cannabinoid receptor affinity for **4** [105]. A more recent meta-analysis from the same group concluded that **4** has very low affinity as an orthosteric ligand for $CB_1$ receptors (Table 2), but may affect $CB_1$ receptor activity in vivo via an indirect mechanism [78]. However, a study recently published showed that **4** can act as a negative allosteric modulator of $CB_1$ receptors [106]. Allosteric modulators alter the potency and efficacy of the orthosteric ligands but do not activate the receptor themselves. The allosteric effects of **4** were studied using an operational model of allosterism [107] and the effects of **4** treatment compared to the well-characterized negative allosteric modulators ORG2759 and PSNGBAM-1 [108–111]. The efficacy of both of the orthosteric ligands, **1** and 2-AG, was reduced by **4** ($<1$ μ*M*) and **4** displayed negative co-operativity for binding of these ligands. Moreover, **4**-treatment reduced G-protein dependent signaling and arrestin 2 recruitment, similar to the effects of the negative allosteric modulators ORG2759 and PSNCBAM-1 [109, 112]. This allosteric modulation of $CB_1$ receptors needs to be validated by further studies, but the results from this study could explain the reported ability of **4** to functionally antagonize some effects of **1** in animal studies and clinical studies in humans (for a review see [113]).

Compound **4** has an effect in vitro of inhibiting anandamide uptake and therefore affecting endocannabinoid tone by increasing availability of anandamide. The concentration at which **4** exerts its half maximal response, however, is higher than what would be relevant for a physiological effect in vivo [80].

## *5.2 Cannabinoid Receptor Independent Activity*

Despite **4** showing very little affinity for $CB_1$ and $CB_2$ receptors, as described above, there is evidence of an antagonist effect of **4** at the novel cannabinoid receptor GPR55 both in vitro and in vivo. At a concentration of 1 μ*M*, **4** suppressed the activation of GPR55 in rat hippocampal slices, thus suppressing excitatory output from pyramidal cells [114]. In a GTPγS-binding assay, **4** had potent antagonist effects at GPR55 with a submicromolar half maximum response (Table 6) [67]. Whyte and co-workers have shown a role for GPR55 in bone physiology, regulating osteoclast formation and function and bone mass [115]. This group reported that administration of **4** (10 mg $kg^{-1}$) to mice three times daily for 8 weeks significantly reduced bone resorption in these mice.

Outside of the endocannabinoid system, **4** has numerous targets and its activity at these targets results in a variety of physiological effects. Some of these physiological effects may be mediated by more than one target, such as the anti-inflammatory and immunosuppressive effect of **4**. These effects are mediated by both adenosine mechanisms and via strychnine-sensitive glycine receptors, as detailed in the following paragraphs.

**Table 6** A comparison of select in vitro studies showing cannabinoid receptor independent activity of cannabidiol (**4**) according to concentrations, assay types, and cell types used

| Target | Concentration/ μ*M* | $EC_{50}$/μ*M* | $IC_{50}$/μ*M* | Assay | Cell line | Ref. |
|---|---|---|---|---|---|---|
| GPR55 | <1 | | ND | Two photon $Ca^{2+}$ imaging | Hippocampal slices/Rats | [114] |
| | <1 | | 0.45 | GTPγS binding assay | HEK293/Human | [67] |
| | <1 | | 0.45 | Rho/ERK1/2 activation | Human osteoclasts | [115] |
| Glycine receptors | | | | | | |
| α1 subunit | 1–300 | 12.3 (allosteric) | | Patch clamp | HEK293/ND | [117] |
| | | 132.4 (direct) | | | | |
| α1β subunit | 1–300 | 18.1 (allosteric) | | Patch clamp | HEK293/ND | |
| | | 144.3 (direct) | | | | |
| α3 subunit | 0.01–50 | 3 (direct) | | Patch clamp | HEK293/ND | [118] |
| $5HT_{1A}$ | <1 | 0.007 | | GTPγS binding assay | Rat brainstem | [123] |
| | >10 | ND | ND | [3H]-8-OH-DPAT ligand binding | CHO/Human | [122] |
| | | | | [35S]-GTPγS assay | | |
| | | | | Forskolin | | |
| $5HT_{2A}$ | >10 | ND | ND | [$^3$H]-Ketanserin | NIH 3 T3 membrane/rat | |
| $5HT_{3A}$ | <1 | | 0.6 | Patch clamp | *Xenopus laevis* oocytes | [135] |
| PPARγ | 1–10 | 5 | | Contraction | Aorta/rat | [166] |

| | | | | | | |
|---|---|---|---|---|---|---|
| Mitochondrial Complex I<br>Complex II<br>Complex IV | 1–10, >10 | | | High resolution respirometry | Brain cortex/Pig | [170] |
| | | | 8.2 | | | |
| | | | 19.1 | | | |
| | | | 18.8 | | | |
| CYP2C19<br>CYP3A5<br>CYP2C9<br>CYP1A1 | 1–10 | | 0.5–2.7 | HPLC, Fluorescence FLUOSTAR OPTIMA | Recombinant/Human liver microsomes | [167, 168, 171, 172] |
| Cav3.1–3.3 T-type | 1–10 | | 0.78–3.7 | Whole cell patch clamp | HEK293/Human, Sensory neurons/Mouse | [173] |
| TRPA1 | <1 | 0.096 | | $Ca^{2+}$ Fluorescence assay | HEK293/Rat | [79] |
| | | 0.11 | 0.06 | | HEK293/Rat | [80] |
| TRPM8 | <1 | | 0.14 | $Ca^{2+}$ Fluorescence | HEK293/Rat | [79] |
| | | | 0.06 | | HEK293/Rat | [80] |
| TRPV1 | 1–10 | 3.5 | | $Ca^{2+}$ Fluorescence assay | HEK293/Human | [136] |
| | 1–10 | 1 | | | HEK293/Human | [80] |
| TRPV2 | 1–10 | 1.25 | | $Ca^{2+}$Fluorescence | HEK293/Rat | |
| | >10 | 22.2 | | $Ca^{2+}$ mobilization | U87MG/Human | [174] |
| TRPV3 | 1–10 | 3.7 | | $Ca^{2+}$ Fluorescence | HEK293/Rat | [81] |
| TRPV4 | 1–10 | 0.8 | | | HEK293/Rat | |
| Adenosine uptake | <1 | | 0.12 | Scintillation counting [$^3$H] Adenosine | EOC-20 microglia | [116] |
| | | | 0.19 | Scintillation counting [$^3$H] Adenosine | RAW264.7 macrophages | |
| | 1–10 | | 3.5 | Dual label counting [$^3$H] Adenosine | Striatal tissue synaptosome/ Mouse, Rat | [175] |

8-OH-DPAT, 8-hydroxy-2-(dipropylamino)-tetralinhydrobromide; HPLC, high-performance liquid chromatography; also see Tables 1 and 2

Activity of **4** at one target may also elicit various physiological effects. This is shown by **4** having anti-inflammatory effects and antiarrhythmic effects both mediated by adenosine mechanisms. Another example refers to the 5HT serotonin receptors of a target where **4** acts to mediate multiple physiological effects such as acute autonomic responses to stress, nausea and vomiting, cerebral infarction and anxiolytic, panicolytic, and antidepressant effects. The sections below will describe in more detail the studies that support evidence for the numerous and varied physiological targets of **4**.

It is known that **4** has anti-inflammatory and immunosuppressive effects, but these effects have been shown to be mediated by multiple pharmacological targets, as detailed below. The mechanisms by which **4** possibly mediate anti-inflammatory and immunosuppressive effects include: activity at $A_{1A}$ and $A_{2A}$ adenosine receptors and the inhibition of the equilibrative nucleoside transporter [116] and the activation of strychnine-sensitive $\alpha_1$ and $\alpha_1\beta$ glycine receptors [117, 118].

The effects of **4** mediated via adenosine have been shown in both in vitro and in vivo studies. Uptake of [$^3$H] adenosine was inhibited by **4** in murine microglia and RAW264.7 macrophages by a mechanism of binding to the equilibrative nucleoside transporter 1 (ENT1) and competitively inhibiting this nucleoside transporter with a $K_i$ value of less than 0.25 $\mu M$ and a submicromolar half maximal response [116] (Table 6). In addition to inhibition of ENT1 uptake of adenosine, the authors also documented in vivo that **4** could bind and activate the $A_{2A}$ receptor, since the effects of **4** on tumor necrosis factor α (TNF α) were abolished by an $A_{2A}$ receptor antagonist and by genetic deletion of this receptor [116]. An in vivo effect of **4** on anti-inflammatory effects mediated by the $A_{2A}$ receptor was shown with lipopolysaccharide-induced inflammation in the rat retina [119] and in the mouse lung [120], both using the $A_{2A}$ receptor antagonist ZM241385. The study by Liou and co-workers that indicated inhibition of adenosine uptake by ENT1 is important in the anti-inflammatory effects of **4** both in vitro and in vivo in the rat retina [119].

These studies clearly indicated that **4** has immunosuppressive effects that are mediated via adenosine mechanisms. This immune-suppressive effect is important in limiting cellular stress and inflammation and perhaps explains the effect of **4** on improving arthritis and multiple sclerosis symptoms. Its immunosuppressive effects in microglia would have considerable benefits for a number of neurodegenerative conditions.

