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Review article

Overview of cannabidiol (CBD) and its analogues: Structures, biological activities, and neuroprotective mechanisms in epilepsy and Alzheimer's disease

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ABSTRACT

Herein, 11 general types of natural cannabinoids from *Cannabis sativa* as well as 50 (-)-CBD analogues with therapeutic potential were described. The underlying molecular mechanisms of CBD as a therapeutic candidate for epilepsy and neurodegenerative diseases were comprehensively clarified. CBD indirectly acts as an endogenous cannabinoid receptor agonist to exert its neuroprotective effects. CBD also promotes neuroprotection through different signal transduction pathways mediated indirectly by cannabinoid receptors. Furthermore, CBD prevents the glycogen synthase kinase 3β (GSK-3β) hyperphosphorylation caused by Aβ and may be developed as a new therapeutic candidate for Alzheimer's disease.

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Abbreviation

2-AG	2-arachidonoylglycerol
AD	Alzheimer's disease
AEA	Anandamide
AML	Acute myeloid leukaemia
Apaf-1	Apoptotic protease activating factor-1
APC	Adenomatosis polyposis coli
APP	Amyloid precursor protein
Aβ	β-amyloid
BDNF	Brain-derived neurotrophic factor
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
CBC	Cannabichromene
CBD	Cannabidiol
CBDV	Cannabidivarin
CNS	Central nervous system
CREB	cAMP-response element binding protein
DS	Dravet syndrome
DSI	Depolarization-induced inhibition
Dvl	Dishevelled
ECS	Endocannabinoid system
ETSP	Epilepsy Therapy Screening Program
FAAH	Fatty acid amide hydrolase
Fz	Fizzled
GIRK	G protein gated inwardly rectifying K^+ channels
Glu	Glutamate
GPCRs	G-protein coupled receptors
GSK-3β	Glycogen synthase kinase 3β
HLMs	Human liver microsomes
KA	Kainic acid
LEF	Lymphocyte enhancement factor
LGS	Lennox-Gastaut syndrome
MEC	Minimum effective concentration
MS	Multiple sclerosis
NINDS	National Institute of Neurological Disorders and Stroke
PD	Parkinson's disease
PKA	Protein kinase A
RA	Rheumatoid arthritis
SAR	Structure-activity relationship
T2DM	Type 2 diabetes mellitus
TCF	T cytokine factor
THC	Tetrahydrocannabinol
THCV	Tetrahydrocannabivarin
TRE	Treatment-resistant epilepsy
VR1	Vanilloid receptor type 1

1. Introduction

Cannabis, which has been cultivated for more than 6000 years, is one of the most historical and traditional crops of mankind (Fig. 1). *Cannabis*'s fibre, seed and stalk are endowed with high economic and medical value and are widely used in paper, textile, industrial oil, functional health food, cosmetics, medicine and other areas [1–3]. Morphologically, *Cannabis* has extensive variation, resulting in controversy on the number of subspecies in taxonomy [4]. Botanists have used morphological features to classify *Cannabis* into three main subspecies: *Cannabis sativa*, *Cannabis indica* and *Cannabis ruderalis*. Among them, *Cannabis indica* usually grows 2–4 feet tall and is compactly branched with broad oblanceolate leaves and dense flowering buds, giving it a bushier appearance. *Cannabis ruderalis* is often short and branchless with typically low tetrahydrocannabinol (THC) content and always symbiotic with other plants. The most well-studied subspecies is *Cannabis sativa*, which is the most commonly occurring subspecies in Western countries. *Cannabis sativa* is a tall, thin-leafed plant that can grow 5–18 feet tall and is often branchless [5]. Recent pharmacological studies have indicated that *Cannabis* has a variety of properties, such as analgesic [6], antibacterial [7], anti-inflammatory [8], anti-allergic [9], anti-hypertensive and anti-thrombotic effects [10]. Clinically, unofficially approved *cannabis* has been widely used for neurological diseases and antitumour applications [11,12].

After Raphael Mechoulam first isolated THC [13], numerous phytocannabinoids were discovered as follows: CBD, cannabigerol (CBG), cannabichromene (CBC), cannabidivarin (CBDV) and tetrahydrocannabivarin (THCV). For a long time, the overwhelming preponderance of research focused on the psychoactive THC. Nevertheless, the strong hallucinatory effects of THC, the most common phytocannabinoid found in *Cannabis*, has limited the application of *Cannabis* and made it illegal in most countries and regions. In contrast, CBD hinders anandamide (AEA) biological uptake and inhibits AEA hydrolysis, thus antagonizing the aversive hallucinatory effect of THC.

Fig. 1. Ancient Chinese illustration of the *Cannabis* plant with text describing its functions. This image was obtained from the Compendium of Materia Medica (16th century AD, Ming Dynasty).

2. Natural cannabinoids from *Cannabis sativa*

There has been considerable progress in research on the natural products of *Cannabis sativa* in recent decades. More than 565 compounds have been isolated from *Cannabis sativa*, and more than 120 of these compounds have been identified as natural cannabinoids, including cannabinoid derivatives and metabolites. These cannabinoids are endowed with a typical C_{21} terpenophenolic skeleton, which has not been found in other plant branches or genera [14]. According to their diverse structure, these natural cannabinoids can be classified into 11 general types, specified in Table 1. Among them, types **1**–**10** have relatively fixed substituents or structural modifications on their terpenophenolic skeletons. Their specific structures are shown in Fig. 2. Notably, due to its structural diversity and complexity as well as its multiplex substituent, the miscellaneous type **11** contains 30 different skeletons, and their structures are presented (**11**–**1~11**–**30**) in Fig. 3.

3. CBD and its analogues

3.1. Overview of CBD

CBD (CAS: 13956-29-1) was first isolated from *Cannabis* by Adams et al., in 1940 [35]. Mechoulam et al. described the CBD structure in 1963 [36]; subsequently, Jones reported the crystal structure of CBD and identified two optical isomers in 1977 [37] (Fig. 4). The main difference between these two configurations is the stereochemistry of the connection between the resorcinol moiety and the terpene core. The aromatic ring and the terpene ring are almost perpendicular to each other. (-)-CBD exists in natural *Cannabis* plants, while (+)-CBD can only be obtained by organic synthesis. From the latest reports, all of the naturally occurring CBD-type cannabinoids have a (-)-trans-(1R,6R) absolute configuration, presumably corresponding to negative optical rotation [38].

There has been a long history of unofficial CBD utilization in epilepsy and many other neurological diseases because most preclinical studies have demonstrated primarily positive neurological effects of CBD. As early as the end of the 19th century, physicians in the United Kingdom and United States reported that *Cannabis* extracts reduced the frequency of epileptic seizures [39]. Iván Izquierdo et al. [40] found that CBD downregulated the susceptibility of rat seizures caused by afferent stimuli. Compared with traditional antiepileptics, CBD is an effective anticonvulsant with a higher specificity but fewer neurotoxic effects [41]. In addition, CBD can reduce the post-discharge duration and amplitude, with high selectivity and no excitability to the central nervous system (CREB) [42]. Further research has found that CBD has antiepileptic and anticonvulsant activity in pentylenetetrazol-, pilocarpine- and penicillin-induced seizure models in a dose-dependent manner [43]. In 2017, The National Institute of Neurological Disorders and Stroke (NINDS) funded the Epilepsy Therapy Screening Program (ETSP) to further investigate the effects of CBD on a variety of epileptic models. The ETSP survey indicated that CBD has anti-seizure characteristics in acute seizure models and corneal ignited mice [44]. Although preclinical studies on other clinical indications are still ongoing, the available evidence supports CDB utilization as an effective antiepileptic. In June 2018, the United States FDA approved the first CBD drug Epidiolex® for the therapy of treatment-resistant epilepsy (TRE), including Lennox-Gastaut syndrome (LGS) and Dravet syndrome (DS). Epidiolex® is the sesame oil soluble oral liquid preparation of *Cannabis*-derived CBD, and its approval is based on clinical data from a double-blind trial of 120 DS and drug-resistant seizure patients taking CBD or placebo [45,46].

