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Innovative Therapeutic Potential of Cannabinoid Receptors as Targets in Alzheimer's disease and Less Well-Known Diseases

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Abstract: The discovery of cannabinoid receptors at the beginning of the 1990s, CB1 being cloned in 1990 and CB2 cloned in 1993, and the availability of selective and potent cannabimimetics could only be justified by the existence of endogenous ligands that are capable of binding to them. Thus, the characterisation and cloning of the first cannabinoid receptor (CB1) led to the isolation and characterisation of the first endocannabinoid, arachidonoylethanolamide (AEA), two years later and the subsequent identification of a family of lipid transmitters known as the fatty acid ester 2-arachidonoylglycerol (2-AG).

The endogenous cannabinoid system is a complex signalling system that comprises transmembrane endocannabinoid receptors, their endogenous ligands (the endocannabinoids), the specific uptake mechanisms and the enzymatic systems related to their biosynthesis and degradation.

The endocannabinoid system has been implicated in a wide diversity of biological processes, in both the central and peripheral nervous systems, including memory, learning, neuronal development, stress and emotions, food intake, energy regulation, peripheral metabolism, and the regulation of hormonal balance through the endocrine system.

In this context, this article will review the current knowledge of the therapeutic potential of cannabinoid receptor as a target in Alzheimer's disease and other less well-known diseases that include, among others, multiple sclerosis, bone metabolism, and Fragile X syndrome.

The therapeutic applications will be addressed through the study of cannabinoid agonists acting as single drugs and multi-target drugs highlighting the CB2 receptor agonist.

Keywords: Alzheimer's disease, CB2 agonist, endocannabinoid system (eCS), bone disorders, Autism disorders, cardiovascular diseases

1. Introduction

The hemp plant has been used medicinally and recreationally from Antiquity. Most authors have located Central Asia as the possible origin of this plant, although the geographic situation is too ambiguous.

It was known in China almost five thousand years ago, when it was used to obtain fibre and oil. The earliest documented uses of hemp in China date from 4000 B.C. [1,2]. Hemp thread and rope have also been found in Turkestan since 3000 B.C. [3]. In addition, hemp cloth and paper were found in the grave of Emperor Wu (Han Dynasty 104-87 B.C.) by Ts'ai Lun. The hemp paper also was frequently used by Tibetans to write their monastic histories due to their durability and quality [3].

Cannabis grain was chosen as one of the "five grains", together with rice, barley, millet and soy beans [2], and is still a source of cooking oil and grain in areas of Nepal [3]. Cannabis may be the oldest plant that is not grown specifically for use as food. However, the location of the first cannabis crops is unclear [3].

There is archaeological evidence that the Scythians of southern Central Asia used the plant during funeral rites as hemp seeds have been in metal tripod censers; for example, Herodotus described this more than 2,000 years ago. The discovery of leaves and fruits of hemp in the Yanghai Tombs (Turpan District in Xinjiang, China), dated by 14C at 2500 years B.C., provides evidence about the ancient utilisation of Cannabis in China for ritual/medicinal purposes [4].

Pen Ts'ao Ching compiled the herbal around 100 A.D., but this was attributed to the legendary emperor Shen-Nung, (2700 B.C.); this provides evidence that the Chinese knew of its psychotropic properties and therefore of medical cannabis use in China [3].

In India, it formed part of certain religious rites, and was used for its curative properties. The medicinal use of *Cannabis* was referred to in India in the medical work *Susrita*, which was compiled around 1000 B.C. and was later recorded in ancient Persia, in about 600 B.C., in the Sanskrit work *Zend Avesta* [3].

The hallucinogenic use of cannabis seems to be associated with indigenous central Asian shamanistic practices. However, medicinal use was probably not originally distinguished from religious use.

In the *Atharva Veda* (1200 to 1000 B.C.), cannabis was collected as one of the five sacred plants. It was traditionally considered sacred in Tibet and used to facilitate meditation and highlight consciousness in all aspects of the ceremony. There is no evidence regarding the use of psychotropic properties of cannabis by the Arabs before the ninth century. However, Arab scholars translated the Greek texts of Dioscorides and Galen, and became familiar with the medicinal properties of cannabis [3, 5]. The Greeks and the Romans also grew cannabis to obtain fibre. Early texts on herbal medicines (*De Materia Medica*) were summarised by Dioscorides (40 - 90 A.D.) and by Galen (129 - 216 A.D.), who wrote of cannabis in the 2nd century A.D. in *"De Alimentorum Facultatibus"*.

Cannabis appeared in the 1788 New England Dispensatory, which included elements of Dioscorides herbal pharmacopoeia; therefore, cannabis was a common pain remedy in Western medicine in the 1800s [6,7], before aspirin was popularised.

In the 19th century, the interest of the therapeutic potential use of cannabis to Western medicine is primarily due to an Irish physician, William Brooke O'Shaughnessy [8]. Thus, the treatment of general pain, muscle spasms, or stomach cramps is one of the main medicinal uses of cannabis in the UK. However, their use declined in the early 20th century, due to their psychoactivity and effects on behaviour; as a result, cannabis was removed from the British Pharmacopoeia in 1932 and prohibited in 1973 by the Misuse of Drugs Regulation, which is classified in Schedule 1.

In spite of restrictions, renewed interest in the pharmacology and potential therapeutic use of natural cannabinoids was induced by the isolation and elucidation of natural cannabinoids from marihuana extract.

From the 1960s, the isolation of pure compounds from mixtures and elucidation of the structure of these compounds were possible due to the availability of novel chromatography and NMR spectrometric methods. Thus, many cannabinoids were isolated, including cannabidiol [9], Δ9−THC, which was reported by Gaoni and Mechoulam in 1964 [10] (**Fig. 1**), and the subsequent discovery of other natural cannabinoids [10, 11].

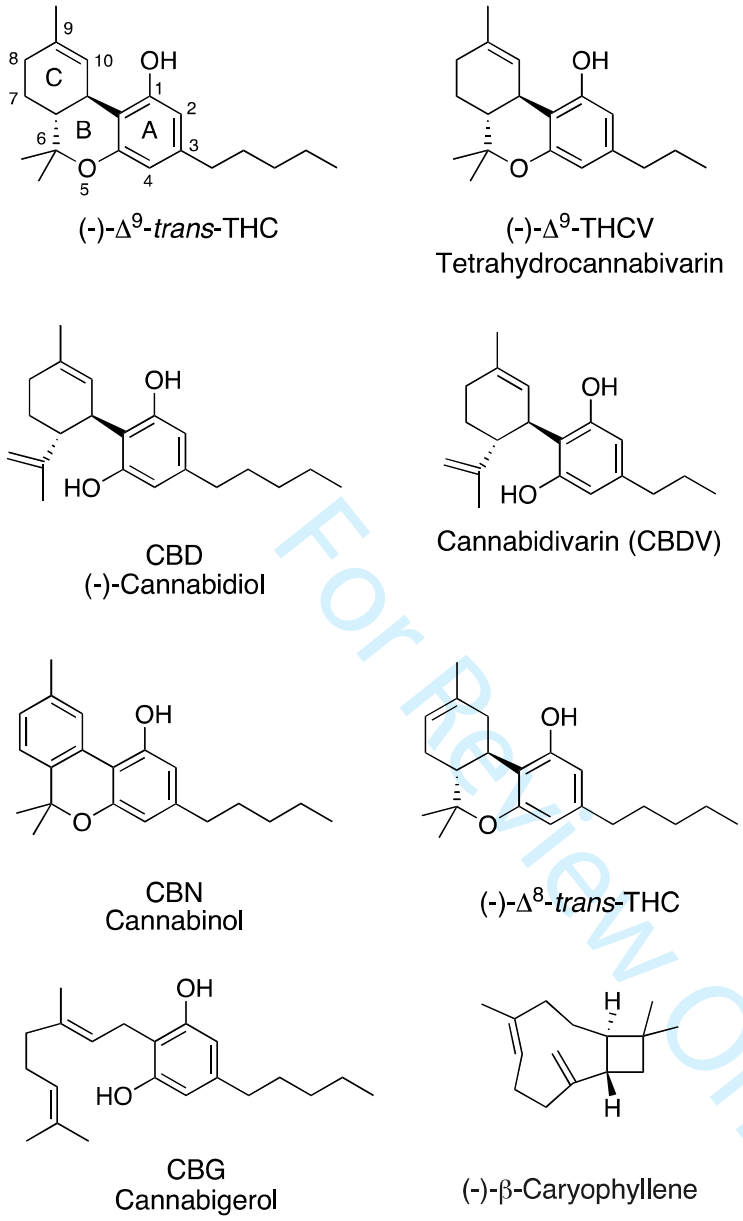

Fig. (1). Phytocannabinoids

Thereafter, the development of synthetic cannabinoid receptor ligands, such as CP55,940 [12] (**Fig. 2**) in the 1980s, and the identification of specific Δ9-THC binding sites in the human CNS [13], led to the identification of the first cannabinoid receptor. The existence of this receptor was confirmed when Howlett showed that cannabinoids decreased cAMP in neuroblastoma cell cultures [14], suggesting mediation by a Gi/o-coupled receptor [15-17].

The discovery of this cannabinoid receptor and the availability of potent cannabimimetics motivated the search for endogenous ligands capable of binding to them. Thus, the identification of the first cannabinoid receptor (CB1), cloned in 1990 [18], led to the isolation and characterisation of the first endocannabinoid two years later, the arachidonoylethanolamide (AEA), named anandamide, from the

Sanskrit 'internal bliss' [19]. The CB2 receptor was identified and cloned in 1993 [20]. Afterwards, a family of lipid transmitters that serve as endogenous ligands for the CB1 and CB2 receptors was identified.

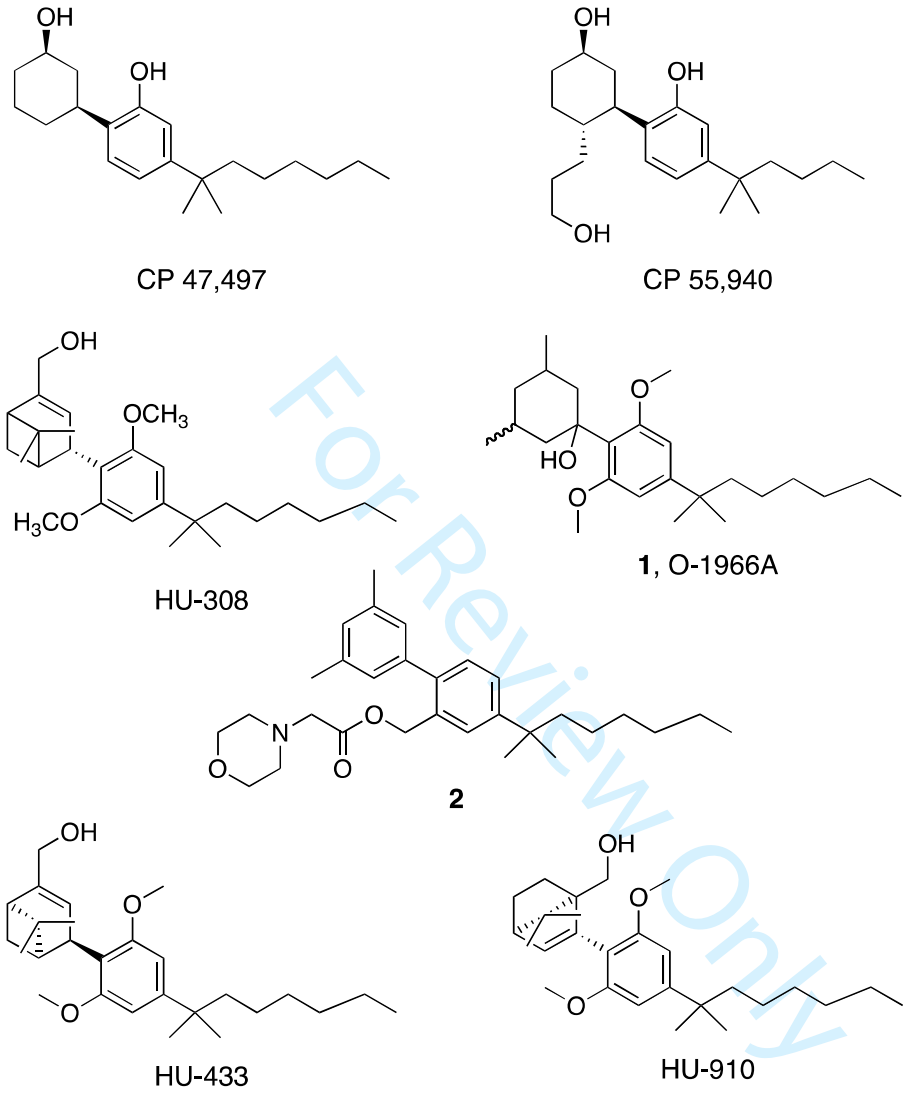

Fig. (2). Selected CP55,950 analogs

In this context, this article will review the current knowledge of the therapeutic potential of cannabinoid receptors as targets in Alzheimer's disease and other less well-known diseases, including multiple sclerosis, bone metabolism, and Fragile X syndrome. The therapeutic applications will be addressed through the study of cannabinoid agonists acting as single drugs and multi-target drugs, highlighting the CB2 receptor agonist.

2. Endocannabinoid system

The endocannabinoid system is a complex endogenous signalling system that comprises transmembrane endocannabinoid receptors, their endogenous ligands (the endocannabinoids), the specific uptake mechanisms and the enzymatic systems related to their biosynthesis and degradation.

The endocannabinoid system (eCS, a term introduced by Di Marzo & Fontana in 1995) [21] plays significant functions in many biological processes, in both normal and pathological conditions that affect the physiology and pathology in the central (CNS) and peripheral [22] nervous systems (for reviews: [22,23]).

The cannabinoid receptors belong to the family of G-protein coupled receptors (GPCRs), one of the largest and most studied superfamily of transmembrane proteins [24]. CBRs have been described in many species, including humans, monkey, pig, dog, rat and mouse, but not in insects [25]. The percentage of identity between both CB1 and CB2 receptors and between different species is very variable. Human CBRs exhibit 68% amino acid sequence identity within the transmembrane region and 44% amino acid sequence identity throughout the total protein [26].

