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A Systematic Review of Minor Phytocannabinoids with Promising Neuroprotective Potential.

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Abstract

Embase and Pubmed were systematically searched for articles addressing the neuroprotective properties of phytocannabinoids, aside from cannabidiol and Δ^9-tetrahydrocannabinol, including Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabidiolic acid (CBDA), cannabidivarin (CBDV), cannabichromene (CBC), cannabichromenic acid (CBCA), cannabichromevarin (CBCV), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabigerivarin (CBGV), cannabigerovarinic acid (CBGVA), cannabichromevarinic acid (CBCVA) cannabidivarinic acid (CBDVA) and cannabinol (CBN). Out of 2,341 studies, 31 articles met inclusion criteria. CBG (range 5 $mg.kg^{-1}$ to 20 $mg.kg^{-1}$) and CBDV (range 0.2 $mg.kg^{-1}$ to 400 $mg.kg^{-1}$) displayed efficacy in models of Huntington's disease and epilepsy. CBC (10-75 $mg.kg^{-1}$), Δ^9-THCA (20 $mg.kg^{-1}$) and Δ^9-THCV (range 0.025-2.5 $mg.kg^{-1}$) showed promise in models of seizure and hypomobility, Huntington's and Parkinson's disease. Limited mechanistic data showed CBG, VCE.003, VCE.003.2 and Δ^9-THCA mediated some of their effects through PPARγ, but no other receptors were probed. Further studies with these phytocannabinoids, and their combinations, are warranted across a range of neurodegenerative disorders.

Introduction

According to the World Health Organisation (WHO), neurodegenerative diseases will be the second most prevalent cause of death by 2040 (Gammon, 2014). The cellular mechanisms of these diseases typically overlap with neuronal dysfunction and neuronal cell death being a common thread, regardless of definitive clinical presentations. Typically, neurodegenerative diseases are categorised as amyloidoses, which includes Alzheimer's disease and British familial dementia; synucleinopathies, which includes lewy body disorders such as Parkinson's; and proteinopathies, which includes amyotrophic lateral sclerosis (ALS) and tauopathies (Kovac, 2018). Other common neurological disorders include epilepsy and stroke, characterised by recurring, unprovoked seizures and vascular pathology respectively. Recently, stroke was re-classified as a neurological disease by the International Classification of Disease (ICD) 11, highlighting that whilst strokes predominantly derive from vascular origin, the neurological consequences are often severe (Shakir, 2018).

Current treatments for neurodegenerative and neurological conditions are often limited and usually rely on managing symptoms rather than making a significant impact on delaying disease progression (Kiaei, 2013). For example, Huntington's disease is managed with tetrabenazine (TBZ) 75-200 mg per day to alleviate chorea (involuntary movement), but because it acts as a vesicular monoamine transporter (VMAT) inhibitor, interfering with both serotonin and dopamine degradation, patients can develop neuropsychiatric symptoms along with other side effects (Hayden *et al.*, 2009; Kaur *et al.*, 2016; Wyant, Ridder and Dayalu, 2017). Other first line treatments, for example L-Dopa in Parkinson's disease, often cause side effects and do not delay disease progression. Finally, cholinesterase inhibitors such as donepezil are only minimally effective in improving cognition for the treatment of Alzheimer's disease. In light of this, there is clearly an urgent need to develop new therapies with more tolerable side effect profiles to combat these debilitating conditions and increase the quality of life of the aging population.

Over 120 different phytocannabinoids have been isolated from *Cannabis Sativa* (ElSohly and Gul, 2015). Of these, Δ^9- tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) are the most abundant and widely studied. Δ^9-THC is responsible for the psychoactive effects of cannabis,

which are mediated through the cannabinoid type 1 receptor (CB_1) Pertwee 2008. Δ^9-THC also interacts with other targets including transient receptor potential (TRP) channels, the orphan G-protein receptor, GPR55, and peroxisome proliferator-activated receptors (PPARs) (Pertwee and Cascio, 2015). CBD has also been shown to modulate a plethora of pharmacological targets including 5-HT_{1A}, PPARγ and TRPV1, but has no psychotropic effects because it does not activate central CB_1 receptors (reviewed in Ibeas Bih et al., 2015 and Russo, E. B., & Marcu, J. 2017). Interaction with these targets has given CBD status as a neuroprotectant, anti-inflammatory agent and antioxidant (Fernandez-Ruiz et al., 2012 and Maroon et al., 2018). These features, along with its favourable safety profile in humans (World Health Organization, 2017; Millar *et al.*, 2019) has made CBD, in many respects, a more desirable drug candidate than Δ^9-THC. CBD has shown promise in several animal models of neurodegeneration as well as clinical trials for Parkinson's, Alzheimer's and amyotrophic lateral sclerosis (ALS) (Iuvone *et al.*, 2009). Furthermore, a CBD: Δ^9-THC (1:1) product is currently licenced by GW Pharmaceuticals under the brand name Sativex® to treat pain and spasticity associated with multiple sclerosis (MS), and Epidiolex® (pure CBD) is licensed to treat Lennox-Gastaut syndrome and Dravet syndrome, which are severe forms of childhood epilepsy. Other cannabis-based medicines (CBMs) are also under development: GW pharmaceuticals has four compounds (structures are not disclosed) in the pipeline for neurological conditions including glioblastoma, schizophrenia and neonatal hypoxic-ischaemic encephalopathy (GW Pharmaceuticals, 2019).

Phytocannabinoids are highly unique compounds, they are promiscuous, modulating a range of pharmacological targets as well as exhibiting high antioxidant capability due to their phenolic structures and the presence of hydroxyl groups (Hampson *et al.*, 1998; Yamaori *et al.*, 2011; Borges *et al.*, 2013). These features, along with their lipophilicity and ability to act as anti-inflammatory agents makes them desirable therapeutic candidates for the treatment of CNS disorders, as they can effectively cross the blood-brain barrier (BBB), modulate the immune response and overall target the many facets of neurodegeneration (Deiana *et al.*, 2012). These characteristics have been well established for Δ^9-THC and CBD but are less known for some of the minor constituents of the plant and in order to understand the full therapeutic potential of cannabis *sativa*, the pharmacology of the lesser-

known components of the plant should be elucidated (Turner et al., 2017). Given the wide-ranging neuroprotective effects of Δ^9-THC and CBD already established, it is not unfounded to suggest other phytocannabinoids may exhibit similar or more potent neuroprotective properties. Therefore, the aim of this systematic review was to collate all available data on the neuroprotective effects of Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabidiolic acid (CBDA), cannabidivarin (CBDV), cannabichromene (CBC), cannabichromenic acid (CBCA), cannabichromevarin (CBCV), cannabigerol (CBG), cannabigerolic acid (CBGA), cannabigerivarin (CBGV), cannabigerovarinic acid (CBGVA), cannabichromevarinic acid (CBCVA) cannabidivarinic acid (CBDVA) and cannabinol (CBN). These phytocannabinoids were selected based on their abundance in the plant, ease of synthesis, efficacy in other fields (e.g as anticancer agents or treatments for inflammatory bowel disease) and similarities in their structure to CBD and Δ^9-THC (which have already shown promise as a neuroprotectants and displayed safety in humans), and are therefore more likely to have neuroprotective potential and exhibit human translatability.

Methods

Data sources and search strategy

An electronic search was conducted using the search engines PubMed and Embase from its inception to June 2019. This was carried out in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Moher D, Liberati A, Tetzlaff J, 2009; Tóth *et al.*, 2010; Shamseer *et al.*, 2015) Search terms included Δ^9-tetrahydrocannabinolic acid, Δ^9-tetrahydrocannabivarin, cannabidiolic acid, cannabidivarin, cannabichromene, cannabichromenic acid, cannabichromevarin, cannabigerol, cannabigerolic acid , cannabigerivarin, cannabigerovarinic acid, cannabichromevarinic acid, cannabidivarinic acid and cannabinol (and their corresponding abbreviations), phytocannabinoids, neurovascular unit, pericytes, neurons, astrocytes, human brain microvascular endothelial cells, brain, neuroinflammation, hyperexcitability, neurodegeneration, Huntington's, Alzheimer's, Parkinson's, epilepsy and stroke. Two

independent reviewers carried out the searches by November 2019 and the reference lists of the final papers were hand searched for any additional studies.

Eligibility and exclusion criteria

Conference abstracts and review articles were excluded. No restrictions were applied to type of study, publication year, or language. Inclusion criteria were as follows: an original, peer reviewed article that involved the application of emerging phytocannabinoids in an *in vivo* or *in vitro* model of neurodegeneration or neuronal damage. Studies that looked at CBG derivatives VCE-003 or VCE-002.3 were also included because current research is targeted at these compounds due to their increased affinity for PPARγ. Studies that assessed CBD, Δ^9-THC, Δ^9-THC: CBD 1:1 (Sativex®) or similar combination of phytocannabinoids (i.e different ratios of phytocannabinoids) were excluded from this review. After duplicates and irrelevant articles were removed, the full text was obtained for the remaining articles and studies were examined for data regarding Δ^9-THCA, Δ^9-THCV, CBDA, CBDV, CBC, CBCA, CBCV, CBG, CBGA, CBGV, CBGVA, CBCVA, CBDVA and CBN application in an *in vitro* and/or *in vivo* model of neuroprotection or neuronal damage. Dose and route of administration were extracted from *in vivo* studies and where possible range and average were calculated. If studies reported mechanistic data, this was also described in the results section.