Additionally, a variety of positive roles of CBD, such as anticonvulsant, anti-nausea, and analgesic effects, have been demonstrated in subsequent preclinical research [47]. Furthermore, CBD was shown to display a more potent antioxidant neuroprotective effect than ascorbate or tocopherol in neurons. CBD is cytotoxic in many tumour cell lines but exhibits cytoprotective effects on normal cells and neurons. In a rodent rheumatoid arthritis (RA) model, CBD, similar to capsaicin, antagonized cell necrosis as a transient receptor potential channel, vanilloid subfamily member 1 (TRPV1) and tumour necrosis factor (TNF)-α agonist, enhancing adenosine receptor A2A signalling by inhibiting adenosine transporters and preventing prion accumulation and neuronal toxicity without COX inhibition. These phenomena indicate that CBD has the potential to be an RA therapeutic [48].

CBD is a G protein-coupled receptor 55 (GPR55) and GPR18 dual antagonist [49,50] and is thus a therapeutic candidate in cell migration disorder diseases, such as endometriosis. However, CBD is a novel GPR12 inverse agonist [51]. Herein, a structure-activity relationship (SAR) analysis of CBD was performed to reveal the structural features required for inversely agonizing GPR12. As shown in Fig. 5, the dihydroxyl groups on C-2′ and C-6′ of the benzene ring are essential for maintaining the affinity of CBD on GPR12, which results in further inverse agonistic activity of CBD on

Table 1
Eleven general types of natural cannabinoids from *Cannabis sativa*.

Type	Cannabinoids	Ref.
1	(-)-Δ9-*trans*-Tetrahydrocannabinol (Δ^9-THC)	[15]
2	(-)-Δ8- *trans*-Tetrahydrocannabinol (Δ^8-THC)	[16]
3	Cannabigerol (CBG)	[17]
4	Cannabidiol (CBD)	[18]
5	Cannabielsoin (CBE)	[19]
6	Cannabichromene (CBC)	[20]
7	Cannabinodiol (CBND)	[21]
8	Cannabicyclol (CBL)	[22]
9	Cannabitriol (CBT)	[23]
10	Cannabinol (CBN)	[20]
11	**Miscellaneous types**	[14]
11−1	Dehydrocannabifuran (DCBF-C_5)	[24]
11−2	Cannabifuran (CBF−C_5)	[25]
11−3	8-Hydroxy-isohexahydrocannabivirin (OH-iso-HHCV-C_3)	[26]
11−4	Cannabichromanone-C_5 (CBCN−C_5)	[27]
11−5	Cannabichromanone-C_3 (CBCN−C_3)	[27]
11−6	10-Oxo-$\Delta^{6a(10a)}$-tetrahydrocannabinol (OTHC)	[25]
11−7	Cannabicitran	[28]
11−8	(-)-Δ^9-*cis*-(6a*S*,10a*R*)-Tetrahydrocannabinol (*cis*-Δ^9-THC)	[14]
11−9	Cannabicoumaronone (CBCON−C_5)	[29]
11−10	Cannabiripsol (CBR)	[30]
11−11	Cannabitetrol (CBTT)	[31]
11−12	(±)-Δ^7-*cis*-Isotetrahydrocannabivarin-C_3 (*cis*-iso-Δ^7-THCV)	[18]
11−13	(-)-Δ^7-*trans*-(1*R*,3*R*,6*R*)-Isotetrahydrocannabivarin-C_3 (*trans*-iso-Δ^7-THCV)	[18]
11−14	(-)-Δ^7-*trans*-(1*R*,3*R*,6*R*)-Isotetrahydrocannabinol-C_5 (*trans*-Iso-Δ^7-THC)	[18]
11−15	Cannabichromanones B	[27]
11−16	Cannabichromanones C	[27]
11−17	Cannabichromanones D	[27]
11−18	(-) - (7*R*)-Cannabicoumarononic acid	[28]
11−19	4-Acetoxy-2-geranyl-5-hydroxy-3-*n*-pentylphenol	[32]
11−20	2-Geranyl-5-hydroxy-3-*n*-pentyl-1,4-benzoquinone	[28]
11−21	5-Acetoxy-6-geranyl-3-*n*-pentyl-1,4-benzoquinone	[32]
11−22	Cannabimovone (CBM)	[33]
11−23	Cannabioxepane (CBX)	[34]
11−24	10α-Hydroxy-$\Delta^{9,11}$-hexahydrocannabinol	[28]
11−25	9β,10β-Epoxy hexahydrocannabinol	[28]
11−26	9α-Hydroxy hexahydrocannabinol	[34]
11−27	7-Oxo-9α-hydroxy hexahydrocannabinol	[34]
11−28	10α-Hydroxy hexahydrocannabinol	[34]
11−29	10a*R*-Hydroxy hexahydrocannabinol	[34]
11−30	9α-Hydroxy-10-oxo-$\Delta^{6a,10a}$-tetrahydrocannabinol	[34]

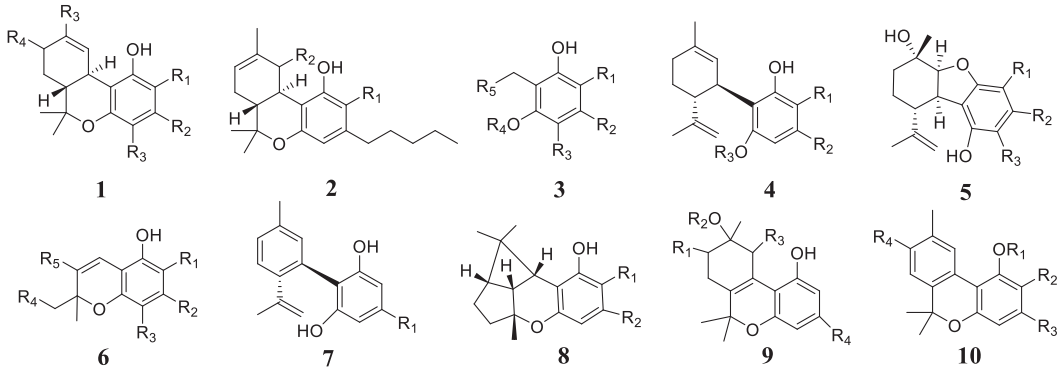

Fig. 2. Skeletons of cannabinoid types 1–10 in Table 1.