Initially, it was described that the distribution of CB1 was predominantly in the brain, whereas CB2 was localised in peripheral cells and tissues derived from the immune system. However, along the years, it has been demonstrated by the scientific community that both receptor are widespread along the human body being involved in a great number of biological processes (Fig. 3). CB1 receptors are the most abundant G protein-coupled receptors in the central nervous system, expressed in both neurons and glial cells, where they regulate neurotransmitter release and act as modulators of excitatory and inhibitory neurotransmission. Regarding the distribution of the CB1 receptor in the CNS [27], this receptor has been found in the hippocampus, some olfactory regions, the caudate, putamen, accumbens nucleus, the substantia nigra pars reticulata, globus palidus, and the horizontal limb of the diagonal band [13, 28]. Moreover, CB1 receptors are also found in peripheral tissues, such as the cardiovascular and reproductive systems, as well as the gastrointestinal tract [22,29-33]. Thus, CB1 is expressed in the heart, uterus, testis, liver and small intestine, as well as in immune cells [22] and adipose tissue [34]. CB1 receptors regulate important brain functions, including cognition and memory, emotion, motor control, feeding, and pain perception [26] playing an important role in energy balance and metabolism [35].

CB2 receptors are mainly expressed in peripheral tissue. Initially, this receptor was localised in cells of the immune system, which are responsible for the release of cytokines. These receptors were localised on immune cells such as monocytes, macrophages, B-cells, and T-cells [36-39].

In relation to the nervous system, CB2 receptors are mainly located in microglia [40], even though relatively low CB2 receptor expression has also been identified in some neurons [41]. CB2 receptors were found also in other peripheral organs, such as the muscle, liver, intestine and testis [22]. Several studies have reported that CB2 receptor expression might be induced in glial cells (reactive microglia) in response to different damaging conditions associated with local inflammation [42].

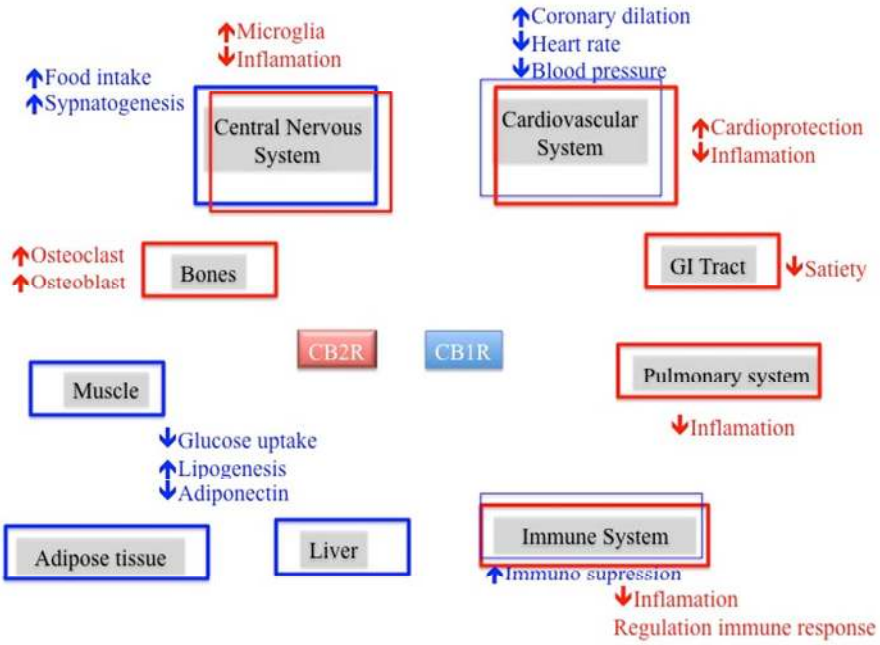

Fig. (3). Schematic representation of the functional role of CB1 and CB2 receptors

In the last decade, increasing evidence has shown that cannabinoid receptors may act as CB1-CB2 receptor heteromers in the brain [43]. In fact, the expression of CB1-CB2 receptor heteromers was determined in a variety of brain regions, such as the nucleus accumbens, pineal gland and globus pallidus [43]. The first-generation of allosteric modulators of CB1 receptors, their structure-activity relationships, signalling pathways and the allosteric binding site(s) on the CB1 receptor have been recently reviewed [44].

In addition, the formation of A2aR-CB1R heteromeric complexes has also been described [45, 46]. Other complexes have been found, as CB1R and 5-HT2aR form heteromers that are expressed and functionally active in specific brain regions involved in memory impairment [45].

The endocannabinoid system is a highly complex organisation where endocannabinoid molecules can interact with 7-transmembrane receptors (CB1, CB2, GPR18, GPR55 ("supposed CB3") [47,48] and GPR119 [49]), nuclear receptor (PPARα, PPARβ/δ and PPARγ) [50, 51] and transmitter-gated channels (TPRV1) [52,53]. The ECS appears to interact with other neurotransmission systems, including the serotonin (5-HT3) receptor, the N-methyl-D-aspartate (NMDA) receptor and nicotinic acetylcholine receptors (nAChRs) [see reviews 54]. Furthermore, the endocannabinoid system has also an important role in the signalling of rewarding events through to the dopaminergic mesolimbic system which is the brain neurotransmitter system that is most clearly involved in this type of process [55], as well as the stimulation or inhibition of GABA release, depending on the dose and brain regions, probably *via* dopaminergic modulation [56].

Modulation of cannabinoid signalling by interactions with various serotonergic receptors, such as 5-HT2A [57] or 5-HT4 receptors [58], has been also demonstrated.

Recently, the role of endocannabinoid signalling in the different effects of acupuncture have been reviewed [59].

2.1. Endocannabinoids

Endocannabinoids are a family of lipid transmitters, which act as endogenous ligands and activate the CB1 and/or CB2 receptors. The brain produces at least five endogenous ligands that possess an affinity for cannabinoid receptors (**Fig. 4**). These endocannabinoids are amides, esters and ethers of long chain polyunsaturated fatty acids, which act as new lipid mediators. The first endogenous ligands for CB receptors were discovered and characterised as arachidonoylethanolamide (Anandamide, AEA) [19]. AEA binds to both CB1 and CB2 receptors [60], but its affinity for the CB2 receptor is approximately four-fold lower than for the CB1 receptor [61] (Table 1). Subsequently, the fatty acid ester 2-arachidonoylglycerol (2-AG) was described [62, 63]. 2-AG is a full agonist of both CB1 and CB2 receptors [64, 65]. Although it exhibits a lower affinity for CB1 than anandamide, it is present in the brain at higher levels. Whereas AEA is a partial CB1 receptor agonist, 2-AG is considered the primary endogenous agonist for CB1 and CB2 receptors with similar affinity for both.

Table 1.

Other possible endocannabinoids have been proposed, such as a fatty acid ether, 2-arachidonylglyceryl ether (Noladin ether) [66], O-arachidonoylethanolamine (Virodhamine) [67] and N-arachidonoyldopamine (NADA) [68], but their natural occurrence and roles are still unclear.

For some years, it has been reported that endocannabinoid ligands are exclusively synthesised on demand to act on cells located near their site of biosynthesis, and subsequently inactivated by the action of specific degradation enzymes. However, recent studies have shown intracellular stores of anandamide in adiposomes with higher level than in the extracellular space [69, 70].

The levels of endocannabinoid ligands are also controlled by specific enzymes as FAAH for AEA and MAGL for 2arachidonoylgycerol [71-75].

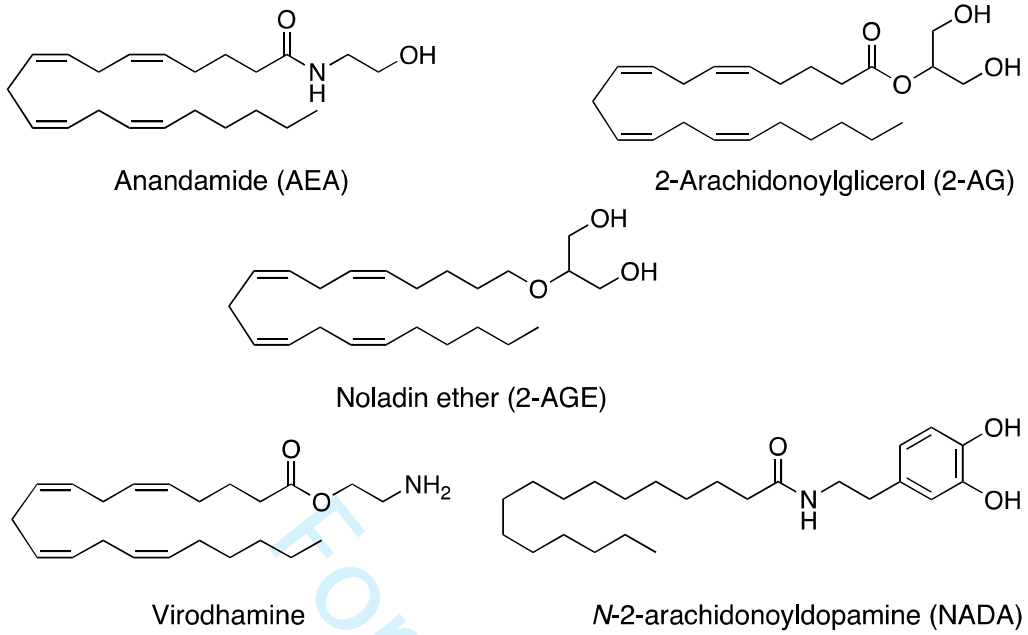

Fig. (4). Endocannabinoids

2.2. Cannabinoid receptor ligands

Taking into account the major interest in cannabinoid agonists for diseases, which are included in this report, this section will focus mainly on these ligands, with special emphasis on the properties of agonists against the CB2 receptor and in the analysis of the most important aspects of their activity and selectivity (For recent reviews see: [76-80]).

Cannabinoid receptor antagonists have also been reviewed elsewhere, especially those compounds of the 1,2-diarylpyrazole class, where the most representative compound is SR141716A (Rimonabant; Acomplia®) reported in 1994 by the Sanofi group as a selective CB1 antagonist [81-84] and AM251 used as a reference derivative in pharmacological assays such as CB1-selective receptor antagonists. In addition to 1,2-diarylpyrazoles, aminoalkylindoles (AAIs) and analogue ligands appeared to be quite interesting, not only due to the selectivity of some derivatives but also due to their agonist or antagonist properties (**Fig. 5**). Thus, the aminoalkylindole AM630 was the first described CB2-selective antagonist derived from this class of compounds [85].

In addition, novel antagonists and inverse agonists of the CB2 receptor have been described; these are, however, outside the scope of the current review (for reviews on antagonist and inverse agonists, see [86-91]).

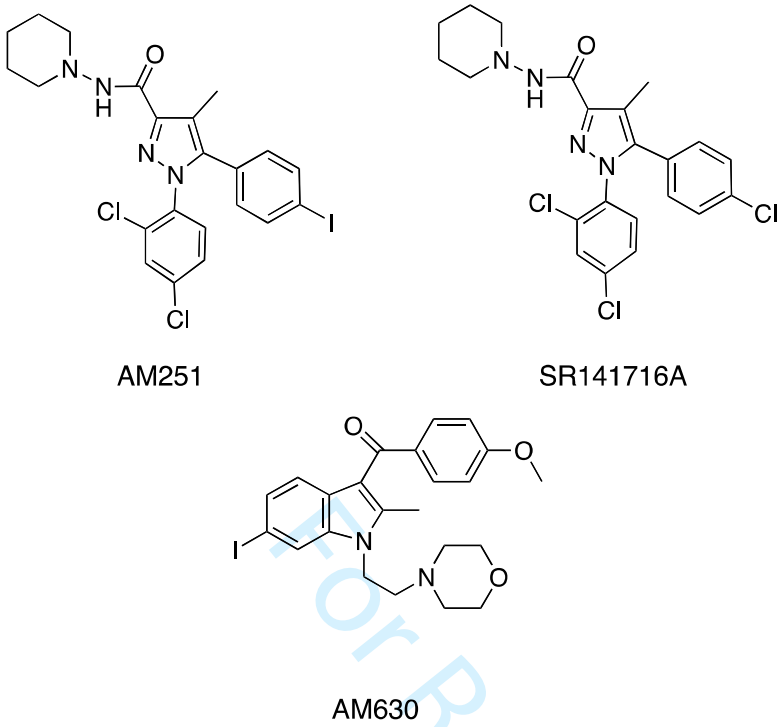

Fig. (5). Selected cannabinoid receptor antagonists

First generation cannabinoid receptor agonists can be classified into different groups: phytocannabinoids (**Fig. 1**), which include the natural products isolated from *Cannabis sativa*, synthetic analogues of natural cannabinoids, usually bicyclic systems lacking the pyran ring such as CP-55,940 [12] (**Fig. 2**), and aminoalkylindoles, with WIN55212-2 being the most representative member [61] (**Fig. 6**). In addition to these major groups, other chemical structures have proven cannabinoid properties and are the subject of intensive research.

In this review, synthetic cannabinoid receptor agonists have been classified into different groups from a chemical point of view as a function of the heterocyclic system.