The anti-inflammatory effects of **4** mediated through strychnine-sensitive $\alpha_1$ and $\alpha_1\beta$ glycine receptors have also been shown in in vitro and in vivo studies but the in vitro study detailed below would be physiologically irrelevant due to the high concentrations used to elicit an effect that would not be achieved in vivo. The study used a whole cell patch clamp technique to show that **4**, at a mid-micromolar range, had positive allosteric modulating effects at these glycine receptor subunits and at higher concentrations showed direct activation of these receptor subunits (Table 6) [117].

It has also been reported that **4** has anti-inflammatory actions and suppresses neuropathic pain in vivo, mediated by glycine receptors. In α3 glycine channel knockout mice injected with Freund's adjuvant into the hind paw, the anti-

inflammatory effects of **4** (50 mg $kg^{-1}$ i.p.) in this model of inflammatory pain were abolished [118].

Another physiological effect of **4** mediated via adenosine mechanisms is its antiarrythmic effect, shown by inhibiting ventricular tachycardia in rats [121]. This effect was shown using a low dose of **4** of 50 μg $kg^{-1}$ and agonism of the $A_{1A}$ receptor by **4** was validated by administration of the selective antagonist, 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), at 100 μg $kg^{-1}$. In the presence of this selective antagonist, these effects of **4** were abolished [121]. Importantly, this effect was also determined to be a molecular effect rather than a functional one since a DCPX-only treatment group showed no effect on the incidence or duration of arrhythmias.

Significant evidence supports **4** producing its effects via serotonin (5-HT) receptors, predominantly the $5HT_{1A}$ receptor subtype but also the $5HT_{3A}$ receptor and less so at the $5HT_{2A}$ receptor. As described above, activity at these receptors mediates a variety of physiological responses.

In two in vitro studies, first in Chinese hamster ovary (CHO) cells [122] and more recently using rat brainstem membranes [123], **4** was found to enhance the ability of a $5HT_{1A}$ agonist, 8-OH-DPAT, to stimulate GTPγS binding at submicromolar concentrations (Table 6).

In vivo, **4** induces various $5HT_{1A}$-mediated physiological responses. These include attenuation of: acute autonomic responses to stress, nausea and vomiting, and cerebral infarction, and induction of anxiolytic, panicolytic, and antidepressant effects [123–133]. Studies with evidence supporting these effects are detailed below.

At doses of 1, 10, or 20 mg $kg^{-1}$ (i.p.) of **4** in male Wistar rats, this compound dose-dependently reduced the acute autonomic response to restraint stress and reduced the anxiety behavior caused by previous exposure to restraint [124]. These effects of **4** were blocked by the $5HT_{1A}$ receptor antagonist, WAY100635 (0.1 mg $kg^{-1}$), while by itself WAY100635 did not have an effect on cardiovascular or anxiogenic responses, indicating this to be a molecular effect [124]. Another study reported that **4** administration directly into the dorsal periaqueductal gray via an implanted cannula in rats elicits panicolytic effects by inhibiting escape responses in the elevated T maze via $5HT_{1A}$ mediated responses. These responses were blocked by treatment with WAY100635 [125]. In both of these studies, a WAY100635-only treatment group was not used and therefore these results are not indicative of a molecular effect.

Activation of $5HT_{1A}$ receptors has been regularly related to the therapeutic effect of antidepressant treatments [130] and a reduced number/affinity of postsynaptic $5HT_{1A}$ receptors in the brains of depressed individuals has been reported by a number of studies [131, 132]. The first study to investigate whether there is a link between these receptors and the antidepressant effects of **4** was conducted quite recently by Zanelati and co-workers [126]. Mice received i.p. injections of 3, 10, 30, and 100 mg $kg^{-1}$ **4** and were then subjected to the forced swimming test. This test is predictive of antidepressant-like activity [133]. Immobility time was reduced by **4** and showed a bell-shaped response, since **4** was only effective at 30 mg $kg^{-1}$

and not at the lower or higher doses [126]. The $5HT_{1A}$ receptor antagonist WAY100635 blocked the effects of **4** on antidepressant-like activity but mediation of this effect by $5HT_{1A}$ receptors was not validated by use of a WAY100635-only treatment group.

Various studies have reported **4** to have antiemetic- and antinausea-like effects and this is thought to be mediated by $5HT_{1A}$ receptors. Unlike **1**, for which the antiemetic effects are mediated by both $CB_1$ receptors and 5HT receptors (as described in Sect. 2.2), it appears that **4** exerts its antiemetic effects primarily through 5HT receptors. Three studies showed that a low dose of **4** (5 mg $kg^{-1}$ i.p.) suppressed nicotine, cisplatin, and lithium chloride-induced vomiting in house musk shrews (*Suncus murinus*) [123, 127, 128] and lithium chloride-conditioned gaping in rats [123]. This suppression of vomiting and conditioned gaping was abolished by pre-treatment with the $5HT_{1A}$ receptor antagonists, WAY100135 and WAY100635 [123], but since an antagonist-only treatment group was not included in this study, an effect of **4** being mediated by $5HT_{1A}$ receptors has not been validated and it only showed a functional effect.

Neuroprotective effects of **4** have been shown through increasing cerebral blood flow and reducing infarct volume in a mouse model of middle cerebral artery occlusion [129]. This effect has been claimed to be $CB_1$ receptor independent [134] and in this study the effects of **4** were opposed by WAY100135 but not by the $CB_1$ receptor antagonist, rimonabant [129]. The neuroprotective effects of **4** have been claimed here to be mediated by $5HT_{1A}$ receptors but since a WAY100135-only treatment group was not included, this effect may be functional rather than molecular.

The only study to date investigating **4** activity at $5HT_{3A}$ receptors in vitro was conducted using *Xenopus laevis* oocytes expressing mouse $5HT_{3A}$ receptors using two electrode voltage clamp techniques [135]. In this study, **4** reversibly inhibited 5HT evoked currents in a concentration-dependent manner, which indicated that **4** is a non-competitive antagonist of $5HT_{3A}$ receptors (Table 6) [135]. This antagonist activity of **4** at $5HT_{3A}$ receptors may also be involved in the control of pain and emesis as described for **1** [46–50].

Activity of **4** at $5HT_{2A}$ receptors seems to be minimal and studies to date are not physiologically relevant, as shown with the high concentrations used in the following study. Using NIH/3 T3 cells expressing rat $5HT_{2A}$ receptors, **4** showed activity as a partial agonist but only at a concentration of 32 μ*M* and furthermore it only had 50% efficacy at displacing [$^3$H]-ketanserin [122]. The concentration used here would not be reached in vivo.

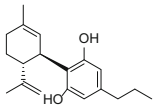
Unlike most of the other phytocannabinoids, **4** has been reported to act at TRP cation channels not just in vitro but in vivo as well. In vitro, **4** has been reported by numerous studies to activate TRPV1, TRPV2, and TRPA1 channels [80, 136–139] in HEK293 cells expressing these channels (Table 6). The TRPA1 channels are potently activated by **4**, with this compound being the second most potent agonist at this channel of all the phytocannabinoids tested in this study (Table 6) [80]. Indeed, all phytocannabinoids mentioned in this chapter, except cannabichromene (**7**), antagonize the $Ca^{2+}$ elevation response induced by the agonist icilin [79, 80], but

**4** is the most potent antagonist at this channel (Table 6) [80]. Moreover, in a recent study using whole cell patch clamp techniques on HEK293 transfected cells, it was shown that **4** (3, 10, 30 μ*M*) dose-dependently activated and rapidly desensitized TRPV1, TRPV2, and TRPA1 channels [139]. The TRPV3 channels are activated by **4** with high efficacy (50% higher than that of ionomycin) and high potency (Table 6) [81].

In vivo, **4** shows possible activity at TRPV1 channels in mice and rats [102–104] and TRPA1 channels in rats [140]. The effects of **4** in one of these studies was blocked by the TRPA1 selective antagonist, AP18, and by the TRPV1 selective antagonist, 5-iodo-resiniferatoxin, indicating involvement of these channels in the tail-flick related antinociception effects of **4** in anesthetized rats [140]. However, these antinociceptive effects were also blocked by the $CB_1$ receptor-selective antagonist, AM251, the $5HT_{1A}$ receptor antagonist, WAY100635, and also the adenosine $A_1$-selective antagonist [140]. This indicated that the descending pathway of antinociception in rats is possibly mediated by various mechanisms and the mechanism by which **4** mediates antinociception needs to be explored further.

The non-psychotropic quality of **4** provides promise for its use in the clinic and its "taming" of the effects of **1** have also proven beneficial in a licensed cannabis extract medication currently on the market in several countries, Sativex®. This medication is for the treatment of spasticity in multiple sclerosis patients and contains equal ratios of **4** and **1**. Here **4** functionally, not molecularly, antagonizes the undesirable effects of **1**, thus increasing its therapeutic index [113]. This reported "antagonism" may be explained by the negative allosteric modulation of $CB_1$ receptors as described in Sect. 5.1. Under the names Epidiolex® [141] and Cannabidiol Oral solution [142], **4** has been granted Orphan Drug designation by the U.S. FDA for treatment of Dravet syndrome and Lennox-Gastaut syndrome, both of which being forms of childhood-onset epilepsy. Epidiolex is in Phase 3 trials for Dravet syndrome and Lennox-Gastaut syndrome and Cannabidiol Oral Solution is in Phase 1 clinical trials for both these syndromes [141, 142]. Epidiolex is also nearing the end of Phase 2 trials for tuberous sclerosis, a genetic disease that results in benign tumor growth in the brain and other vital organs. Novagant Corp. has released GoldenCBD™ in capsule and liquid form. This is cannabidiol-rich hemp oil that is being marketed as medical marijuana for people living outside the states of Washington and Colorado in the USA [143].