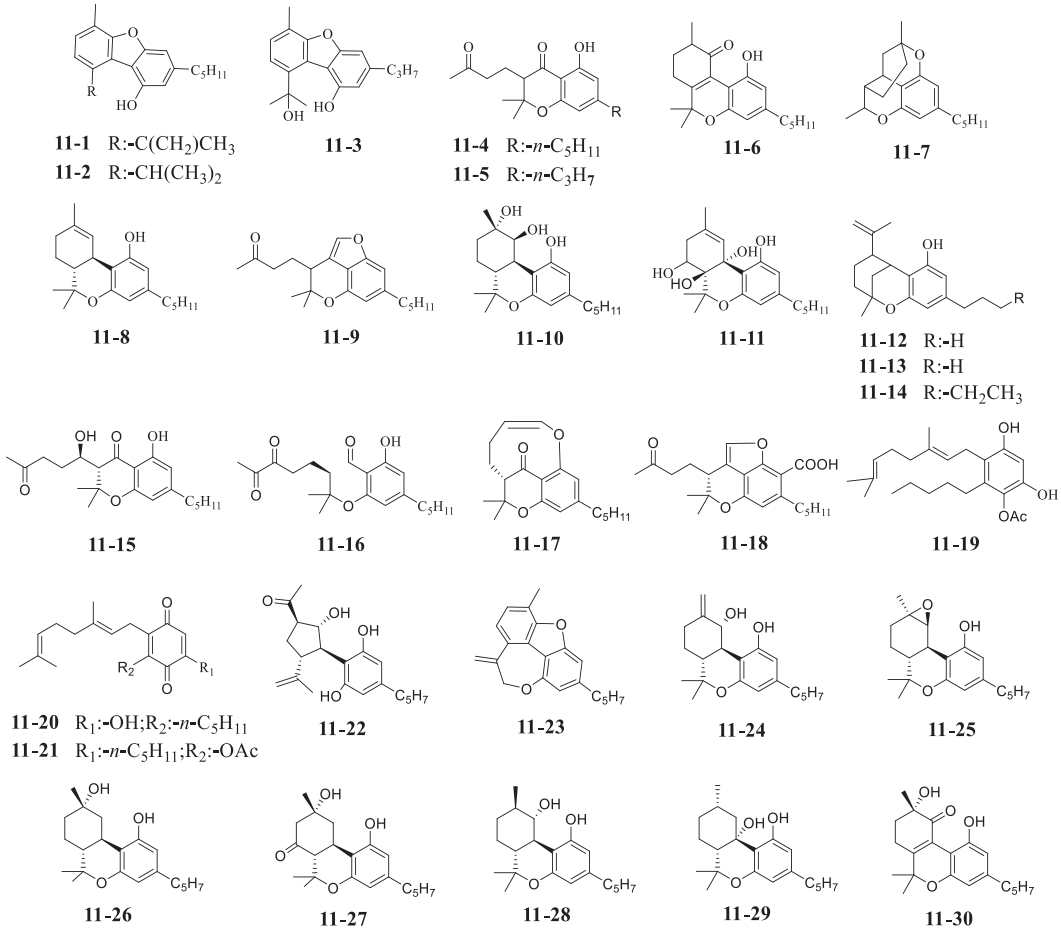

Fig. 3. Structures of type 11 miscellaneous cannabinoids.

GPR12. Elimination of the hydroxyl group or shrinkage of the C-4' side chain directly reduced GPR12-mediated cAMP accumulation. Moreover, as shown in Table 2, CBD exerts obvious inhibitory effects on different cancer cells, including human breast adenocarcinoma cells [52], colon adenocarcinoma cells [53] and prostate carcinoma cells [54]. In the field of oncology therapy, CBD has been shown to enhance gamma radiation sensitivity in human glioblastoma cells by mediating the TNF/TNF receptor 1 (TNFR1) and TNF-related apoptosis-inducing ligand (TRAIL)/TRAIL-R2 signalling pathways [55]. During the process of lipid metabolism, CBD downregulates the intracellular lipid levels, which suggests that CBD might be used as a new therapeutic agent for obesity, hepatic steatosis and metabolic syndrome [56].

3.2. Classification based on the (-)-CBD analogue structure

(-)-CBD has promising pharmacological activities and surprisingly low addictive, hallucinogenic, and toxic side effects. However, due to the solubility and chemical and metabolic instability of (-)-CBD, many researchers have investigated novel (-)-CBD analogues, including metabolites, natural compounds and synthetic derivatives. According to the modifications on multiple reactive

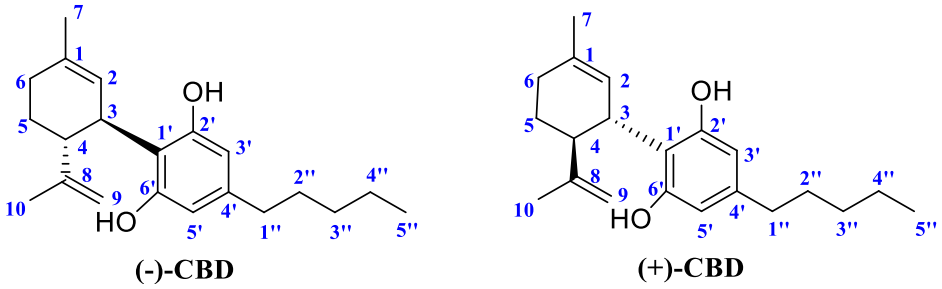

Fig. 4. Two optical isomers of CBD.

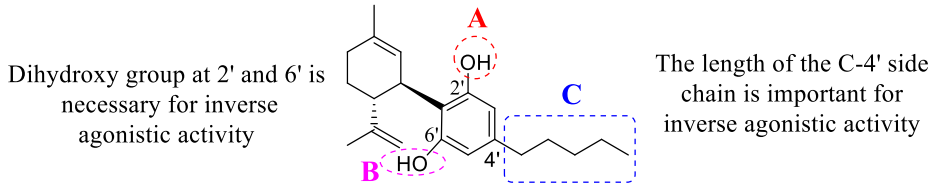

Fig. 5. SAR of CBD as an inverse agonist for GPR12.

Table 2
CBD under trial.

Drug Name	Mechanism of Action	Pharmacological Activity	Method	Parameter	Ref.
CBD	Inducible nitric oxide synthase (NOS-2) inhibitor	Cytotoxicity (4T1 mouse mammary cancer cells)	MTT assay	IC_{50} = 1.80 μM	[52]
	TRPM8 antagonist	Cytotoxicity @ 24 h (Caco-2 human colon adenocarcinoma cells)	MTT assay	IC_{50} = 3.73 μM	[53]
	Apoptosis induction	Cytotoxicity @ 72 h (DU145 human prostate carcinoma cells)	MTT assay	IC_{50} = 5.40 μM	[54]
	MAPK p38 inhibitor	Apoptosis (radiation-induced U87 human glioblastoma cells)	Propidium iodide assay	MEC≤5.00 μM	[55]
	AMPK agonist	Induction of decreases in triglyceride levels (3T3L1 mouse adipocytes)	Fluorescent assay	MEC≤5.00 μM	[56]

MEC: minimum effective concentration.

sites of the (-)-CBD terpineol skeleton, its analogues can be divided into three categories: 1) (-)-CBD terpene moiety-modified analogues, 2) (-)-CBD benzene ring-modified analogues, and 3) (-)-CBD analogues with simultaneous modifications in both the benzene ring and the terpene moiety. All three types of compounds are summarized in Table 3, and their structures are shown in Fig. 6.

3.2.1. (-)-CBD terpene moiety-modified analogues

Compounds **12**–**21** (Table 3) are **(-)-**CBD analogues with modifications to a portion of the terpene moiety. Of these, compounds **12**–**14** are hydroxyl metabolites of (-)-CBD. The first step of mammalian metabolism of (-)-CBD is hydroxylation mainly at the C-7 position, producing (-)-7-OH-CBD (**12**), followed by hydroxylation at the C-6 position, producing (-)-6,7-di–OH–CBD (**14**). Martin et al. [57] reported that (-)-CBD was oxidized by rat liver supernatant to form (-)-7-OH-CBD as the main metabolite, among other metabolites; (-)-6α–OH–CBD/(-)-6β–OH–CBD (**13**) results from C-6 hydroxylation. In addition, compounds **13** and **14** are (-)-CBD metabolites produced by human liver microsomes (HLMs), and CYP3A4 and CYP2C19 in HLMs play key roles in the formation of 6α-OH, 6β-OH and 7-OH [58]. (-)-CBD-7-oic acid (**15**) is produced by further oxidation of **12** and is another major metabolite of (-)-CBD. At present, **12** [59] and **15** [60] can be obtained by a synthetic method. Some of the biological effects observed are hypothesized to be due to active metabolites rather than (-)-CBD itself; regrettably, however, the pharmacological activities of these hydroxyl metabolites have not yet been revealed.