Results

The preliminary search retrieved 2,341 studies, which after duplicates were removed left 1,851. A total of 107 cannabinoid studies were retrieved, once exclusion criteria were applied, 26 articles were considered to be potentially relevant and their full texts obtained. After additional screening (including reviewing reference lists for any potential studies) 28 studies were included in this review, see figure 1. Table 1a summarises the *in vitro* data included in this review and table 1b summarises the *in vivo* data. Within the 28 studies, the neuroprotective models were epilepsy (n=7), Huntington's disease (n=6), Parkinson's (n=4), amyloid lateral sclerosis (ALS) (n=3), neuroprotection (not disease specific, n=2), multiple Sclerosis (MS) (n=1), Rett Syndrome (n=2), neuroinflammation (n=1), Alzheimer's (n=1), and oxidative stress (n=1). Fifteen papers studied CBG or its derivatives, 5 studies used CBN, 8 studies used CBDV and 4 studies used CBC. Only 2 studies used Δ^9-THCV and 3 used Δ^9-

THCA. CBDA was only included in one study. No data on the neuroprotective effects of, CBGA, CBGV, CBCA, CBCV, CBCVA, CBGVA, or CBDVA were identified. Figure 2 depicts some of the minor phytocannabinoids structures with CBD and Δ^9-THC for reference and table 2 summarises the neurological conditions for which emerging cannabinoids have shown therapeutic potential.

Cannabigerol (CBG) and its derivatives

Nine studies included *in vitro* data and 8 included *in vivo* data on CBG and its derivatives which are formed by the oxidation of CBG (Carrillo-Salinas *et al.*, 2014; Díaz-Alonso *et al.*, 2016; García *et al.*, 2018). VCE-003 and VCE-003.2 have displayed increased affinity for PPARγ, thus maintaining their anti-inflammatory properties whilst having little affinity for CB_1 and CB_2 (VCE-003 Ki >40 μM for CB_1 and >1.76 μM CB_2 (Granja *et al.*, 2012) and VCE-003.2 Ki >40 μM for both CB_1 and CB_2 (García *et al.*, 2018). All studies except one, reported a positive effect of CBG, VCE-003 or VCE-002.3 vs control in the disease model being studied. In an *in vivo* 3-nitropropionic acid (3-NP) model to induce Huntington's disease, CBG (10 $mg.kg^{-1}$ per day i.p) significantly attenuated the upregulation of COX-2, iNOS, IL-6 and TNF-α (Valdeolivas *et al.*, 2015). CBG treatment also prevented 3-NP-induced neuronal loss, recovered catalase, superoxide dismutase and glutathione versus control, as well as downregulating genes that were directly associated with Huntington's disease including sgkl, Cd44 and normalised huntingtin associated protein-1. Aggregation of mutant Huntingtin protein was diminished and motor deficits such as hindlimb clasping and dystonia and general locomotor activity were also improved (Valdeolivas *et al.*, 2015). Hill et al., (2014) assessed the anti-convulsant potential of CBG (50-200 $mg.kg^{-1}$ i.p) when administered prior to the initiation of pentylenetetrazole (PTZ) seizures, however despite being able to block Nav channel activity CBG had no effect on seizure severity. No antagonist experiments were conducted in these studies, but Valdeolivas et al., (2015) did show that CBG dose-dependently activated PPARγ in cultured striatal cells (WT and mutant huntingtin; supplementary data). Four studies reported that the CBG derivatives VCE-003 (5 $mg.kg^{-1}$i.p) and VCE-003.2 (10 $mg.kg^{-1}$oral/i.p) successfully reduced immune cell activation in macrophages, microglia and infiltrating neutrophils in models of EAE (to model MS), Huntington's and LPS induced Parkinson's disease (PD) (Carrillo-Salinas *et al.*, 2014; Díaz-

Alonso *et al.*, 2016; García *et al.*, 2018; Aguareles *et al.*, 2019). In the *in vivo* PD model, García et al., (2018) found that PPARγ antagonist T0070907 (5 $mg.kg^{-1}$) blocked VCE-003.2 mediated decreases in TNF-α, IL-1β and iNOS mRNA levels, but no other antagonists were investigated. In a follow up study by the same group, 20 $mg.kg^{-1}$ (but not 10 $mg.kg^{-1}$) oral VCE-003.2 promoted a trend towards recovery in the basal ganglia of LPS lesioned mice and was associated with decreases in IL-1β gene expression, lysosomal-associated membrane protein-1 (LAMP-1) and glial fibrillary acidic protein (GFAP) immunostaining (Burgaz et al., 2019). Orally dosed VCE-003.2 (10 $mg.kg^{-1}$) promoted neurogenesis in mice subjected to mutant Huntingtin expression in a Huntington's disease model (Aguareles *et al.*, 2019). In another model of Huntington's disease VCE-003.2 (10 $mg.kg^{-1}$ i.p) prevented neuronal loss, indicated by increases in Nissl and NeuN staining and at the same dose improved RotaRod performance and reduced astrogliosis in mice, measured by attenuated levels of GFAP and ionized calcium binding adaptor molecule 1 (Iba-1) (Díaz-Alonso *et al.*, 2016). Rodrigueuz-Cueto et al., (2018) found that VCE-003.2 10 $mg.kg^{-1}$ i.p successfully improved neuropathological deterioration and normalised CB_2 receptor and IL-1β levels, in an experimental model of ALS, but again no mechanisms of action were probed.

In vitro, Schubert et al., (2019) reported that CBG (100 nM) prevented MC65 neurons from accumulating toxic amyloid beta (Aβ) protein in an Alzhiemer's disease model. CBG also preserved neuronal trophic factors in primary rat cortical neurons (EC_{50} 1.5 μM) and prevented oxytosis in mouse HT22 hippocampal nerve cells (EC_{50} 1.9 μM). Although no mechanisms were explored in this study, neither MC65 neurons nor HT22 cells express CB_1 or CB_2, thus authors concluded that these effects were mediated independently of these receptors. In N2a cells, VCE-003.2 (10, 25 μM) prevented excitotoxicity induced by glutamate and in models of LPS induced PD and ALS (García *et al.*, 2018; Rodríguez-Cueto *et al.*, 2018). Similarly, VCE-003 (0.1-25 μM) dose dependently protected neuronal cells in a model of multiple sclerosis (MS), while VCE-003.2 (500 nM) promoted neuronal differentiation when dosed for 21 days in an *in vitro* model of Huntington's disease, but no antagonist experiments were conducted to explain these effects (Granja *et al.*, 2012; Aguareles *et al.*, 2019). In a model of neuroinflammation, pre-treatment with CBG (7.5 μM) improved viability in cells treated medium from LPS stimulated macrophages and while

authors reported that CBG treatment resulted in PPARγ downregulation, no direct mechanistic probing was conducted (Gugliandolo et al., 2018). Granja et al., (2012) and Carillos-Salinas et al., (2014) found that treatment with VCE-003 (1, 2.5 μM and 1, 10,25 μM) blocked the secretion of a number pro-inflammatory mediators including IL-6, TNF-α, IL-1β and MIP-1α in macrophages and primary microglia. VCE-003.2 also attenuated TNF-α and L-1β secretion but from BV2 mouse microglial cells (5 μM) and astroglial cells (1, 5 μM) (Díaz-Alonso *et al.*, 2016; García *et al.*, 2018; Rodríguez-Cueto *et al.*, 2018). Díaz-Alonso et al., (2016) and Concepción García et al., (2018) deduced that VCE-003.2 did not mediate its protective effects via CB_1/CB_2 due to poor binding affinity (K_i >40 μM) and both groups found that VCE-003.2 was an agonist at PPARγ (IC_{50} of 1.2 μM).

Cannabidivarin (CBDV)

All *in vivo* cannabidivarin (CBDV) studies evaluated the anti-epileptic properties of the compound in models of Rett syndrome and MES seizures (Hill *et al.*, 2012, 2013; Amada *et al.*, 2013; Vigli *et al.*, 2018; Zamberletti *et al.*, 2019). Doses in these studies ranged from 0.2 to 400 $mg.kg^{-1}$ per day in rodents with efficacy in reducing tremors was observed between 2-200 $mg.kg^{-1}$ per day. Two studies reported that 200 $mg.kg^{-1}$ i.p CBDV significantly decreased PTZ seizure severity and mortality in rats (A. J. Hill et al., 2012; T. D. M. Hill et al., 2013). Hill et al., (2012) found that 90% of animals remained seizure free at a dose of 200 $mg.kg^{-1}$ CBDV i.p per day, however lower concentrations of CBDV were ineffective (0.2 mg.kg) and CBDV had no effect on the severity of pilocarpine convulsions at any tested concentration (50-200 $mg.kg^{-1}$ day). CBDV (400 $mg.kg^{-1}$ oral gavage) suppressed PTZ seizures, significantly decreasing seizure severity ($P<0.05$) but had no effect on expression of epilepsy related genes (Amada *et al.*, 2013). Another study reported that 20 $mg.kg^{-1}$ i.p CBDV dosed for 14 days improved brain weight in Rett syndrome (RTT) mice vs WT mice, but had no effect on neurotrophin levels (Vigli *et al.*, 2018). None of the above *in vivo* studies conducted antagonist experiments to elucidate CBDVs anticonvulsant effects.

In HEK293 cells transfected with TRPV1, 2 and 3 channels, CBDV caused a concentration-dependent bidirectional current at TRPV1 similar to capsaicin and capsazepine (TRPV1 antagonist) blocked this effect. Furthermore, 5'-Iodoresiniferatoxin (5'-IRTX), a selective

TRPV1 antagonist counteracted the effect of CBDV in the duration but not amplitude of neuronal burst. These data suggest that CBDV acts as an agonist at TRPV1, but some of CBDVs effects are mediated independently of this receptor. However, no other antagonists were tested to establish which receptors were responsible for CBDVs other effects (Iannotti *et al.*, 2014). Hill et al., (2012) reported that CBDV (10 and 100 μM) decreased the amplitude and duration of local field potentials (LFPS) in hippocampal brain slices, with an anti-epileptiform effect observed in the CA1 region (100 μM). CBDV also showed efficacy in an *in vitro* model of Alzheimer's disease (AD), preventing oxytosis and energy loss in HT22 cells (EC_{50} 1.1 μM and 90 nM respectively), as well as reducing Aβ toxicity (EC_{50} 100 nM) and trophic withdrawal (EC_{50} 350 nM), however no mechanistic data were reported to determine how these effects were mediated (Schubert et al., 2019).

