Review

Non-psychotropic plant cannabinoids: new therapeutic opportunities from an ancient herb

Angelo A. Izzo[1,4], Francesca Borrelli[1,4], Raffaele Capasso[1,4], Vincenzo Di Marzo[2,4] and Raphael Mechoulam[3]

[1] Department of Experimental Pharmacology, University of Naples Federico II, Naples, Italy
[2] Institute of Biomolecular Chemistry, National Research Council, Pozzuoli (NA), Italy
[3] Department of Medicinal Chemistry and Natural Products, Hebrew University Medical Faculty, Jerusalem, Israel
[4] Endocannabinoid Research Group, Italy

Δ^9-tetrahydrocannabinol binds cannabinoid (CB_1 and CB_2) receptors, which are activated by endogenous compounds (endocannabinoids) and are involved in a wide range of physiopathological processes (e.g. modulation of neurotransmitter release, regulation of pain perception, and of cardiovascular, gastrointestinal and liver functions). The well-known psychotropic effects of Δ^9-tetrahydrocannabinol, which are mediated by activation of brain CB_1 receptors, have greatly limited its clinical use. However, the plant *Cannabis* contains many cannabinoids with weak or no psychoactivity that, therapeutically, might be more promising than Δ^9-tetrahydrocannabinol. Here, we provide an overview of the recent pharmacological advances, novel mechanisms of action, and potential therapeutic applications of such non-psychotropic plant-derived cannabinoids. Special emphasis is given to cannabidiol, the possible applications of which have recently emerged in inflammation, diabetes, cancer, affective and neurodegenerative diseases, and to Δ^9-tetrahydrocannabivarin, a novel CB_1 antagonist which exerts potentially useful actions in the treatment of epilepsy and obesity.

Introduction

The plant *Cannabis sativa* produces over 421 chemical compounds, including about 80 terpeno-phenol compounds named phytocannabinoids that have not been detected in any other plant [1–4]. For obvious reasons, most attention has been paid to Δ^9-tetrahydrocannabinol (Δ^9-THC), which is the most psychotropic component and binds specific G-protein-coupled receptors named cannabinoid (CB_1 and CB_2) receptors [5,6]. The discovery of a specific cell membrane receptor for Δ^9-THC was followed by isolation and identification of endogenous (animal) ligands termed endocannabinoids. The two main endocannabinoids are anandamide (which is metabolized mostly by fatty acid amide hydrolase (FAAH)) and 2-arachidonoylglycerol (which is mostly degraded by monoglyceride lipase (MAGL)) [5,6]. Cannabinoid receptors, endogenous ligands that activate them, and the mechanisms for endocannabinoid biosynthesis and inactivation constitute the "endocannabinoid system". With its ability to modulate several physiological and pathophysiological processes (e.g. neurotransmitter

Corresponding author: Izzo, A.A. (aaizzo@unina.it).

Glossary

Transient receptor potential (TRP): Transient receptor potential (TRP) is a superfamily of non-selective, ligand-gated cation channels. They can be subdivided in six main subfamilies: the TRPC ('Canonical'), TRPV ('Vanilloid'), TRPM ('Melastatin'), TRPP ('Polycystin'), TRPML ('Mucolipin') and the TRPA ('Ankyrin') group. At least six TRPs (TRPV1, TRPV2, TRPV3, TRPV4, TRPM8 and TRPA1) have been shown to be expressed in primary afferent nociceptors, where they act as transducers for thermal, chemical and mechanical stimuli. Many TRPs are activated by natural compounds, such as capsaicin (TRPV1), cannabidiol (TRPV2), incensole acetate (TRPV3), menthol (TRPM8) and mustard oil isothiocyanates (TRPA1)

Adenosine uptake: Uptake of adenosine is a primary mechanism of terminating adenosine signalling. Adenosine is a multifunctional, ubiquitous molecule that activate four known adenosine receptors (A_1, A_{2A}, A_{2B} and A_3). Adenosine A_{2A} receptor is an important regulator of inflammation.

GPR55: GPR55 is an orphan G-protein-coupled receptor originally identified in silico from the expressed sequence tags database. GPR55 may be activated by plant and synthetic endocannabinoids as well as by anandamide-related acylethanolamides and may be antagonized by cannabidiol. Possible role in antinociception.

Peroxisome proliferator-activated receptors (PPARs): Peroxisome proliferators-activated receptors (PPARs) belong to a family of nuclear receptors comprising three isoforms: α, β and γ. Among these, PPARγ is involved in the regulation of cellular glucose uptake, protection against atherosclerosis and control of immune reactions. Activation of PPARγ attenuates neurodegenerative and inflammatory processes.

Lipoxygenase (LOX): Lipoxygenases are non-heme iron-containing enzymes that catalyze the dioxygenation of polyunsaturated fatty acids, such as arachidonic acid and linolenic acids. Three major LOX isoforms have been discovered (i.e., 5-, 12-, and 15-LOX). 5-LOX is responsible for the production of leukotrienes-inflammatory lipid mediator. 15-LOX oxygenates not only free fatty acids but also complex substrates such as phospholipids, cholesterol ester, and the cholesterol ester in the low density lipoprotein particle, with a role in atherosclerosis and inflammation

Glycine receptors: Glycine receptors, which belong to the superfamily of transmitter-gated ion channels - are pentamers formed either from α subunits alone, or from both α and β subunits. They are activated by glycine, one of the major inhibitory neurotransmitters in posterior areas of the vertebrate central nervous system. Glycine receptors are also involved in inflammation, immune response and cytoprotection.

Abnormal-cannabidiol receptor: The abnormal-cannabidiol receptor is a putative receptor expressed in the endothelium of rat mesenteric bed, which can be activated by abnormal-cannabidiol (abn-cbd), a synthetic analogue of cannabidiol. This endothelial receptor, distinct from the currently known cannabinoid receptors, has also been suggested to mediate anandamide-induced relaxation in the whole mesenteric bed of the rat.

5-HT_{1A} receptor: The 5-HT_{1A} receptor is one of the best-characterized 5-HT receptors. This G protein-coupled receptor is involved a number of physiological or pathophysiological processes, including anxiety, mood, depression, vasoreactive headache, food intake, immune regulation, and cardiovascular regulation.

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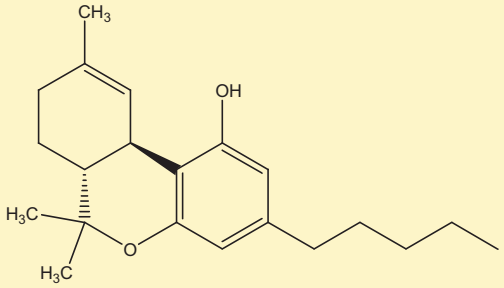
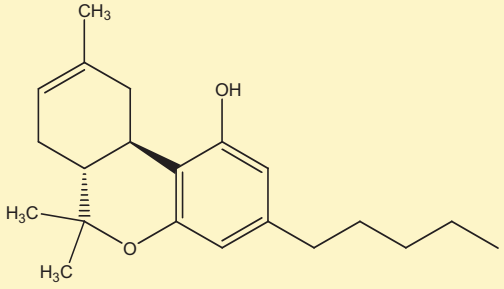
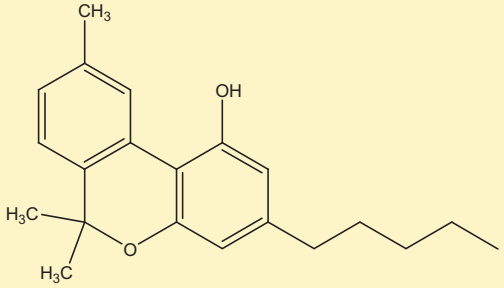
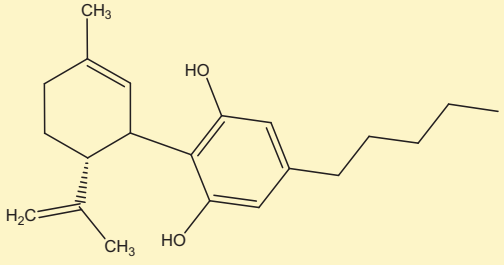
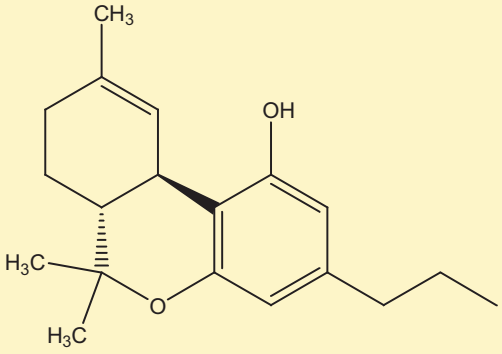
release in the central and peripheral nervous system, pain perception, and cardiovascular, gastrointestinal and liver functions), the endocannabinoid system represents a potential target for pharmacotherapy [6]. Strategies to improve the efficacy and/or the risk–benefit ratio of drugs that manipulate the endocannabinoid system include the targeting of cannabinoid receptors located outside the blood–brain barrier with selective cannabinoid ligands or compounds that prevent endocannabinoid degradation (e.g. inhibitors of FAAH or MAGL) [5,6].