Compounds **16**–**21** are synthetic derivatives of (-)-CBD, of which compounds **16**–**18** are hydrogenated products of (-)-CBD reported by Shimon Ben-Shabat et al. [61]. Mild hydrogenation of (-)-CBD by catalytic PtO_2 resulted in a mixture of (-)-1,2-H_2-CBD (**16**, C-1 position) and (-)-8,9-H_2-CBD (**17**, C-8 position), while hydrogenation of both the C-1 position and C-8 position created compound (-)-H_4-CBD (**18**). Biological screening using mouse macrophages has shown that **18** exerts an enhanced inhibitory effect on the release of nitric oxide. Compared with (-)-CBD (Ki > 10 μM), **18** exhibited good affinity to cannabinoid receptor 1 (CB1) with a Ki of 145 nM. Compounds **19** and **20** are endowed with hydroxyl groups at the C-6 and C-7 positions, respectively, whereas the C-8 and C-9 positions are hydrogenated. The C-2″ and C-6″ hydroxyl groups of **17** can be acetylated by acetic anhydride to obtain (-)-8,9-H_2-CBD-diacetate (**57**), which can be further catalysed by SeO_2 to obtain a mixture of (-)-8,9-H_2-6-OH-CBD-diacetate (**58**) and (-)-8,9-H_2-7-OH-CBD-diacetate (**59**). Finally, a mixture of compounds **19** and **20** can be obtained after deacetylation by $NaBH_4$ [62]. Although compound **20** does not exhibit a very satisfying binding capacity to CB1 or CB2, it has a slightly higher affinity to CB1 than does (-)-CBD. Since many endogenous ingredients and launched drugs have been activated by introducing fluorine atom-containing substituents, the synthesis of fluorinated CBD derivatives has attracted much effort from medicinal chemists. Compound **21** is a representative compound with a fluorine substitution on the C-7 methyl group of the (-)-8,9-H_2-CBD terpene ring and has been identified to have antidepressant potential [63].

Table 3
Three types of (-)-CBD analogues.

No.	Compound Name	Categories	Compound Type
12	(-)-7-OH-CBD	(-)-CBD terpene moiety-modified analogues	Metabolite
13	(-)-6α−OH−CBD/(-)-6β−OH−CBD		Metabolite
14	(-)-6,7-di−OH−CBD		Metabolite
15	(-)-CBD-7-oic acid		Metabolite
16	(-)-1,2-H_2-CBD		Synthetic derivative
17	(-)-8,9-H_2-CBD		Synthetic derivative
18	(-)-H_4-CBD		Synthetic derivative
19	(-)-8,9-H_2-6-OH-CBD		Synthetic derivative
20	(-)-8,9-H_2-7-OH-CBD		Synthetic derivative
21	(-)-8,9-H_2-7-fluoro-CBD		Synthetic derivative
22	(-)-1″−OH−CBD	(-)-CBD benzene ring-modified analogues	Metabolite
23	(-)-2″−OH−CBD		Metabolite
24	(-)-3″−OH−CBD		Metabolite
25	(-)-4″−OH−CBD		Metabolite
26	(-)-5″−OH−CBD		Metabolite
27	(-)-CBD-C_3, acid		Metabolite
28	(-)-CBD-dimethyl ether		Metabolite
29	(-)-CBD-monomethyl ether (CBDM-C_5)		Natural compound
30	(-)-Cannabidiorcol (CBD-C_1)		Natural compound
31	(-)-Cannabidivarin (CBDV-C_3)		Natural compound
32	(-)-Cannabidiol-C_4 (CBD-C_4)		Natural compound
33	(-)-Cannabidiolic acid (CBDA-C_5)		Natural compound
34	(-)-Cannabidivarinic acid (CBDVA-C_3)		Natural compound
35	(-)-CBD-DMH		Synthetic derivative
36	(-)-6″-azidohex-2″-yne-CBD		Synthetic derivative
37	(-)-4′-fluoro-CBD		Synthetic derivative
38	Abnormal-CBD		Synthetic derivative
39	(-)-3″,7-di−OH−CBD	(-)-CBD analogues with simultaneous modifications in both the benzene ring and the terpene moiety	Metabolite
40	(-)-4″,6-di−OH−CBD		Metabolite
41	(-)-4″,7-di−OH−CBD		Metabolite
42	(-)-5″,6-di−OH−CBD		Metabolite
43	(-)-2″,6,7-tri−OH−CBD		Metabolite
44	(-)-4″,6,7-tri−OH−CBD		Metabolite
45	(-)-5″,6,7-tri−OH−CBD		Metabolite
46	(-)-1″-OH,7-COOH-CBD		Metabolite
47	(-)-2″-OH,7-COOH-CBD		Metabolite
48	(-)-3″-OH,7-COOH-CBD		Metabolite
49	(-)-4″-OH,7-COOH-CBD		Metabolite
50	(-)-5″-OH,7-COOH-CBD		Metabolite
51	(-)-1,2-H_2-CBD-DMH		Synthetic derivative
52	(-)-8,9-H_2-CBD-DMH		Synthetic derivative
53	(-)-H_4-CBD-DMH		Synthetic derivative
54	(-)-7-OH-CBD-DMH		Synthetic derivative
55	(-)-7-COOH-CBD-DMH		Synthetic derivative
56	(-)-1-COOH-CBD-DMH		Synthetic derivative
57	(-)-8,9-H_2-CBD-diacetate		Synthetic derivative
58	(-)-8,9-H_2-6-OH-CBD-diacetate		Synthetic derivative
59	(-)-8,9-H_2-7-OH-CBD-diacetate		Synthetic derivative
60	(-)-10-hydroxy-CBD-diacetate		Synthetic derivative
61	(-)-10-fluoro-CBD-diacetate		Synthetic derivative

3.2.2. (-)-CBD benzene ring-modified analogues

Compounds **22**—**38** in Table 3 are (-)-CBD analogues with modifications to the benzene ring moiety. Compounds **22**—**26** are monohydroxy metabolites of the pentyl side chain. Meanwhile, compounds **29**—**34** are natural CBD analogues isolated from *Cannabis*.

ElSohly et al. summarized six natural CBD analogues, including (-)-CBD-monomethyl ether (**29**), (-)-cannabidiorcol (**30**), (-)-cannabidivarin (**31**), (-)-cannabidiol-C_4 (**32**), (-)-cannabidiolic acid (**33**) and (-)-cannabidivarinic acid (**34**) [64]. Among them, **31** was observed to dose-dependently inhibit Caco-2 cell viability through antagonism of the TRP cation channel subfamily M member 8 (TRPM8) receptor [53]. Yedukondalu Nalli et al. [65] evaluated the cytotoxicity of CBDV on several cancer cell lines (A549, HepG2, OVCAR, MCF7, PC-3, SH-SY5Y and HCT-116) by MTT assay. The experimental data showed that **31** showed potent antiproliferative activity on the abovementioned cancer cells (Table 4). In a mechanistic study, **31** reduced the activity of Top-Flash reporter genes in HepG2 cells and blocked the transmission of the Wnt/β-catenin signalling pathway. Furthermore, GW Pharmaceuticals has investigated the therapeutic potential of **31** in the field of autism spectrum disorders and focal seizures. In addition, the efficacy and safety of **31** have been evaluated in a phase II placebo-controlled study, but the results have been suboptimal and require further exploration. Compound **33** was the first *Cannabis* acid isolated in 1955 by Krejčí Z et al. [66] and has been shown to inhibit the migration of highly invasive MDA-MB-231 mastadenoma cells by cAMP-dependent protein kinase A (PKA) inhibition and RhoA activation [67]. Activation of the RhoA signalling pathway results in mobility inhibition of various cancer cells [68]. Compared with (-)-CBD, **33** demonstrated more potent inhibition in a rat vomiting/nausea model by enhancing 5-HT_{1A} receptor activation [69], implying that **33** may be an effective treatment for acute unexpected nausea and appetite disorders [70].