Cannabichromene (CBC)

In a model of electroshock seizure, CBC (10-75 $mg.kg^{-1}$ i.p per day) significantly depressed motor activity during the first 10-minute interval, but subsequently only the highest dose was effective (Davis & Hatoum, 1983). *In vitro* Shinjyo & Di Marzo, (2013) found that 1 μM CBC increased viability of adult nestin positive neuronal stem cells when applied in medium without growth factors (B27 medium), by inducing extracellular signal-regulated kinase (ERK) phosphorylation. No antagonist data was presented in these studies.

Cannabinol (CBN)

Only one *in vivo* study assessed CBN (5 $mg.kg^{-1}$ per day) in an SOD1 model of Amyotrophic lateral sclerosis (ALS). CBN delayed motor abnormalities at day 17 in the chronic treatment regimen, vs vehicle control, but disease progression was not affected (Weydt *et al.*, 2005). In a model of Huntington's disease, Aiken, Tobin, & Schweitzer, (2004) found that CBN reduced lactate dehydrogenase (LDH) activity in PC12 cells (20 and 100 μM), but authors did not probe the mechanism of this effect. CBN displayed potent antioxidant activity in primary cerebral granule cells under oxidative stress conditions, however no antagonist data was presented on this cannabinoid (Marsicano et al., 2002).

Δ^9-THCV

Male Sprague Dawley rats and CB_2 knockout mice were dosed with 2 $mg.kg^{-1}$ per day Δ^9-THCV over a period of 14 days in a model of PD, induced by 6-hydroxydopamine (6-OHDA)

or lipopolysaccharide (LPS) (García et al., 2011). Δ^9-THCV reduced slow motor movements induced by 6-OHDA and enhanced mean velocity of movement with a potency similar to rimonabant. Chronic Δ^9-THCV dosing reduced microglial activation and preserved nigrostriatal dopaminergic neurons after 6-OHDA application and in the LPS model of PD, Δ^9-THCV preserved tyrosine hydroxylase positive neurons, mirroring the effects of CB_2 agonist HU-308. Thus, authors speculated that Δ^9-THCV mediated at least some of its effects in the LPS model via CB_2(García et al., 2011). Authors also reported that 2 $mg.kg^{-1}$ Δ^9-THCV blocked the effects of the CB_1 agonist, CP55,940, suggesting it acts as an antagonist at this receptor, however no data was presented assessing if Δ^9-THCV's antagonistic properties at CB_1 mediated its protective effects in the 6-OHDA or LPS models of PD. Hill et al., (2010) studied Δ^9-THCV in a seizure model induced by 80 $mg.kg^{-1}$ PTZ and found that at a dose of 0.25 $mg.kg^{-1}$ i.p Δ^9-THCV, with 33% of animals having a complete absence of seizures. Although no direct mechanistic probing was investigated, receptor binding assays were performed on rat cortical membranes and Δ^9-THCV was found to act as a CB_1 ligand (CB_1 Ki∼290 nM; [^{3}H]SR141716A displacement but no agonist stimulation using [^{35}S] GTPγS binding) (Hill *et al.*, 2010).

Δ^{9}-THCA

In an acute 3-NPA model of Huntington's disease, Nadal et al., (2017) observed a significant improvement in hindlimb dystonia (uncontrollable hindlimb muscle contraction) and locomotor activity in male, C57BL/6 mice treated with Δ^9-THCA (20 $mg.kg^{-1}$ per day i.p). Δ^9-THCA also prevented astrogliosis, microgliosis and attenuated the upregulation of pro-inflammatory mediators induced by 3-NPA, these effects were blocked when mice were co-administered with PPARγ antagonist T0070903 (with the exception of IL-6) (Nadal et al., 2017*)*. *In vitro*, N2a cells infected with the huntingtin polyQ repeats resulted in toxicity, which was significantly reduced by treatment with Δ^9-THCA, as well as decreased expression of inflammatory mediators: TNF-alpha, iNOS, IL-6, COX-2. Δ^9-THCA also improved neuronal viability post serum deprivation and this effect was prevented by GW9662, a PPARγ antagonist. No other antagonists were used in this study (Nadal et al., 2017*).*

Δ^9-THCA (0.01-10 μM) displayed no pro-survival effect on dopaminergic neurons but had a significant, positive effect on cell count (123%) when compared to the control in an *in vitro* model of PD (Moldzio et al., 2012).

Discussion

To our knowledge, this is the first systematic review on the neuroprotective effects of lesser-known minor phytocannabinoids in various models of neurological disease. Data obtained from our search revealed that CBG, VCE.003, VCE.003.2 and CBDV were the most promising candidates as neuroprotectants, while Δ^9-THCV, Δ^9-THCA, CBC and CBN have limited but encouraging data as neuroprotectants. CBG, VCE.003, VCE.003.2 and Δ^9-THCA mediated their neuroprotective effects at least in part by the nuclear receptor PPARγ. CBDV was found to mediate some of its antiepileptic effects via TRPV1 and Δ^9-THCV was found to be a CB_1 ligand and a possible CB_2 agonist, but no experiments were conducted to establish whether its neuroprotective action was mediated by CB_1 or CB_2. No other receptors were investigated, and no studies assessed the neuroprotective potential of CBDA, CBGA, CBGV, CBCV, CBGVA, or CBDVA.

CBG was first isolated in 1964 by the same group that reported the structure of Δ^9-THC (Gaoni and Mechoulam, 1964), it possesses antioxidant and anti-inflammatory properties, whilst displaying no psychotropic effects as it is a poor CB_1 agonist (Gauson *et al.*, 2007; Rosenthaler *et al.*, 2014; Navarro *et al.*, 2018). CBG is a partial agonist towards CB_2, a potent α2-adrenoceptor (A2A) agonist (EC_{50} 0.2 nM) and a moderate 5-HT_{1A} receptor antagonist, as well as interacting with various TRP isoforms including TRPV1 and 2 (Cascio et al., 2010; De Petrocellis et al., 2012). Studies included here show that these compounds have significant anti-inflammatory effects, including attenuating cytokine release and decreasing the activation of immune cells, an effect observed in both *in vitro* and *in vivo* models.

CBG and its derivatives were particularly effective in models of Huntington's disease, targeting multiple facets of the disease including gene expression, easing motor symptoms, reducing microglial activation and attenuating the inflammatory response. Huntington's disease pathophysiology, like other neurodegenerative disorders, exhibits uncontrolled

microglial activation which is a key part of the neuroinflammatory response. In early stages of HD, positron emission tomography (PET) imaging has revealed marked microglial activation, which was correlated with impairments of neuronal activity (Tai *et al.*, 2007). Microglial activation along with increases in pro-inflammatory mediators have also been detected in post-mortem HD brains (Palpagama *et al.*, 2019). Interestingly, microglial mediated neuroinflammation was suppressed with the activation of CB_2 receptors (Ehrhart *et al.*, 2005). However, given VCE-003 and VCE.003.2's protective effects were likely to be CB_1 and CB_2 independent, their effects on microglial activation are likely to be via a different mechanism, possibly through the activation of PPARy which has an important role in regulating the inflammatory response, especially in the CNS (reviewed in Bright et al., 2008 and Villapol., 2018). It is also worth noting that microglial activation can be protective, preserving neurons by secreting anti-inflammatory cytokines such as IL-4 and IL-10 as well as various trophic factors (Reviewed in Le, Wu and Tang, 2016 and Pöyhönen *et al.*, 2019). In line with these observations, there effectively needs to be a balancing act between enabling some degree of microglial activation to protect neurons, whilst limiting their overactivation which would ultimately lead to damage. Given that HD symptoms are currently managed using VMAT inhibitors (TBZ) to decrease levels of monoamines, it would be useful to assess whether CBG and its derivates have any efficacy as VMAT inhibitors, or whether their protective effects in models of Huntington's disease are independent of this mechanism. If the latter is the case, future studies should investigate low dose VMATs (to minimise neuropsychiatric side effects) together with CBG or its derivatives as an adjuvant therapy to assess if there is a cumulative protective effect of these compounds.

Long-term dose tolerability and a lack of accumulation in tissue are both essential features of neuroprotective agents as these drugs are typically taken for life after disease onset. In a study conducted by Deiana *et al.*, (2012), CBG was found to have similar PK profiles in rats and mice but exhibited slower brain penetration in mice. Both animals also had higher concentrations of CBG following i.p injection compared to oral administration, but interestingly in rats this did not equate to higher concentrations in brain tissue (Deiana *et al.*, 2012). In our review, CBG, VCE-003 and VCE.003.2's dosing was well tolerated and ranged from just 3 days to 10 weeks with two studies dosing CBG orally vs 7 studies dosing intraperitoneally. Whilst Diena et al., (2012) reported that animals tolerated CBG

administration better via i.p rather than orally, in humans, i.p dosing is not a viable means of regular administration and all drugs given orally have a larger side effect profile. Moreover, patients receiving certain oral therapies for neurological conditions, such as levodopa for Parkinson's, must also take medications to minimise peripheral effects (Fahn, 2008). Therefore, dose formulation and route of administration for these compounds should be carefully assessed based on thorough ADME profiling and feasibility of long-term dosing.

CBG exhibited positive effects in two Huntington's disease models, despite one study administering the compound orally and the other by i.p. Of note, CBD has already been trialled in Huntington's disease patients, CBD (10 $mg.kg^{-}$; 700 mg average daily dose) was dosed for 6 weeks, which resulted in a consistent plasma level of 5.9-11 ng.mL and once treatment had stopped, elimination was between 2 and 5 days, suggesting CBD does not accumulate and remain in plasma longer than 5 days in Huntington's disease patients (Consroe *et al.*, 1991). Future studies should elucidate whether CBG and its derivatives display efficacy in humans and clarify whether their activation of PPARy corresponds to their neuroprotective properties and if other receptors are involved. More data is also needed on CBGs PK profile in older mice and larger mammals and to establish whether it exhibits a similar elimination to CBD in humans. These factors would aid in the translation of this compound as a treatment for neurodegenerative conditions.