## 6 Cannabidivarin




**5**

Cannabidivarin (**5**) is the *n*-propyl analog of **4**, therefore being part of the **4** chemical class type (Table 1) and like **4** it is non-psychotropic. Little is known about the pharmacological properties of **5** [103] and how it exerts its therapeutic benefits. It was first isolated in 1969 by Vollner and co-workers [103], but, since its classification, relatively few studies have been conducted to determine its pharmacological profile.

## *6.1 Activity at Cannabinoid Receptors*

Pure **5** and **5**-enriched cannabis extracts are known to be $CB_1$ independent due to the lack of effect on motor function in a battery of motor tasks [2, 144]. Additionally, in a CP-55940 radioligand-binding assay using MF1 whole mouse brain and in CHO cells expressing human $CB_1$ receptors, pure **5** only displaced CP-55940 at the highest concentration tested (10 μ*M*) and a **5**-enriched extract showed very weak affinity for $CB_1$ receptors, displacing CP-55940 only weakly [2]. For a summary of **5** binding affinities to $CB_1$ and $CB_2$ receptors, see Table 2.

## *6.2 Cannabinoid Receptor Independent Activity*

De Petrocellis and co-workers showed **5** to have agonist and antagonist effects at (TRP) cation channels. At human TRPA1 channels, **5** is a potent agonist and a less potent agonist at human TRPV1 and TRPV2 channels [80]. In this study, when **5** was given to TRPM8 transfected HEK293 cells, it antagonized the $Ca^{2+}$ elevation response elicited by the agonist icilin. With the same potency, **5** induced intracellular $Ca^{2+}$ elevation at the TRPV4 channel and is also an agonist at TRPV3 channels (Table 7) [81].

A recent study using whole cell patch clamp techniques on HEK293 transfected cells reported that **5** (3, 10, 30 μ*M*) dose-dependently activated, and rapidly desensitized, TRPV1, TRPV2, and TRPA1 channels [139]. Previous work has shown **5** to have antiepileptiform activity in rat hippocampal slices [144] and Iannotti and co-workers showed there to be significant TRPV1 transcript expression in rat hippocampal slices [139]. This group therefore conducted multi-electrode array (MEA) experiments, which showed that **5** and the TRPV1 agonist, capsaicin,

**Table 7** A comparison of selected in vitro studies showing cannabinoid receptor independent activity of cannabidivarin (**5**) according to concentrations, assay types, and cell types used

| Target | Concentration/ μ*M* | $EC_{50}$/ μ*M* | $IC_{50}$/ μ*M* | Assay | Cell type | Ref. |
|---|---|---|---|---|---|---|
| GPR55 | <1 | 0.4 | | ERK1/2 MAPK phosphorylation | HEK293/ Human | [69] |
| TRP cation channels | | | | | | |
| TRPA1 | 1–10 | ND | ND | Whole cell patch clamp | Hipocampal slices/Rat | [139] |
| | <1 | 0.42 | | $Ca^{2+}$ Fluorescence assay | HEK293/Rat | [80] |
| TRPM8 | | | 0.9 | | HEK293/Rat | |
| TRPV1 | 1–10 | 3.6 | | | HEK293/ Human | |
| TRPV2 | 1–10 | ND | ND | Whole cell patch clamp | Hippocampal slices/Rat | [139] |
| | 1–10 | 7.3 | | $Ca^{2+}$ Fluorescence assay | HEK293/Rat | [80] |
| TRPV3 | | 1.7 | | | HEK293/Rat | [81] |
| TRPV4 | <1 | 0.9 | | | HEK293/Rat | |

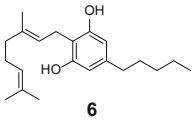
See Tables 1 and 2

produced similar effects on epileptiform activity induced in rat hippocampal slices. The effects of capsaicin on burst amplitude were reversed by the selective TRPV1 antagonist, IRTX, but the effects of **5** were not. This indicated that the anti-epileptiform effects of **5** are not mediated by activity at TRPV1 channels [139].

In vivo, **5** has been shown to display anticonvulsant properties in various acute animal models of seizure [144] and is currently in Phase 2 clinical trials as an antiepileptic drug [141]. The mechanism of action underlying these effects, however, is yet to be determined. In the pentylenetetrazole (PTZ) model of acute seizure, **5** (400 mg $kg^{-1}$ p.o.) exhibited anticonvulsant effects by significantly reducing PTZ-induced seizure activity, in male Wistar rats, which was correlated with changes in gene expression of various epilepsy-related genes [145]. Of note is the clinical relevance of the route of administration used in this study (per os) compared to other in vivo studies where administration is via non-clinically relevant routes. The mechanism by which **5** induces changes in these epilepsy-related genes requires investigation.

Apart from currently being in clinical trials for epilepsy, **5** is also in clinical trials for glioma, type-2 diabetes and schizophrenia and has received U.S. FDA Orphan Drug Designation for neonatal hypoxic-ischemic encephalopathy [141]. This phytocannabinoid, like **4**, is therefore proving to be a promising therapeutic constituent of cannabis.

## 7 Cannabigerol




**6**

Cannabigerol (**6**) is another non-psychotropic phytocannabinoid and its chemical class type is the second most abundant in the cannabis plant, making up 16.3% of the phytocannabinoid content [1]. The carboxylic acid form of this phytocannabinoid, cannabigerolic acid (CBGA), is very important for the synthesis of other phytocannabinoids. In fresh cannabis plant material, phytocannabinoids are present in their carboxylic acid forms [146]. Cannabigerolic acid is the precursor to the acid forms of three phytocannabinoids: $\Delta^9$-tetrahydrocannabinolic acid ($\Delta^9$-THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA) [147–149]. Cannabigerovaric acid (CBGVA) is the precursor of the *n*-propyl analogues of the carboxylic acid derivatives $\Delta^9$-THCVA, CBDVA, and CBCVA [146]. Upon heating and storage of cannabis plant material these acid forms undergo decarboxylation to produce the non-acid forms, such as **1** and **2** [150]. Furthermore, under prolonged storage and drying some of these non-acid forms undergo oxidative catabolism to other phytocannabinoids. An example of this is oxidative catabolism of **1** to **3**, as described in Sect. 4 [84]. This phytocannabinoid was first isolated by Gaoni and Mechoulam in 1964 [151] and since then only a few studies have been conducted to investigate its pharmacological actions.

### *7.1 Activity at Cannabinoid Receptors*

The non-psychotropic effect of **6** is explained by its low affinity for $CB_1$ receptors (Table 2) [103] and it has been shown in vivo to not produce psychotropic effects like **1** [152]. It does however affect endocannabinoid tone indirectly by inhibiting anandamide uptake, thereby increasing levels of anandamide, as shown in Table 8.

**Table 8** A comparison of selected in vitro studies showing cannabinoid receptor independent activity of cannabigerol (**6**) according to concentrations, assay types, and cell types used

| Target | Concentration/ μ*M* | $EC_{50}$/ μ*M* | $IC_{50}$/ μ*M* | Assay | Cell type | Ref. |
|---|---|---|---|---|---|---|
| GPR55 | 1–10 | 2.16 | | ERK1/2 MAPK phosphorylation | HEK293/ Human | [69] |
| α2 adrenoceptor | <1 | 0.0002 | | GTPγS binding assay | Brain membranes/Mouse | [153] |
| | | 0.072 | | Electrically invoked contractions | Vas deferens/ Mouse | |
| TRP cation channels | | | | | | |
| TRPA1 | 1–10 | 3.4 | | $Ca^{2+}$ Fluorescence assay | HEK293/Rat | [79] |
| | <1 | 0.7 | | | HEK293/Rat | [80] |
| TRPM8 | | | 0.16 | | HEK293/Rat | [79] |
| | | | 0.16 | | HEK293/Rat | [80] |
| TRPV1 | 1–10 | 1.3 | | | HEK293/ Human | |
| TRPV2 | | 1.72 | | | HEK293/Rat | |
| TRPV3 | | 1.0 | | | HEK293/Rat | [81] |
| TRPV4 | | 5.1 | | | HEK293/Rat | |
| Anandamide uptake | | | 11.3 | [$^{14}$C]-AEA uptake | RBL-2H3 cells/Rat | [80] |

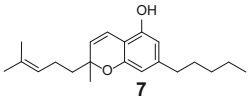
AEA, anandamide (arachidonoylethanolamine); also see Tables 1 and 2

## *7.2 Cannabinoid Receptor Independent Activity*

Despite the relatively few investigational studies conducted, there is evidence of pharmacological actions at a number of targets. In a study using mouse brain membranes, **6** acted as a potent α2 adrenoceptor agonist [153]. The same study found **6** to moderately block $5HT_{1A}$ receptors with a $K_B$ value of 0.0519 μ*M*. This effect is opposite to that of **4** on $5HT_{1A}$ receptors and explains the ability of **6** to antagonize the antinausea and antiemetic effect of **4** [154].