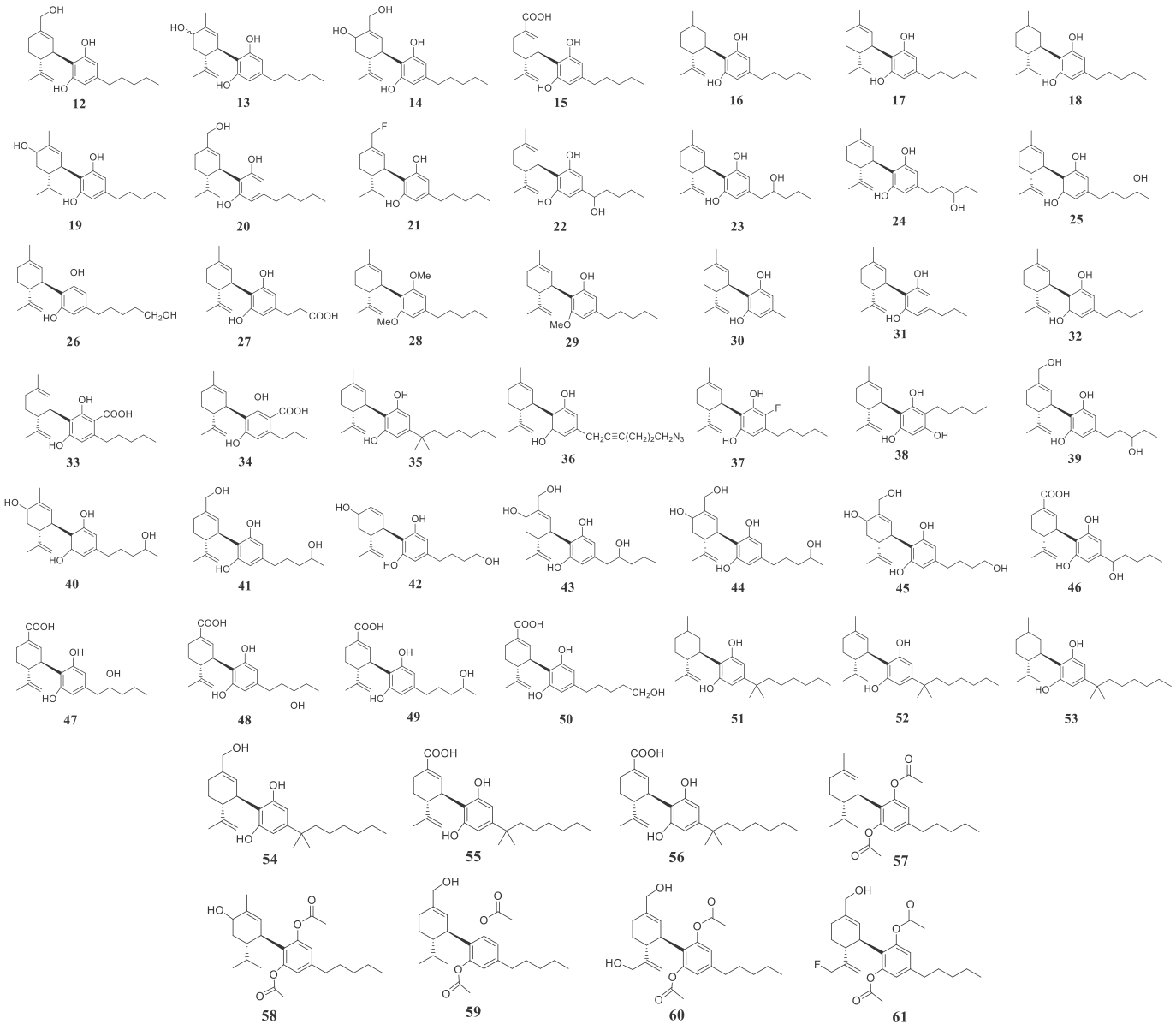

Fig. 6. Structures of (-)-CBD analogues.

Compounds **35**–**38** are synthetic derivatives of (-)-CBD. Compound **35** has a dimethylheptyl side chain instead of the pentyl side chain and has been demonstrated to induce apoptosis in the human acute myeloid leukaemia (AML) HL-60 cell line and bind weakly to CB2 [71]. Compared to the existing CB1 antagonists, compound **36**, in which the C-4′ pentyl is replaced by 6″-azido-2″-hexyne, exhibits stronger CB1 antagonism. Moreover, since **36** did not enhance the electrically evoked contraction amplitudes in the mouse vas deferens, further experiments are needed to determine if **36** is indeed a neutral cannabinoid CB1 antagonist [72]. The hydrogen of the phenyl ring at the C-3' position was altered by fluorine in **37**, and a preliminary biological test indicated that this fluorinated (-)-CBD derivative may be further developed as an anti-anxiolytic, antidepressant and antipsychotic new chemical entity. Another synthetic cannabinol **38** was shown to bind to the GPR55 receptor and reduce endoplasmic reticulum stress-induced pancreatic β-cell line (MIN6 and β-TC-6 cell) apoptosis, indicating that **38** might be effective for the treatment of type 2 diabetes mellitus (T2DM) [73].

3.2.3. (-)-CBD analogues with simultaneous modifications in both the benzene ring and the terpene moiety

Compounds **39**–**61** (Table 3) are CBD analogues with simultaneous modifications of the benzene ring and terpene moiety. Among them, compounds **39**–**42** are bihydroxy metabolites of (-)-CBD. The metabolic sites are mainly C-6/C-7 and the pentyl side chain of the benzene ring. Compounds **43**–**45** are trihydroxy metabolites of (-)-CBD. Varying the positions of the side-chain hydroxylation of **15** results in derivatives **46**–**50**; specifically, the hydroxylated positions are C-1" (**46**), C-2" (**47**), C-3" (**48**), C-4" (**49**), and C-5" (**50**) of the benzene ring.

Compounds **51** and **52** are obtained by partial hydrogenation of (-)-CBD-DMH **35** under PtO_2 catalysis, wherein **52** is the main epimer. Compound **53** significantly inhibits the production of TNF-α and exhibits much more potent anti-inflammatory activity than (-)-CBD [61]. Compound **54** inhibits anandamide uptake; thus, it may be utilized as a cannabinoid receptor antagonist. Compound **55** exhibited anti-inflammatory effects in an otitis model. In addition, **54** and **55** were demonstrated to have anti-diarrhoeal and/or

Table 4
(-)-Cannabidivarin and (-)-cannabidiolic acid under trial.

Drug Name	Highest Phase Reached	Mechanism of Action	Pharmacological Activity	Method	Parameter	Ref.
(-) Cannabidivarin (31)	Phase II	Transient receptor potential cation channel subfamily M member 8 (TRPM8) antagonist	Cytotoxicity @ 24 h (Caco-2 human colon adenocarcinoma cells)	MTT assay	IC_{50} = 10.1 μM	[53]
		Wnt/β-catenin inhibitor	Cytotoxicity @ 72 h (A549 human non-small-cell lung carcinoma cells)	MTT assay	IC_{50} = 39 μM	[65]
			Cytotoxicity @ 72 h, (HepG2 human hepatoblastoma cells)	MTT assay	IC_{50} = 64 μM	
			Cytotoxicity @ 72 h (OVCAR human ovarian carcinoma cells)	MTT assay	IC_{50} = 57 μM	
			Cytotoxicity @ 72 h (MCF7 human breast adenocarcinoma cells, hormone-dependent)	MTT assay	IC_{50} = 53 μM	
(-) Cannabidivarin (**31**)	Phase II	Wnt/β-catenin inhibitor	Cytotoxicity @ 72 h (PC3 human prostate adenocarcinoma cells)	MTT assay	IC_{50} = 44 μM	[65]
			Cytotoxicity @ 72 h (SHSY5Y human dopaminergic neuroblastoma cells)	MTT assay	IC_{50} = 55 μM	
			Cytotoxicity @ 72 h (HCT116 human colon carcinoma cells)	MTT assay	IC_{50} = 13 μM	
		CB1 receptor inverse agonist	Protection against nausea and vomiting prophylaxis (rats, male)	Conditioned gaping assay	MED = 0.200 g/kg i.p.	[74]
(-)-Cannabidiolic acid (**33**)	Preclinical	PKA inhibitor	Inhibition of cell migration (MDAMB231 cells)	Transwell chamber assay	MIC≤5.00 μM	[67]
		5-HT1A receptor agonist	Protection against nausea and vomiting prophylaxis (mouse)	[35S]-GTPgammaS binding assay	Kb = 1.80 μM	[69]
		/	Protection against nausea and vomiting prophylaxis (rats, male)	Conditioned gaping assay	MED = 2.50 μg/kg	[70]