Cannabidivarin (CBDV) is a structural analogue to CBD, with the molecule shortened by two methylene bridges (Vollner, L., Bieniek, D., and Korte, 1969; Morales, Hurst and Reggio, 2017). From our search, *in vivo* studies consistently reported 200 $mg.kg^{-1}$ i.p CBDV having anti-epileptic effects and a 400 $mg.kg^{-1}$ oral dose also showing promise. Like CBD, CBDV is a agonist at TRPV1/2 and TRPA1, and an antagonist at TRPM8, which may explain similarities in their neuroprotective properties, particularly CBDV's action as an agonist at TRPV1 (Scutt and Williamson, 2007; De Petrocellis *et al.*, 2011; Iannotti *et al.*, 2014). In our review, studies showed that CBDV did not affect neurotrophic levels or epilepsy related gene expression, thus it can be assumed that CBDV mediates its protective effects independent of these (Amada *et al.*, 2013; Vigli *et al.*, 2018). Deiana *et al.*, (2012) reported that CBDV was rapidly absorbed in mice and rats but there was a higher drug concentration in plasma and brain following oral treatment in rats compared to mice. Furthermore, whilst i.p injection resulted

in similar PK profiles in the two species, brain concentrations in rats were higher. This brings into question the differences in the amount of CBDV delivered to the brain in the studies conducted in mice vs rats presented in this review and whether this influenced study outcomes. Only two studies reported chronic CBDV dosing both in models of Rett syndrome, highlighting the need for future studies to assess the long-term tolerability of CBDV as an anti-epileptic agent and how different species exhibit different bioavailability of this compound, as these will both affect CBDVs translatability to humans.

Although out of the scope of this review, it is worth noting that CBDV has already been trialled as an anti-convulsant by GW Pharmaceuticals in a phase 2a, placebo-controlled study of 162 adult patients (clinical trial number: NCT02369471/ NCT02365610). The drug GWP42006 (which features CBDV as its main ingredient) was dose titrated (over two weeks) up to an 800 mg twice daily dose for a 6 week stable treatment period, however focal seizures were inadequately controlled with this dose and GWP42006 displayed no difference in efficacy to the placebo control group (Schultz, 2018). Whilst this may cast doubt on the translatability of the evidence presented in this review, it is worth highlighting that the maximum dose in humans from the GW study would be considerably less than if the same dose regimens as the *in vivo* studies were followed for a 60 kg human. Furthermore, Morano *et al.*, (2020) speculated that CBDVs inability to control seizures was in part due to an extremely high response from the placebo group and that the use of purified CBDV may have also influenced the study outcome. Therefore, it is important to exercise caution when extrapolating the findings from the *in vitro* and *vivo* data presented here and what doses may constitute as effective in clinical trials.

Cannabichromene (CBC) was first isolated in 1966 by Gaoni and Mechoulam and is a non-psychotropic cannabinoid that does not interact with CB_1 (Gaoni and Mechoulam, 1966). CBC is an agonist at CB_2 and TRP channels, acting potently at TRPA1 as well as displaying some activity at TRPV3 and TRPV4 (De Petrocellis *et al.*, 2008, 2011; de Petrocellis *et al.*, 2012; Cascio and Pertwee, 2015; Udoh *et al.*, 2019). CBC (0.001–1 μM) exhibited promising anti-inflammatory effects in an *in vitro* model of colitis, decreasing LPS increased nitrite levels and attenuating INF-γ and IL-10 secretion in peritoneal macrophages (Romano *et al.*, 2013). More recently CBC acted as a CB_2 agonist in AtT20 cells transfected with CB_2 and was

confirmed by application of CB_2 antagonist AM630, which blocked CBCs effects (Udoh et al.,2019). We found only two papers related to neuroprotective effects of CBC; *in vivo* CBC suppressed motor activity while *in vitro* CBC improved viability of neural stem cells (Davis and Hatoum, 1983; Shinjyo and Di Marzo, 2013). CBCs anti-inflammatory effects may translate to this compound acting as a neuroprotectant as inflammation and overactivation of the immune response is an important feature in neurodegenerative conditions. Thus, future research should assess this compound in neuro-inflammatory conditions where it may have potential.

Cannabinol (CBN) is an oxidative product of Δ^9-THC and was the first cannabinoid to be discovered and isolated (Wood, Spivey & Easterfield., 1899). Like Δ^9-THC it has been shown to activate CB_1 (K_i 211.2 nM) but with lower potency, as well as acting as an agonist at TRPV2 (Rhee *et al.*, 1997; Russo and Marcu, 2017). CBN (1 mg/mL) was recently shown to reduce mechanical sensitization and sensitivity of afferent muscle fibers in an *in vivo* model of myofascial pain, but no mechanism of action was investigated (Wong and Cairns, 2019). From our search, limited data showed that CBN decreased cell damage and acted as a potent antioxidant in a cell-based Huntington's disease model (Aiken et al., 2004). CBNs activity as an antioxidant is a characteristic feature of cannabinoids, which as previously mentioned, is thought to be due to the presence of phenolic ring and carboxyl moieties, as well as the ability to increase antioxidant defences. CBD has already shown extensive antioxidant properties, including increasing the levels and activity of antioxidants, capturing reactive oxygen species (ROS) and transforming them into less active forms, as well as activating nuclear erythroid 2-related factor (NrF2) which governs the transcription of many antioxidant genes (Reviewed in Atalay, Jarocka-karpowicz and Skrzydlewskas, 2020). Oxidative stress is a key feature of neurodegenerative disorders including PD and AD. In Alzheimer's disease, Aβ deposits contain a significant number of binding sites for biometals (zinc, copper, iron) which have been shown to contribute to oxidative stress in AD patients (Kozlowski *et al.*, 2009; Huang, Zhang and Chen, 2016). Furthermore, AD patients have decreased levels of antioxidant enzymes and increased products of oxidative stress, such as peroxidised lipids and oxidised proteins in brain tissue (Kim *et al.*, 2006; Sultana *et al.*, 2011). Also, large amounts of ROS are generated by reactive microglial cells, with studies showing superoxide produced by microglia directly contributing to the death of

dopaminergic neurons in PD (Hernandes, Café-Mendes and Britto, 2013). It is clear that more information is needed on the pharmacology of CBN, especially its antioxidant potential. Moreover, the ability of CBDV, CBG, CBC and CBN to reduce Aβ deposits *in vitro* is also noteworthy and will be of interest to examine the antioxidant and anti-inflammatory potential of these compounds in *in vivo* AD models and whether these compounds act through similar mechanisms to CBD.

Δ^9-THCV is a homologue of Δ^9-THC differing by just a propyl side chain and studies have suggested that Δ^9-THCV acts as a CB_1 receptor agonist, sharing similar properties to Δ^9-THC albeit with less potency (Gill, Paton and Pertwee, 1970; Pertwee, 2008). They exhibit similarities in their *in vivo* effects such as inducing catalepsy in mice and Δ^9-THC-like effects in humans (Gill, Paton and Pertwee, 1970; Hollister, 1974). We found two studies where Δ^9-THCV showed promise as an anti-epileptic agent and protected neurons in two models of PD, while García *et al.*, (2011) suggested Δ^9-THCV mediated some of its protective effects by acting at CB_1 and CB_2, Δ^9-THCVs mechanism of action was largely unexplored (Hill *et al.*, 2010; García *et al.*, 2011). In an earlier study, Thomas *et al.*, (2005) found that Δ^9-THCV displaced [^{3}H]CP55940 from specific sites in mouse brain and CHO-hCB_2 cell membranes (K_i values 75.4 nM and 62.8 nM respectively) and along with data from GTPγS-binding experiments authors concluded Δ^9-THCV acted as a CB_1 and CB_2 receptor antagonist. Other groups have shown Δ^9-THCV can block CB_1 activity in murine cerebellar slices and at 5.8 μM increase GABA release from neurons, sharing the same properties as AM251, a CB_1 receptor antagonist (Ma *et al.*, 2008; Pertwee, 2008). Thus, whilst there is evidence to suggest Δ^9-THCV mediates some of its protective effects via CB_1 and CB_2, the data remains largely unclear, and there is also a lack of investigation into the potential of Δ^9-THCV to act at other known cannabinoid targets.

Microglial activation and the presence of neuroinflammatory factors are well known characteristics of Parkinson's and well documented amongst PD sufferers (Mogi *et al.*, 1994; Qian *et al.*, 2011). Moreover, studies have demonstrated that microglial over-activation leads to deleterious effects and the exacerbation of the immune response, especially the release of pro-inflammatory mediators. Like CBG derivative VC-003.2, Δ^9-THCV reduced microglial activation, inducing a protective effect by dampening the immune response.

Studies have already demonstrated CBDs ability to modulate the immune response by acting an agonist of PPARγ and altering nuclear factor-κB (NFκB) signalling, which is upregulated in both microglia and astrocytes of PD patients. Furthermore, activation of PPARγ leads to inhibition of NFκB signalling and decreases mRNA levels of proinflammatory mediators TNF-a, IL-1β, IL-6 and iNOS (Vallée *et al.*, 2017). Therefore, it would be of interest to determine whether Δ^9-THCVs ability to reduce microglial activation is carried out by the same mechanism as CBD, involving the activation of PPARγ.

Limited pharmacokinetic data on Δ^9-THCV has shown it exhibits rapid absorption in rats and mice when administered either by i.p or orally, but is rapidly eliminated when orally administered (<1.5hrs) compared to i.p where its elimination rate is >5hrs (Deiana *et al.*, 2012). Interestingly, Δ^9-THCV exhibited extensive brain penetration (exceeding plasma levels), regardless of the route of administration, meaning it can effectively cross the BBB. At 24hrs Δ^9-THCV was no longer detected, suggesting that it exhibits a lack of accumulation in brain tissue (Deiana *et al.*, 2012). Altogether these features, along with evidence collected in this study, support Δ^9-THCV as a neuroprotective agent, however clearly more data is needed, especially to assess Δ^9-THCVs safety in chronic dosing and whether this compound exhibits tolerance with long term use.