Like many phytocannabinoids, **6** interacts with numerous TRP cation channels. It is a potent TRPA1 agonist, a weak agonist at TRPV1 and TRPV2 and a potent TRPM8 antagonist (Table 8) [80].

## 8 Cannabichromene




7

Cannabichromene (**7**) is one of the most abundant phytocannabinoids naturally occurring in the cannabis plant, with its chemical class type making up the same percentage as that of the **4** chemical class type (Table 1) [1, 151, 155]. It was discovered independently by Claussen and co-workers and Gaoni and Mechoulam in 1966 [103].

### *8.1 Activity at Cannabinoid Receptors*

Cannabichromene has not been found to have significant affinity for $CB_1$ or $CB_2$ receptors as shown in Table 2 but it does, however, affect endocannabinoid tone indirectly by inhibiting cellular uptake of anandamide (Table 9) [80].

### *8.2 Cannabinoid Receptor Independent Activity*

The most notable pharmacological action of **7** to date is most likely its effect at TRP cation channels. At TRPA1 channels, **7** was found to be the most potent agonist of all the phytocannabinoids tested and also desensitized the TRPA1 channel to activation by the agonist allyl isothiocyanate [80]. At a lower potency, but still within the lower micromolar range, **7** was able to activate TRPV3 and TRPV4 channels and also desensitize TRPV4 channels to an agonist (9.9 $\mu M$) [81]. At the TRPV2 channel, **7** was only found to desensitize the channel and although **7** was found to block TRPM8 channel activation, this was at a very low potency [80] and would not be deemed physiologically relevant in vivo (Table 9) [23].

At a concentration of 1 $\mu M$, **7** has also been reported to act via ATP upregulation and adenosine signaling to raise the viability of adult mouse neural stem/progenitor cells (NSPCs) during differentiation [156]. The adenosine $A_{1A}$ receptor selective antagonist, DPCPX, countered the stimulation of ERK1/2 phosphorylation by **7** and the upregulation of the astrocyte marker nestin by **7**.

**Table 9** A comparison of selected in vitro studies showing cannabinoid receptor independent activity of cannabichromene (**7**) according to concentrations, assay types, and cell types used

| Target | Concentration/μ*M* | $EC_{50}$/μmol | $IC_{50}$/μ*M* | Assay | Cell type | Ref. |
|---|---|---|---|---|---|---|
| TRP cation channels | | | | | | |
| TRPA1 | <1 | 0.06 | | $Ca^{2+}$ Fluorescence assay | HEK293/Rat | [79] |
| | | 0.09 | | | HEK293/Rat | [80] |
| TRPM8 | >10 | | 40.7 | | HEK293/Rat | |
| TRPV1 | | 24.2 | | | HEK293/Human | |
| TRPV3 | 1–10 | 1.9 | | | HEK293/Rat | [81] |
| TRPV4 | <1 | 0.6 | | | HEK293/Rat | |
| Anandamide uptake | >10 | | 12.3 | [$^{14}$C]-AEA uptake | RBL-2H3 cells/Rat | [80] |
| Adenosine $A_{1A}$ receptor | | ND | | MTT (viability) assay | Neuroprogenitor cells (NSPCs)/Mouse | [156] |

See Tables 1 and 2

## 9 Conclusions

This chapter has reviewed the molecular pharmacology of the seven most thoroughly studied phytocannabinoids and demonstrated that each has a diverse set of pharmacological targets with varying therapeutic, recreational and toxicological effects. Even slight structural differences between the phytocannabinoids can produce very diverse and competing physiological effects. Investigations into some of the phytocannabinoids have produced conflicting results, as mentioned in this chapter. Thus, it is critical to take into account the differences in assays used, the species from which the target is taken and the concentrations used in in vitro studies in order to predict the pharmacology of the phytocannabinoids at the system level. It is important that the concentrations used to elicit a response in vitro are indicative of the levels that will be reached after administration in animal models or in the clinic, otherwise no predictions can be made on the physiological relevance of results from in vitro studies. As highlighted by McPartland and co-workers, it is imperative that when analyzing the results of various studies one takes into account interspecies differences in receptor distribution and differences among different tissues and cell types [105]. Moreover, it is also important when designing experiments to look at the therapeutic benefits of a phytocannabinoid that the species used, route of administration of the compound, and concentrations used are clinically relevant, i.e. applicable to the end target species. It is therefore important to assess species differences in receptor orthologues and distribution, remembering that there are molecular divergences between human and rodent orthologues such as, for example, within the endocannabinoid system [157].

This chapter has also highlighted the importance of each individual phytocannabinoid in mediating the therapeutic and recreational effects of cannabis. Two phytocannabinoids, **4** and **5**, may prove to be clinically useful constituents of cannabis. Both phytocannabinoids have been granted Orphan Drug designation by the U.S. FDA for a number of seizure-related disorders and, as a result, Phase II and III clinical trials are underway [141–143]. The conduct of formal clinical trials using these non-$\Delta^9$-tetrahydrocannabinol phytocannabinoids could stimulate new research of cannabis and its constituents and see additional phytocannabinoids objectively assessed for therapeutic potential. Even though research on individual phytocannabinoids has been conducted for many years, still much more research is warranted. The cannabis plant contains about 120 phytocannabinoids, which shows, in reality, how little research has been conducted on these compounds. Further research on the "known" phytocannabinoids as well as the "unknown" phytocannabinoids would greatly advance our understanding of these substances alone as well as in conjunction with each other or as part of a whole in cannabis.

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## References


1. Elsohly M, Gul W (2014) Constituents of *Cannabis sativa*. In: Pertwee RG (ed) Handbook of Cannabis. Oxford University Press, Oxford, pp 3–22
2. Hill TDM, Cascio MG, Romano B, Duncan M, Pertwee RG, Williams CM, Whalley BJ, Hill AJ (2013) Cannabidivarin-rich cannabis extracts are anticonvulsant in mouse and rat via a CB1 receptor-independent mechanism. Br J Pharmacol 170:679
3. Li H-L (1974) An archaeological and historical account of *Cannabis* in China. Econ Bot 28:437
4. Touw M (1981) The religious and medicinal use of *Cannabis* in China, India and Tibet. J Psychoactive Drugs 13:23
5. Gaoni Y, Mechoulam R (1971) The isolation and structure of delta-1-tetrahydrocannabinol and other neutral cannabinoids from hashish. J Am Chem Soc 93:217
6. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI (1990) Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346:561
7. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, Gibson D, Mandelbaum A, Etinger A, Mechoulam R (1992) Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258:1946
8. Mechoulam R, Ben-Shabat S, Hanus L, Ligumsky M, Kaminski NE, Schatz AR, Gopher A, Almog S, Martin BR, Compton DR (1995) Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. Biochem Pharmacol 50:83
9. Sugiura T, Kondo S, Sukagawa A, Nakane S, Shinoda A, Itoh K, Yamashita A, Waku K (1995) 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem Biophys Res Commun 215:89
10. Baker D, Pryce G, Giovannoni G, Thompson AJ (2003) The therapeutic potential of *Cannabis*. Lancet Neurol 2:291
11. Noyes R, Brunk SF, Avery DA, Canter AC (1975) The analgesic properties of delta-9-tetrahydrocannabinol and codeine. Clin Pharmacol Ther 18:84
12. Martin WJ, Coffin PO, Attias E, Balinsky M, Tsou K, Walker JM (1999) Anatomical basis for cannabinoid-induced antinociception as revealed by intracerebral microinjections. Brain Res 822:237
13. Darmani NA (2001) Delta-9-tetrahydrocannabinol differentially suppresses cisplatin-induced emesis and indices of motor function via cannabinoid CB1 receptors in the least shrew. Pharmacol Biochem Behav 69:239
14. Tramèr MR, Carroll D, Campbell FA, Reynolds DJ, Moore RA, McQuay HJ (2001) Cannabinoids for control of chemotherapy induced nausea and vomiting: quantitative systematic review. BMJ 323:16
15. Westfall RE, Janssen PA, Lucas P, Capler R (2006) Survey of medicinal *Cannabis* use among childbearing women: patterns of its use in pregnancy and retroactive self-assessment of its efficacy against "morning sickness". Complement Ther Clin Pract 12:27
16. O'Shaugnessy WB (1840) On the preparations of the Indian hemp, or gunjah (*Cannabis indica*). Trans Med Phys Soc Bengal 5:71
17. Lozano I (1997) Therapeutic use of *Cannibis sativa* L. in Arab medicine. Asclepio 49:199
18. Porter BE, Jacobson C (2013) Report of a parent survey of cannabidiol-enriched *Cannabis* use in pediatric treatment-resistant epilepsy. Epilepsy Behav 29:574
19. Ellison JM, Gelwan E, Ogletree J (1990) Complex partial seizure symptoms affected by marijuana abuse. J Clin Psychiatry 51:439
20. Grinspoon L, Bakalar JB (1997) Marihuana, the forbidden medicine. Yale University Press, New Haven, CT
21. Consroe PF, Wood GC, Buchsbaum H (1975) Anticonvulsant nature of marihuana smoking. JAMA 234:306
22. Lal S, Prasad N, Ryan M, Tangri S, Silverberg MS, Gordon A, Steinhart H (2011) *Cannabis* use amongst patients with inflammatory bowel disease. Eur J Gastroenterol Hepatol 23:891