In addition to pharmacological modulation of the endocannabinoid system, a different approach to minimize the well-known psychotropic side effects of *Cannabis* is the use of phytocannabinoids with very weak or no psychotropic effects. These include cannabidiol (CBD), cannabigerol (CBG), cannabichromene (CBC), Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabidivarin (CBDV) as well as cannabinoid acids such as Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA) and cannabidiolic acid (CBDA) (Box 1). These compounds exert multiple actions through mechanisms

Box 1. Chemical structures and key (including historical) information of the main phytocannabinoids

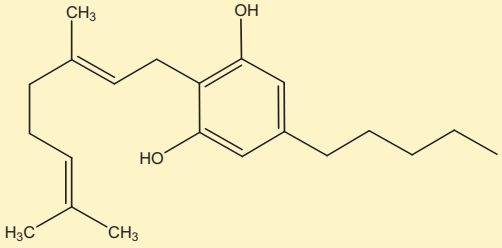
Phytocannabinoid	Key information*
 Δ^9-Tetrahydrocannabinol (Δ^9-THC)	Isolated in 1964 by Gaoni and Mechoulam at the Weizmann Institute in Rehovot, Δ^9- THC is the primary psychotropic ingredient of *Cannabis*. It is a partial agonist at CB_1 and CB_2 receptors (K_i approx. 20–40 nM). Δ^9- THC also activates PPAR-γ (at nanomolar concentrations) and TRPA1 (at micromolar concentrations) [2]. It is therapeutically used as an antiemetic and to boost appetite in AIDS patients. A *Cannabis* based-extract with approx 1:1 ratio of Δ^9-THC and CBD (Sativex®) is marketed in Canada for the symptomatic relief of neuropathic pain in adults with multiple sclerosis and as an adjunctive analgesic treatment for adult patients with advanced cancer [76].
 Δ^8-Tetrahydrocannabinol (Δ^8-THC)	In general, Δ^8-THC is regarded as an artefact because it results from the isomerization of Δ^9-THC. Δ^8-THC concentration in *Cannabis* is usually minuscule, and it does not contribute significantly to the activity of the plant extract. Δ^8-THC is considered less expensive to prepare and more stable than Δ^9-THC. The pharmacology of Δ^8-THC is similar to that of Δ^9-THC, although it may be less active [3]. It is as active as Δ^9-THC in antiemetic studies, although it is not marketed (apparently for purely commercial reasons).
 Cannabinol (CBN)	Isolated in 1896 by Wood and colleagues in Cambridge, CBN represents the first natural cannabinoid to be obtained in pure form. Its correct structure was later determined by Adams and colleagues in 1940. It was initially—and incorrectly—assumed to be the active psychotropic ingredient of *Cannabis*. It is a relatively minor constituent in fresh *Cannabis* because it is a product of Δ^9-THC oxidation. CBN content increases as Δ^9-THC degrades in storage. It is a weak CB_1 and CB_2 partial agonist, with approximately 10% of the activity of Δ^9-THC [2]. It has potential therapeutic application in diseases in which cannabinoid receptors are up-regulated [2].
 Cannabidiol (CBD)	CBD, a major non-psycotropic cannabinoid, was first isolated in 1940 by Adams and coworkers, but its structure and stereochemistry were determined in 1963 by Mechoulam and Shvo. CBD exerts a plethora of pharmacological effects, mediated by multiple mechanisms (Table 1, Figure 1). It has been clinically evaluated in anxiety, psychosis, and movement disorders, and to relieve neuropathic pain in patients with multiple sclerosis (in combination with Δ^9-THC as a 1:1 mixture, i.e. Sativex®) [76].

Box 1 (*Continued*)



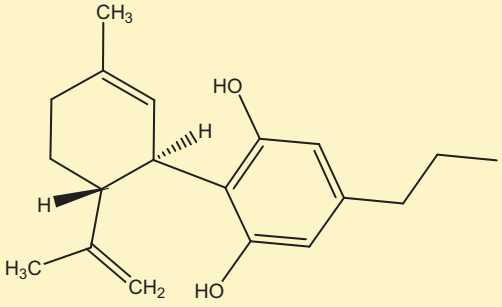
Δ^9-tetrahydrocannabivarin (Δ^9-THCV)

Δ^9- THCV[a] was detected in 1970 by Edward Gil and colleagues from a tincture of *Cannabis* BPC (then a licensed medicine in the UK). It is particularly abundant in Pakistani hashish. Δ^9-THCV at low doses (<3 mg/kg) antagonises Δ^9-THC effects, but it acts as a CB_1 agonist at higher doses (10 mg/kg) *in vivo* in mice[b] [2,25]. Δ^9-THCV shares the ability of synthetic CB_1 antagonists to reduce food intake in mice [62].



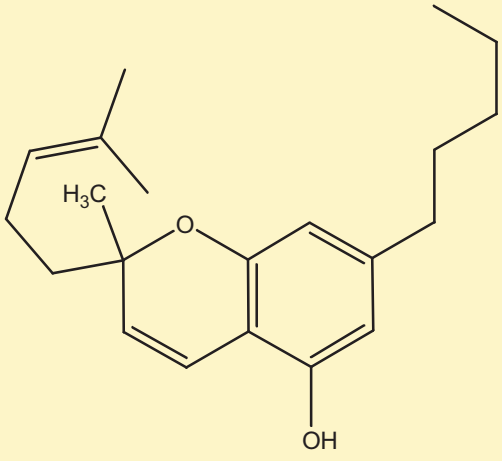
Cannabigerol (CBG)

Non-psychotropic cannabinoid obtained in 1964 by Gaoni and Mechoulam when they separated a hexane extract of hashish on Florisil. It exerts anti-proliferative and antibacterial activity. It is a potent TRPM8 antagonist [14], a TRPV1, TRPA1 and cannabinoid agonist, and an anandamide reuptake inhibitor in the low micromolar range [11,14].



Cannabidivarin (CBDV)

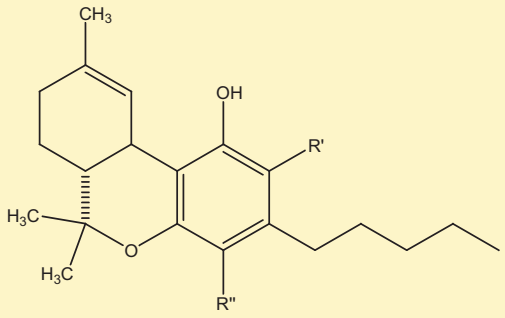
CBDV[a] was isolated from hashish by Vollner and coworkers in 1969. Little information on its pharmacology has been reported and a mode of action has not been proposed.