Δ^9-THCA is Δ^9-THCs acidic precursor and competition binding assays revealed that this compound was unable to achieve displacement of [^{3}H]-CP55,940 (CB_1 and CB_2 agonist) up to 10 µM, suggesting Δ^9-THCA exhibits poor affinity for CB_1 or CB_2 (McPartland *et al.*, 2017). Results from this study also showed that Δ^9-THCA has little efficacy at these receptors as it exhibited no inhibition of forskolin mediated cAMP, compared to Δ^9-THC which acted as an agonist in this assay. Our search revealed that Δ^9-THCA had anti-inflammatory effects improved neural viability in a model of Huntington's, but interestingly it did not affect the survival of dopaminergic neurons in a model of Parkinson's disease (Moldzio *et al.*, 2012; Nadal *et al.*, 2017). In a recent study, Anderson *et al.*, (2019) reported that Δ^9-THCA had extremely poor brain penetration (an optimistic brain−plasma ratio of 0.15) in both vehicles tested. Furthermore, studies have shown that Δ^9-THCA has poor stability and rapidly decarboxylates to Δ^9-THC, bringing into question whether the ability of Δ^9-THCA to act as a

neuroprotectant in the studies presented here is merely due to nearly unavoidable contamination with Δ^9-THC (McPartland *et al.*, 2017; Anderson *et al.*, 2019). Overall, these data warrant further investigation into Δ^9-THCA as a potential neuroprotective and anti-inflammatory agent, however caution should be advised, and future studies should include purity data on Δ^9-THCA to enhance robustness of experimental data.

There were no studies identified in this review that looked at the potential neuroprotective effects of other cannabinoid varins or their acidic forms such as CBGV, CBGVA, CBDVA, CBCV and CBCVA. This may be due to the lack of commercial availability of these compounds due to their low concentrations in the plant, costly synthetic production or that these compounds are not very stable. CBDA was only used in one study on Huntington's disease, where it had no protective effects. This compound, however, has shown promise in other conditions including breast cancer migration, inflammatory pain and nausea (Takeda *et al.*, 2012; Bolognini *et al.*, 2013; Rock, Limebeer and Parker, 2018), with groups suggesting that CBDA is 1000 times more potent at the 5-HT_{1A} receptor than CBD (Bolognini *et al.*, 2013). Activation of the 5-HT_{1A} receptor has been shown to be protective both *in vitro* in Parkinsonian models and *in vivo* in models of hypoxia ischaemia (Miyazaki *et al.*, 2013; Pazos *et al.*, 2013). Although Anderson *et al.*, (2019) concluded that CBDA displayed poor brain penetration in an oil-based formulation uptake was increased when CBDA was formulated in a tween-based vehicle. Authors also found CBDA was anti-convulsant at 10 and 30 $mg.kg^{-1}$ displaying greater potency compared to CBD (100 $mg.kg^{-1}$). These data support CBDA's efficacy in the brain, as well as highlighting its potential as an anticonvulsant. (Anderson *et al.*, 2019). Considering these points, CBDA may be also protective in conditions such as ischaemic stroke and Parkinson's disease and warrants further investigation. Recent studies have also shown that CBDA, CBGV and CBGA interact with various TRP channel isoforms including TRPV1, TRPV2, TRPA1 and TRPM8. Of note, CBGV and CBGA were also potent desensitizers of TRPV3 and TRPV4 respectively (De Petrocellis et al., 2012). Whilst the extent of the role of TRP channels in neuroprotection has yet to be fully understood, studies have shown that these receptors are involved in a wide range of neurological disorders. For example, TRPA1 deficient mice were more likely to sustain damage post ischaemia and TRPA1 activation in AD may have a crucial role in regulating astrocyte-mediated inflammation (Lee *et al.*, 2016; Pires and Earley, 2018). Conversely, TRPV1 activity has been

implicated in epilepsy having a role in neuronal excitability and synaptic transmission (Nazıroglu, 2015). Therefore, CBDA, CBGV and CBGA interactions at TRP channels may be beneficial in conditions that implicate these channels in their pathophysiology.

Translatability of these data and viability of minor phytocannabinoids as neuroprotectants will also rely on understanding and perhaps manipulating their bioavailability and pharmacokinetic properties. In a recent systematic review conducted by our group, Millar *et al.*, (2018) highlighted discrepancies regarding CBD bioavailability, Cmax, Tmax and half-life ($t_{1/2}$) in humans depending on the route of administration, formulation and whether CBD was dosed in a fed or fasted state. That being said, studies conducted in piglets (Garberg *et al.*, 2017) and rodents (Long *et al.*, 2012; Hammell *et al.*, 2016) have shown a dose-dependent relationship between CBD administration and brain and plasma concentrations. Limited data extracted by Millar et al., (2018), showed that CBD administration in humans also led to dose-dependent increases in plasma concentrations, suggesting the same may apply to brain concentrations in man.

Information on the human metabolites of CBD, Δ^9-THC and other phytocannabinoids is scarce, with the majority of research has focusing on CBDs extensive first pass metabolism and the identification of its urinary metabolites. Of interest, a patent filed by Mechoulam, Tchilibon and Fride, (2010) described that CBDs two major metabolites, 7-hydroxy (7-OH) CBD and 7-carboxy (7-COOH), are both anti-inflammatory and dose dependently inhibit TNF-α, nitric oxide and ROS. However, this data has yet to be reported in academic studies or found to be true of other phytocannabinoids. In addition, the cytochrome P450 (CYP) superfamily is responsible for metabolising 60-80% of CNS drugs; 23% by CYP3A4 and 38% CYP2C19, both of which CBD is a known substrate (Cacabelos, 2010; Iffland and Grotenhermen, 2017). Altogether these findings highlight that there are major gaps in the ADME of phytocannabinoids, as well as a lack of identification of metabolites and whether they have biological effects. In phase 2 trials, the minor phytocannabinoids presented in this review will in all likelihood be used alongside current therapies to see if they can augment survival of neurons and/or symptom burden, rather than being used as a single agent. In light of the above, it will be essential to consider the interactions that these compounds may have when administered in conjunction with conventional drug therapies (where they

exist) and to establish potential synergistic or deleterious effects. Looking forward, initial ADME data will be incredibly important to ascertain whether these compounds have true clinical potential and essential for their subsequent formulating and administration.

Conclusions

This review aimed to collate and summarise all current data on the neuroprotective potential of phytocannabinoids other than Δ^9-THC and CBD. Despite the lack of studies available in this area, we found that all phytocannabinoids tested displayed neuroprotective properties in a range of disorders. CBG and its derivatives displayed significant anti-inflammatory effects and were particularly effective in Huntington's disease models. CBDV, Δ^9-THCV and CBC were effective as anti-seizure agents, while CBN displayed antioxidant activity and Δ^9-THCA had anti-inflammatory effects. CBG and Δ^9-THCA, like CBD, mediate their anti-inflammatory effects through PPARγ. Many of the studies were screening studies that conducted no mechanistic probing, suggesting that research into these compounds is still in its early stages. Extensive pharmacokinetic and pharmacodynamic data in larger mammals is also necessary on these compounds, given that all *in vivo* studies in this review were conducted in mice and rats. This would provide more evidence for the facilitation of these compounds as therapies in man. Future studies are required to investigate the full neuroprotective potential of these compounds particularly the mechanisms in which they mediate their protective effects, as well as exploring whether their combinations may enhance their capabilities as neuroprotectants. Whilst we have focused on a select number of minor phytocannabinoids, based predominantly on their shared physical and biological similarities to CBD, there are over 100 phytocannabinoids and terpenes present in the cannabis plant that could potentially display neuroprotective potential.

Nomenclature of Targets and Ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding et al., 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20 (Alexander et al., 2019).

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NS and SOS wrote the paper. NS and AM conducted the searches. All authors contributed to editing the paper and approving the final version.

Conflicts of Interest

Saoirse O'Sullivan is the Chief owner of CanPharmaConsulting. She is on the advisory board for Artelo Biosciences, as well as acting as the science lead for the Centre for Medicinal Cannabis (CMC). Other authors declare that they have no conflict of interest in relation to this review.

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Table 1a: Summary of included *in vitro* studies.