23. Deiana S, Watanabe A, Yamasaki Y, Amada N, Arthur M, Fleming S, Woodcock H, Dorward P, Pigliacampo B, Close S, Platt B, Riedel G (2012) Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarin (CBDV), delta(9)-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behavior. Psychopharmacology 219:859
24. Howlett AC, Qualy JM, Khachatrian LL (1986) Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. Mol Pharmacol 29:307
25. Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC (1990) Cannabinoid receptor localization in brain. Proc Natl Acad Sci U S A 87:1932
26. Felder CC, Veluz JS, Williams HL, Briley EM, Matsuda LA (1992) Cannabinoid agonists stimulate both receptor- and non-receptor-mediated signal transduction pathways in cells transfected with and expressing cannabinoid receptor clones. Mol Pharmacol 42:838
27. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG (2002) International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. Pharmacol Rev 54:161
28. Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC (1988) Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacol 34:605
29. Pertwee RG (1999) Pharmacology of cannabinoid receptor ligands. Curr Med Chem 6:635
30. Pertwee RG (2005) Pharmacological actions of cannabinoids. Handb Exp Pharmacol 168:1
31. Martin BR, Compton DR, Thomas BF, Prescott WR, Little PJ, Razdan RK, Johnson MR, Melvin LS, Mechoulam R, Ward SJ (1991) Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. Pharmacol Biochem Behav 40:471
32. Varvel SA, Bridgen DT, Tao Q, Thomas BF, Martin BR, Lichtman AH (2005) Delta9-tetrahydrocannabinol accounts for the antinociceptive, hypothermic, and cataleptic effects of marijuana in mice. J Pharmacol Exp Ther 314:329
33. Wiley JL, Jefferson RG, Grier MC, Mahadevan A, Razdan RK, Martin BR (2001) Novel pyrazole cannabinoids: insights into CB(1) receptor recognition and activation. J Pharmacol Exp Ther 296:1013
34. Hillard CJ (2000) Endocannabinoids and vascular function. J Pharmacol Exp Ther 294:27
35. Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D, Ferrara P, Soubrie P, Breliere JC, Le Fur G (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 350:240
36. Sharir H, Abood ME (2010) Pharmacological characterization of GPR55, a putative cannabinoid receptor. Pharmacol Ther 126:301
37. Savinainen JR, Saario SM, Niemi R, Järvinen T, Laitinen JT (2003) An optimized approach to study endocannabinoid signaling: evidence against constitutive activity of rat brain adenosine A1 and cannabinoid CB1 receptors. Br J Pharmacol 140:1451
38. De Petrocellis L, Bisogno T, Maccarrone M, Davis JB, Finazzi-Agrò A, Di Marzo V (2001) The activity of anandamide at vanilloid VR1 receptors requires facilitated transport across the cell membrane and is limited by intracellular metabolism. J Biol Chem 276:12856
39. Farrimond JA, Hill AJ, Whalley BJ, Williams CM (2010) *Cannabis* constituents modulate delta9-tetrahydrocannabinol-induced hyperphagia in rats. Psychopharmacology 210:97
40. Bayewitch M, Rhee MH, Avidor-Reiss T, Breuer A, Mechoulam R, Vogel Z (1996) (−)-Delta9-tetrahydrocannabinol antagonizes the peripheral cannabinoid receptor-mediated inhibition of adenylyl cyclase. J Biol Chem 271:9902
41. Bolognini D, Cascio MG, Parolaro D, Pertwee RG (2012) AM630 behaves as a protean ligand at the human cannabinoid CB 2 receptor. Br J Pharmacol 165:2561
42. Paronis CA, Nikas SP, Shukla VG, Makriyannis A (2012) Delta(9)-tetrahydrocannabinol acts as a partial agonist/antagonist in mice. Behav Pharmacol 23:802

43. Morrison PD, Zois V, McKeown DA, Lee TD, Holt DW, Powell JF, Kapur S, Murray RM (2009) The acute effects of synthetic intravenous delta9-tetrahydrocannabinol on psychosis, mood and cognitive functioning. Psychol Med 39:1607
44. Barann M, Molderings G, Brüss M, Bönisch H, Urban BW, Göthert M (2002) Direct inhibition by cannabinoids of human 5-HT3A receptors: probable involvement of an allosteric modulatory site. Br J Pharmacol 137:589
45. Shi B, Yang R, Wang X, Liu H, Zou L, Hu X, Wu J, Zou A, Liu L (2012) Inhibition of 5-$HT_3$ receptors-activated currents by cannabinoids in rat trigeminal ganglion neurons. J Huazhong Univ Sci Technolog Med Sci 32:265
46. Aapro MS (1991) 5-HT3 receptor antagonists. An overview of their present status and future potential in cancer therapy-induced emesis. Drugs 42:551
47. Mitchelson F (1992) Pharmacological agents affecting emesis. A review (Part II). Drugs 43:443
48. Karim F, Roerig SC, Saphier D (1996) Role of 5-hydroxytryptamine3 (5-HT3) antagonists in the prevention of emesis caused by anticancer therapy. Biochem Pharmacol 52:685
49. Voog O, Alstergren P, Leibur E, Kallikorm R, Kopp S (2000) Immediate effects of the serotonin antagonist granisetron on temporomandibular joint pain in patients with systemic inflammatory disorders. Life Sci 68:591
50. Simpson K, Spencer CM, McClellan KJ (2000) Tropisetron: an update of its use in the prevention of chemotherapy-induced nausea and vomiting. Drugs 59:1297
51. Hejazi N, Zhou C, Oz M, Sun H, Ye JH, Zhang L (2006) Delta9-tetrahydrocannabinol and endogenous cannabinoid anandamide directly potentiate the function of glycine receptors. Mol Pharmacol 69:991
52. Betz H, Kuhse J, Schmieden V, Laube B, Kirsch J, Harvey RJ (1999) Structure and functions of inhibitory and excitatory glycine receptors. Ann N Y Acad Sci 868:667
53. Lynch JW (2004) Molecular structure and function of the glycine receptor chloride channel. Physiol Rev 84:1051
54. Molander A, Soderpalm B (2005) Accumbal strychnine-sensitive glycine receptors: an access point for ethanol to the brain reward system. Alcohol Clin Exp Res 29:27
55. Molander A, Soderpalm B (2005) Glycine receptors regulate dopamine release in the rat nucleus accumbens. Alcohol Clin Exp Res 29:17
56. O'Sullivan SE, Tarling EJ, Bennett AJ, Kendall DA, Randall MD (2005) Novel time-dependent vascular actions of delta9-tetrahydrocannabinol mediated by peroxisome proliferator-activated receptor gamma. Biochem Biophys Res Commun 337:824
57. O'Sullivan SEO, Kendall DA, Randall MD (2006) Further characterization of the time-dependent vascular effects of delta-9-tetrahydrocannabinol. J Pharmacol Exp Ther 317:428
58. Vara D, Morell C, Rodríguez-Henche N, Diaz-Laviada I (2013) Involvement of PPARγ in the antitumoral action of cannabinoids on hepatocellular carcinoma. Cell Death Dis 4:618
59. Ferre P (2004) The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. Diabetes 53:43
60. Rangwala SM, Lazar MA (2004) Peroxisome proliferator-activated receptor gamma in diabetes and metabolism. Trends Pharmacol Sci 25:331
61. Alarcon de la Lastra C, Sanchez-Fidalgo S, Villegas I, Motilva V (2004) New pharmacological perspectives and therapeutic potential of PPAR-gamma agonists. Curr Pharm Des 10:3505
62. McHugh D, Page J, Dunn E, Bradshaw HB (2012) Δ9 tetrahydrocannabinol and *N*-arachidonyl glycine are full agonists at GPR18 receptors and induce migration in human endometrial HEC-1B cells. Br J Pharmacol 165:2414
63. McHugh D, Roskowski D, Xie S, Bradshaw HB (2014) Delta(9)-THC and *N*-arachidonoyl glycine regulate BV-2 microglial morphology and cytokine release plasticity: implications for signaling at GPR18. Front Pharmacol 4:162
64. Pertwee RG, Howlett AC, Abood ME, Alexander SPH, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA (2010) International Union of

Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. Pharmacol Rev 62:588
65. Pertwee RG (1972) The ring test: a quantitative method for assessing the "cataleptic" effect of *Cannabis* in mice. Br J Pharmacol 46:753
66. Yin H, Chu A, Li W, Wang B, Shelton F, Otero F, Nguyen DG, Caldwell JS, Chen YA (2009) Lipid G protein-coupled receptor ligand identification using beta-arrestin PathHunter assay. J Biol Chem 284:12328
67. Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J, Elebring T, Nilsson K, Drmota T, Greasley PJ (2007) The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol 152:1092
68. Kapur A, Zhao P, Sharir H, Bai Y, Caron MG, Barak LS, Abood ME (2009) Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. J Biol Chem 284:29817
69. Anavi-Goffer S, Baillie G, Irving AJ, Gertsch J, Greig IR, Pertwee RG, Ross R (2012) Modulation of L-α-lysophosphatidylinositol/GPR55 mitogen-activated protein kinase (MAPK) signaling by cannabinoids. J Biol Chem 287:91
70. Bolognini D, Costa B, Maione S, Comelli F, Marini P, Di Marzo V, Parolaro D, Ross RA, Gauson LA, Cascio MG, Pertwee RG (2010) The plant cannabinoid delta9-tetrahydrocannabivarin can decrease signs of inflammation and inflammatory pain in mice. Br J Pharmacol 160:677
71. Garcia C, Palomo-Garo C, Garcia-Arencibia M, Ramos J, Pertwee R, Fernandez-Ruiz J (2011) Symptom-relieving and neuroprotective effects of the phytocannabinoid delta(9)-THCV in animal models of Parkinson's disease. Br J Pharmacol 163:1495
72. 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
73. Thomas A, Stevenson LA, Wease KN, Price MR, Baillie G, Ross RA, Pertwee RG (2005) Evidence that the plant cannabinoid delta9-tetrahydrocannabivarin is a cannabinoid CB1 and CB2 receptor antagonist. Br J Pharmacol 146:917
74. Dennis I, Whalley BJ, Stephens GJ (2008) Effects of delta9-tetrahydrocannabivarin on [$^{35}$S] GTPgammaS binding in mouse brain cerebellum and piriform cortex membranes. Br J Pharmacol 154:1349
75. Pertwee RG, Griffin G, Lainton JA, Huffman JW (1995) Pharmacological characterization of three novel cannabinoid receptor agonists in the mouse isolated vas deferens. Eur J Pharmacol 284:241
76. Ma Y-L, Weston SE, Whalley BJ, Stephens GJ (2008) The phytocannabinoid delta9-tetrahydrocannabivarin modulates inhibitory neurotransmission in the cerebellum. Br J Pharmacol 154:204
77. 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
78. McPartland JM, Duncan M, Di Marzo V, Pertwee RG (2015) Are cannabidiol and Δ(9)-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br J Pharmacol 172:737
79. De Petrocellis L, Vellani V, Schiano-Moriello A, Marini P, Magherini PC, Orlando P, Di Marzo V (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
80. De Petrocellis L, Ligresti A, Moriello AS, Allara M, Bisogno T, Petrosino S, Stott CG, Di Marzo V (2011) Effects of cannabinoids and cannabinoid-enriched *Cannabis* extracts on TRP channels and endocannabinoid metabolic enzymes. Br J Pharmacol 163:1479
81. De Petrocellis L, Orlando P, Moriello AS, Aviello G, Stott C, Izzo AA, Di Marzo V (2012) Cannabinoid actions at TRPV channels: effects on TRPV3 and TRPV4 and their potential relevance to gastrointestinal inflammation. Acta Physiol 204:255

82. Billeter AT, Hellmann JL, Bhatnagar A, Polk HC (2014) Transient receptor potential ion channels: powerful regulators of cell function. Ann Surg 259:229
83. Moreira FA, Grieb M, Lutz B (2009) Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: focus on anxiety and depression. Best Pract Res Clin Endocrinol Metab 23:133
84. Harvey DJ (1990) Stability of cannabinoids in dried samples of cannabis dating from around 1896–1905. J Ethnopharmacol 28:117
85. Rhee MH, Vogel Z, Barg J, Bayewitch M, Levy R, Hanuš L, Breuer A, Mechoulam R (1997) Cannabinol derivatives: binding to cannabinoid receptors and inhibition of adenylylcyclase. J Med Chem 40:3228
86. Showalter VM, Compton DR, Martin BR, Abood ME (1996) Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. J Pharmacol Exp Ther 278:989
87. Felder CC, Joyce KE, Briley EM, Mansouri J, Mackie K, Blond O, Lai Y, Ma AL, Mitchell RL (1995) Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. Mol Pharmacol 48:443
88. MacLennan SJ, Reynen PH, Kwan J, Bonhaus DW (1998) Evidence for inverse agonism of SR141716A at human recombinant cannabinoid CB1 and CB2 receptors. Br J Pharmacol 124:619
89. Kenakin T (1995) Inverse, protean, and ligand-selective agonism: matters of receptor conformation. FASEB J 15:598
90. Booker L, Naidu PS, Razdan RK, Mahadevan A, Lichtman AH (2009) Evaluation of prevalent phytocannabinoids in the acetic acid model of visceral nociception. Drug Alcohol Depend 105:42
91. Farrimond JA, Whalley BJ, Williams CM (2012) Cannabinol and cannabidiol exert opposing effects on rat feeding patterns. Psychopharmacology (Berl) 223:117
92. Salamone JD, McLaughlin PJ, Sink K, Makriyannis A, Parker LA (2007) Cannabinoid CB1 receptor inverse agonists and neutral antagonists: effects on food intake, food-reinforced behavior and food aversions. Physiol Behav 91:383
93. Carai MAM, Colombo G, Gessa GL (2005) Rimonabant: the first therapeutically relevant cannabinoid antagonist. Life Sci 77:2339
94. Buckley JL, Rasmussen EB (2014) Rimonabant's reductive effects on high densities of food reinforcement, but not palatability, in lean and obese Zucker rats. Psychopharmacology (Berl) 231:2159
95. Darmani NA, Pandya DK (2000) Involvement of other neurotransmitters in behaviors induced by the cannabinoid CB1 receptor antagonist SR 141716A in naive mice. J Neural Transm 107:931
96. Järbe TUC, Andrzejewski ME, DiPatrizio NV (2002) Interactions between the CB1 receptor agonist $\Delta^9$-THC and the CB1 receptor antagonist SR-141716 in rats: open-field revisited. Pharmacol Biochem Behav 73:911
97. Parker LA, Mechoulam R, Schlievert C, Abbott L, Fudge ML, Burton P (2003) Effects of cannabinoids on lithium-induced conditioned rejection reactions in a rat model of nausea. Psychopharmacology (Berl) 166:156
98. Darmani NA (2001) Delta-9-Tetrahydrocannabinol and synthetic cannabinoids prevent emesis produced by the cannabinoid CB1 receptor antagonist/inverse agonist SR 141716A. Neuropsychopharmacology 24:198
99. Després J-P, Golay A, Sjöström L (2005) Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. N Engl J Med 353:2121
100. Taglialatela-Scafati O, Pagani A, Scala F, De Petrocellis L, Di Marzo V, Grassi G (2010) Cannabimovone, a cannabinoid with a rearranged terpenoid skeleton from hemp. Eur J Org Chem 11:2023
101. Elsohly S, Slade D (2005) Chemical constituents of marijuana: the complex mixture of natural cannabinoids. Life Sci 78:539

102. Mechoulam R, Peters M, Murillo-Rodriguez E, Hanus LO (2007) Cannabidiol-recent advances. Chem Biodivers 4:1678
103. Izzo AA, Borrelli F, Capasso R, Di Marzo V, Mechoulam R (2009) Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb. Trends Pharmacol Sci 30:515
104. 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
105. McPartland JM, Glass M, Pertwee RG (2007) Meta-analysis of cannabinoid ligand binding affinity and receptor distribution: interspecies differences. Br J Pharmacol 152:583
106. Laprairie RB, Bagher AM, Kelly MEM, Denovan-Wright EM (2015) Cannabidiol is a negative allosteric modulator of the type 1 cannabinoid receptor. Br J Pharmacol 172:4790
107. Keov P, Sexton PM, Christopoulos A (2011) Allosteric modulation of G protein-coupled receptors: a pharmacological perspective. Neuropharmacology 60:24
108. Price MR, Baillie GL, Thomas A, Stevenson LA, Easson M, Goodwin R, McLean A, McIntosh L, Goodwin G, Walker G, Westwood P, Marrs J, Thomson F, Cowley P, Christopoulos A, Pertwee RG, Ross RA (2005) Allosteric modulation of the cannabinoid CB1 receptor. Mol Pharmacol 68:1484
109. Horswill JG, Bali U, Shaaban S, Keily JF, Jeevaratnam P, Babbs AJ, Reynet C, Wong Kai In P (2007) PSNCBAM-1, a novel allosteric antagonist at cannabinoid CB1 receptors with hypophagic effects in rats. Br J Pharmacol 152:805
110. Wang X, Horswill JG, Whalley BJ, Stephens GJ (2011) Effects of the allosteric antagonist 1-(4-chlorophenyl)-3-[3-(6-pyrrolidin-1-ylpyridin-2-yl)phenyl]urea (PSNCBAM-1) on CB1 receptor modulation in the cerebellum. Mol Pharmacol 79:758
111. Ahn KH, Mahmoud MM, Shim J-Y, Kendall DA (2013) Distinct roles of beta-arrestin 1 and beta-arrestin 2 in ORG27569-induced biasedsignaling and internalization of the cannabinoid receptor 1 (CB1). J Biol Chem 288:9790
112. Cawston EE, Redmond WJ, Breen CM, Grimsey NL, Connor M, Glass M (2013) Real-time characterization of cannabinoid receptor 1 (CB1) allosteric modulators reveals novel mechanism of action. Br J Pharmacol 170:893
113. Russo E, Guy GW (2005) A tale of two cannabinoids: the therapeutic rationale for combining tetrahydrocannabinol and cannabidiol. Med Hypotheses 66:234
114. Sylantyev S, Jensen TP, Ross RA, Rusakov DA (2013) Cannabinoid- and lysophosphatidylinositol-sensitive receptor GPR55 boosts neurotransmitter release at central synapses. Proc Natl Acad Sci U S A 110:5193
115. Whyte LS, Ryberg E, Sims NA, Ridge SA, Mackie K, Greasley PJ, Ross RA, Rogers MJ (2009) The putative cannabinoid receptor GPR55 affects osteoclast function in vitro and bone mass in vivo. Proc Natl Acad Sci U S A 106:16511
116. Carrier EJ, Auchampach JA, Hillard CJ (2006) Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. Proc Natl Acad Sci U S A 103:7895
117. Ahrens J, Demir R, Leuwer M, De La Roche J, Krampfl K, Foadi N, Karst M, Haeseler G (2009) The nonpsychotropic cannabinoid cannabidiol modulates and directly activates alpha-1 and alpha-1-beta glycine receptor function. Pharmacology 83:217
118. Xiong W, Cui T, Cheng K, Yang F, Chen SR, Willenbring D, Guan Y, Pan HL, Ren K, Xu Y, Zhang L (2012) Cannabinoids suppress inflammatory and neuropathic pain by targeting alpha 3 glycine receptors. J Exp Med 209:1121
119. Liou GI, Auchampach JA, Hillard CJ, Zhu G, Yousufzai B, Mian S, Khan S, Khalifa Y (2008) Mediation of cannabidiol anti-inflammation in the retina by equilibrative nucleoside transporter and A2A adenosine receptor. Investig Ophthalmol Vis Sci 49:5526
120. Ribeiro A, Ferraz-de-Paula V, Pinheiro ML, Vitoretti LB, Mariano-Souza DP, Quinteiro-Filho WM, Akamine AT, Almeida VI, Quevedo J, Dal-Pizzol F, Hallak JE, Zuardi AW,