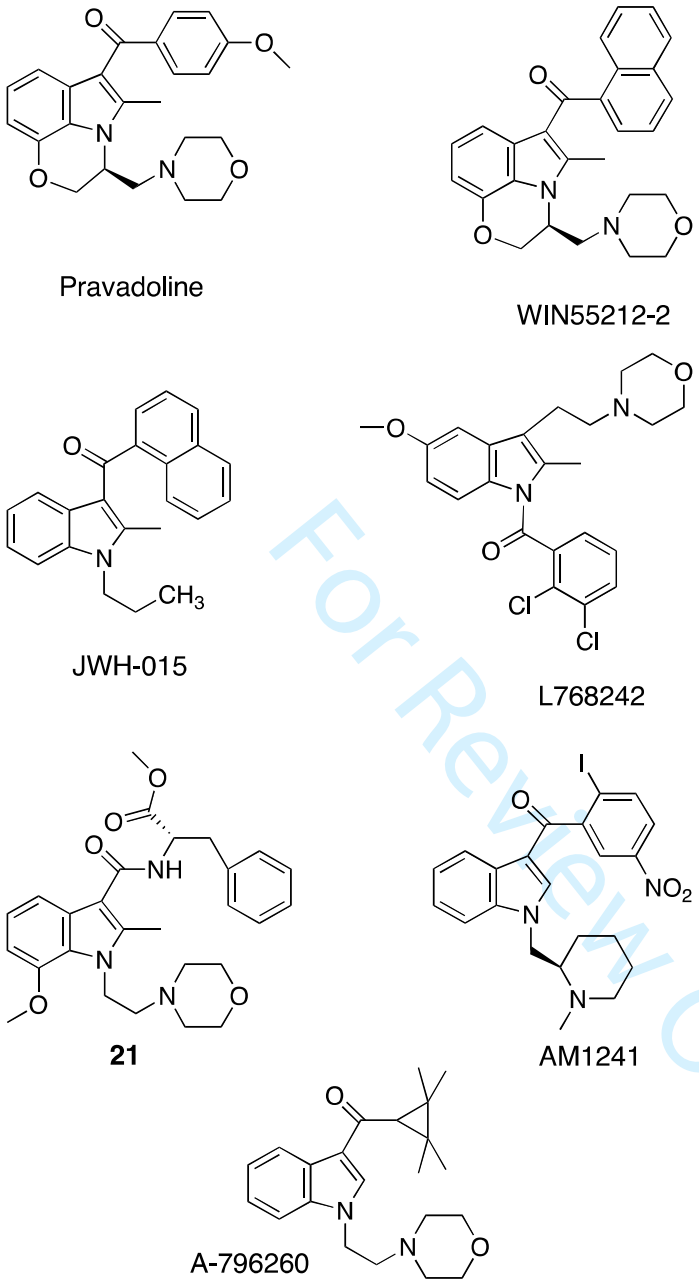

Fig. (6). Indole derivatives

Phytocannabinoids

The plant genus *Cannabis* is within the plant family *Cannabaceae* and produces annual herbs, which are mostly dioecious and rarely monoecious. It comprises a single but variable subspecies, or varieties such as *C. sativa*, *C. indica* and *C. ruderalis*. Its taxonomy is controversial and some authors designate these varieties as distinct species [4].

The hemp plant *Cannabis sativa* is very complex, according to the chemical composition, as it contains over 560 phytochemical compounds [80] among 18 different chemical classes, and contain at least 104 identified phytocannabinoids [92, 93].

The compounds derived from a diterpene structure can be classified into different groups (**Fig. 7**): Cannabigerol (CBG), Cannabichromene (CBC), Cannabidiol (CBD), (−)-Δ9-trans-Tetrahydrocannabinol (Δ9-THC), (−)-Δ8-*trans*-Tetrahydrocannabinol (Δ8-THC), Cannabicyclol (CBL), Cannabielsoin (CBE), Cannabinol (CBN), Cannabinodiol (CBND), Cannabitriol (CBT) and miscellaneous types [92]. The term cannabinoid was first used to describe the tricyclic natural compounds isolated from *Cannabis sativa,* of which (-)-Δ9-trans-THC (THC) is the principal psychoactive component.

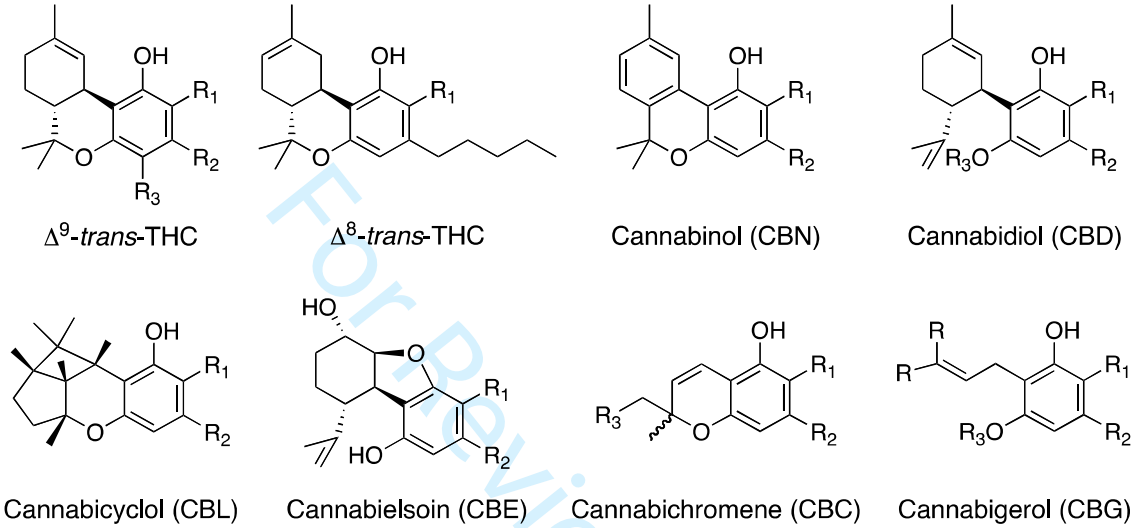

Fig (7). Structures of general types of *Cannabis sativa L*.

The phytocannabinoids are tricyclic terpenoid derivatives bearing a benzopyran moiety that include the natural product Δ9-THC (THC) [10] and other pharmacologically active constituents of the plant *Cannabis Sativa,* as well as analogue tricyclics lacking the pyran ring of THC (**Fig. 1** and **Fig. 2**). THC, the main psychotropic constituent of cannabis, is a CB1 and CB2 receptor partial agonist. Cannabinol binds with higher affinity in the nM range to both receptors, showing a modest selectivity [94], whereas the Δ9-THCV behaves as a potent CB2 receptor partial agonist [95]. In this regard, the most therapeutically attractive phytocannabinoids include cannabidiol (CBD) [96-98] [7], cannabigerol (CBG), Δ9-tetrahydrocannabivarin (Δ9-THCV) and cannabidivarin (CBDV) (**Fig. 1**). More recently, the (−)-β-caryophyllene [99], a natural bicyclic sesquiterpene of *Cannabis sativa*, has been examined in depth.

Synthetic analogues of natural cannabinoids

In 1982, a derivative of 3-phenylcyclohexanol (CP-47,497) was described as a cannabimimetic agent, with 3 to 28-times greater potency than THC [100] (**Fig. 2**). Subsequently, other cannabimimetics were developed by Pfizer based upon the dibenzopyran structure of THC, which includes the well-known ligand CP-55,940 [101]. This compound was found to be more potent than THC, is considered the prototypical example of this class and is extensively used for the evaluation of potential cannabinoid ligands in binding assays [13, 102]. A high number of tricyclic analogues of Δ9-THC and

analogues of CP-55,940, where the oxygen-containing pyran ring of THC has been removed, were synthesised and studied by different groups (**Fig. 2**).

Thus, a great number of tricyclic compounds with cannabinoid properties has been described, being Nabilone [103] and HU-210 [104, 105] the most representatives (**Fig. 8**). This last compound, bearing a hydroxymethyl group at C-9, was synthesised by Mechoulam *et al.* and is one of the most potent cannabinoid receptor agonists [106] (Table 1). Subsequently, some tricyclic compounds have been reported, along with structural analogues of Δ9-THC and Δ8-THC (**Fig. 1,** Table **1**) and CP55,940 analogues **(Fig. 2)**, which have shown selectivity to the CB2 receptor. Based on the similar effect (*in vitro* and *in vivo*) that Δ8-THC derivatives show in relation to Δ9-THC and taking into account that they are synthetically easier to prepare due to the increased stability of the Δ8-double bond, several analogues of the tricyclic Δ8-THC have been synthesised and studied, allowing a broad spectrum of compounds with cannabinoid properties that show selectivity for the CB2 receptor to be obtained. It is worth mentioning that compounds JWH-133 [107, 108] (**Fig. 8**) and the peripheral CB2 receptor agonist HU-308 [109] (**Fig. 2**) are the most representative members of this family, showing very high affinity for the CB2 receptor and low affinity for the CB1 receptor (Table **1**). The tricyclic compound JWH-133 has been characterised as a full agonist in a GTPγS-binding assay at the human CB2 receptor, and HU-308 has been also described as a selective full agonist in human CB2 cyclic GMP assays.

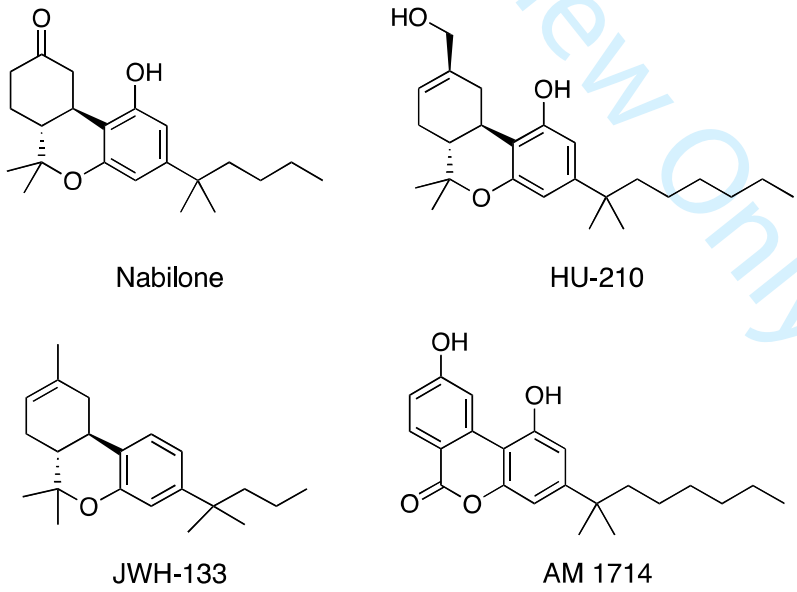

Fig. (8). Synthetic analogs of THC

Subsequently, new tricyclic derivatives of benzo[c]chromen-6-one have been published where the importance of lactone functionality in the observed CB2 selectivity is highlighted. Optimal receptor subtype selectivity of 490-fold and subnanomolar affinity for the CB2 receptor is exhibited by the 9-

hydroxyl analogue, AM1714 (**Fig. 8**). In functional assays, this compound behaves as an agonist, with activities comparable to WIN55212-2 and with analgesic activity [110].

Regarding CP-55,940 analogues (**Fig. 2**), some derivatives of resorcinol dimethyl ether have been reported. Among them, it is worth mentioning compound **1**, O-1966 (**Fig.** 6, Table **1**) since it has a selectivity of 220-fold for the CB2 receptor and very low affinity for the CB1 receptor [111].

Other groups have described new biaryl cannabinoids, where the phenol group has been replaced by a methyl morpholino acetate group, leading to compound **2** (**Fig. 2**), a 500-fold selective CB2 receptor agonist [112].

More recently, a structurally similar compound HU-910 (95, Fig. 5) has been reported as a potent CB2 agonist, which may exert protective effects in diseases related to inflammation and tissue injury [113]. The enantiomer of HU-308, called HU-433 (**Fig. 2,** Table **1**), shows higher potency than HU-308, although it has a lower affinity [114].

Five-member heterocyclic scaffold

Pyrazole Derivatives. Regarding heterocyclic derivatives of five atoms, a lot of compounds have been reported with cannabinoid properties with high selectivity for the CB2 receptor (Table **1**, **Fig. 9**). Thus, a series of derivatives obtained from the modification of heterocyclic system that include imines of oxazole, isothiazole and isoxazole have led to pyrazol-5-ylidene benzamide **3** (CBS0550), which exhibited high affinity and selectivity for the CB2 receptor and a greater aqueous solubility **[115]**. The Merck Research Laboratory has published different derivatives based on a 2,4-diaryl-1*H*-imidazole scaffold. Compound **4** behaves as a potent CB2 full agonist with improved pharmacokinetic properties [116].

Isoxazole Derivatives. Moreover, a series of derivatives that include the isoxazole ring has been described. The substituents on the heterocycle system define the activity on CB2 receptor or on FAAH enzyme. SAR studies of isoxazole-3-carboxamide derivatives have shown that the presence and position of the alkoxy chain influence the biological activity and the K_i affinity (Table 1, **Fig.** 9). Thus, the adamatylcarboxamide **5** (ALIAE809) and cycloheptyl carboxamide **6** have shown *in vivo* anti-inflammatory activities in a colitis mouse mode [117].

1,3-Thiazole Derivatives. Taisho Pharmaceuticals has previously published different thiadiazol-2-ylidene derivatives [118]. Thiazole **7** (Table **1**, **Fig. 9**) with the cyclopropylmethyl group at the 3-position and the presence of a bulky lipophilic group like *tert*-butyl at the 5-position exhibited higher CB2 affinities with a selectivity of 270 and excellent pharmacokinetics in rats [119].

1,2,4-Oxadiazole Derivatives. In order to optimise the pharmacokinetic properties of the 2-pyridone **8** (S-777469) (**Fig. 10**), a lot of derivatives of oxadiazole have been synthesised and SAR studies have been performed. The results led to the preparation of a potent and selective agonist of the CB2 receptor, the 5-propylamine oxadiazol **9** with excellent pharmacokinetic properties [120].

Structural modifications of the substituent at 5-position of 1,2,4-oxadiazole led to the oxadiazole **10** (Table **1**, **Fig. 9**) as a conformationally constrained molecule by the replacement of a propylamine

group of **9** by 4-piperidinyl, which improved the stability with a lower potency and greater selectivity [121].

Bicycles of Pyrazole Derivatives. Regarding condensed bicycles of pyrazole, Arena Pharmaceuticals has described cyclopenta[*c*]pyrazole-3-carboxamide derivatives. Through the introduction of a variety of alkyl and aryl substituents, (5*R*,6*R*)-tetrahydrocyclopenta[*c*]pyrazole-3-carboxamide derivative **11** (Table **1**, **Fig. 9**) was obtained. This derivative showed good activity and selectivity (> 8100) for CB2 receptor. In addition, this compound exhibited favourable ADME properties and was efficacious at reversing inflammatory pain after oral administration in the rat [122].