Cannabichromene (CBC)

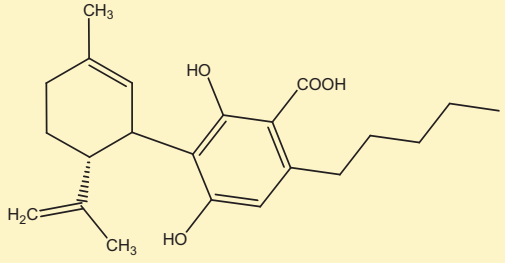
The discovery of CBC, a non-psychotropic cannabinoid, was independently reported by Claussen and coworkers, and Gaoni and Mechoulam in 1966. CBC, together with Δ^9-THC, is the major cannabinoid in freshly harvested dry-type material. CBC is nearly 2.5-times more toxic than Δ^9-THC and, like Δ^9-THC, it may cause hypothermia, sedation and hypoactivity in mice [3]. CBC exerts anti-inflammatory, antimicrobial and modest analgesic activity [3,32,39,75]. It is a potent TRPA1 agonist and weak anandamide reuptake inhibitor [11,14].

Box 1 (*Continued*)



Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA)
R' = COOH; R'' = H D^9-THCA A
R' = H; R''= COOH D^9-THCA B

Δ^9-THC has two acidic analogs: Δ^9-THCA A and Δ^9-THCA B[c]. Δ^9-THCA A, first extracted by Korte and coworkers (1965), was isolated as a pure compound in 1967 by Nishioka's group. In 1969, Mechoulam and coworkers reported the isolation of Δ^9-THCA B. Δ^9-THCA (unknown as to whether it is Δ^9-THCA A or Δ^9-THCA B) is a potent TRPA1 agonist and TRPM8 antagonist [14] and has been shown to exert anti-proliferative [11] and anti-spasmodic [3] actions.



Cannabidiolic acid (CBDA)

CBDA, the first cannabinoid acid[c] to be discovered, was isolated in 1955 by Krejci and Santavy. Together with CBD, CBDA is the main component of glandular hairs (up to 15%). In fresh plant material, 95% of CBD exists as its acid. It is a selective COX-2 inhibitor [22], TRPA1 and TRPV1 agonist and TRPM8 antagonist in the low micromolar range [11,14]. It exerts anti-proliferative actions [11].

Abbreviations: CBD, cannabidiol; Δ^9-THCV, Δ^9-tetrahydrocannabivarin; CBC, cannabichromene; CBG, cannabigerol; Δ^9-THCA, Δ^9-tetrahydrocannabinolic acid;, CBDA, cannabidiolic acid; Δ^9-THC, Δ^9-tetrahydrocannabinol; CBN, cannabinol; peroxisome proliferator-activated receptor γ (PPARγ), TRPV1, transient receptor potential vanilloid type 1; TRPV2, transient receptor potential vanilloid type 2; TRPA1, transient receptor potential ankyrin type 1; TRPM8, transient receptor potential melastatin type 8; COX-2, cycloxygenase-2.

[*]Chemical and historical data were extracted from refs. 3-4.

[a]the suffix ''varin'' indicates replacement of *n*-pentyl side chain with an *n*-propyl.

[b]Because Δ^9-THCV does not display detectable CB_1 receptor efficacy *in vitro*, CB_1 agonism is probably due to a Δ^9-THCV metabolite. Thus, high doses of Δ^9-THCV can produce anti-nociception and cataleptic behavior in mice and induce THC-like effects in humans, although with a potency in mouse and humans 4–5-times lower than that of Δ^9-THC [2].

[c]It has been suggested that cannabinoid acids are the original cannabinoids formed in the plant, to be subsequently decarboxylated to yield the better known neutral cannabinoids, but this hypothesis is controversial. None of the cannabinoid acids possess psychotropic activity [4].

which are only partially related to modulation of the endocannabinoid system [1,2]. The most promising of this class of safe compounds is CBD. CBD exerts several positive pharmacological effects that make it a highly attractive therapeutic entity in inflammation, diabetes, cancer and affective or neurodegenerative diseases [1,2,7,8]. More recently, Δ^9-THCV has been shown to express the pharmacological profile of a CB_1 antagonist [9], with potential use in obesity treatment [2].

Here, we focus on recent developments in the preclinical pharmacology of non-psychotropic phytocannabinoids. We highlight the novel biochemical/pharmacological advances, mechanisms of action, and possible therapeutic uses of these plant-derived compounds.

Molecular basis of the pharmacological action of non-psychotropic cannabinoids

Non-psychotropic phytocannabinoids exert multiple pharmacological effects via different mechanisms. The most recently investigated mechanisms are modulation of the endocannabinoid system, transient receptor potential (TRP) channels (see Glossary), the peroxisome proliferator-activated receptor γ (PPARγ) GPR55, the putative abnormal-CBD receptor 5-hydroxytryptamine receptor subtype 1A ($5\text{-}HT_{1A}$), glycine α1 and α1β receptors, the adenosine membrane transporter phospholipase A_2, lipoxygenase (LOX) and cyclooxygenase-2 (COX-2) enzymes, and Ca^{2+} homeostasis (Table 1) [9–26]. For example, CBD, CBG and CBC, which have very low affinity for cannabinoid CB_1 and CB_2 receptors, might enhance endocannabinoid-mediated actions through their ability to inhibit anandamide inactivation [11]. Δ^9-THCV behaves as a potent CB_2 partial agonist *in vitro* and as a CB_1 antagonist *in vivo* and *in vitro* [2,9,25]. CBD and CBG activate TRPV1, whereas CBD, CBC, CGB, and CBDA activate TRPA1 and, except for CBC, are TRPM8 antagonists [11,14].

CBD might also exert its pharmacological effects via modulation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). CBD increases $[Ca^{2+}]_i$ in hippocampal neurons [18] through modulation of intracellular Ca^{2+} stores— specifically via mitochondrial Ca^{2+} uptake and release—and L-type voltage-gated Ca^{2+} channels [19]. Interestingly, under pathological conditions such as high neuronal-excitability conditions, CBD reduces $[Ca^{2+}]_i$ [19]. Despite the fact that CBD has potent antioxidant activity, the increase in $[Ca^{2+}]_i$ in tumor cells causes generation of reactive oxygen species (ROS) and cell apoptosis [11,27] (see the section below on cancer). It has been suggested that CBD hydroxyquinone, formed during hepatic microsomal metabolism of CBD, is capable of generating ROS and inducing cytotoxicity [28].

Table 1. Proposed molecular mechanisms of the actions of non-psychotropic phytocannabinoids