Phytocannabinoid	Compound	Concentration/ Incubation period	Neuro Model	Cells used	n number	Results-primary endpoints	Study
Cannabigerol (CBG)	Cannabigerol derivative VCE-003.2	500 nM for 21 days.	Huntington's disease	Mouse embryonic stem cells (R1 line)/P19 neurospheres.	*n*=3	VCE-003.2 increased CTIP-2 positive cells, promoted neuronal like-differentiation and significantly larger P19 neurospheres vs vehicle treated cells (P<0.01).	Aguareles J, *et al*., 2019
	Cannabigerol derivative VCE-003	1,5,10 µM (Human T-cells). 1, 2.5 µM (RAW 264.7 cells) for 3 days post stimulation.	Autoimmune Encephalomyelitis to model multiple sclerosis (MS).	Jurkat, BV2 RAW 264.7 cells.Human peripheral T-cells.	[a]*n*=3	1 µM reduced expression of iNOS in BV2 microglial cells. Antagonists AM630 (CB_2) and GW9662 (PPARγ) blocked these effects. Prevented T cell division at 1 µM and 5 µM and inhibition of the release of all soluble mediators (T-cells).	Carrillo Salinas *et al*., 2014
	Cannabigerol derivatives; VCE-003 and VCE-003.2.	1- 50 µM (N2a) for 24hrs 50 nM-50 µM (HiB5) 30, 10, 3 µM for 6hrs.	Huntington's Disease	N2a cells/HiB5 cells) Immortalised striatal neuroblasts expressing huntingtin/ mutant repeats.	[a]*n*=3	VCE-003.2 improved cell viability (10 and 25 µM) and prevented excitotoxicity in N2a cells. VCE-003.2. reduced the number of cells with aggregates (neuroblasts) and improved neuronal viability post serum deprivation.	Diaz-Alonso *et al*., 2016
	VCE-003 Cannabigerol Quinone derivative	0.1, 1, 10, 25 µM CBG/VCE-003 (HTT cells-24hrs) (Microglia-18hrs) (hippocampal cells; mice treated 15 days 5 mg.kg i.p VCE-003[b])	Multiple Sclerosis	HEK293 cells and primary microglial cells. HT22 Mouse hippocampal cells.	[a]*n*=3	VCE-003 protected neuronal cells from excitotoxity. Reduction in IL-1beta, IL-6, TNF-alpha, PGE_2 and MIP-1-alpha in microglia (1, 10 and 25 µM) VCE-003 ameliorated MS symptoms induced by TMEV.	Granja *et al*., 2012
	VCE-003.2 Cannabigerol derivative	BV2 cells 5 µM VCE-003.2 for 21hrs. VCE-003.2 (M-213 cells) Vehicle (0.1% DMSO) vs 0.1,0.5, 1 µM for 40hrs.	Parkinson's disease model induced by LPS. (Conditioned medium from BV2 cells added to M-213 cells).	Mouse microglial BV2 cells. M-213 (striatal cell line) neuronal cells.	BV2 cells: *n*=14, 7 repeats.	In BV2 cells, VCE-003.2 significantly decreased TNF-alpha COX-2 and iNOS mRNA. Attenuated TNF-alpha and IL-1beta secreted in medium of BV2 cells (5 µM).	Garcia. C *et al.,* 2018
	Cannabigerol	MTT assay: 1,2.5,5,7.5,10,12.5,15,20 µM pre-treated 24hrs. NSC-34:Pre-treated with 7.5 µM	Neuroinflammation-medium from LPS stimulated macrophages.	NSC-34 motor neurons.	*n*=3 repeats	CBG at 2.5 and 7.5 µM increased cell viability approximately 20% compared to control. CBG pre-treatment inhibited apoptosis and reduced; IL-1β, TNF-alpha, INF-Y (NSC-34 motor neurons). CBG restored decreased Nrf2 levels.	Gugliandolo A, *et al*., 2018
	Cannabidiol* and Cannabigerol	Electropsyiology: 1/10 µM 20 minutes. hNAv cells- 1 nM-200 µM for 100 seconds.	PTZ Seizures	Transverse hippocampal slices, SH-SY5Y, hNAv cell lines.	SH-SY5Y-*n*=6 Mouse cortical neurons *n*=8 hNAv *n*=3 drug/	10 µM CBG significantly reduced peak Nav current in SH-SY5Y cells and mouse cortical neurons. CBG was also effective as a low affinity Nav channel blocker.	Hill, A *et al*., 2014

					concentration		
	Cannabigerol derivative VCE-003.2	0.1,0.5,1, and 5 μM added 1hr prior to LPS, for 24hrs.	Amyotrophic lateral sclerosis (ALS)	Astroglial cells (mutant SOD1 mice).	*n*=4, 6 samples per group	VCE.003.2 at 1 and 5 μM attenuated levels of TNF-alpha and IL-1β, elevated due to LPS stimulation.	Rodrígueuz-Cueto C *et al.*, 2018
	Cannabigerol	6hrs-supplementary information cannot be accessed.	Huntington's disease	Immortalized striatal progenitor cells: $STHdh^{Q7/Q7}$ And $STHdh^{Q111/Q111}$ cells	*n*=3 repeats	CBG dose-dependently activated PPARγ.	Valdeoliva, S *et al.*, 2015
	Cannabigerol	1 μM 24hrs ATP assay/viability and differentiation for 2days.	Neuroprotection	Adult neural stem cells/progenitor cells (NSPC).	*n*=6	CBG had no significant effect on any of the endpoints measured.	Shinjyo and Di Marzo 2013
Cannabidivarin (CBDV)	Cannabidivarin	1, 10, 100 μM 30 mins after epileptiform activity for 30 mins.	Epilepsy-spontaneous local field potentials (LFPs)	Transverse hippocampal slices male/ female Kyoto rats.	*n*>5 slices from *n*>5 animals	CBDV decreased amplitude and duration of LFPs and increased Mg2+ free induced LFPs frequency (>10 μM).	Hill *et al.*, 2012
	Cannabidivarin (+CBD)	3, 10, 30 μM 30-40 mins after control readings for 1 min.	Epilepsy	Human embryonic kidney cells (HEK293) transfected with TRPV1, TRPV2, TRPA1.	*n*=4	CBDV was anticonvulsant and TRPV1 antagonist capsazepine blocked this effect. 10 μM CBDV tended to increase phosphorylation at the S800 site of TRPV1.	Iannotti *et al.*, 2014
Cannabichromene (CBC)	Cannabichromene	1 μM 24hrs ATP assay/viability and differentiation for 2days.	Neuroprotection	Adult Neural stem cells/progenitor cells (NSPC)	*n*=6	CBC raised viability in B27 medium. CBC had no significant effect on proliferation. In B27 medium CBC upregulated nestin, but reduced GFAP.	Shinjyo and Di Marzo 2013
Cannabinol (CBN)	Cannabinol/ delta 8 THC	100, 20, 4, 0.8, 0.16, or 0 μM for 48hrs.	Huntington's Disease	PC12 cells expressing polynucleotide repeats (103 glutamines).	*n*= 2 repeats, average 3-4 wells	Cannabinol reduced LDH activity in medium at 20 and 100 μM. At 100 μM CBN decreased LDH release by 84%. Protective EC_{50} of CBN was determined to be 30 μM in this model.	Aiken, C *et al.*, 2004
	Cannabinol (+THC and CBD)	0.1, 1, 2.5,5 10 μM for 24hrs.	Oxidative stress and neuroprotection	Primary cerebral granule cells (rats/mice), CB1 expressing cell lines. PC12 and HT22 cell lines.	*n*=3	Cannabinol was shown to be a potent antioxidant.	Marsicano G *et al.*, 2002
Tetrahydrocannabidivarin(Δ^9-THCV)	Δ^9-THCV	0,5 10, 20, 40, 50 μM applied directly after epileptiform activity. 20 min pre-treatment at 10 μM.	*In vitro* electrophysiology (epileptiform bursting)	Brain slices obtained from male and female outbred rats.	*n*=5	Δ^9-THCV (20-50 μM) decreased burst incidence, PDS amplitude and frequency. The most significant effect was at 50 μM. Δ^9-THCV also decreased epileptiform burst speed (40 μM). Δ^9-THCV was found to act as a CB_1 ligand in receptor binding assays.	Hill *et al.*, 2010

Tetrahydrocannabidiolc acid(Δ⁹-THCA)	Δ⁹-THCA	0.01, 0.1, 1 and 10 μM for 48hrs	Parkinson's disease	Dopaminergic neuronal cell culture.	*n*=3-4 four wells/treatment	Δ⁹-THCA had no effect on the survival of dopaminergic neurons, but at 10 μM led to an increased cell count (123%) and morphology was ameliorated vs control cultures.	Moldzio *et al*., 2012
Mixed	Δ⁹Tetrahydrocannabinolic acid (Δ⁹-THCA) and cannabidiolic acid (CBDA), cannabigerol (CBG.	0, 0.5 and 1 μM (Δ⁹-THCA) N2a cells-48hrs.0, 0.1-15 μM (Δ⁹-THCA, CBDA, CBGA in HEK-293T cells-6hrs. 1-10 μM Δ⁹THCA STHdh$^{Q7/Q7}$ cells 1hr./CB	Huntington's disease/ neurodegeneration	HEK-293T Neuro-2a STHdh$^{Q7/Q7}$ And STHdh $^{Q111/Q111}$ cells	*n*=5 repeats	Δ⁹-THCA increased neuronal cell viability post serum deprivation and increased mitochondrial mass. This effect was blocked by a PPARγ antagonist GW9662. All cannabinoid acids induced PPARγ transcriptional activity in HEK293 cells.	Nadal *et al*., 2017
	Cannabichromene, cannabidiol,cannabidivarin, cannabigerol, cannabinol, Δ⁹ tetrahydrocannabinol,Δ⁹Tetrahydrocannabinolic acid.	0, 0.1, 1, 10 μM for 48hrs.	Neuroprotection	N18TG2 cells (neuroblastoma cell line)	In triplicate with 2-5 repeats.	Emerging phytocannabinoids did not affect the number of dopaminergic neurons. CBG and CBC decreased glutathione levels (0.1, 1 μM and 1 and 10 μM). 0.1 μM CBDV reduced glutathione levels by 9.6%, THC, THCA and CBN has no effect. CBDV and CBN decreased resazurin reduction at 10 μM (32.9 and 38.9%) and affected PI uptake at all concentrations. CBG also affected PI uptake at 0.1 and 10 μM.	Rosenthaler *et al*., 2014
	Cannabigerol, Cannabichromene, Cannabidivarin, cannabinol (as well as THC, CBD and CBD derivative DMCBD*)	250 nM-10 μM Oxytosis assay, 30 minutes. Energy loss assay: 22hrs. Trophic factor withdrawal, 48hrs.	Alzheimer's Disease	MC65 cells (human nerve cell line), Ht22 cells (mouse hippocampal cell line) and BV2 microglial cell line.	n=6 (twice in triplicate)	CBG, CBDV, CBC, CBN, THCA prevented oxytosis. CBG, CBDV, CBC and CBN preserved trophic factors. THCA was toxic to MC65 cells at 1 μM, however CBDV, CBC, CBN and CBDA prevented amyloid toxicity at ≤100 nM.CBDV, CBG, CBC and CBN (100 nM) prevented MC65 neurons from accumulating amyloid beta (Aβ).	Schubert *et al*., 2019

Table 1b: Summary of included *in vivo* studies.