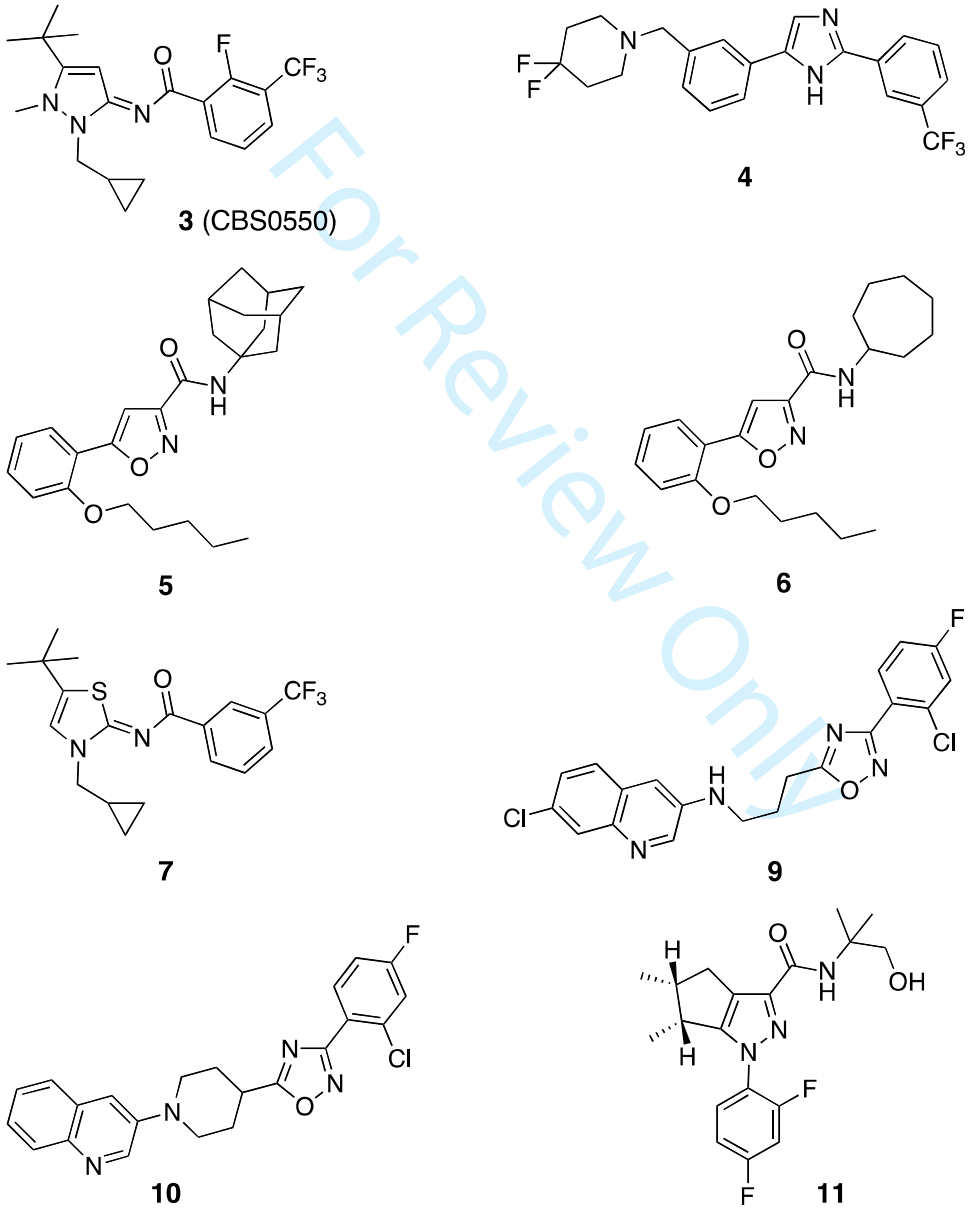

Fig. (9). Pyrazole, imidazole, oxazole, thiazole and oxadiazole derivatives

Six-member heterocyclic scaffold

Pyridine Derivatives. A different series of six-member heterocycles has been reported with cannabinoid properties with high selectivity for the CB2 receptor (**Fig. 10** and **11**).

The replacement with a pyridine ring (Table **1**, **Fig. 10**) of the central pyrimidine core of compound GW842166X (Table **1**, **Fig. 11**) [123, 124] used as a model led to the pyridine-3-carboxamide CB2 agonist **12** (Table **1**, **Fig. 10**), which demonstrated efficacy in an *in vivo* model of inflammatory pain [125].

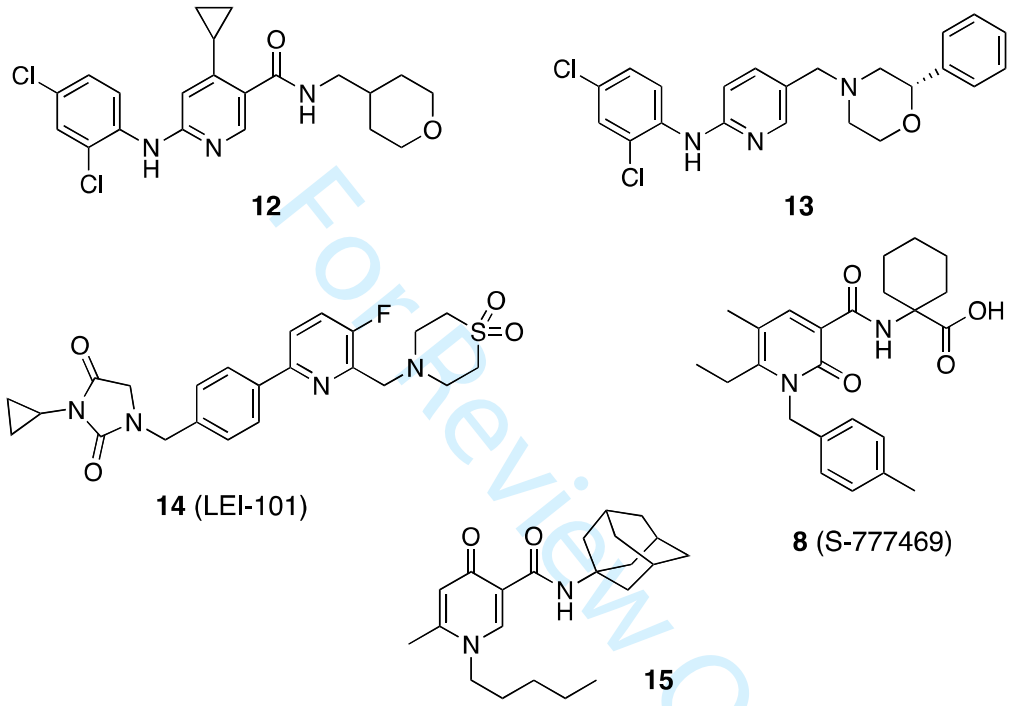

Fig. (10). Pyridine derivatives

Boehringer Ingelheim also discovered a series of 2-aminopyridines bearing a chiral morpholine ring. Compound **13,** where the carboxamide group is replaced by a methylamine group, was reported as a potent and selective CB2 receptor agonist and showed efficacy comparable to prednisolone in a mouse model of inflammation [126].

An orally available and peripherally restricted selective cannabinoid CB2 receptor agonist **14**, LEI-101 (Table **1**, **Fig. 10**), which prevents cisplatin-induced nephrotoxicity, has been described. This compound showed therapeutic potential in diseases that are associated with inflammation and/or oxidative stress, including kidney disease [127]. Other CB2 ligands based on the 3-carbamoyl-2-pyridone derivatives have been reported by changing the size of side chain at the 1-, 5- and 6-positions. The structure-activity relationship around this series led to the 2-pyridone derivative **8** (**S-777469**), as a selective CB2 receptor agonist [128].

Finally, another series of 3-carbamoyl-**4-pyridones**, as exemplified by the adamantyl-1,4-dihydropyridine-3-carboxamide **15** (Figure 9, Table **1**), has also been described as potent and selective

CB2 receptor agonists [129].

Pyrimidine Derivatives. Another interesting ligand derivative of *5-pyrimidinecarboxamide,* GW-842166X, has been described by GlaxoSmithKline as a selective CB2 receptor agonist. The pyrimidine-5-carboxamide GW-842166X (Table **1, Fig. 11**) is the most interesting compound for the treatment of inflammatory pain [123, 124].

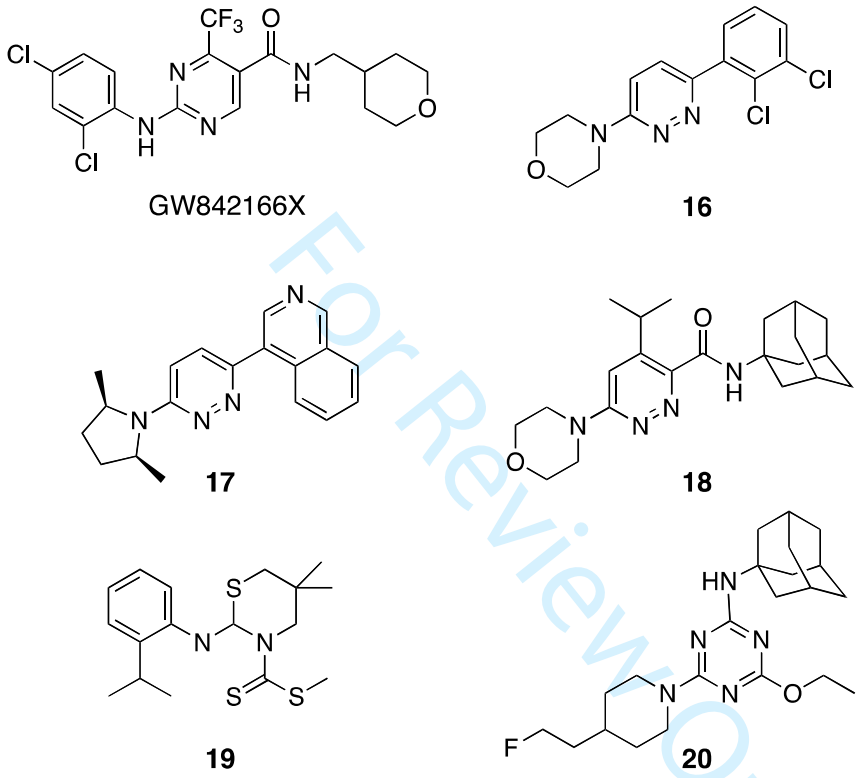

Fig. (11). Pyrimidine, pyridazine, thiazine and triazine derivatives

Pyridazine Derivatives. A series of 2-Amino-5-arylpyridazine-*3-carboxamides* had been identified from high-throughput screening studies as CB2 agonists. Substitution of the pyridine ring [130] with a pyridazine led to the discovery of a number of analogues with metabolic stability (compound **16** as representative example). The introduction of the cis-2,5-dimethylpyrrolidine group in pyridazine ring (compound **17)** (Table **1, Fig. 11**) led to an increase in CB2 activity [131].

The design of a series of CB2-selective agonist derivatives of pyridazine-3-carboxamides has been performed using GW842166x as a model. Several derivatives obtained exhibited enhanced potency and high selectivity at the CB2 receptor over the CB1 receptor. Specifically, *N*-adamantanylpyridazine-3-carboxamide **18** (Table **1, Fig.11**) showed the highest CB2 agonist activity and remarkable CB2 selectivity (Selectivity Index > 2700) [132].

1,3-Thiazines Derivatives. A series of 1,3-thiazines has been synthesised with the objective of optimising cannabinoid properties. The most potent compound, 2-phenylimino-1,3-thiazine **19** (**Fig.**

11), displays *ki* values of >5000 and 9 nM for the CB1 and CB2 receptors, respectively (Table **1**). Functional assays indicate that this thiazine behaves as a cannabinoid receptor agonist with analgesic activity due to activation of the CB2 receptor [133, 134].

1,3,5-Triazine Derivatives. Finally, a series of 2,4,6-Trisubstituted 1,3,5-triazines was identified as a potent CB2 agonist by 3D ligand-based virtual screening [135]. Subsequently, an additional series with enhanced water solubility was described. The most interesting compound was the adamantanylamino-1,3,5-triazine **21** (**Fig. 11,** Table **1**), which induced decreased cell viability in prostate and leukaemia cell lines and diminished the proliferation of C8161 melanoma cells [136].

Bicyclic [6+5] heterocyclic core

Indole Derivatives. In relation to first generation cannabinoids, other classes of cannabimimetics include the aminoalkyldindoles developed by Sterling Winthrop as potential non-steroidal anti-inflammatory agents. In 1991, the unexpected inhibition of the electrically simulated mouse vas deferens by Pravadoline was published [137]. Other indole derivatives were developed with increased cannabinoid potency, like WIN55212-2, which it is a potent CB1 and CB2 receptor agonist [61, 138]. A great number of indole derivatives have been synthesised by structural modifications of all positions of the ring [139-142].

Several groups have worked in the development of selective ligands for the CB2 receptor. These efforts have allowed the description of two different series of cannabimimetic indoles, with the most representative compounds being 2-methyl-1-propyl-3-(1-naphthoyl)indole (JWH-015) [143] and 1-(2,3-dichlorobenzoyl)-2-methyl-3-(2-[1-morpholino]ethyl)-5-methoxyindole (L768242) [144]. These derivatives have high affinity for the CB2 receptor and low affinity for the CB1 receptor (**Fig. 7**, Table 1). Functionally, L768242 displays partial agonist activity in human CB2 receptor-mediated inhibition of forskolin-stimulated cAMP release assays [145]. Makriyannis' group also discovered another interesting cannabimimetic aminoalkylindole, AM1241, which has been found to exhibit high affinity for human, rat and mouse CB2 receptors [146]. The efficacy of AM1241 in models of inflammatory [147] and neuropathic pain [148] has been shown to be mediated by the CB2 receptor, since the effects are blocked by CB2 receptor antagonists but not by CB1 antagonists.

Other series of indole derivatives have been published by Bristol-Myers Squibb, with the most interesting being the indole-3-carboxamide **21** (Table 1, **Fig.** 6). This derivative has been described as a very selective CB2 agonist that inhibits pro-inflammatory responses in a murine model of acute inflammation [149].

Moreover, a new series of indolylketones has been described by Abbot [150, 151]. Thus, an interesting new ligand derivative of indole, A-796260 **(Fig. 6,** Table **1)** has been described as a potent and selective CB2 agonist with anti-hyperalgesic and anti-allodynic properties in models of chronic inflammatory and neuropathic pain [150].