Phytocannabinoid	Mechanism [reference]	Quantitative data	Assay	Pharmacological Relevance [reference]
CBD	Antagonist of CB_1/CB_2 agonists [10]	K_B (nM); 79 (CB_1) and 138 (CB_2)	[^{35}S]GTPγS binding to mouse brain membranes (CB1) and to hCB_2-CHO cell membranes	CBD antagonises cannabinoid-induced antispasmodic effect in the isolated vas deferens as well as the *in vivo* responses to Δ^9-THC in animals and humans [2,8,10]
	CB_2 inverse agonist [10]	EC_{50}: 503 nM	[^{35}S]GTPγS binding to hCB_2-CHO cell membranes	To be determined. Potential role in CBD-induced anti-inflammatory effects
	FAAH inhibition [11]	IC_{50}: 28 μM	Measurement of [^{14}C]ethanolamine released from [^{14}C]anandamide by membranes prepared from N18TG2 cells	CBD reduces FAAH expression in the inflamed intestine and, probably via this mechanism, reduces inflammation-induced intestinal hypermotility in mice [57,58]
	Anandamide reuptake inhibitor [11]	IC_{50}: 28 μM	[^{14}C]anandamide uptake by basophilic leukaemia or MDA-MB-231 cells	To be determined
	GPR55 antagonist [12]	IC_{50}: 445 nM	Antagonism of CP55970-induced activation of [^{35}S]GTPγS binding to transfected HEK293S cells	To be determined
	positive allosteric modulator at α1 and α1β glycine receptors. [13]	EC_{50} (μM): 12.3 (α_1) and 18.1 ($\alpha_{1\beta}$)	Measurement of the current response to glycine in HEK 293 cells expressing α1 or $\alpha_{1\beta}$ receptors	To be determined. In the dorsal horn of the spinal cord, glycine acts as an inhibitory postsynaptic neurotransmitter, with a role in chronic pain after inflammation or nerve injury
	μ opioid receptor ligand [see ref. 2]	IC_{50}: 7 μM	Inhibition of [^{3}DNM] (μ opioid receptor ligand) binding to rat brain synaptosomal membranes	To be determined. CBD could potentially enhance the effects of opiates
	Positive Allosteric modulator at μ and δ opioid receptors [see ref. 2]	pE_{50}: 4.38 (μ) and 4.10 (δ)	H^3-DAMGO and H^3-naltrindole (μ and δ opioid receptor ligand) binding to rat cerebral cortical membranes	The effect occurs at very high concentrations and cannot be expected to contribute to the *in vivo* action of CBD
	TRPA1 agonist [14]	EC_{50}: 96 nM	Increase of $[Ca^{2+}]_i$ in TRPA1-HEK-293 cells	To be determined. Potential role in CBD analgesic effects
	TRPM8 antagonist [14]	EC_{50}: 80-140 nM	Antagonism of icilin- or menthol-induced increase in $[Ca^{2+}]_i$ in TRPM8-HEK-293 cells	To be determined. Potential role in CBD analgesic effects. Potential role in prostate carcinoma
	TRPV1 agonist [14]	EC_{50}: 1-3 μM	Increase of $[Ca^{2+}]_i$ in TRPV1-HEK-293 cells	To be determined. TRPV1 is involved in CBD antipsychotic and analgesic effects [30,50]
	TRPV2 agonist [15]	EC_{50}: 3.7 μM	Ca^{2+} mobilization in TRPV2-HEK-293 cells	The effect is shared by Δ^9-THC and CBN [15]. TRPV2 activation by CBD may mediate CGRP release from cultured rat dorsal root ganglion neurons [15]
	adenosine uptake competitive inhibitor[a] [16]	IC_{50}: 120 nM	[^{3}H]adenosine uptake in murine microglia and macrophages	CBD decreases TNF-α in wild-type but not in A_{2A} receptor-deficient mice [16]. Its anti-inflammatory effects in the retina are linked to the inhibition of adenosine uptake [65]
	PPARγ agonist [17]	IC_{50} approx 5 μM	Reporter gene assay, competition-binding assay and adipogenesis assay	CBD induces vasorelaxation and stimulation of fibroblasts into adipocytes via PPARγ activation [17]
	5-HT_{1A} agonist [see ref. 2]	Approx 80% displacement at 16 μM	Displacement of [^{3}H]8-OH-DPAT in CHO cells transfected with 5-HT_{1A} receptors; [^{35}S]GTPγS binding to transfected CHO cells	5-HT_{1A} is involved in CBD-induced antischemic and anxiolytic properties [34,35]

Table 1 (*Continued*)

Phytocannabinoid	Mechanism [reference]	Quantitative data	Assay	Pharmacological Relevance [reference]
	Antagonist of the putative abnormal-CBD receptor [see ref. 2]	Effect at 1 μM	Antagonism of the vasodilator response of abnormal-CBD	CBD attenuates the vasodilator response to anandamide [2]
	Regulator of intracellular $[Ca^{2+}]$[b] [18,19]	Effect at 1 μM	Ca^{2+} imaging experiments in hippocampal cultures	To be determined. Potential basis for the neuroprotective and antiepileptic properties of CBD
	T-type Ca^{2+} channel inhibitor [20]	IC_{50}: approx 1 μM	Electrophysiological recordings in transfected HEK293 cells and sensory neurons	To be determined. Potential role in CBD-induced nociception and antiepileptic effects
	Suppressor of tryptophan degradation [21]	IC_{50}: 1.2-2.4 μg/ml	Measurements in human peripheral blood mononuclear cells	To be determined. Tryptophan is a precursor of 5-HT. Potential role in pain, inflammation and depression
	5-Lipoxygenase inhibitor [22]	IC_{50}: 73.73 μM	Enzymatic assay in a cell-free system	The effect is observed at very high concentrations. However, the 5-lipoxygenase pathway may be involved in CBD-induced antimitotic effect in glioma cells [69]. CBD decreases 5-lipoxygenase in tumour tissues *in vivo* [69]
	15-Lipoxygenase inhibitor [22]	IC_{50}: 2.56 μM	Enzymatic assay in a cell-free system	To be determined. 15-Lipoxygenase is involved in developing atherosclerosis
	Phospholipase A_2 modulator[c] [23]	EC_{50}: 6.4 μM (activation); IC_{50}: 134 μM (inhibition)	Enzymatic assay in a cell-free system	CBD exerts a biphasic stimulation of PGE_2 release in human synovial cells [23]. CBD exerts anti-inflammatory effects in rodents [1,7]
Δ^9-THCV	CB_1 antagonist [9,24, see also ref. 2]	K_i: 46-75 nM (brain membranes); pA_2 7.62 (cerebellum) - 7.44 (piriform cortex)	Antagonism of cannabinoid agonist-induced [^{35}S]GTPγS binding to mouse whole brain, cerebellar and piriform cortical membranes	Δ^9-THCV increases central inhibitory neurotransmission [31] - with a therapeutic potential in epilepsy - and decreases food intake through CB_1 antagonism [62]. Δ^9-THCV attenuates Δ^9-THC-induced hypothermia and antinociception in vivo [2,25]
	CB_2 partial agonist [see ref. 2]	NR	Inhibition of forskolin-induced stimulation of cAMP production by hCB_2-CHO cells.	Δ^9-THCV stimulates mesenchymal stem cells via CB_2 receptors [67]
CBG	CB_1 and CB_2 partial agonist [see ref. 2]	K_i (nM): 439 (CB_1), 337 (CB_2)	Displacement of [^{3}H]CP55,940 from mouse brain membranes of hCB_2-CHO cell membranes. [^{35}S]GTPγS binding to mouse brain membranes (CB1) and to hCB_2-CHO cell membranes	To be determined.
	Anandamide reuptake inhibitor [11]	IC_{50}: 15 μM	[^{14}C]anandamide uptake by basophilic leukaemia or MDA-MB-231 cells	To be determined. Potential applications similar to those of inhibitors of endocannabinoid degradation
	TRPA1 agonist [14]	EC_{50}: 3.4 μM	Increase of $[Ca^{2+}]_i$ in TRPA1-HEK-293 cells	To be determined. Potential role in analgesia
	TRPV1 agonist [11]	EC_{50}: 10 μM	Increase of $[Ca^{2+}]_i$ in TRPV1-HEK-293 cells	To be determined. Potential role in analgesia
	TRPM8 antagonist [14]	EC_{50}:140-160 nM	Antagonism of icilin- or menthol-induced increase in $[Ca^{2+}]_i$ in TRPM8-HEK-293 cells	To be determined. Potential role in analgesia and in the treatment of prostate carcinoma.
	Phospholipase A_2 modulator[c] [23]	EC_{50}: 9.5 μM; IC_{50}: 55 μM	Enzymatic assay in cell-free system	CBG reduces PGE_2 release in human synovial cells [23].