Phytocannabinoid	Compound	Dose/route/time	Neuro Model	[a]Animals	n number	Results	Study
Cannabigerol (CBG)	Cannabigerol derivatives VCE-003 and VCE-003.2	10 mg.kg^{-1} of body weight i.p per day until sacrifice.	Two models of Huntington's Disease.	**M** CD1 mice (12 weeks)	*n*=7 each group	QA model: VCE-003.2 RotaRod performance, prevented neuronal loss, microglial activation and reduced astrogliosis. 3NP model: VCE-003.2 improved motor deficits, reduced all pro-inflammatory mediator release and prevented neuronal loss.	Alonso-Diaz *et al.*, 2016
	Cannabigerol derivative VCE-003.2	10 mg.kg^{-1} oral once daily for 3 days before sacrifice.	Huntington's Disease.	**M** C57/6N mice (10 weeks)	*n*=3-6 mice/condition	VCE-003.2 promoted neurogenesis, increased GFAP positive cells and reduced microglial activation. Mice performed better on the RotorRod test drug treated vs vehicle.	Aguareles J, *et al.*, 2019
	Cannabigerol derivative VCE-003.2	Oral 10 mg.kg^{-1}, 20 mg.kg^{-1}, 16hrs after LPS for 28 days daily.	LPS induced Parkinson's disease.	C57BL/6 **F** mice, 7-11 months old	*n*=6 mice per group	20 mg.kg^{-1} partly corrected altered cylinder rearing test but poor activity in rotarod and CAA tests. VCE -003.2 attenuated TNF-a, IL-1b (greatest effect at ^dose missing mg.kg^{-1}) and recovered tyrosine hydroxylase nigrostriatal neurons.	Burgaz S *et al.*,2019
	Cannabigerol derivative VCE-003	Daily 5 mg.kg^{-1} i.p for 21 days.	Autoimmune Encephalomyelitis (EAE) to model MS.	**F** C57BL/6 mice	*n*= 6 animals per group.	5 mg.kg^{-1} of VCE-003 decreased EAE symptoms. VCE-003 decreased microglial/macrophage activation, reduced demyelination, maintained myelin structure and reduced axonal damage lesions. Significant decrease in all measured inflammatory mediators.	Carrillo-Salinas *et al.*, 2014
	VCE-003 Cannabigerol Quinone derivative	15 days 5 mg.kg^{-1} i.p VCE-003 treated 60 days after infection.	Multiple sclerosis (MS) induced by TMEV.	SJL/J mice	*n*=12	Clinical score (0-5) was significantly improved with VCE-003 treatment. VCE-003 completely recovered motor activities to normal levels.	Granja A.G *et al.*, 2012
	VCE-003.2 Cannabigerol derivative	10 mg.kg^{-1} i.p 16hr post LPS and then daily for 21 days.	Parkinson's disease model- LPS induced.	**M** C57BL/6 mice	*n*=4-6 subjects per group.	VC-003.2 prevented nigrostriatal neuronal loss and reduced microgliosis. Elevation in iNOS was decreased by VC-003.2 vs control.	Garcia. C *et al.*, 2018
	Cannabigerol	50-200 mg.kg^{-1} i.p 1hr before PTZ seizures.	PTZ seizure model (85 mg.kg i.p).	**M** Wistar Kyoto rats	*n*=72	CBG had no effect on seizure severity, incidence or timing and did not alter animal mortality. CBG displayed no anti-convulsant effects.	Hill, A *et al.*, 2014
	Cannabigerol derivative VCE-003	10 mg.kg^{-1} i.p animals 60 days old up to age 18 weeks.	Amyotrophic lateral sclerosis (ALS),	**M** B6SJL-Tg (SOD1*G93A)1Gur/J vs WT	*n*=5-6 animals per group	In SOD1 mice, VCE-003.2 delayed disease progression and reduced a number of neuropathological signs.	Rodriguez-Cueto

						Weight loss was reduced, as were anomalies in clinical score.	C *et al.,*2018
	Cannabigerol (CBG)	4 i.p injections every 24hrs at a dose of 10 mg.kg$^{-1.}$ for 6 weeks (4 weeks after birth to 10 weeks).	Huntington's disease induced by 3NP/R6/2 variant mice.	16 week old **M** C57BL/6 mice/ 4-10 week old R6/2 mice.	*n*=6-8 animals/experiment	CBG improved motor activities, prevented neuronal loss, increased GFAP staining and decreased Iba-1 staining. CBG downregulated Huntington associated genes and decreased inflammatory mediators.	Valdeolivas S *et al.*, 2015
Cannabidivarin (CBDV)	Cannabidivarin (CBDV)	Pre-treatment Vehicle vs 400 mg.kg^{-1} CBDV oral for 3.5 hrs.	Seizures induced by PTZ 95 mg.kg.	Wistar-Kyoto rats (3/4 weeks old).	*n*=51	400 mg.kg^{-1} CBDV significantly decreased seizure severity and increased latency to first signs of seizure. CBDV did not significantly affect gene expression changes induced by PTZ.	Amanda *et al.*, 2013
	Cannabidivarin (CBDV)	50, 100, 200 mg.kg^{-1} i.p injection 1hr/ 30 mins before induced seizures. 400 mg.kg oral gavage 13.5/3.5hr before i.p PTZ.	Epilepsy (mES seizures; 30 mA, 100 Hz for 200 ms or generalized seizures 85 mg.kg PTZ injected i.p.	**F/M** adult Wistar Kyoto rats. Non-Agouti (DBA/) mice 3-4 weeks, ICR (CD-1) mice 5 weeks old.	*n*=80 (10/ group). 640Wistar rats 3-4 weeks old (*n*=15 /group).	200 mg.kg^{-1} CBDV- 90% of mice remained seizure free. In rats, CBDV significantly decreased PTZ seizure severity and rodent mortality (200 mg·kg-1) and delayed seizure onset. On co-administration experiments, 2.9 % of rats (n=7) exhibited a fatal reaction to CBDV administration.	Hill *et al.*, 2012
	Cannabidivarin (only data from purified CBDV is reported here)	1hr pre-treatment 50, 100, 200 mg.kg^{-1} i.p (rats) 10-200 mg.kg^{-1} i.p (mice).	PTZ seizures (85 mg.kg-1) or pilocarpine (380 mg.kg-1).	**M** Wistar Kyoto rats, **M** MF1 mice, DBA/2 mice 3-4 weeks	*n*=10 mice *n*=15 rats.	CBDV significantly affected observed seizure severity >50 mg.kg^{-1}. Mortality was reduced by CBDV administration and suppressed seizure activity (100 mg.kg^{-1})	Hill *et al.*, 2013
	Cannabidivarin (CBDV)	2, 20, 100 mg.kg^{-1} vs vehicle control, daily i.p for 14 consecutive days.	Rett Syndrome	5-month-old MeCP2-308 (B6.129S-MeCP2tm1Heto/J	*n*=70	20 mg.kg^{-1} CBDV improved motor learning ability. Brain weight was increased with CBDV treatment. CBDV had no effect on GPR55 levels and neurotrophin levels.	Vigil, D *et al.*, 2018
	Cannabidivarin (96.4% CBDV, 3.6 % CBD) (started on postnatal day 28, lasting until day 67)	0.2, 2, 20, 200 mg.kg^{-1} i per day i.p initiated postnatal day (PND) 28 until PND 67.	Rett syndrome model; (WT vs Mecp2 KO).	Mecp2-Mouse (WT vs KO).	*n*=>5 per treatment group total *n*=112	2-200 mg.kg^{-1} per day CBDV reduced tremors, 0.2 mg.kg^{-1} per day was ineffective. CBDV reduced hind limb clasping but again not at the lowest dose tested. CBDV improved breathing and gait abnormalities, reduced total symptom score and improved neurological motor deficits.	Zamberletti *et al.*, 2019
Cannabichromene (CBC)	CBC	0.01 mL/g 25, 50,75 mg.kg^{-1} i CBC (mice), 1.0 mL/kg, 10-75 mg.kg^{-1} i CBC (rats) i.p for 1hr prior to electroshock.	Electroshock seizure test; 50 mA intensity for 0.2 seconds.	**M** ICR albino mice or Male Sprague-Dawley rats	*n*=90 (mice), 193 mice, 106 rats	CBC/THC had no effect on tonic hindlimb extension. CBC did not alter latency. CBC (lowest dose) shortened the duration of extension. All doses of CBC depressed motor activity (first 10 min interval).	Davis and Hatoum *et al.*, 1982
Cannabinol (CBN)	CBN	5mg.kg.day subcutaneous pouch (25g mouse). 28 days up to 12 weeks.	Amyotrophic lateral sclerosis (ALS) SOD1 model.	**M** Tg (SOD1-G93A) 2Gur (11) mice.	*n*=18	Motor abnormalities were delayed by CBN vs vehicle. No significant difference for PaGE test assessment or the age at which animals reached end stage.	Weydt *et al.*, 2005

				Assigned 6 weeks of age.			
Tetrahydrocannabidivarin (Δ^9-THCV)	Δ^9-THCV	2 mg.kg^{-1} i.p for 14 days.	Parkinson's Disease (by 6-hydroxytryptamine -6-HT) or LPS.	**M** Sprague-Dawley rats/ CB2 knockout mice.	*n*=5-6 rats per group	THCV improved motor activities, reduced neuronal loss and reduced microglial activation. THCV was able to preserve tyrosine hydroxylase positive neurons (LPS model).	Garcia, C *et al.*, 2011
	Δ^9-THCV	0.025, 0.25, 2.5 mg.kg^{-1} i.p +vehicle prior to initiating seizures.	Seizures induced by 80 mg.kg PTZ.	**M** Wistar rats	64 rats in total; *n*=16 per group.	Median seizure severity, duration, progression or latency was unaffected by any dose of THCV. 33% of animals exhibited a complete absence of seizures at a dose of 0.25 mg.kg^{-1} THCV.	Hill *et al.*, 2010
Tetrahydrocannabidiolc acid(Δ^9-THCA)	Δ^9-THCA	20.mg.kg^{-1} i.p 30 mins before 3NPA, every 24hrs for 4 days.	Huntington's disease (3 NPA model).	**M** C57BL/6 mice	*n*=70; 9 animals per group.	THCA improved hindlimb dystonia and locomotor activity. THCA downregulated all pro-inflammatory mediators.	Nadel *et al.*, 2017

Table 2: Summary of the conditions where emerging cannabinoids have been studied. A tick or cross represents whether a cannabinoid showed efficacy in a condition or not. A dash means that a cannabinoid has yet to be studied in a condition.