Benzo[d]imidazole Derivatives. New series of benzo[d]imidazole CB2-receptor agonists have been described (**Fig. 12**). The group at the 2-position determines the level of agonism, ranging from inverse

agonism to partial agonism or full agonism [152]. The most promising derivative is the 5-sulphonylbenzo[*d*]imidazole **22** (**Fig. 12,** Table **1**), that showed activity as potent CB2 receptor agonist and excellent selectivity (>4000-fold) over the CB1 receptor [152].

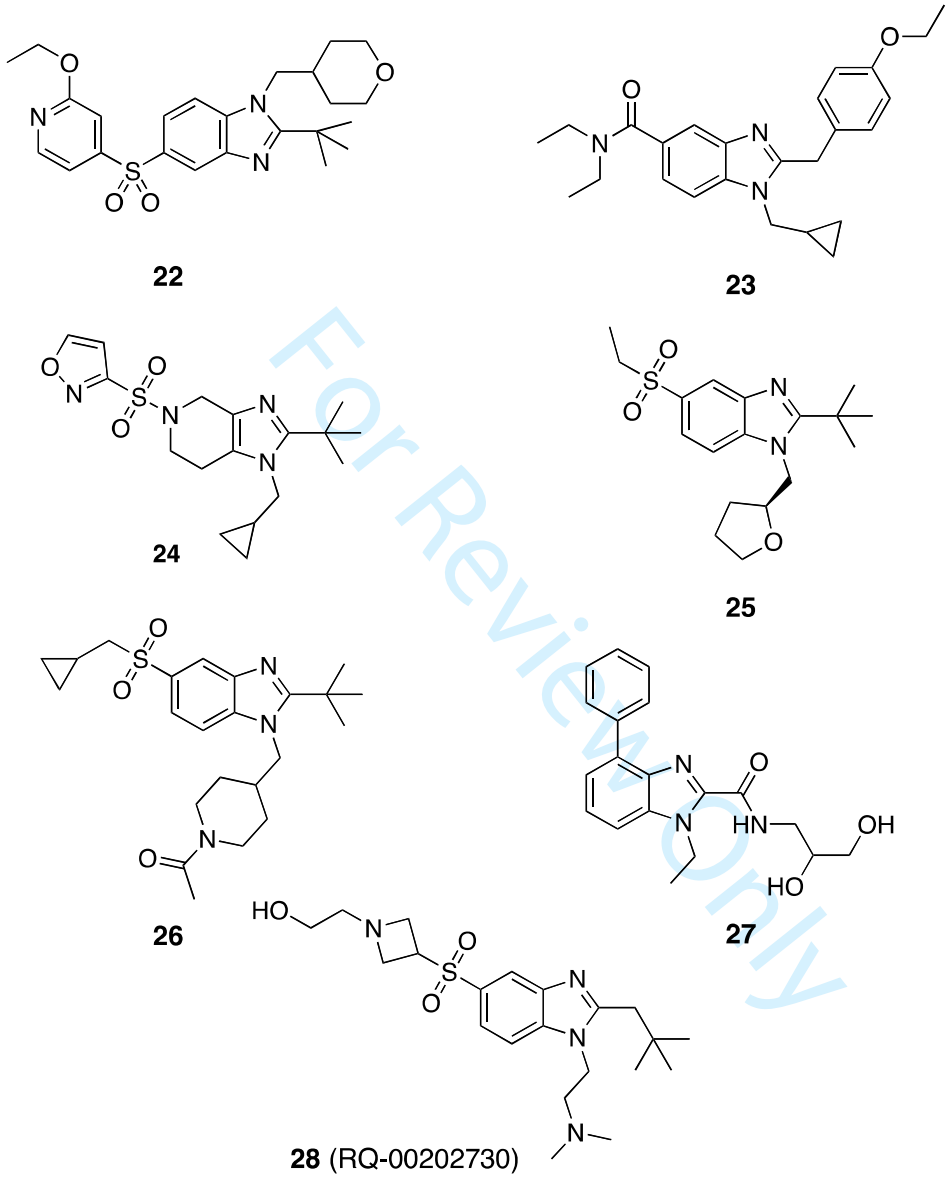

Fig. (12). Benzo[*d*]imidazole derivatives

Other CB2 agonists based on benzo[d]imidazole template have been reported by AstraZeneca from HTS studies. SAR studies around benzo[*d*]imidazole derivatives modifying the N-1 position with alkyl and aromatic groups led to the *1*-cyclopropylmethyl derivative **23** with optimal potency and selectivity [153].

Further studies by Pfizer on the benzimidazole core led to the preparation of a large number of sulphonyl derivatives. The introduction of a neopentyl group at the 2 position together with a

sulphonyl group at the 5 position led to one of the best compounds of these series, the 5-sulphonyl tetrahydorbenzo[*d*]imidazole **24**, (Table 1, **Fig. 12**), which behaves as a selective and potent full agonist of the CB2 receptor [154].

Other studies of the optimisation of the benzo[*d*]imidazole substituents led to the discovery of the fully CNS penetrant CB2 agonist 5-ethylsulphonylbenzo[*d*]imidazole **25** (Table **1**, **Fig. 12**) with reduced human hERG activity [155].

Moreover, other series of sulphonylbenzo[*d*]imidazole CB2 receptor agonists have been developed by Janssen Pharmaceuticals. Thus, the 5-sulphonylbenzo[*d*]imidazole cannabinoid agonists **26** (Table **1**, **Fig. 12**), showed an excellent binding affinity and selectivity for CB2. In addition, this compound displayed good, sustained activity in a chronic model of neuropathic pain, which delayed the onset of clinical symptoms in an experimental model for multiple sclerosis [156].

Other new series of benzo[*d*]imidazole-2-carboxamide derivatives have been described. The most potent 2-carboxamide **27** (**Fig. 12**) in this series was obtained by the introduction of an aliphatic amide bearing two hydroxyl groups [157].

Regarding indazole series, it is worth highlighting compound **28** (RQ-00202730, **Fig. 12**), which bears an aazetidin-3-ylsulphonyl group at position 5 of the benzo[*d*]imidazole ring. RQ-00202730 demonstrated the best overall profile in terms of potency and selectivity of this series over the CB1 receptor, showing a dose-dependent analgesic effect on TNBS-induced visceral hypersensitivity in rats by oral administration [158].

Pyrazolo[3,4-b]pyridine Derivatives. A series of heteroaryl-4-oxopyridine and 7-oxopyrimidine derivatives has been described [159]. An interesting condensed heterocycle was the pyrazole ring exemplified by the *N*-adamantanyl-4-oxopyrazolo[3,4-*b*]pyridine-5-carboxamide **29** (Table **1, Fig. 13**) that acts as a partial agonist.

Imidazo[1,5-a]pyridine Derivatives. Other series of condensed pyridine derivatives relate to imidazo[1,5-*a*]pyridines, which have been described by analogy with indole ligands as CB2 agonists. The 3-morpholinoimidazo[1,5-*a*]pyridine-1-carboxamides **30** and **31** (Table **1**, **Fig. 13**) were the most interesting derivatives, since they are selective for CB2 and show analgesic effects [160].

Purine Derivatives. Lilly Research Laboratories have described a series of purine derivatives as CB2 receptor agonists by analogy with the thieno[2,3-*d*]pyrimidine scaffold**.** The selectivity for CB2 and the metabolic profile were further optimised by the modification of a substituent at the 9 position of the purine ring. The (*R*)-4-methylpiperazin-1-ylpurine **32** and the 9-tetrahydropyranpurine **33** (**Fig. 13, Table 1**) showed potent oral activity in a preclinical model of joint pain [161,162.

Benzofurane Derivatives. Finally, it is worth highlighting another CB2 receptor agonist, **34** (**MDA7,** 1-((3-benzyl-3-methyl-2,3-dihydro-1-benzofuran-6-yl)carbonyl)piperidine) [163] (Table **1**, **Fig. 13**). This compound was active in a model of neuropathic pain and has been studied as a novel therapeutic target in the treatment of AD, as will be discussed later.

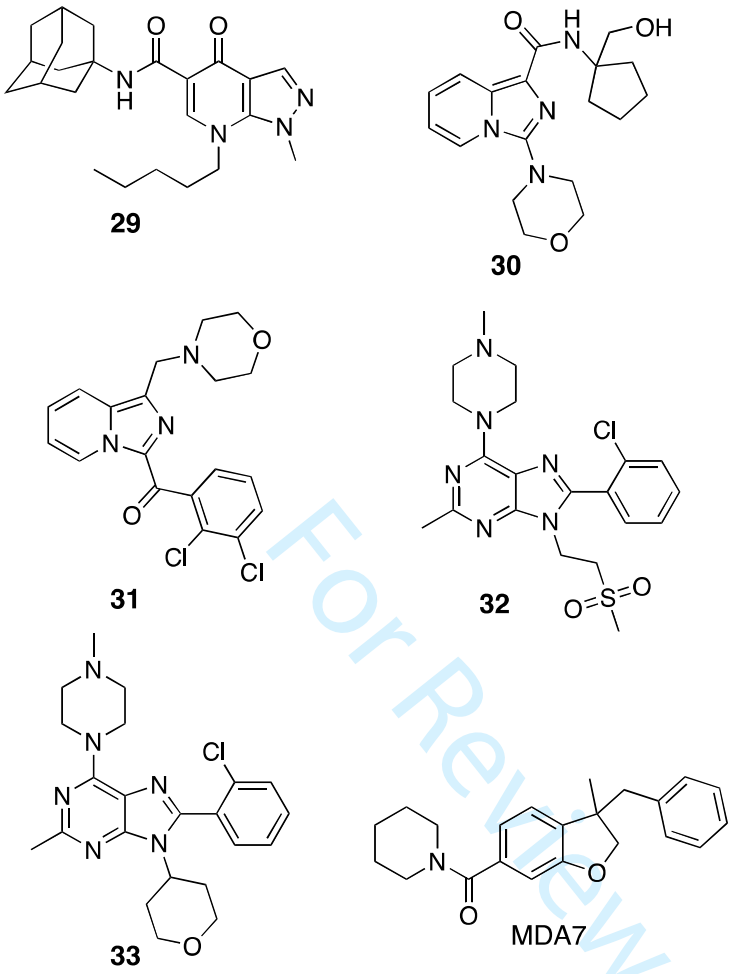

Fig. (13). Pyrazolo[3,4-*b*]pyridine, imidazo[1,5-*a*]pyridine, purine and benzofurane derivatives

Bicyclo [6+6] scaffold

Quinoline Derivatives. One of the earliest series of synthetic CB2 receptor agonists included bicyclo [6+6] scaffolds such as 4-oxoquinoline. Thus, it was reported that compound **35** shows a high affinity for the CB2 receptor with a selectivity that is >300-fold (Table 1, **Fig. 14**). This compound behaves as an agonist according to [35S]GTPg binding assays and functional studies [164]. Besides, other series of 4-oxo-1,4-dihydroquinoline-3-carboxamides have been reported. The most interesting derivative, **36**, is reported as a CB2 agonist with high selectivity for the CB2 receptor [165] (Table 1, **Fig.** 14).

The substituents at different positions of the heterocyclic system were studied; one of the most potent CB2 agonists of these series was **37** (**Fig. 14, Table 1**), which demonstrated analgesic effects in a mouse formalin test of acute peripheral and inflammatory pain [166].

Other published derivatives related to the decahydroquinoline CB2 agonists were reported by the Merck Research Laboratories. Optimisation of the amide substituent led to the selective CB2 agonist **38** (Table **1**, **Fig. 14**), which displayed a dose-dependent analgesic effect [167].

1,8-Naphthyridine Derivatives. The CB2 receptor agonists also include 4-oxo-1,8-naphthyridine-3-carboxamide derivatives [168] [165]. Different series of 1,8-naphthyridin-2(1*H*)-on-3-carboxamide analogues were described as CB2 receptor agonists. Thus, the naphthyridine **39** [164] (CB13, **Fig. 14**) induces apoptosis in colon cancer cells [169].

In another series of cannabinoid receptor agonists, the *2-oxo*-1,8-naphthyridine-3-carboxamide **40** (Table **1, Fig. 14**) exerted a CB2-mediated inhibitory action on immunological human basophil activation [170].

Finally, the structure–activity relationships for 4-oxo-1,8-naphthyridine-3-carboxamides were described with different substituents in position N-1 or C-6. The substituents in C-6 determined the functionality as agonist or antagonist/inverse agonist. An example of cannabinoid receptor agonist is derivative **41** (Table 1, **Fig. 14**) which showed good results for both CB2 affinity and selectivity [171].