Table 1 (*Continued*)

Phytocannabinoid	Mechanism [reference]	Quantitative data	Assay	Pharmacological Relevance [reference]
CBC	TRPA1 agonist [14]	EC_{50}: 60 M	Increase of $[Ca^{2+}]_i$ in TRPA1-HEK-293 cells	To be determined. Potential role in analgesia
	Anandamide reuptake inhibitor [11]	IC_{50}: 13 μM	[^{14}C]anandamide uptake by basophilic leukaemia or MDA-MB-231 cells	To be determined. Potential applications similar to those of inhibitors of endocannabinoid degradation
Δ^9-THCA	TRPA1 partial agonist [14]	EC_{50}: 240 nM	Increase of $[Ca^{2+}]_i$ in TRPA1-HEK-293 cells	To be determined. Potential role in analgesia
	TRPM8 antagonist [14]	EC_{50}: 70-140 nM	Antagonism of icilin- or menthol-induced increase in $[Ca^{2+}]_i$ in TRPA1-HEK-293 cells	To be determined. Potential role in analgesia and in the treatment of prostate carcinoma.
CBDA	TRPA1 partial agonist [14]	EC_{50}: 12 μM	Increase of $[Ca^{2+}]_i$ in TRPA1-HEK-293 cells	To be determined. Potential role in analgesia
	TRPV1 agonist [11]	EC_{50}: 10 μM	Increase of $[Ca^{2+}]_i$ in TRPV1-HEK-293 cells	To be determined. Potential role in analgesia
	TRPM8 antagonist [14]	EC_{50}: 0.9-1.9 μM	Antagonism of icilin- or menthol-induced increase in $[Ca^{2+}]_i$ in TRPA1-HEK-293 cells	To be determined. Potential role in analgesia and in the treatment of prostate carcinoma.
	COX-2 inhibitor [26]	IC_{50}: 2.2 μM	Enzymatic assay	To be determined. The effect is not shared by Δ^9-THC or CBD; Δ^9-THCA weakly active at 100 μM [26]. Potential role in inflammation

NR = not reported.
Abbreviations: CBD, cannabidiol; Δ^9-THCV, Δ^9-tetrahydrocannabivarin; CBC, cannabichromene; CBG, cannabigerol; Δ^9-THCA, Δ^9-tetrahydrocannabinolic acid;, CBDA, cannabidiolic acid; Δ^9-THC, Δ^9-tetrahydrocannabinol; CBN, cannabinol; TRPV1, transient receptor potential vanilloid type 1; TRPV2, transient receptor potential vanilloid type 2; TRPA1, transient receptor potential ankyrin type 1; TRPM8, transient receptor potential melastatin type 8; COX-2, cycloxygenase-2; 5-HT_{1A}, 5-hydroxytryptamine receptor subtype 1A; FAAH, fatty acid amide hydrolase.
[a]denotes that the effect occurs via the equilibrate nucleoside transporter.
[b]denotes that the effect occurs via mitochondrial uptake and release or via L-type voltage gated $[Ca^{2+}]$ channel.
[c]denotes activation at low concentrations, inhibition at higher concentrations.

As a consequence, CBD hydroxyquinone reduces colon cancer growth in nude mice [29]. The multiple pharmacological targets of phytocannabinoids, most notably those of CBD, result in a wide range of pharmacological actions with potential therapeutic interest.

Pharmacological actions and potential therapeutic applications

Non-psychotropic phytocannabinoids exert multiple pharmacological actions in the central nervous system and in the periphery. Among these compounds, CBD has been more thoroughly investigated. CBD effects (e.g. analgesic/anti-inflammatory, antioxidant, neuroprotective and pro-apoptotic) might predict possible future use for the treatment of pain, neurodegenerative disorders, ischemia and cancer. Many effects of CBD (e.g. anxiolytic, anti-inflammatory, neuroprotective, anti-ischemic) follow a bell-shaped dose–response curve [1,7,8], suggesting that dose is a key factor in CBD pharmacology.

Psychosis

Preliminary reports have demonstrated the antipsychotic action of CBD in human models of psychotic symptoms induced in volunteers and in psychotic patients [1,7,8]. The pharmacological profile of the antipsychotic action of CBD, investigated in animal models using behavioral and neurochemical techniques, was shown to be similar to that of atypical antipsychotics such as clozapine, and different from that of "typical" antipsychotics such as haloperidol, in that it was associated with fewer unwanted side effects such as catelepsy. Three important points are worth noting. First, CBD, like clozapine and haloperidol, attenuated some dopaminergic effects associated with apomorphine (i.e. stereotypy, prolactin secretion, and palpebral ptosis) and reduced hyperlocomotion induced by amphetamine and ketamine in mice. However, in these experiments, haloperidol (but not CBD or clozapine) caused catalepsy [7]. Second, CBD, like clozapine (but not like haloperidol) increased Fos protein expression in the nucleus accumbens, but not in the striatum, indicating that CBD produces neuronal activation in mesolimbic but not in motor control areas [7]. Third, CBD reversed, in a TRPV1 antagonist-sensitive manner and similar to clozapine, the sensorimotor gating deficits induced by a NMDA receptor antagonist [30], which is relevant in the light of the observation that sensorimotor gating is deficient in patients with psychotic disorders such as schizophrenia.

In summary, CBD is the only phytocannabinoid to have been evaluated for possible antipsychotic effects. Experimental results suggest that it exerts antipsychotic actions and is associated with fewer adverse effects compared with "typical antipsychotics".

Epilepsy

The clinical efficacy of CBD with respect to epilepsy is uncertain [7], but this compound has been shown to attenuate convulsions induced in animals by various procedures [1,7,8] and to reduce Ca^{2+} oscillations under high-excitability in cultured hippocampal neurons [19]. The molecular basis for the antiepileptic action of CBD might involve a reduction of $[Ca^{2+}]_i$, via interaction with the mitochondrial Na^{2+}/Ca^{2+}-exchanger [19].

Another phytocannabinoid that might exert antiepileptic actions is Δ^9-THCV. This compound acts in a manner similar to "standard" CB_1 receptor antagonists to increase—in a $GABA_A$ antagonist-sensitive manner—miniature inhibitory postsynaptic currents at interneuron–Purkinje cell synapses, and to decrease Purkinje cell spike firing in the mouse cerebellum *in vitro* [31]. Collectively, such results suggest that Δ^9-THCV acts to limit excitation via increase in GABA release, an idea that is consistent with its efficacy in an experimental model of epilepsy [2]. An early report showed that CBC produced minor effects on the latency and duration of electroshock-induced seizures [32].

In summary, CBD (via reduction of $[Ca^{2+}]_i$) and Δ^9-THCV (via CB_1 antagonism) have been suggested to exert antiepileptic actions in experimental studies.

Anxiety and sleep

Preliminary studies in healthy volunteers suggest that CBD has an anxiolytic action [1,7,8]. Experimentally, the anxiolytic-like properties of CBD (which are benzodiazepine receptor-independent) have been demonstrated in different animal models such as the conditioned emotional response, the Vogel conflict test, and the elevated plus-maze [7,33]. CBD might exert anxiolytic-like effects by activating post-synaptic 5-HT_{1A} receptors in the periaqueductal gray matter [34]. Furthermore, CBD attenuated the acute autonomic response (i.e. increased blood pressure and heart rate) associated with restraint stress in rats in a 5-HT_{1A} antagonist-sensitive manner [35]. Preclinical studies also suggest the potential use of CBD as an adjuvant in exposure-based psychotherapies for anxiety disorders related to inappropriate retention of aversive memories. Bitencourt and colleagues recently found that CBD facilitated the extinction of contextual fear memory in rats, possibly through indirect activation of the CB_1 receptor [36].

CBD has been shown to exert alerting and sleep-inducing actions. Its systemic administration prolonged pentobarbitone sleep in mice [37] and reduced ambulation and operant behavior in rats [1,7,8]. However, when CBD was directly administered into specific wake-related areas, such as the lateral hypothalamus or dorsal raphe nuclei, an enhancement in rat alertness was observed [38]. Notably, the effect of CBD in humans is biphasic, exhibiting alerting properties at low doses and sedative actions at high doses [7]. Early studies showed that CBC, like Δ^9-THC, prolonged hexobarbital hypnosis in mice [3,39].