anti-inflammatory potential for the treatment of inflammatory bowel disease and cystic fibrosis [75]. (-)-1-COOH-CBD-DMH (**56**) has the 1,2-dimethyl-heptyl (DMH) moiety inserted in the (-)-CBD C-4' position instead of the n-pentyl chain and the C-1 methyl superseded with a carboxyl group. These dramatic structural changes of **56** abolish the cannabinoid-mediated vanilloid receptor type 1 (VR1)-related physiological response [76].

3.3. Neuroprotective mechanism of CBD

Preclinical and clinical studies have shown that CBD can exert neuroprotective effects in a variety of ways for the treatment of epilepsy, multiple sclerosis (MS), Parkinson's disease (PD), Alzheimer's disease (AD) and other systemic diseases caused by degeneration or abnormalities of the nervous system. CBD has already been a common focus of research studying neurodegenerative diseases and their pathomechanism. Herein, the neuroprotective effects of CBD as well as the molecular mechanisms involved in CBD are summarized and analysed to obtain an extensive understanding of CBD.

3.3.1. CBD interacts with endogenous cannabinoid receptors (CB1 and CB2)

The endocannabinoid system (ECS) is a complex endogenous signal network that has multiple functions under both physiological and pathological conditions. The ECS consists of cannabinoid receptors, endogenous fatty acid ligands, and proteins responsible for the biosynthesis and degradation of endocannabinoids. The main receptors of the ECS, CB1 and CB2, are seven-transmembrane G protein-coupled receptors (GPCRs) distributed in different tissues [77]. Both CB1 and CB2 receptors can be activated by endocannabinoids but exhibit different affinities and irrelevant biological effects. The CB1 receptor is widely distributed in the CNS. Specifically, the CB1 receptor is highly distributed in the substantia nigra, pale Globus pallidus, cerebellar molecular layers, hippocampus and cerebral cortex. CB2 receptors are mainly distributed in the peripheral regions of the spleen, on the surface of immune cells and in the tonsils [78]. An increasing number of studies have revealed that CB2 receptors are also expressed in the CNS and peripheral nervous system and exert a variety of neurophysiological effects [79,80]. Among these receptors, the CB1 receptor has been widely reported as an antiepileptic drug target in animal models [81].

Physiological and pathological studies have shown that endocannabinoids are able to improve neuronal hyperexcitation-related symptoms. In an MS model, activation of the CB1 receptor significantly reduced spasms and tremors [82]. In a rat pilocarpine-ignited epilepsy model, Wallace et al. found that endocannabinoids reduced seizures more effectively than phenobarbital or even phenytoin sodium [83]. These researchers also proposed that endocannabinoids regulate the duration and termination of seizures by activating the CB1 receptor. In addition, by testing variations in endocannabinoid content in the hippocampus of seizure mice induced by kainic acid (KA), another group provided evidence supporting the involvement of endocannabinoids in seizure prevention [84]. Neurophysiology research has confirmed that glutamic acid is the main CNS stimulant. Excessive glutamate (Glu) transmission causes excitability toxicity and seizures in rodents and primates. The main result of CB1 receptor activation is reducing presynaptic Glu release [85]. CBD provides neuroprotection against acute excitotoxicity by indirectly activating CB1. As shown in Fig. 7, postsynaptic membrane depolarization is a characteristic event that occurs during seizures [86]. Endocannabinoids are thought to be the reverse mediator of depolarization-induced inhibition (DSI) [87]. Fatty acid amide hydrolase (FAAH), an amidase signature family member, is an integral membrane enzyme that degrades lipid amides, including endocannabinoids such as AEA and 2-arachidonoylglycerol (2-AG). CBD effectively increases the AEA and 2-AG levels in tissue by inhibiting the activity of FAAH [88]. Subsequently, activation of presynaptic CB1 receptors by endocannabinoids leads to voltage-sensitive N-type and P/Q-type

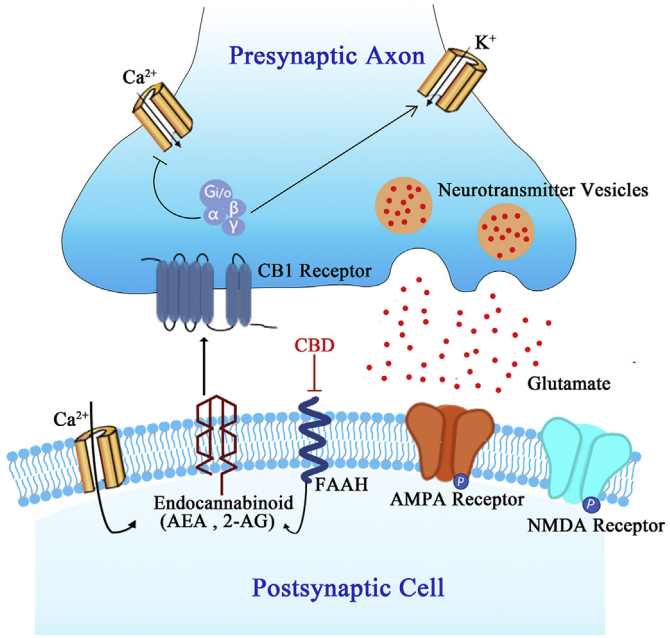

Fig. 7. As a FAAH inhibitor, CBD increases the AEA and 2-AG concentrations to activate retrograde signals.

calcium channel inhibition, resulting in a reduction in Ca^{2+}-dependent glutamic acid release. Less glutamatergic transmission induces an anticonvulsant effect for epilepsy therapy. Additionally, endocannabinoids activate G protein-gated inwardly rectifying K^+ channels (GIRKs). By stabilizing the membrane potential and reducing other factors involved in epileptiform discharge, K^+ channel activation can reduce neuronal excitability to prevent seizures [89].

In addition, CBD might also promote neurite outgrowth by indirectly stimulating CB1 and CB2 receptors to trigger different signal transduction pathways. Recent research has demonstrated that activation of CB1 receptors expressed on neuronal somata increases extracellular signal-regulated kinase (ERK) activity and induces brain-derived neurotrophic factor (BDNF) expression [90]. BDNF is implicated in long-term potentiation in the adult hippocampus [91]. At the same time, when the CB1 receptor is activated, Gi/o-βγ transduction activates PKC to initiate the inositol triphosphate (IP3)/mitogen-activated protein kinase (MAPK) pathway to upregulate ERK1/2 protein and finally enable a key transcription factor, cAMP-response element binding protein (CREB), to promote the growth of neurites [92]. The CB2 receptor is also coupled to Gi/o protein in the CNS to activate adenylate cyclase through Gi/o-α transduction, thereby reducing the cAMP concentration and igniting the PI3K/Akt and ERK pathways to promote the growth of neurites [93]. The growth of neurites is a highly differentiated complex process that is adjusted by a variety of nerve growth factors and neurotransmitters, as well as electrophysiological activity. CBD relies on intermediary endocannabinoids to activate the CB1 receptor, stimulating a protective cascade against neuronal excitatory toxicity. Overall, these observations indicate that CBD could become a promising therapeutic for neuro-related diseases characterized by hyperexcitability and neuro-excitotoxic-related events.