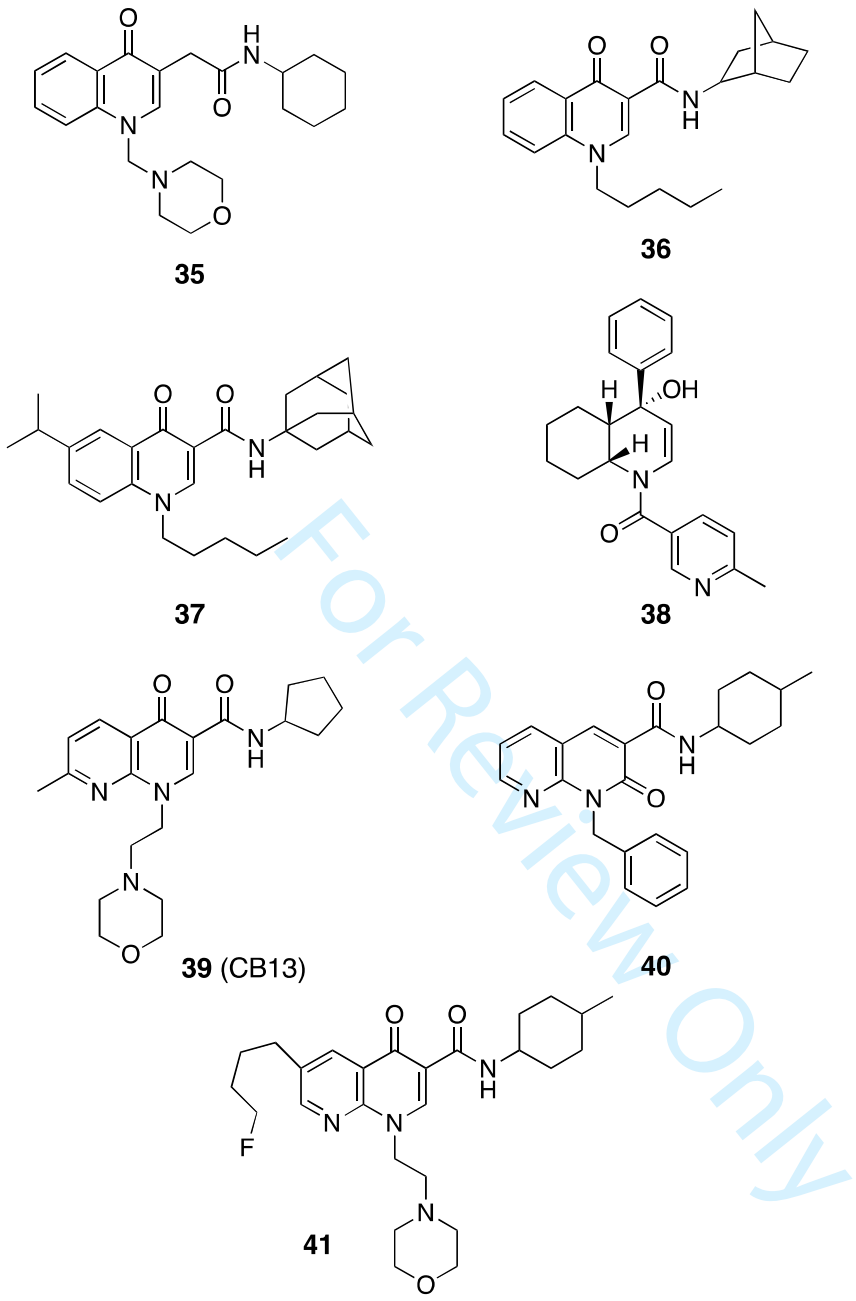

Fig. (14). Quinoline and naphthyridine derivatives

Tricyclic Heterocycle scaffold

Pyrido[4,3-b]indole Derivatives. The γ-carboline series was identified by a scaffold from the bicyclic benzimidazole core as mixed CB and CB2 agonists [172]. This new structural class of cannabinoid agonists showed good physicochemical properties and low CNS penetration. The ethylsulphonyl derivative **42** (Table **1**, **Fig. 15**) behaves as an agonist (Table 1) exhibiting significant anti-hyperalgesia in a rat inflammatory pain model with low levels of CNS penetration [172].

Pyrazolo[4,3-c]quinoline Derivatives. Very recently, the 2*H*-pyrazolo[4,3-*c*]quinolin-3(5*H*)-one scaffold was developed as constrained analogues of the 4-oxo-1,4-dihydroquinoline-3-carboxamide series with improved affinity and selectivity for the CB2 receptor. The focus of this series was the adamantanylmethyl-3-oxo-pyrazolo[4,3-*c*]quinoline **43** (Table 1, **Fig. 15**), which was found to protect mice against experimental colitis after oral administration [173].

Benzo[b]quinolizine Derivatives. Other tricyclic cannabinoid receptor agonist derivatives of quinolizin-6-one were described. Sch35966 **44** (8,10-bis[(2,2-dimethyl-1-oxopropyl)oxy]-11-methyl-1,2,3,4-tetrahydro-6H-benzo[b]quinolizin-6-one) displays high affinity for the CB2 receptor and good selectivity for CB2 receptor (Table 1, **Fig. 15**) [174].

Oxazino[2,3,4-ij]quinoline Derivatives. A tricyclic system based on the 4-oxoquinoline-3-carboxamide (**Fig.**14) and the indole scaffolds (**Fig. 6**) is the oxazino[2,3,4-ij]quinoline scaffold. Compound **45** (MT178) (**Fig. 15, Table 1**), in which a pyrrolidine moiety was introduced in position 10, showed high affinity and selectivity at the CB2 receptor (Table 1) [175]. MT178 produced analgesia in inflammatory and chronic pain models via CB2 receptors [176].

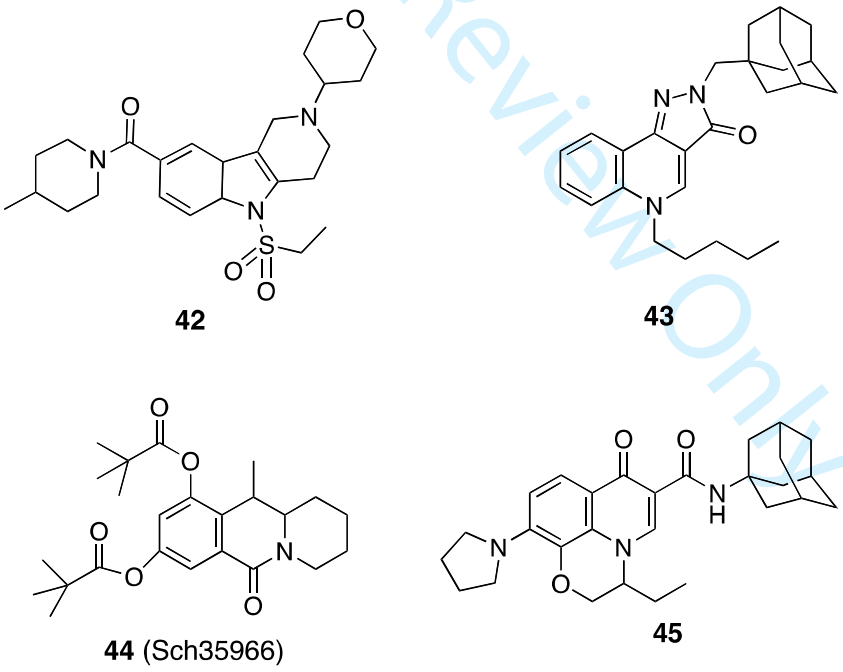

Fig. (15). Derivatives of tricyclic heterocycles

Other synthetic cannabinoid structures

Lastly, other different structures with high selectivity to CB2 receptors, such as sulphone [177], arylsulphonamide, [178] amidosulphone [179], 1,4-Diazepane and proline [180,181] derivatives should be mentioned, which behave as cannabinoid receptor agonists **(Fig. 14).**

Multi-target Cannabinoid Receptor Agonists

In the past, drug discovery was based on the premise that a promising drug candidate should exhibit high potency and high selectivity of action. However, the complexity of human diseases often requires multiple approaches for effective treatment, as it is well known for the treatment of different types of cancer. Nowadays, there is increasing recognition of the limitations of the premise that high selectivity and high potency are the most desirable properties for discovery of new therapeutic agent. Thus, during the last decade, a new paradigm, called multi-target directed ligand (MTDL), multiple ligands (DMLs) or multiple ligand strategy (MLS) has been developed as an innovative approach dedicated for complex diseases [182-184].

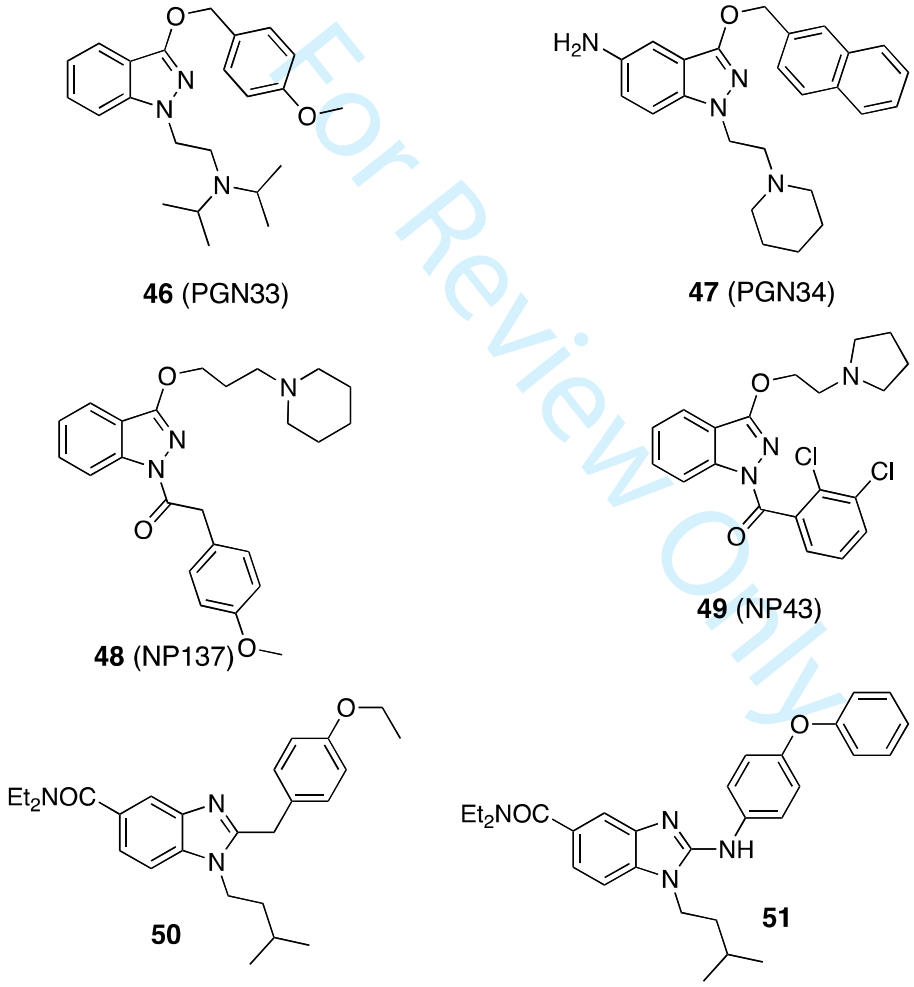

Fig. (16). Multitarget cannabinoid receptor agonists

In this respect, a novel series of indazole ether derivatives have been described as cannabinoid receptor agonists with simultaneous affinity towards other receptors (**Fig. 16**). Thus, using docking studies, a set of indazol ethers that behave as CB2 cannabinoid agonists and BuChE inhibitors have been designed. In particular, compounds **46** (PGN33) and **47** (PGN34) have emerged as promising

candidates as novel CB2 receptor agonists that simultaneously inhibit BuChE by a non-competitive or mixed mechanism, respectively. On the other hand, in addition, both molecules show antioxidant properties [185].

Subsequently, other series of indazole derivatives have been described as multi-target ligands (**Fig. 16**). Thus, some indazolylketone derivatives as **48** (PGN43) and **49** (PGN137) have been describe that behave as CB2 cannabinoid agonists and simultaneously shown BuChE and/or BACE-1 inhibition [186]. Moreover, new derivatives of aminobenzimidazole agonists as a second-generation of compound **50** have been designed as cannabinoid agonists with the inhibitory activity of AChE/BuChE (**Fig. 16**). Thus, derivative 51 shows micro- or sub-micromolar activities at both targets and excellent selectivity over CB1 and AChE, respectively [187].

3. Cannabinoid System in Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder defined as the loss of cognitive functions of sufficient severity to impair social and occupational functioning. It affects intellectual abilities such as memory, thinking, orientation, comprehension, calculation, learning capacity, language, and judgement. AD is usually chronic and irreversible and is currently the most common cause of dementia in the elderly, accounting for probably 60-70% of all dementias worldwide, followed by vascular dementia, mixed dementia, and Lewy body dementia.

In 2015, epidemiological data estimated that the worldwide prevalence of dementia is around 47 million people and there are almost 9,9 million new cases annually, according to WHO data. According to these data, the number of people affected by 2030 will be 75 million and will be 132 million by 2050 (see [188] and references therein).

It is, therefore, to be expected that the cost of taking care of these patients can be enormous, considering the actual level of incidence and future generations of patients affected by AD. Physical, emotional and economic pressures can cause great stress to families and carers, and support is required from the health, social, financial and legal systems. Besides the monetary cost, it is impossible to quantify the suffering of the families that take care of people with AD during years. Nevertheless, in 2015, the total global cost of dementia was estimated at US$ 818 billion. Despite the devastating effects of this disease, therapeutic options for treating AD remain limited, even though the drugs commonly prescribed today as a treatment for AD only provide temporary benefits related to improvement of symptoms, but do not prevent disease progression with only temporary benefit.

Currently, cholinergic drugs like acetylcholinesterase (AChE) inhibitors, including donepezil, rivastigmine, and galantamine (**Fig. 17**), are currently the most prescribed pharmaceuticals for AD [189-192], even though their therapeutic use remains limited to a symptomatic approach. The only alternative for the treatment of the Alzheimer's disease in the moderate to severe stages is Memantine, a non-competitive antagonist of the glutamatergic NMDA receptor [193].

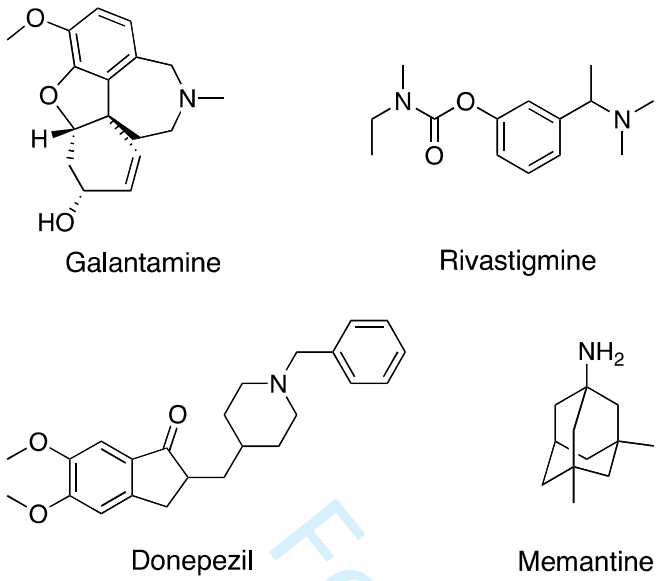

Fig. (17). Therapeutic drug used to treatment Alzheimer's disease

Alzheimer's disease is a process characterised morphologically by the presence of senile plaques (fibrillar β-amyloid) and by the presence of neurofibrillary tangles (hyperphosphorylated and nitrated tau proteins).