In summary, CBD has been shown to exert anxiolytic actions—possibly via 5-HT_{1A} receptor activation—and to facilitate the extinction of contextual fear memory—perhaps via indirect activation of CB_1 receptors—in rodents. Sleep-inducing actions have been described for CBC and CBD, although centrally administered CBD may also have alerting properties.

Neuroprotection and neurodegenerative diseases

CBD is a well-known antioxidant, exerting neuroprotective actions that might be relevant to the treatment of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). CBD may prove beneficial in preventing apoptotic signalling in neurons via restoration of Ca^{2+} homeostasis [18].

CBD exerts a combination of neuroprotective, anti-oxidative and anti-apoptotic effects against the neuronal damage induced by the β-amyloid peptide (Aβ). It inhibits Aβ-induced neurotoxicity in PC12 cells and this effect is mediated by the Wnt–β-catenin pathway [40], an important finding in light of the observation that disruption of the Wnt pathway by Aβ represents a pivotal event in the neuronal apoptosis occurring in AD. Moreover, in a mouse model of AD-related neuroinflammation induced by the intra-hippocampal inoculation of Aβ *in vivo*, CBD attenuated the expression of several glial pro-inflammatory proteins, including glial fibrillary acidic protein, inducible nitric oxide synthase (iNOS) and interleukin 1β (IL-1β) [41], which are major contributors to the propagation of neuroinflammation and oxidative stress.

By using a rat model of PD generated by unilateral injection of 6-hydroxydopamine into the medial forebrain bundle, it was shown that CBD can attenuate dopamine depletion and tyrosine hydroxylase deficits, which are indicative of the degree of neurodegeneration of nigrostriatal dopaminergic projections [1,7]. The neuroprotective action of CBD in animal models of PD is in accord with the strong positive correlation between the *N*-acetylaspartate/total creatine ratio (which is suggestive of increased neurogenesis or synaptogenesis) and CBD levels measured in the putamen/globus pallidus of recreational users of *Cannabis* [42]. Further studies investigating the mode of action of CBD showed that this plant compound counteracted the decrease in copper-zinc superoxide dismutase (a key enzyme in endogenous defences against oxidative stress) induced by 6-hydroxydopamine in the rat substantia nigra [43].

CBD has been shown to reduce rat striatal atrophy generated by the administration of 3-nitropropionic acid (a mitochondrial toxin that replicates some of the biochemical alterations occurring in HD). This ability seems to be based on the antioxidant properties of CBD, and is independent of the activation of cannabinoid, TRPV1 and adenosine A_{A2} receptors [44]. Such neuroprotective effects might be relevant to HD, which is characterized by the preferential loss of striatal projection neurons due, at least in part, to the generation of ROS species caused by mitochondrial failure and complex II deficiency typical of patients with HD.

In summary, CBD, possibly due to its extraordinary antioxidant properties and to its modulation of Ca^{2+} homeostasis, exerts positive effects on a wide range of pathophysiological processes implicated in neurodegenerative diseases. CBD is also effective in experimental models of AD, PD and HD.

Cerebral and myocardial ischemia

CBD can reverse brain damage caused by cerebral ischemia in mice and in gerbils [1,7]. The cerebroprotectant effect of CBD is different from that of Δ^9-THC in that it is: i) cannabinoid receptor-independent, ii) long-lasting, iii) observed when the drug is administered pre- and post-ischemia, and iv) not associated with the development of tolerance [45–47]. Importantly, CBD reduced cerebral hemodynamic impairment, improved brain metabolic activity post-insult, and reduced brain edema and seizures associated with temporary occlusion of carotid arteries and hypoxia in newborn gerbils [48]. These neuroprotective effects were associated with extracerebral benefits such as cardiac, hemodynamic and ventilatory improvements [48]. The mechanism of the cerebroprotectant effect of CBD might involve an increase in cerebral blood flow mediated by the 5-HT_{1A} receptor [1,7] and/or be secondary to its cannabinoid receptor-independent anti-inflammatory action [46]. The anti-inflammatory action of CBD is associated with inhibition of monocyte/macrophages expressing high-mobility group (a non-histone DNA-binding protein which is known to induce neurofinallation and microglial activation in the post-iscremic brain) in the infarct area (including the striatum), and to a reduction in the number of Iba1-positive and glial fibrillary acidic protein-positive cells in the striatum [47].

CBD is also promising for treatment of myocardial ischemia. It caused a reduction in infarct size in an *in-vivo* rat model of ischemia and reperfusion, and the effect was associated with a reduction of myocardial inflammation and interleukin (IL)-6 levels [49]. CBD was ineffective in the isolated rat heart model 49, so it is possible that its cardioprotective effects are mediated by systemic immunomodulatory effects or by a CBD metabolite.

In summary, CBD is a promising agent for treatment of cerebral and myocardial ischemia. CBD increases cerebral flow via the 5-HT_{1A} receptor.

Inflammation, pain and the immune response

Early reports suggested that CBC exerted anti-inflammatory effects [39] and modest analgesic activity [32] in rodents. CBC was superior to the non-steroidal anti-inflammatory drug phenylbutazone in carrageenan-induced rat paw edema and in the erythrocyte membrane stabilization method [39].

More recently, CBD was shown to be effective in well-established experimental models of analgesia (neuropatic and inflammatory pain) [50] as well as in acute (carrageenan-induced rat paw edema) and chronic (e.g. collagen-induced murine arthritis) models of inflammation [1,7] in rodents. It is believed that the analgesic effect of CBD is mediated, at least in part, by TRPV1 [50] and that its anti-arthritic action is due to a combination of immunosuppressive and anti-inflammatory effects. This idea is based on several lines of evidence (summarized in Box 2) [1,2,7,8,51,52,53].

The effect of CBD on T-cells was investigated in detail. It was found that the cannabinoid exerted a generalized immunosuppressive effect through a pro-apoptotic mechanism involving oxidative stress-dependent activation of caspase-8 [52,54]. It was also proposed that CBD-induced T-cell suppression might be linked to its ability to suppress the transcriptional activity of activator protein-1 and nuclear factor of activated T-cells, both of which are critical regulators of IL-2 and interferon-γ (IFN-γ) [55].

Box 2. Evidence supporting the anti-inflammatory and immunosuppressive actions of cannabidiol (CBD)

- ▶CBD suppresses the collagen-type-II-specific proliferation of lymph-node cells from arthritic mice [1].
- ▶ CBD suppresses T-cell response and decreases TNF-α release from synovial cells isolated from mouse arthritic knee joints [1]. This finding suggests that the therapeutic action of CBD in arthritis includes the suppression of TNF-α.
- ▶ CBD decreases TNF-α production in LPS-treated mice via A_{2A} adenosine receptor activation [16].
- ▶ CBD suppresses the production of IL-8 and of the chemokines MIP-1α and MIP-1β in a human B cell line [1].
- ▶ CBD inhibits the release of ROS by zymosan-stimulated neutrophils and blocks nitric oxide production by peritoneal macrophages [1].
- ▶ CBD increases IL-12 and decreases IL-10 production—in a cannabinoid antagonists-sensitive manner—in murine macrophages [1].
- ▶ CBD attenuates—in a cannabinoid antagonists-insensitive manner—phorbol ester/calcium ionophore-stimulated IL-2 production in mouse splenocytes [1].
- ▶ CBD inhibits neutrophil migration induced by fMLP by activating a target, distinct from CB_1 and CB_2 receptors, which is antagonized by the endogenous compound N-arachidonoyl-L-serine [51].
- ▶ CBD attenuates serum production of antigen-specific antibodies in ovalbumin-sensitized mice and suppresses T-cell proliferation and the production of IL-2, IL-4 and IFN-γ by splenocytes [52].
- ▶ CBD decreases IFN-γ release in phytohemagglutinin-stimulated human peripheral mononuclear cells [21] and in lymph-node cells [1].
- ▶ CBD induces apoptosis in immature and immortalized T-cells, with ROS generation having a pivotal role [53].