3.3.2. Neuroprotective effects of CBD on β-amyloid-induced Alzheimer disease therapy

AD, first described by German psychiatrist and pathologist Alois Alzheimer in 1906, is a chronic neurodegenerative disease usually with a concealed onset and unobvious progress over a long time [94]. Neuropathological studies have shown that the disease process is associated with plaques and neurofibrillary tangles in the brain, mainly due to the combination of β-amyloid (Aβ) aggregation and changes in *tau* protein leading to the disintegration of microtubules in brain cells [95–97]. The Aβ hypothesis is considered the most important and pivotal for the pathogenesis of AD because it spearheads a cascade of pathological events that are detrimental to neurons in the brain [98]. The development of AD is driven by the accumulation and deposition of Aβ peptide aggregates in the brain. As shown in Fig. 8-A, amyloid precursor protein (APP) in neurons is degraded by three proteases (α-, β-, and γ-secretases). A sustained imbalance between the production and clearance of Aβ40–42 (major metabolic peptides found in the Aβ plaques) fragments by β- and γ-secretases leads to the accumulation of Aβ peptide monomers and oligomers. An increase in the production or a decrease in the clearance of Aβ peptides leads to the formation of Aβ aggregates in the brain and ultimately large aggregated Aβ plaques. These peptides produced by neurons and other brain cells eventually stick together and aggregate (formation of β-sheet structures) into a variety of toxic assemblies, neurofibrillary tangles and neuritic plaques, which “gum up” the parenchymal space between neurons in the brain. In the *tau* hypothesis model (Fig. 8-B), hyperphosphorylated tau begins to pair with other threads of *tau*, which eventually leads to the formation of neurofibrillary tangles inside nerve cell bodies. When this phenomenon occurs, the microtubules disintegrate, destroying the structure of the cell’s cytoskeleton, collapsing the neuronal transport system and leading to a large amount of hippocampal neuron loss. This process initially results in biochemical communication malfunctions between neurons and then neuronal apoptosis.

The most recent research has demonstrated that when hippocampal and cortical neurons are exposed to Aβ peptide, glycogen synthase kinase 3β (GSK-3β) is activated through phosphorylation and then defunctionalizes the entire Wnt signalling pathway [99]. Moreover, under pathological conditions, phosphorylated GSK-3β is responsible for the massive *tau* protein hyperphosphorylation and relative neurofibrillary tangle formation observed in the brains of AD patients [100]. Giuseppe Esposito demonstrated that CBD could rejuvenate PC12 cells from Aβ peptide-stimulated toxicity through the Wnt/β-catenin pathway [101,102]. On the one hand, CBD regulates T cytokine/lymphocyte enhancement factor (TCF/LEF)-activated transcription in the nucleus by affecting the Wnt/β-catenin pathway. As shown in Fig. 9, CBD upregulates Wnt/β-catenin signalling by inhibiting GSK-3β phosphorylation. When the Wnt/β-catenin signal is activated, the Wnt ligand binds to the Fizzled (Fz) and LRP5/6 receptor proteins, Dishevelled (Dvl) is summoned to the cell membrane, and the DIX and PDZ domains of Dvl decrease the activity of the GSK-3β disruption complex, which is structurally formed by adenomatosis polyposis coli (APC), Axin, and GSK-3β. The inhibition of β-catenin phosphorylation by the destruction complex allows unphosphorylated β-catenin to accumulate in the cytoplasm and translocate to the nucleus; hereafter, β-catenin combines with TCF/LEF to activate transcription factors, which are responsible for initiating the transcription of genes encoding the neuronal survival response and homeostasis [103–105]. The above mechanism illustrates that CBD exerts neuroprotective effects in the Aβ-stimulated PC12 cellular AD model by rescuing the Wnt/β-catenin pathway.

On the other hand, studies have indicated that CBD exerts a protective effect on the nervous system by inhibiting neuronal apoptosis [106]. As shown in Fig. 9, caspase activation is the core step leading to apoptosis. When Aβ stimulates the adrenergic receptor to mobilize the apoptotic signal pathway, caspase 8 and

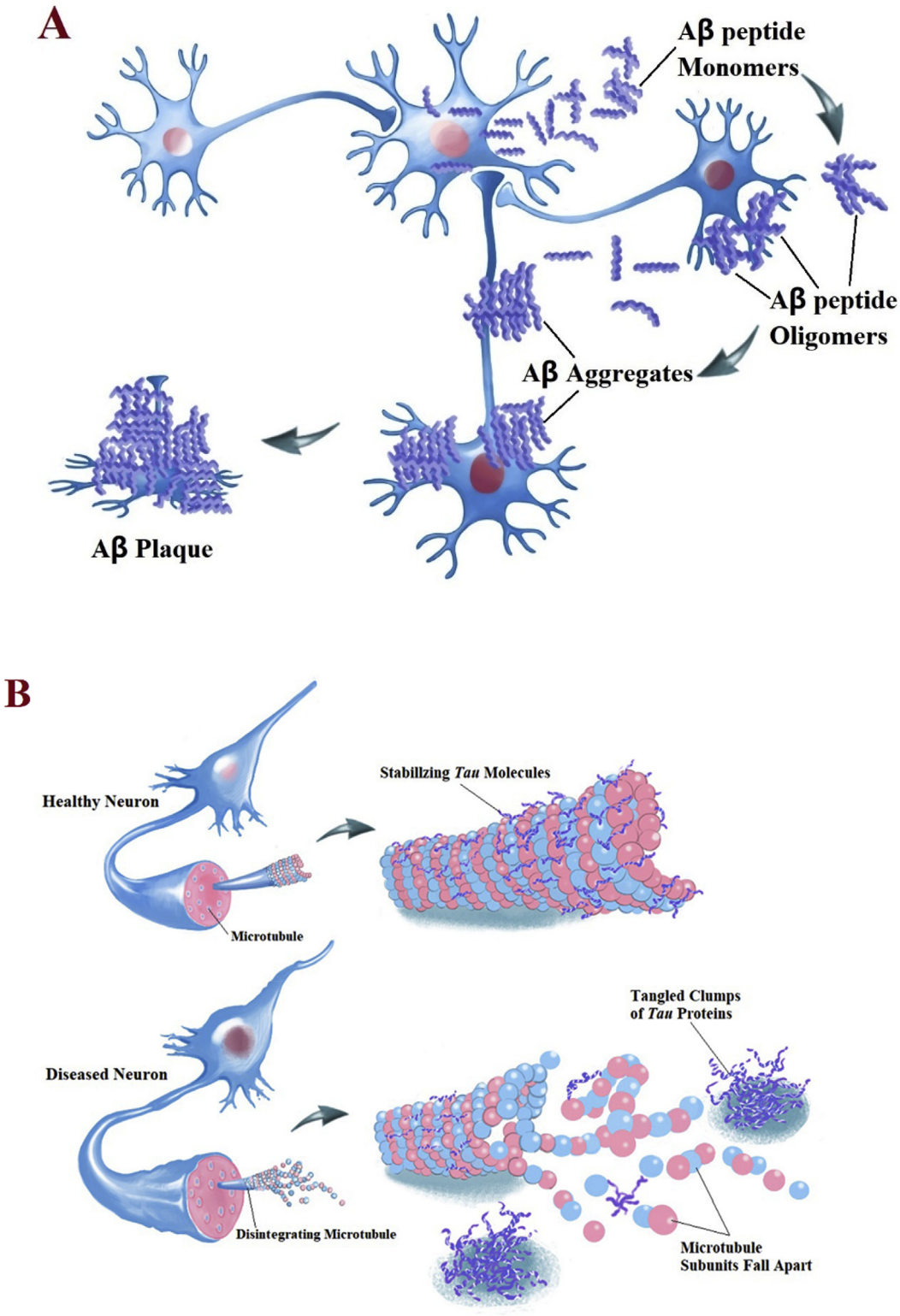

Fig. 8. (A) β-amyloid hypothesis model; (B) *Tau* hypothesis model.