Neuritic plaques are extracellular lesions composed of a central core of aggregate amyloid-β peptide whose accumulation in the brain that is produced proteolytically from the amyloid precursor protein (APP) initiates a cascade of events that originate neuronal dysfunction, neurodegeneration and dementia. Neurofibrillary tangles NFTs are intracellular aggregated hyperphosphorylated forms of the microtubule-associated protein tau. Although the aetiology of these diseases has not been completely defined, both Aβ and hyperphosphorylated tau promote the progression of the pathological process, as these abnormal proteins are accumulated in the brain [194, 195]. Additionally other factors that include neuroinflammation, excitotoxicity and mitochondrial dysfunction that increase the production of reactive oxygen and nitrogen species (ROS and RNS) are involved in the pathogenesis of AD, meaning they could be used therapeutically.

Endocannabinoid signalling has been demonstrated to modulate the main pathological effects occurring during the neurodegenerative process that include protein misfolding, neuroinflammation, excitotoxicity, mitochondrial dysfunction, and oxidative stress [196].

3.1. Cannabinoid Agonists on Alzheimer´s disease

The endocannabinoid system suffers profound changes during neurodegenerative and neuroinflammatory disorders. The expressions of CB1 and CB2 receptors as well as biosynthesis of endocannabinoids are deeply affected (for reviews see [195, 197-200]).

Regarding CB1 receptors, a progressive loss of specific populations of neurons that express CB1 receptors during neurodegenerative processes, particularly in the hippocampus and basal ganglia, has been described [201],[202]. In line with these results, a decrease in CB1 receptor expression in the

triple transgenic mouse model of AD has been reported [203].

A large number of studies support the fact that the CB2 receptors are involved in AD pathology. In this sense, the physiological response mediated by the Endocannabinoid system is to counteract inflammation and therefore the CB2R activation [204, 205]. CB2 receptors are generally less expressed in the neurons of healthy brains, but their expression increases dramatically in reactive microglia and activated astrocytes during neuroinflammation [196, 206].

Thus, post-mortem brains from patients with AD have shown that CB2 receptors are up-regulated in cells that are associated with Aβ-enriched neuritic plaques [201, 202, 207, 208].

Moreover, transgenic models of AD have also revealed the over-expression of CB2 receptors in brain areas affected by AD-pathology [209]. Moreover, an increase in CB2 receptors was also observed in rats and C6 astroglioma cells pre-treated with Aβ42 [197, 210].

During the last few years, interesting and elegant studies have allowed new perspectives to be opened in the prevention and/or treatment of AD, with particular focus on cannabinoid receptor ligands (see reviews: [195, 197-199, 211-214]).

The reduction in CB1 receptors associated with neuronal loss along with the psychotropic effects of CB1 receptor agonists are the reasons why they are not usually considered plausible targets for neuroprotection.

In this context, this review will focus on the most recent and relevant publications of cannabinoid receptor agonists from the medicinal chemistry point of view published highlighting the last years, underlining the effect of CB2 agonists as candidates for AD.

A great variety of studies have provided experimental evidence about the potential therapeutic properties of cannabinoid receptor agonists in cellular models that simulate the different effects of AD. Most of these assays are focused on studying the capacity of CB2 receptors to modulate Aβ and hyperphosphorylated tau levels and the anti-inflammatory properties of CB2 agonists.

3.1.1. Effect of cannabinoids agonist on Aβ

Many *in vitro* studies in models of neuronal damage about the neuroprotective properties of endogenous and exogenous cannabinoids have been reported. Thus, endocannabinoids such as anandamide (AEA) and noladin ether, directly supplied to the cell culture or augmenting their availability through the administration of endocannabinoid reuptake inhibitors, are potent *in vitro* inhibitors of Aβ-toxicity, being effective at nanomolar concentrations [215]. However, the authors have concluded that these effects are exerted through a CB1-dependent, MAPK-mediated mechanism [215]. Other *in vitro* studies have shown that the endocannabinoid anandamide (AEA) protects neurons from inflammatory damage by CB1/CB2 receptor-mediated rapid induction of mitogen-activated protein kinase phosphatase-1 (MAKP-1) in microglial cells [216].

Regarding the phytocannabinoids, several *in vitro* studies have suggested a direct effect of THC on the reduction of Aβ aggregation and on the promotion of Aβ degradation [217, 218]. Similar results about the survival of neuronal cultures exposed to Aβ peptide were obtained with CBD [219] and with 2-AG

[218].

Moreover, several studies have shown that the mixed CB1/CB2 receptor agonists WIN55,212-2 (**Fig. 6**) and HU-210 (**Fig. 8**), and the selective agonists JWH-015 (**Fig. 6**), JWH-133 (**Fig. 8**), and HU-308 (**Fig. 2**) reduce the release of pro-inflammatory cytokines in microglial cell cultures exposed to different toxic Aβ peptides [220,221].

Thus, HU-210 was capable of preventing Aβ1-40-induced changes in microglial morphology and HU-210, WIN55,212-2 and JWH-133 significantly inhibited the microglial production of TNF-α, a known cytokine that participates in Aβ-triggered damage [201]. Moreover, JWH-133 and WIN55,212-2 promote microglial migration, which facilitates the phagocytosis of aggregated Aβ [221].

The protective properties of WIN55,212-2 were also demonstrated in Aβ-induced neurodegeneration in the rat hippocampus. WIN55,212-2 significantly improved memory functions and decreased the elevated levels of neuroinflammatory markers like TNF-α, activated caspase-3, and nuclear NFκB [222]. Moreover, WIN55,212-2, through CB2 receptors, inhibited iNOS and NO production, the release of chemokines (CXCL10, CCL2, and CCL5) and TNF-α from IL-1β-activated human foetal astrocytes [223].

Regarding the selective CB2 agonist, JWH-015 induced a decrease in CD40 receptor expression by mouse microglial cells in primary culture after exposure to interferon-gamma and prevented the Aβ-triggered production of pro-inflammatory cytokines [220]. In this sense, activation of CB2 receptors with the selective agonist JWH-015 facilitates Aβ phagocytosis by human macrophages in brain sections obtained from AD patients [224].

3.1.2. Effect of cannabinoid agonist on Tau hyper-phosphorylation

Different studies have been carried out to clarify the role of CB2 receptors in the modulation of tau hyper-phosphorylation. Thus, studies performed in cell cultures demonstrated that CBD inhibits hyperphosphorylation of the tau protein in Aβ-stimulated PC12 neuronal cells [225].

Other studies have shown that THC is able to reduce tau phosphorylation in N2a/APPswe cells [226]. Moreover, it has been shown that pre-treatment with CBD prevented the expression of proteins that are potentially involved in tau phosphorylation and A-beta production in GMSCs [227].

Early studies performed in cell cultures demonstrated that the mixed CB1–CB2 agonist WIN55,212-2 inhibited tau protein hyper-phosphorylation in Aβ-stimulated PC12 neuronal cells, but that this effect was mainly mediated by CB1 receptors. Moreover, a selective CB2 agonist JWH015 failed to modify tau hyper-phosphorylation under the same experimental conditions [228]. Nevertheless, JWH133, a selective agonist of CB2R, reduces the phosphorylation of tau and GSK3ß activity in HEK293 tau cells, but the effects of JWH133 on phosphorylation of tau and GSK3ß disappeared with compound C (AMPK inhibitor) or Prkaa2-RNAi [229].

3.1.3. Effect of cannabinoid agonist involved in other mechanism of AD

In addition to the above-described effects, other non-receptor-mediated roles of cannabinoids have been published. Thus, in relation to the cholinergic receptor, several studies have been published.

Thus, THC competitively inhibits AChE, preventing AChE-induced Aβ aggregation by binding in the peripheral anionic site of AChE, the critical region involved in amyloidogenesis [217].

Other studies have shown that cannabinoid agonists as THC and JWH133 are AChE inhibitors, showing a mixed type inhibition mode of action [230]. The non-selective agonists THC and the synthetic analogue CP-55,940 behave as inhibitors of AChE/BuChE, with marked selectivity for AChE or BuChE, respectively. The non-selective agonist CP-55,940 showed non-competitive inhibition, suggesting that this cannabinoid only binds to the peripheral site.

Nevertheless, analogues of natural cannabinoids, such as JWH-133 and indazole JWH-015, both with a high degree of selectivity for CB2 receptors, have shown inhibition only against AChE or BuChE, respectively [230].

3.1.4. Cannabinoids Agonists toward the clinic

A considerable number of biological studies have been performed with a small group of cannabinoid agonists with different animal models (Table **2**). The tested cannabinoids include endocannabinoids such as anandamide and noladin ether, phytocannabinoids such as Δ9-THC, cannabidiol (CBD) (**Fig. 1**), and synthetic cannabinoids such as WIN55,212-2 (**Fig. 6**), JWH-133, HU-210 (**Fig. 8**), ß-caryophyllene (**Fig. 1**) and MDA7 (**Fig. 13**).

Several studies have been performed using Aβ administration directly into the CNS in different transgenic animal models. Thus, endocannabinoids such as anandamide (AEA) and noladin ether, have shown that reduced Aβ-induced memory impairment in rats [231].

The neuroprotective properties of phytocannabinoids have also been demonstrated to prevent memory deficits in Aβ-injected rats and mice for mixed CB1/CB2 receptor agonists such as Δ9-THC and cannabidiol (CBD).

Thus, it has been shown that Δ9-THC significantly reduced Aβ and neurodegeneration in 5XFAD transgenic mice and that these effects are preserved in the presence of COX-2 inhibition and also elevates expression of neprilysin, an important endopeptidase for Aβ degradation [232].

On the other hand, it has been shown that the combination of THC and CBD exhibits a better therapeutic profile than each separately [233]. Thus, a combination of Δ9-THC and CBD reduced learning impairment and caused a significant decrease in soluble Aβ42 peptide levels and a change in plaque composition in AβPP/PS1 mice [233].

Moreover, a reduction of free radicals and mitochondrial activity was also suggested in a mouse model of tauopathy exposed to chronic treatment with the Sativex® mixture of Δ9-THC and CBD [234], although no evidence of the direct implication of CB2 receptors or other receptors in such effects was provided. Therefore, Δ9-THC and CBD natural mixture present in Sativex®, even after a short administration in animals with present behavioural and pathological abnormalities, improves the phenotype, oxidative stress, and deposition of proteins.

Additionally, CBD is capable of attenuating Aβ evoked neuroinflammatory responses. Thus, CBD inhibited GFAP mRNA and protein expression in mice inoculated with human Aβ (1-42) peptide into

the right dorsal hippocampus. Also, CBD impaired iNOS and IL-1beta protein expression, and the related NO and IL-1beta release [235].

Likewise, different studies with synthetic cannabinoid agonists have demonstrated their neuroprotective properties. Thus, both CBD and WIN55,212-2, after subchronic administration for 3 weeks, were able to prevent the learning of a spatial navigation task and cytokine gene expression in β-amyloid-injected mice. The studies show that CBD is able to modulate microglial cell function *in vitro* and induce beneficial effects in an *in vivo* model of AD [221].

Other studies have reported that the CB1/CB2 cannabinoid agonist WIN55,212-2 prevented the cognitive impairment and loss of neuronal markers induced by βA25-3 i.c.v. administration to Wistar rats. In addition to this, microglial activation was also prevented by WIN55,212-2 administration. It is currently well-known that microglia play a pivotal role in the response to amyloid deposition, modulating the immunological response of the brain. It seems reasonable to think that the reported prevention of microglial activation also collaborates in the neuroprotective effect of WIN55,212-2 in this rat model of AD [201].

Other studies with WIN55212-2 have shown decreased Aβ-induced neuroinflammation in rats through the activation of both CB1 and CB2 cannabinoid receptors and the PPAR-gamma pathway [222]. This agonist significantly improved memory functions and decreased the elevated levels of neuroinflammatory markers like TNF-α, activated caspase-3, and nuclear NFκB. The use of antagonists confirmed that neuroprotective effects were partially mediated by CB1 and CB2 receptors [222]. Moreover, JWH-133 and WIN55,212-2 significantly reduced the levels of COX-2, TNF-α, and increased Aβ clearance (Martin-following prolonged oral administration in Tg APP mice [236]).

However, a study where WIN55,212-2 and the selective CB2 cannabinoid receptor agonist JWH-133 were evaluated indicated that JWH-133 was able to reduce cognitive impairments and decrease microglial activation in Tg2576 mice, while WIN55,212-2 was ineffective [236].

The vascular response of the CB1/CB2 cannabinoid receptor agonist WIN 55,212-2 and the CB2 selective agonist JWH-133 has been studied in Tg APP mice. The results have shown that both cannabinoid agonists were able to prevent decreased ACh relaxation in the presence of Aβ [237].

On the other hand, the selective CB2 cannabinoid receptor agonist JWH-133 has been evaluated in double AβPP/PS1 transgenic mice, a genetic model of Alzheimer's disease. The results obtained indicate that JWH-133 was able to reduce cognitive impairments associated with decreased microglial reactivity and the reduced expression of pro-inflammatory cytokines IL-1β, IL-6, TNF-α, and IFN-γ. In addition, JWH-133 lowered tau hyper-phosphorylation in the vicinity of Aβ plaques. Moreover, JWH-133 produced a decrease in the expression of hydroxynonenal adducts derived from lipid peroxidation, and enhanced the expression of SOD1 and SOD2 around plaques. In contrast, the chronic treatment with JWH-133 failed to modify the Aβ production or deposition in cortex and hippocampus [238].

Surprisingly, a study performed with a very potent CB1/CB2 receptor agonist cannabinoid HU-210

showed that it did not improve water maze performance and a contextual fear conditioning task in an APP23/PS45 double transgenic mouse model of AD. Furthermore, HU-210 did not show any effect on APP processing and Aβ generation, as well as neuritic plaque formation in the brains of transgenic mice [239].

A different full selective CB2 receptor agonist as ß-caryophyllene has been studied in APP/PS1 mice [240]. This CB2 agonist prevented cognitive impairment associated with reduced Aβ burden in both the hippocampus and the cerebral cortex. Moreover, beta-caryophyllene reduced astrogliosis and microglial activation as well as the levels of COX-2 protein and the mRNA levels of the pro-inflammatory cytokines TNF-α and IL-1β in the cerebral cortex [240]. The results obtained indicate that the anti-inflammatory effect of the β-caryophyllene involves CB2 receptor activation and the PPAR-γ pathway since the effect is reversed by the CB2 antagonist AM630 or the PPAR-γ antagonist GW9662 [240].