Abbreviations: fMLP, formyl-methionyl-leucyl-phenylalanine; IFN-γ, interferon-γ, IL, interleukin; LPS, lipopolysaccharide; MIP-1, Macrophage Inflammatory Protein-1; ROS, reactive oxygen species; TNF-α, tumor necrosis factor α.

Psoriasis is an inflammatory disease characterized by epidermal keratinocyte hyper-proliferation. The most significant mediators involved in this disorder are those associated with a dominant Th1 cytokine profile. Δ^9-THC, CBN and CBD were shown to inhibit keratinocyte proliferation in the low micromolar range and in a cannabinoid receptor-independent manner. Although the mechanism is incompletely understood, these results support a therapeutic potential of non-psychotropic cannabinoids for the treatment of psoriasis [56].

CBD was shown to normalize motility in an experimental model of intestinal inflammation [57]. This protective action might involve down-regulation of the endocannabinoid-degrading enzyme FAAH in the inflamed gut [57,58].

In summary, CBD exerts anti-arthritic actions through a combination of immunosuppressive and anti-inflammatory effects. CBD may exert protective actions in other inflammatory conditions (e.g. psoriasis and gut inflammation). The anti-inflammatory effect of CBC requires further investigation.

Emesis

CBD was effective in animal models of anticipatory nausea and vomiting (conditioned retching reaction in the musk shrew, a model in which standard antiemetics such as 5-HT_3 antagonists are ineffective) [59], as well as in models of nausea and/or vomiting (i.e. lithium-induced conditioned gaping in rats and vomiting in musk shrews, cisplatin-induced emesis in the musk shrew) [1,60]. Such results suggest a potential use of CBD in the treatment of chemotherapy-induced nausea and anticipatory nausea. In musk shrews, CBD showed a biphasic effect, being antiemetic at low doses (1–5 mg/kg) and pro-emetic at higher doses (25–40 mg/kg) [1]. By contrast, CBD was ineffective in an experimental model of motion-induced emesis in the musk shrew [61], suggesting that this compound (unlike Δ^9-THC) does not act as a broad-spectrum antiemetic.

Food intake

Δ^9-THCV, at doses as low as 3 mg/kg, shares the ability of synthetic CB_1 antagonists to reduce food intake and body weight in mice [62]. At similar doses, Δ^9-THCV attenuated Δ^9-THC-induced hypothermia and antinociception, confirming its efficacy as a CB_1 receptor antagonist [2,9,25]. Under similar conditions, CBD induced a small non-significant reduction of food intake and weight gain [62].

Type-1 diabetes and diabetic complications

CBD prevents the initiation of diabetes in non-obese diabetic (NOD) mice [1,7] and, importantly, ameliorates the manifestations of the disease in NOD mice, which are either in a latent diabetes stage or with initial symptoms of diabetes [63]. CBD treatment induced qualitative modification of the pancreatic islets infiltrated by mononuclear cells, and inhibited the specific destruction of the islets [63]. Levels of the pro-inflammatory cytokine IL-12 produced by splenocytes were significantly reduced, whereas those of the anti-inflammatory IL-10, were elevated after CBD treatment [63].

CBD also exerts significant therapeutic benefits against diabetic complications because it significantly reduces oxidative stress and prevents retinal cell death and vascular hyperpermeability in the diabetic retina in an experimental model of diabetic retinopathy [1,7]; in addition, CBD exerts anti-inflammatory and neuroprotective effects in retinal microglial cells [64]. It has been proposed that the protective effect of CBD against diabetes-induced retinal damage may be linked to inhibition of adenosine uptake [65]. In human coronary artery endothelial cells (HCAECs), CBD attenuates high glucose-induced mitochondrial superoxide generation, nuclear factor κB (NF-κB) activation, nitrotyrosine formation, up-regulation of iNOS and adhesion molecules ICAM-1 and VCAM-1, trans-endothelial migration of monocytes and monocyte-endothelial adhesion, while preserving HCAECs from disruption of endothelial barrier functions [66].

In summary, CBD exerts beneficial actions against diabetes and some of its complications (e.g. retinal damage). The anti-inflammatory, antioxidant and neuroprotective actions of CBD could contribute to these protective effects.

Bone formation

Mesenchymal stem cells (MSCs) have a central role in a series of physiological and pathophysiological processes, including bone formation and fracture healing. CBDV, CBG, CBN, CBD, Δ^9-THC, and Δ^9-THCV stimulated the recruitment of quiescent MSCs present in bone marrow [67]. The effect varied from a relatively small stimulation of about 20% by CBG to as much as 100% after treatment with CBDV or Δ^9-THCV. The effect of Δ^9-THCV was CB_2-antagonist sensitive and MSCs are cannabinoid receptor-negative cells, so it was believed that Δ^9-THCV may stimulate the recruitment of MSCs from the bone marrow indirectly via a mechanism mediated by a CB_2-expressing accessory cell [67].

CBD also controls bone resorption during the progression of experimental periodontitis in rats. In this case, morphometrical analysis of alveolar bone loss demonstrated that CBD-treated animals had reduced alveolar bone loss and lower expression of the activator of the NF-κB ligand RANKL/RANK [68]. Moreover, gingival tissues from the CBD-treated group showed reduced neutrophil migration associated with lower production of IL-1β and tumor necrosis factor-α [68].

Overall, the phytocannabinoids CBDV, Δ^9-THCV and CBD may exert beneficial effects on bone formation and fracture healing.

Cancer

Δ^9-THC, CBD, CBG, CBC, Δ^9-THCA and CBDA have been shown to exert anti-proliferative/pro-apoptotic effects (IC_{50} in the range 5–25 μM) in a panel of tumor cell lines: human breast carcinoma, human prostate carcinoma, human colorectal carcinoma, human gastric adenocarcinoma, C6 rat glioma, rat basophilic leukemia and transformed thyroid cells. CBD exhibited the highest potency with IC_{50} values between 6 μM and 10.6 μM, and maximal efficacy at 25 μM, followed by CBG and CBC [11]. CBDA was the least effective compound, being active against only breast, thyroid and glioma cells. Furthermore, prostate carcinoma cells were found to be quite resistant to the action of phytocannabinoids, with only CBD and CBG exerting anti-proliferative effects [11]. More in-depth studies showed that CBD inhibited glioma, leukaemia and breast cancer, as detailed below.

1) CBD exerted cannabinoid-independent anti-metastatic and pro-apoptotic effects on human glioma cells and tumor regression *in vivo* [1,7,27]. CBD-induced apoptosis of human glioma cells involves early production of ROS and concomitant activation of initiator caspase-8 and caspase-9, converging into the activation of the downstream effector caspase-3 [27]. *In vivo*, CBD induced glioma growth inhibition through specific modulation of the pro-carcinogenic LOX pathway [69].
2) CBD induced a CB_2-mediated reduction in viability and apoptosis in leukemia cells, and reduced tumor burden and increased the number of apoptotic tumours in EL-4-bearing mice *in vivo*; the effect was associated with increased production of ROS, which was mediated through regulation of Nox4 and p22phox [70].
3) CBD inhibited the growth of xenograft tumours obtained by subcutaneous injection of human breast