Crippa JA, Palermo-Neto J (2012) Cannabidiol, a non-psychotropic plant-derived cannabinoid, decreases inflammation in a murine model of acute lung injury: role for the adenosine A2A receptor. Eur J Pharmacol 678:78
121. Gonca E, Darici F (2014) The effect of cannabidiol on ischemia/reperfusion-induced ventricular arrhythmias: the role of adenosine A1 receptors. J Cardiovasc Pharmacol Ther 20:76
122. Russo EB, Burnett A, Hall B, Parker KK (2005) Agonistic properties of cannabidiol at 5-HT1a receptors. Neurochem Res 30:1037
123. Rock EM, Bolognini D, Limebeer CL, Cascio MG, Anavi-Goffer S, Fletcher PJ, Mechoulam R, Pertwee RG, Parker LA (2012) Cannabidiol, a nonpsychotropic component of cannabis, attenuates vomiting and nausea-like behaviour via indirect agonism of 5-HT1A somatodendritic autoreceptors in the dorsal raphe nucleus. Br J Pharmacol 165:2620
124. Resstel LB, Tavares RF, Lisboa SF, Joca SR, Correa FM, Guimaraes FS (2009) 5-HT1A receptors are involved in the cannabidiol-induced attenuation of behavioral and cardiovascular responses to acute restraint stress in rats. Br J Pharmacol 156:181
125. 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. Behav Brain Res 213:225
126. 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
127. Kwiatkowska M, Parker LA, Burton P, Mechoulam R (2004) A comparative analysis of the potential of cannabinoids and ondansetron to suppress cisplatin-induced emesis in the *Suncus murinus* (house musk shrew). Psychopharmacology (Berl) 174:254
128. Parker LA, Kwiatkowska M, Burton P, Mechoulam R (2004) Effect of cannabinoids on lithium-induced vomiting in the *Suncus murinus* (house musk shrew). Psychopharmacology (Berl) 171:156
129. Mishima K, Hayakawa K, Abe K, Ikeda T, Egashira N, Iwasaki K, Fujiwara M (2005) Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1A receptor-dependent mechanism. Stroke 36:1077
130. Savitz J, Lucki I, Drevets WC (2009) 5-HT1A receptor function in major depressive disorder. Prog Neurobiol 88:17
131. Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, Messa C, Meyer J, Gunn RN, Grasby PM, Cowen PJ (2000) Brain serotonin1A receptor binding measured by positron emission tomography with [11C]WAY-100635: effects of depression and antidepressant treatment. Arch Gen Psychiatry 57:174
132. Szewczyk B, Albert PR, Burns AM, Czesak M, Overholser JC, Jurjus GJ, Meltzer HY, Konick LC, Dieter L, Herbst N, May W, Rajkowska G, Stockmeier CA, Austin MC (2009) Gender-specific decrease in NUDR and 5-HT1A receptor proteins in the prefrontal cortex of subjects with major depressive disorder. Int J Neuropsychopharmacol 12:155
133. Porsolt RD, Le Pichon M, Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. Nature 266:730
134. Hayakawa K, Mishima K, Abe K, Hasebe N, Takamatsu F, Yasuda H, Ikeda T, Inui K, Egashira N, Iwasaki K, Fujiwara M (2004) Cannabidiol prevents infarction via the non-CB1 cannabinoid receptor mechanism. Neuroreport 15:2381
135. Yang K-H, Galadari S, Isaev D, Petroianu G, Shippenberg TS, Oz M (2010) The nonpsychoactive cannabinoid cannabidiol inhibits 5-hydroxytryptamine3A receptor-mediated currents in *Xenopus laevis* oocytes. J Pharmacol Exp Ther 333:547
136. Bisogno T, Hanus L, De Petrocellis L, Tchilibon S, Ponde DE, Brandi I, Moriello AS, Davis JB, Mechoulam R, Di Marzo V (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
137. Ligresti A, Moriello AS, Starowicz K, Matias I, Pisanti S, De Petrocellis L, Laezza C, Portella G, Bifulco M, Di Marzo V (2006) Antitumor activity of plant cannabinoids with

emphasis on the effect of cannabidiol on human breast carcinoma. J Pharmacol Exp Ther 318:1375
138. 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
139. Iannotti FA, Hill CL, Leo A, Alhusaini A, Soubrane C, Mazzarella E, Russo E, Whalley BJ, Di Marzo V, Stephens GJ (2014) Nonpsychotropic plant cannabinoids, cannabidivarin (CBDV) and cannabidiol (CBD), activate and desensitize transient receptor potential vanilloid 1 (TRPV1) channels in vitro: potential for the treatment of neuronal hyperexcitability. ACS Chem Neurosci 5:1131
140. Maione S, Piscitelli F, Gatta L, Vita D, De Petrocellis L, Palazzo E, de Novellis V, Di Marzo V (2011) Non-psychoactive cannabinoids modulate the descending pathway of antinociception in anaesthetized rats through several mechanisms of action. Br J Pharmacol 162:584
141. GW Pharmaceuticals Press Release (2015) http://www.gwpharm.com/news.aspx
142. Insys Therapeutics, Inc. Cannabidiol oral solution 2015. http://www.insysrx.com/products/cannabidiol-oral-solution-cbd/
143. Novagant Golden CBD$^{TM}$ (2015) http://www.novagant.com/goldencbd/
144. Hill AJ, Mercier MS, Hill TDM, Glyn SE, Jones NA, Yamasaki Y, Futamura T, Duncan M, Stott CG, Stephens GJ, Williams CM, Whalley BJ (2012) Cannabidivarin is anticonvulsant in mouse and rat. Br J Pharmacol 167:1629
145. Amada N, Yamasaki Y, Williams CM, Whalley BJ (2013) Cannabidivarin (CBDV) suppresses pentylenetetrazole (PTZ)-induced increases in epilepsy-related gene expression. PeerJ 1:214
146. Potter D (2009) The propagation, characterisation and optimisation of *Cannabis sativa* L. as a phytopharmaceutical. PhD thesis, King's College London, pp 17–18
147. Fellermeier M, Eisenreich W, Bacher A, Zenk MH (2001) Biosynthesis of cannabinoids. Incorporation experiments with $^{13}$C-labeled glucoses. Eur J Biochem 268:1596
148. Taura F, Morimoto S, Shoyama Y (1996) Purification and characterization of cannabidiolic-acid synthase from *Cannabis sativa* L. Biochemical analysis of a novel enzyme that catalyzes the oxidocyclization of cannabigerolic acid to cannabidiolic acid. J Biol Chem 271:17411
149. Gaoni Y, Mechoulam R (1971) The isolation and structure of delta-1-tetrahydrocannabinol and other neutral cannabinoids from hashish. J Am Chem Soc 93:217
150. De Meijer EP, Bagatta M, Carboni A, Crucitti P, Moliterni VM, Ranalli P, Mandolino G (2003) The inheritance of chemical phenotype in *Cannabis sativa* L. Genetics 163:335
151. Turner CE, Elsohly MA, Boeren EG (1980) Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. J Nat Prod 43:169
152. Grunfeld Y, Edery H (1969) Psychopharmacological activity of the active constituents of hashish and some related cannabinoids. Psychopharmacologica 14:200
153. Cascio MG, Gauson LA, Stevenson LA, Ross RA, Pertwee RG (2010) Evidence that the plant cannabinoid cannabigerol is a highly potent α2 adrenoceptor agonist and moderately potent 5HT1A receptor antagonist. Br J Pharmacol 159:129
154. Rock EM, Goodwin JM, Limebeer CL, Breuer A, Pertwee RG, Mechoulam R, Parker LA (2011) Interaction between non-psychotropic cannabinoids in marihuana: effect of cannabigerol (CBG) on the anti-nausea or anti-emetic effects of cannabidiol (CBD) in rats and shrews. Psychopharmacology (Berl) 215:505
155. Russo EB (2011) Taming THC: potential cannabis synergy and phytocannabinoid-terpenoid entourage effects. Br J Pharmacol 163:1344
156. Shinjyo N, Di Marzo V (2013) The effect of cannabichromene on adult neural stem/progenitor cells. Neurochem Int 63:432
157. McPartland JM, Matias I, Di Marzo V, Glass M (2006) Evolutionary origins of the endocannabinoid system. Gene 370:64