	Cannabigerol (CBG)/derivatives	Cannabidivarin (CBDV)	Cannabichromene (CBC)	Cannabinol (CBN)	Cannabidiolic acid (CBDA)	Δ^9THCV	Δ^9-THCA
Huntington's	✓	-	-	✓	X	-	✓ PPARγ[a]
Multiple Sclerosis	✓	-	-	-	-	-	-
Autoimmune Encephalomyelitis	✓ PPARγ/CB_2[a]	-	-	-	-	-	-
Parkinson's	✓ PPARγ[a]	-	-	-	-	✓	✓
Neuroinflammation /neuroprotection	✓	✓	✓	✓	-	✓	✓
Epilepsy/seizure	X	✓ TRPV1 [a]	✓	-	-	✓	-
Amyotrophic sclerosis (ALS)	✓	-	-	✓	-	-	-
Oxidative stress	-	-	-	✓	-	-	-

Rett syndrome	-	✓	-	-	-	-	-
Alzheimer's disease	✓	✓	✓	-	-	-	-

[a] Some of the compounds neuroprotective effects were mediated by this receptor, but no other receptors were probed.

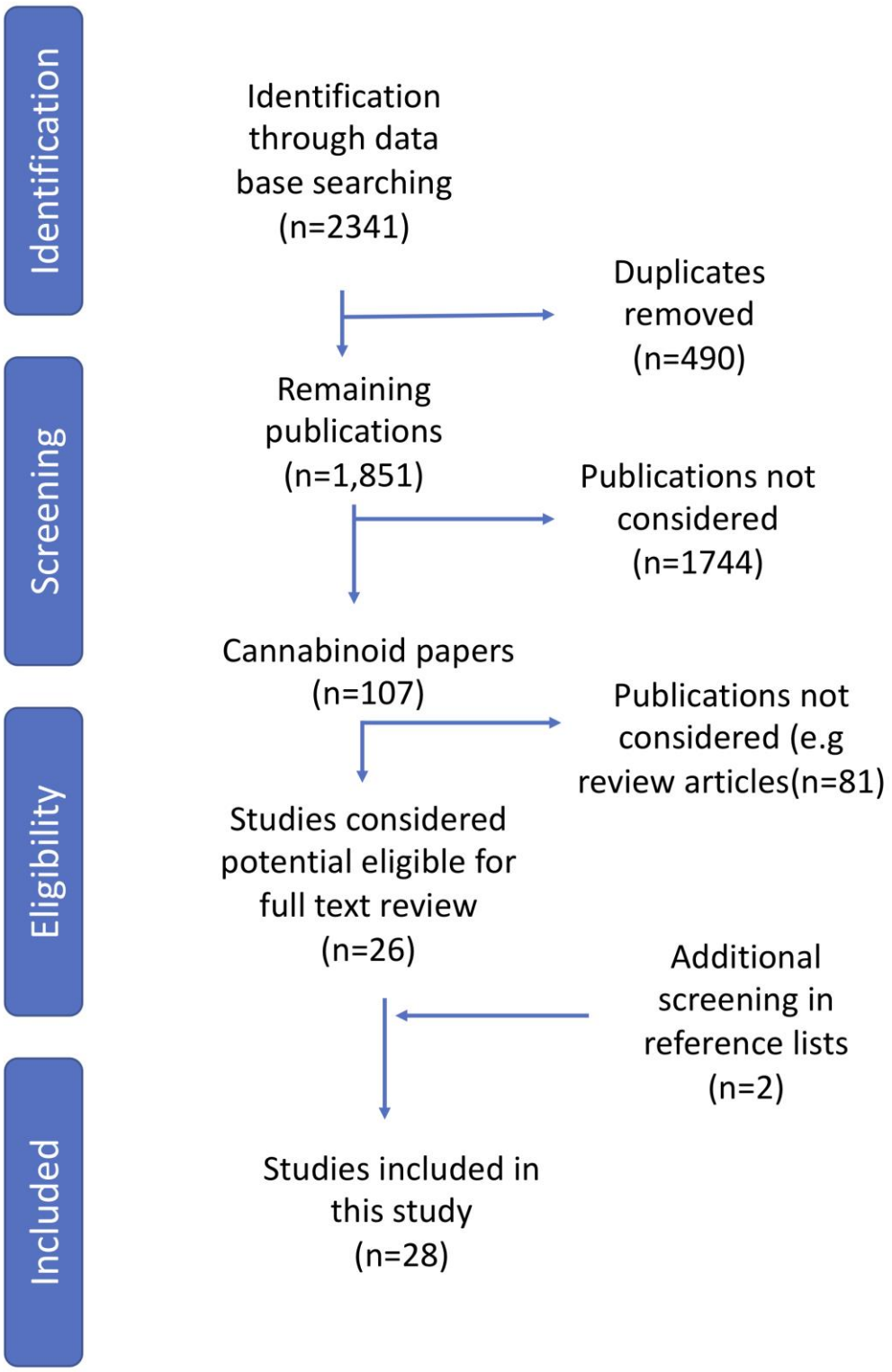

Figure 1: Overview of methodology used in the search process, identification, screening, eligibility and inclusion.

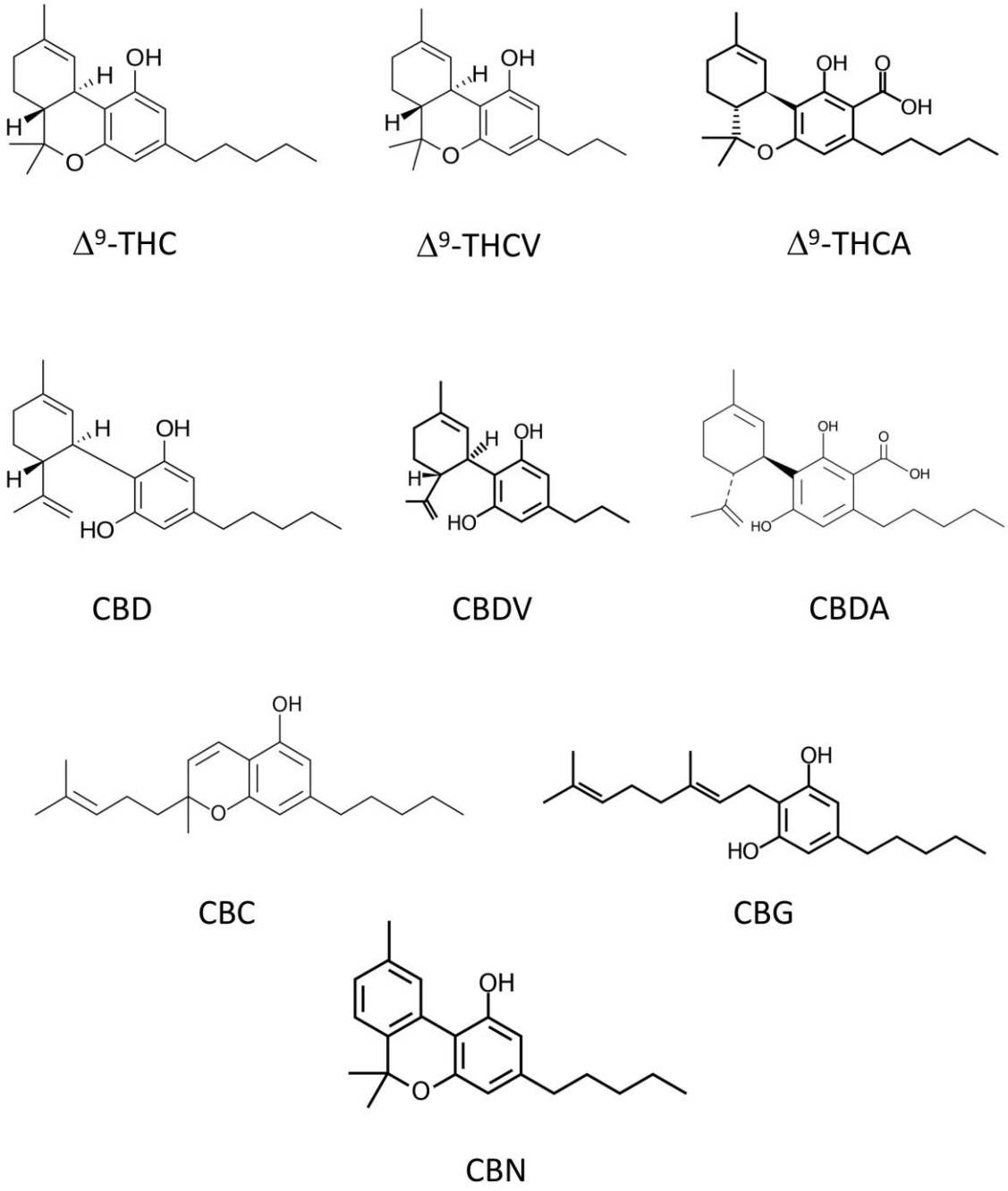

Figure 2: Structures and pharmacological profiles of some of the minor phytocannabinoids with cannabidiol (CBD) and tetrahydrocannabidiol (Δ^9-THC) included for reference: Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA), Δ^9-tetrahydrocannabivarin (Δ^9-THCV), cannabidivarin (CBDV), cannabidiolic acid (CBDA), cannabichromene (CBC) , cannabigerol (CBG) and cannabinol (CBN).