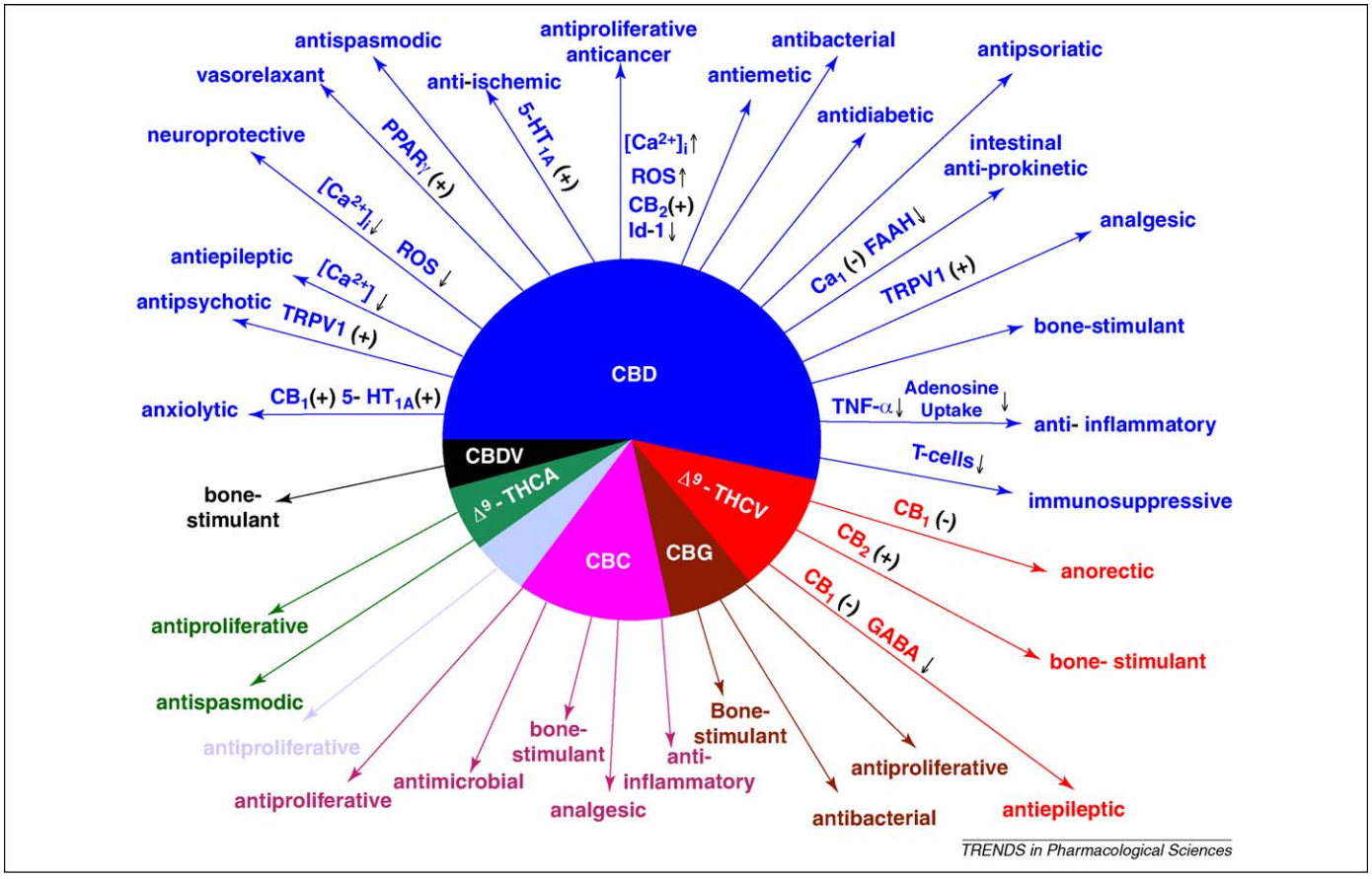


Figure 1. Pharmacological actions of non-psychotropic cannabinoids (with the indication of the proposed mechanisms of action).
Abbreviations: Δ^9-THC, Δ^9-tetrahydrocannabinol; Δ^8-THC, Δ^8-tetrahydrocannabinol; CBN, cannabinol; CBD, cannabidiol; Δ^9-THCV, Δ^9-tetrahydrocannabivarin; CBC, cannabichromene; CBG, cannabigerol; Δ^9-THCA, Δ^9-tetrahydrocannabinolic acid; CBDA, cannabidiolic acid; TRPV1, transient receptor potential vanilloid type 1; PPARγ, peroxisome proliferator-activated receptor γ; ROS, reactive oxygen species; 5-HT_{1A}, 5-hydroxytryptamine receptor subtype 1A; FAAH, fatty acid amide hydrolase.
(+), direct or indirect activation; ↑, increase; ↓, decrease.

carcinoma cells into athymic mice [11]. Studies investigating the mode of action showed that CBD down-regulated the expression of Id-1 (a key regulator of the metastatic potential of breast and other carcinomas) in metastatic human breast cancer cells, leading to reduction of tumour aggressiveness [71].

Phytocannabinoids have been shown to inhibit ATP-binding cassette (ABC) transporters, which play a part in the multi-drug resistance of tumor cells. Specifically, P-glycoprotein (ABCB1) was inhibited by CBD, but not by Δ^9-THCV, Δ^9-THCA or CBN [72]; multi-drug resistance-related protein 1 (ABCC1/MRP1) and breast cancer resistance protein were inhibited by CBD, CBN and Δ^9-THC (order of potency: CBD > CBN > Δ^9-THC) [73].

CBD was shown to attenuate oxidative/nitrosative stress, inflammation, and cell death induced by the anticancer drug cisplatin in the mouse kidney [74]. Nephrotoxicity is a common complication of cisplatin chemotherapy, which limits its clinical use.

In summary, the phytocannabinoids CBD, CBG and CBC have shown interesting pro-apoptotic properties in cancer cell lines. The most studied phytocannabinoid is CBD. CBD induces increases in $[Ca^{2+}]_i$, thereby stimulating ROS production and causing apoptosis. In vivo, CBD inhibits glioma growth and experimental breast carcinoma.

Microbial growth

Preparations from *Cannabis sativa* were extensively investigated in the 1950s as highly active topical antiseptic agents for the oral cavity and the skin, and as anti-tubercular agents. Cannabinoid acids, which can be precursors of the neutral cannabinoids, were shown to be antibiotic 4 and were used in veterinary medicine in Czechoslovakia in the 1960s. An early report showed that CBC exerted antifungal and, to a lesser degree, antibacterial activity [39]. Recently, five major cannabinoids (Δ^9-THC, CBN, CBD, CBC and CBG) showed potent activity against various methicillin-resistant *Staphylococcus aureus* strains of current clinical relevance. No substantial difference in potency was observed, with a minimum inhibitory concentration in the range 0.5–2 μg/mL [75].

Conclusions

Recent developments suggest that non-psychotropic phytocannabinoids exert a wide range of pharmacological effects (Figure 1), many of which are of potential therapeutic interest. The most studied among these compounds is CBD, the pharmacological effects of which might be explained, at least in part, by a combination of mechanisms of action (Table 1, Figure 1). CBD has an extremely safe profile in humans, and it has been clinically evaluated (albeit in a preliminary fashion) for the treatment of

anxiety, psychosis, and movement disorders. There is good pre-clinical evidence to warrant clinical studies into its use for the treatment of diabetes, ischemia and cancer. The design of further clinical trials should: i) consider the bell-shaped pattern of the dose–response curve that has been observed in pre-clinical pharmacology, and ii) establish if CBD is more effective or has fewer unwanted effects than other medicines. A sublingual spray that is a standardized *Cannabis* extract containing approximately equal quantities of CBD and Δ^9-THC (Sativex®), has been shown to be effective in treating neuropathic pain in multiple sclerosis patients [76].

The pharmacology of Δ^9-THCV (i.e. CB_1 antagonism associated with CB_2 agonist effects) is also intriguing because it has the potential of application in diseases such as chronic liver disease or obesity—when it is associated with inflammation—in which CB_1 blockade together with some CB_2 activation is beneficial. Concerning obesity treatment, it will be important in future studies to establish if Δ^9-THCV is more effective or has fewer unwanted effects than rimonabant. Rimonabant was the first clinically available CB_1 receptor antagonist, but was withdrawn from the market because of the increased risk of depression.

The plant *Cannabis* is a source of several other neglected phytocannabinoids such as CBC and CBG. Although the spectrum of pharmacological effects of these compounds is largely unexplored, their potent action at TRPA1 and TRPM8 might make these compounds new and attractive tools for pain management.

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