158. Iwamura H, Suzuki H, Ueda Y, Kaya T, Inaba T (2001) In vitro and in vivo pharmacological characterization of JTE-907, a novel selective ligand for cannabinoid CB2 receptor. J Pharmacol Exp Ther 296:420
159. Rosenthaler S, Pöhn B, Kolmanz C, Nguyen Huu C, Krewenka C, Huber A, Kranner B, Rausch W-D, Moldzio R (2014) Differences in receptor binding affinity of several phytocannabinoids do not explain their effects on neural cell cultures. Neurotoxicol Teratol 46:49
160. Hill AJ, Weston SE, Jones NA, Smith I, Bevan SA, Williamson EM, Stephens GJ, Williams CM, Whalley BJ (2010) Delta(9)-tetrahydrocannabivarin suppresses in vitro epileptiform and in vivo seizure activity in adult rats. Epilepsia 51:1522
161. Thomas BF, Gilliam AF, Burch DF, Roche MJ, Seltzman HH (1998) Comparative receptor binding analyses of cannabinoid agonists and antagonists. J Pharmacol Exp Ther 285:285
162. Nye JS, Seltzman HH, Pitt CG, Snyder SH (1985) High-affinity cannabinoid binding sites in brain membranes labeled with [$^3$H]-5′-trimethylammonium delta 8-tetrahydrocannabinol. J Pharmacol Exp Ther 234:784
163. Compton DR, Rice KC, De Costa BR, Razdan RK, Melvin LS, Johnson MR, Martin BR (1993) Cannabinoid structure-activity relationships: correlation of receptor binding and in vivo activities. J Pharmacol Exp Ther 265:218
164. Jones NA, Hill AJ, Smith I, Bevan SA, Williams CM, Whalley BJ, Stephens GJ (2010) Cannabidiol displays antiepileptiform and antiseizure properties in vitro and in vivo. J Pharmacol Exp Ther 332:569
165. Lauckner JE, Jensen JB, Chen H-Y, Lu H-C, Hille B, 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
166. O'Sullivan SE, Sun Y, Bennett AJ, Randall MD, Kendall DA (2009) Time-dependent vascular actions of cannabidiol in the rat aorta. Eur J Pharmacol 612:61
167. Yamaori S, Kushihara M, Yamamoto I, Watanabe K (2010) Characterization of major phytocannabinoids, cannabidiol and cannabinol, as isoform-selective and potent inhibitors of human CYP1 enzymes. Biochem Pharmacol 79:1691
168. Yamaori S, Koeda K, Kushihara M, Hada Y, Yamamoto I, Watanabe K (2011) Comparison in the in vitro inhibitory effects of major phytocannabinoids and polycyclic aromatic hydrocarbons contained in marijuana smoke on cytochrome P450 2C9 activity. Drug Metab Pharmacokinet 27:294
169. Cascio MG, Zamberletti E, Marini P, Parolaro D, Pertwee RG (2015) The phytocannabinoid, delta-9-tetrahydrocannabivarin, can act through 5-HT1A receptors to produce antipsychotic effects. Br J Pharmacol 172:1305
170. Fišar Z, Singh N, Hroudová J (2014) Cannabinoid-induced changes in respiration of brain mitochondria. Toxicol Lett 231:62
171. Jiang R, Yamaori S, Okamoto Y, Yamamoto I, Watanabe K (2013) Cannabidiol is a potent inhibitor of the catalytic activity of cytochrome P450 2C19. Drug Metab Pharmacokinet 28:332
172. Yamaori S, Ebisawa J, Okushima Y, Yamamoto I, Watanabe K (2011) Potent inhibition of human cytochrome P450 3A isoforms by cannabidiol: role of phenolic hydroxy groups in the resorcinol moiety. Life Sci 88:730
173. Ross HR, Napier I, Connor M (2008) Inhibition of recombinant human T-type calcium channels by delta9-tetrahydrocannabinol and cannabidiol. J Biol Chem 283:16124
174. Nabissi M, Morelli MB, Santoni M, Santoni G (2013) Triggering of the TRPV2 channel by cannabidiol sensitizes glioblastoma cells to cytotoxic chemotherapeutic agents. Carcinogenesis 34:48
175. Pandolfo P, Silveirinha V, Dos Santos-Rodrigues A, Venance L, Ledent C, Takahashi RN, Cunha RA, Köfalvi A (2011) Cannabinoids inhibit the synaptic uptake of adenosine and dopamine in the rat and mouse striatum. Eur J Pharmacol 655:38

**Sarah E. Turner** is undertaking her Ph.D. in neuropharmacology at the University of Reading under the guidance of Prof. Claire M. Williams and Prof. Benjamin J. Whalley. Her work involves investigation of the antiepileptic mechanism of action of two phytocannabinoids. She received a B.Sc. (Hons) degree in Animal Science from the University of Pretoria, South Africa in 2009. Since moving to the United Kingdom in 2010, she worked as a research technician at the University of Reading for three years in Prof. Benjamin J. Whalley and Prof. Claire M. Williams's research group. She has extensive experience in behavioral neuroscience, investigating the effects of phytocannabinoids on acute seizures and epilepsy in various animal models and investigating the effects of phytocannabinoid treatment and the comorbidity effects of epilepsy on motor function and cognition. This work led to being named as a co-author on two peer-reviewed papers. Her main interests lie in investigating the therapeutic benefits of phytocannabinoids in various diseases and disorders and driving phytocannabinoid treatment through clinical trials.

**Claire Williams** is a behavioral pharmacologist with research interests that explore the role of natural phyto-(plant derived) chemicals for biomedical research and developing novel dietary or therapeutic strategies to improve health. After studying for a B.Sc. (Hons) degree in Applied Biology from Nottingham Trent University, Claire moved to the School of Psychology and Clinical Language Sciences at the University of Reading in 1996 to study for a Ph.D. investigating the role of the cannabinoid system in feeding behavior, graduating in 2000. After receiving her Ph.D., she began a postdoctoral research position at the University of Reading continuing the work from her Ph.D. on cannabinoid-induced feeding. In October 2002 she was awarded tenure working as a Lecturer (2002–2010), Associate Professor (2010–2015) and Professor (2015–present) within the School of Psychology and Clinical Language Sciences at the University of Reading. She has been awarded grant funding from several UK research councils and has established extensive research collaborations with industry. To date, Professor Williams has published over 40 research articles in peer-reviewed journals, four invited book chapters, and has been named as co-inventor on several patents.

**Leslie Iversen, Ph.D., F.R.S, C.B.E.** is Visiting Professor at the Department of Pharmacology, University of Oxford. He was previously Director of the Wolfson Centre for Research on Age Related diseases at Kings College London (1999–2004), Director of the Neuroscience Research Centre for Merck & Co. Inc. in the UK (1983–1995), and Director of the Medical Research Council Neurochemical Pharmacology Unit in Cambridge, England (1970–1983). He is well known for his research on how drugs interact with chemical messengers in the nervous system and has published more than 300 scientific papers on this topic and is the author of "The Science of Marijuana, 2nd Ed.", 2007; "Speed, Ecstasy, and Ritalin: the Science of Amphetamines", 2007; "Introduction to Neuropsychopharmacology", 2008; and "Very Short Introduction Drugs" (2nd Edition), 2016, Oxford University Press. He is a Fellow of the Royal Society London, and a Foreign Associate of the US National Academy of Sciences.

**Benjamin J. Whalley** is a Professor of Neuropharmacology at the Reading School of Pharmacy. He received a B. Pharm. (Hons) degree from the London School of Pharmacy, University of London in 1992. Since registering as a pharmacist since 1993, he worked in community and primary care trust pharmacy for several years before undertaking a Ph.D. in Neuroscience at the London School of Pharmacy, University of London. He was subsequently appointed as a Lecturer at the Reading School of Pharmacy in 2005. He is a member of the UK Advisory Council on the Misuse of Drugs. His research interests lie in investigating neuronal processes that underlie complex physiological functions such as neuronal hyperexcitability states and their consequential disorders (e.g. epilepsy and ataxia). This work has helped to determine cellular mechanisms that underlie the effects of antiepileptic drugs in clinical use or currently in clinical trials, particularly non-psychoactive components of cannabis drugs for which he received the 2014 Royal Pharmaceutical Society's Science Award. A further significant component of his research has been the development of experimental platforms that combine biological (neuronal cell culture, including human stem cell-based networks) and machine systems to produce tools for investigating the cellular correlates of complex CNS function and dysfunction.