caspase 10 are initially activated by self-cleavage. Subsequently, BID in the downstream Bcl-2 family is cleaved to produce an activated t-BID. t-BID is transferred to the outer mitochondrial membrane to form pore channels such that Bax and Bak can be inserted, facilitating cytochrome C release from mitochondria [107]. Once cytochrome C is released, it binds with apoptotic protease-activating factor-1 (Apaf-1), which then binds to caspase 9 to create a protein complex known as an apoptosome. The apoptosome further cleaves the inactive precursor molecule pro-caspase 3, which in turn activates the effector caspase 3. Caspase

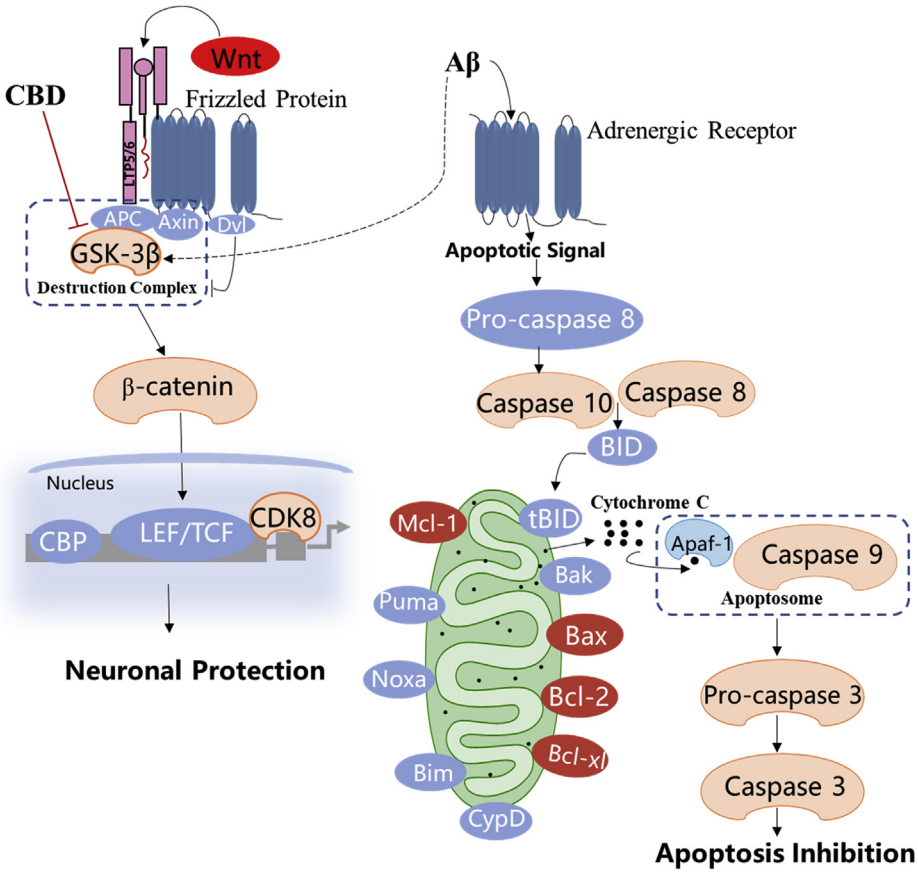

Fig. 9. Two signalling pathways related to the neuroprotective mechanism of CBD.

activation is the key step leading to apoptosis. Experiments have confirmed that CBD inhibits apoptosis by upregulating pro-caspase 3 levels and simultaneously downregulating caspase 3 levels in Aβ-treated PC12 cells. CBD also inhibits the expression of other neuronal apoptosis pathway-related proteins to implement clinically relevant neuroprotective effects. In addition, investigators have also demonstrated that CBD can counteract the Aβ-induced Ca^{2+} increase, consistent with the antioxidant properties of CBD reported in other articles [108].

4. Conclusion

Traditional herbalists use *Cannabis* to treat various diseases, such as earache, arthritis, and convulsions [109,110]. CBD is a phytocannabinoid found in *Cannabis*, although at low concentrations in modern-day strains. Contrary to THC, CBD has no euphorigenic or psychedelic properties [111]. In addition, CBD participates in many pathological processes, such as controlling neurotransmitter release and regulating pain and gastrointestinal function, and has shown potential as an antipsychotic. Epilepsy is a disease of the CNS and is characterized by uncontrollable seizures in the upper or lower limbs [112]. As the pathogenesis of epilepsy is extremely complicated, our current knowledge is not sufficient to fully understand epilepsy at the molecular level. Although the increasing and widespread utilization of *Cannabis* and *Cannabis* extracts is associated with an increased risk of psychosis, the *Cannabis* constituent monomer CBD may remedy epilepsy and neurodegenerative diseases, including AD, through its neuroprotective effects.

A summary of the existing CBD research literature revealed the following three major findings: 1) In the case of neuron damage, CBD inhibits the release of Glu to generate a protective response, which may be triggered by CBD-induced retrograde signal transmission between synapses in the ECS. CBD is one of the phytocannabinoids that interacts with the ECS. The ECS consists of cannabinoid receptors, endogenous cannabinoids and several enzymes that control the activation and availability of these endocannabinoids. Five endogenous cannabinoids have been identified that bind to CB1 or CB2 receptors [113]. However, so far, only the two most relevant endocannabinoids seem to play a relevant role in ECS functioning, namely, 2-AG and AEA. 2) The characteristics of the two cannabinoid receptors CB1 and CB2 differ significantly. The CB1 receptor, the most prominent G protein-coupled endocannabinoid receptor in the CNS, is a transmembrane receptor that converts extracellular stimuli into downstream intracellular signalling pathways. CB1 receptors inhibit the release of excitatory neurotransmitters such as acetylcholine and Glu. CBD activation of CB1 and CB2 receptors may induce multiple signalling pathways, such as the PKC, PI3K/Akt, and ERK pathways, to promote the growth of neurites. However, the further downstream effects of these signalling pathways are complex and have not been fully explored. Neurite function is regulated by various factors, and whether the CBD-related signal transduction pathway is involved in neurite regulation requires further study. 3) CBD is expected to become an emerging therapy for AD treatment, and CBD can rescue the production of neurofibrillary tangles caused by Aβ stimulation by upregulating the Wnt/β-catenin pathway. In addition, existing data indicate that CBD downregulates the level of caspase 3 and inhibits neuronal apoptosis. CBD has potent anti-proliferation properties in several tumour cell lines and can be used as a

small-molecule chemotherapeutic. Although some molecular signalling pathways and related targets of CBD have been revealed and summarized in the present review, unresolved issues remain.

In this review, the structures and function of CBD-represented cannabinoids are preliminarily clarified, but other key active ingredients of *Cannabis*, such as flavonoids, stilbenoids, terpenoids, lignans and alkaloids, have not been fully examined. Moreover, the underlying mechanism of the pharmacological activities of CBD remains to be studied. Last but not least, natural CBD analogues, various metabolites and synthetic derivatives endowed with significant biological activities and relatively low psychotoxicity should be studied for the treatment of epilepsy, cancer, AD, and neuropathic pain. Mechanistic studies need to be performed to elucidate how CBD regulates a variety of nerve growth factors and neurotransmitters, as well as electrophysiological activity.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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