Other selective CB2 receptor agonists such as the **MDA7** have been performed in a model where the effects are induced by the bilateral microinjection of Aβ(1-40) fibrils into the hippocampal CA1 area of rats [241]. The results indicated an improvement of the expression of CD11b (microglia marker) and glial fibrillary acidic protein (astrocyte marker), and a reduction of the secretion of IL-1β, and the increase of CB2 receptors. Also, MDA7 promoted Aβ clearance and an improvement in synaptic plasticity, cognition, and memory [241].

Finally, the protective properties of multi-target CB2 cannabinoid agonist NP137 were also demonstrated in Aβ-induced neurodegeneration in TgAPP mice. Moreover, NP43 reduced the levels of iNOS and COX-2 protein, and the levels of the pro-inflammatory cytokines TNF-α and IL1b in the cerebral cortex using the 5xFAD mice as model of AD [186].

4. Cardiovascular disorder and atherosclerosis

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels, including cerebrovascular disease, atherocleroccoronary heart, and rheumatic heart among others. Heart attacks and strokes are usually acute events and are mainly caused by a blockage that prevents blood from flowing to the heart or brain. The most common reason for this is a build-up of fatty deposits on the inner walls of the blood vessels that supply the heart or brain. Strokes can also be caused by bleeding from a blood vessel in the brain or from blood clots. The cause of heart attacks and strokes are usually the presence of a combination of risk factors, such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol, hypertension, diabetes and hyperlipidaemia. CVDs are still the leading cause of death worldwide. An estimated 17.7 million people died from CVDs in 2015, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke (WHO 2017).

During the last years, an important number of studies have provided evidence, on the one hand, the role of over-production of endocannabionid levels in cardiovascular pathophysiology such as hypotension, different forms of shock, and also in the cardiovascular abnormalities that accompany

cirrosis is reported [242]. On the other hand, cannabinoids have also been associated with cardiovascular beneficial effects, such as a protective role in the progression of atherosclerosis and after myocardial ischaemia [243].

Endocannabinoids in the cardiovascular system cause hypotension, bradycardia mediated *via* CB1 receptors and negative inotropy [244]. In the cardiovascular system CB2 receptor expression has been established in the myocardium and cardiomyocytes, cardiomyoblasts and in human coronary artery endothelial and smooth muscle cells [245, 246]. The CB2 receptor has a compensatory role. Activation of the CB2 receptor exerts an anti-inflammatory, anti-oxidative, and anti-atherogenic role, contributing to cardiovascular protection [247]. For instance, inflammatory processes or tissue injury show increased levels of CB2 expression. CB2 signalling in the heart and vasculature may activate cardioprotective mechanisms and protect against inflammation.

4.1. Atherosclerosis

As commented on above, CVDs are one of the leading cause of death and disability worldwide and can be largely attributed to atherosclerosis. It is a chronic inflammation of the arteries characterised by the presence of the lesions that contain immune cells, lipid-laden macrophages (foam cells), cholesterol, smooth muscle cells, and collagen fibres. The physical rupture of the plaques causes the occlusion of arteries, which can lead to tissue infarction [245, 248-250]. The key role of the inflammation in atherosclerosis has gained relevance during the last years (see reviews [251- 253])

In this sense, the CB2 receptor is known to decrease the production of numerous chemokines and to inhibit leukocyte migration *in vitro* and *in vivo*; it emerged as a potential target to treat atherosclerosis. Since the initial studies performed by Steffens *et al*. in 2005 [254] proving the atheroprotective capacity of the Δ9-THC in mice model via CB2 receptor (Table 3), a great number of studies has been performed [243, 255] in experimental animal model (male ApoE-/- mice) in order to study both the CB2 receptor role in the diseases and the atheroprotective capacity of some agonist cannabinoids such as WIN55212-2 [256] (**Fig. 6)**. In all of these studies, the beneficial effects of the treatment with the CB2 agonist were blocked by the CB2 antagonist, showing that CB2 receptor mechanisms (Table 3). Although most of the published studies confirm that the cannabinoid agonist shows an anti-atherosclerotic effect through CB2 receptor activation, there are still some controversial results [257]. In conclusion, the clinical translation of CB2-based therapies still requires research to clarify the role of cannabinoid agonists in atherosclerosis.

4.2. Acute cardiac ischaemic/reperfusion

Ischaemic injury occurs when there is an interruption in the blood supply to an area of tissue. Tissue injury and/or death is determined primarily by the magnitude and duration of the interruption in the blood supply, and then subsequent damage induced by reperfusion [258]. The incidence of ischaemic/reperfusion (I/R) injury is vast: myocardial infarction, stroke, and other thrombotic events. It has been proposed that oxidative stress and inflammatory responses play an important role in the initiation of vulnerable plaque disruption and subsequent development of acute miocardial ischaemic

[259]. During recent years, different studies have proven that the endocannabinoid system is involved in different forms of ischaemia/reperfusion injury. On the one hand, there is an over-production of endocannabinoids contributing to the cardiovascular depressive state associated with these diseases [260]. On the other hand, endocannabinoids have been proposed to protect against myocardial ischemia/reperfusion injury through CB2 receptor in protecting against myocardial, cerebral and hepatic ischemia/reperfusion injury [243, 260, 261]. The activation of CB2 receptor in injured myocardium produces different protectives responses. It has been shown that the activation of CB2 receptor produces the suppression of the inflammatory response as shown in decreased expression of adhesion molecules, secretion of chemokine, leukocyte chemotaxis among others [255, 260, 262]. Likewise, the CB2 receptor activation produces a protective role regarding stress oxidative decreasing levels of reactive oxygen species [259, 263, 264].

Table 3 collets the different experimental studies of the role of agonists CB2 in myocardial ischemia/reperfusion injury *in vivo* models showing both an inflammatory response as well as oxidative stress protection [259, 265-270].

4.3. Acute ischaemic stroke injury

The human costs of stroke are very large and growing. Survivors are often faced with a loss of ability to function independently. The interruption of cerebral blood flow, regardless of the cause, results in a reduction of nutrient delivery to the brain, ultimately leading to ischaemic cell death [271].

In ischaemic stroke, an acute obstruction of blood cerebral flow, followed by reperfusion, unleashes a cascade of molecular and cellular inflammatory successes, which may lead to neuronal death. In the majority of cases, the obstruction is transient and cell death does not occur immediately. The delay between the initial insult and the cellular damage is variable, from hours to days or even weeks, depending upon the nature of the insult and the brain region affected. An important implication of this delay is that the introduction into the brain of protective therapeutic agents during this critical period could disrupt the cascade of damaging events and salvage vulnerable brain tissue.

Various research groups have investigated the role of CB2 receptors studying the effects of different CB2 ligands or employing knockout mice in experimental models of stroke [272-276] (Table 3). Similarly to the effects observed in myocardial ischaemia/reperfusion injury, CB2 receptor activation limited cerebral infarct size in experimental stroke by attenuating endothelial cell activation, chemokine signalling, inflammatory cell infiltration, glial activation, oxidative/nitrative stress and consequent cell death. Most of these beneficial effects could be blocked by CB2 receptor antagonists and/or were absent in CB2 knockout mice, evidencing the protective role of the endocannabinoid system through CB2 receptors.

Very recently, Bravo-Ferrer *et al*. have shown that CB2 receptor is fundamental for driving neuroblast migration suggesting that an endocannabinoid tone is required for post-stroke neurogenesis by promoting neuroblast migration toward the injured brain tissue, increasing the number of new cortical neurons and, conceivably, enhancing motor functional recovery after stroke [277].

5. Autism spectrum disorders

Autism spectrum disorder (ASD) is formed of a heterogeneous group of neurological disorders characterised by impaired social interaction and communication skills, and is often accompanied by other behavioural symptoms such as repetitive or stereotyped behaviour and abnormal sensory processing [278, 279]. Individual symptoms and cognitive functioning vary across the autism spectrum disorders.

The diagnosis is made during the first three years of age; however, the onset of disease can be from birth or usually during the second year. Some cases of ASD display genetic or chromosomal abnormalities, for instance Fragile X syndrome or Down syndrome. Most ASD cases have an unknown aetiology, although the existence of an abnormal development of brain connectivity has been accepted by the scientific community. Different studies have shown genetic evidence supporting a model of dysregulated axonal growth and guidance as key developmental processes underlying the clinical manifestations of ASD [280].

Neuroinflammation and immune system abnormalities have been shown to be associated with ASD. An active neuroinflammatory process was demonstrated in the post-mortem brain, mainly in the cerebral cortex, white matter, and cerebellum. Different studies have shown a key microglia activation and an increase of the inflammatory cytokine and chemokine production in the brain tissue and cerebral spinal fluid. It has been shown that autistic individuals have immune system abnormalities, specifically for antibodies against brain and CNS proteins and maternal proteins [278, 280]. Regarding the endocannabinoid system, both CB1 and CB2 receptors are involved in autism in different ways. CB1 receptors are expressed in those regions in the brain implicated as dysfunctional in autism, such as the cerebellum, hippocampus and basal ganglia. In addition, during development, CB1 receptors drive axon guidance and are responsible for synaptogenesis.

The CB2 receptors modulate immune cell functions, both in cellular and animal models of inflammatory diseases [281]. CB2R are responsible for responses to inflammatory injury, controlling the different inflammatory cells [281]. Zoppis *et al.* showed the regulatory role of the CB2R and agonist CB2 as JWH-133 in a stress-induced neuroinflammation mice model. They used three different models, wild type (WT), transgenic overexpressing CB2 receptors (CB2xP) and CB2 receptor knockout (CB2-KO) mice exposed to different stress mechanisms to check the role of CB2 receptor and JWH-133 (Fig. 8). JWH-133 prevented the stress-induced increase in pro-inflammatory cytokines (TNF-α and CCL2), in NF-κB, and in NOS-2 and COX-2, and in the consequent cellular oxidative and nitrosative damage (lipid peroxidation). CB2xP mice exhibited anti-inflammatory or neuroprotective actions similar to those in JWH-133 pre-treated animals. Conversely, a lack of CB2 receptors (CB2-KO mice) exacerbated stress-induced neuroinflammatory responses and confirmed that the effects of JWH-133 were mediated through CB2 receptors [282].

6. Bone disorders

Bones, the highly specialised supporting framework of the body, are characterised among other aspects by the power of regeneration. Bones have two components: the *cortical bone* which is dense, solid, and surrounds the marrow space and the *trabecular bone* which is composed of a honeycomb-like network of trabecular plates and rods interspersed in the bone marrow compartment.

Bone is composed of support cells, namely, *osteoblasts and osteocytes*; remodelling cells, namely, *osteoclasts*; and non-mineral matrix of *collagen* and non-collagenous proteins called *osteoid*, with inorganic mineral salts deposited within the matrix. During life, the bones undergo processes of longitudinal and radial growth, modelling (reshaping), and remodelling [283]. Bone remodelling can be divided into the following six phases (Fig. 18), namely, quiescent, activation, resorption, reversal, formation and mineralisation. Bone is a living tissue that is constantly being broken down and replaced; thus, the major common cause of most bone disorders is a mismatch between bone formation and bone resorption.

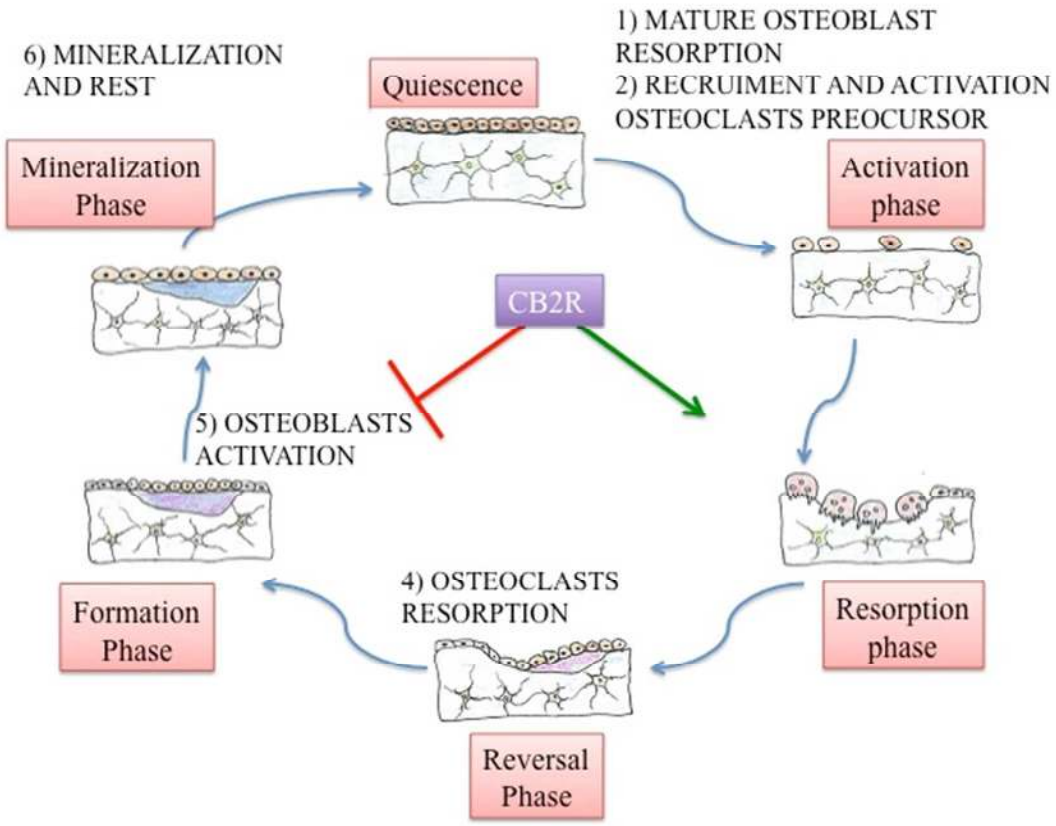

Fig. (18). Bone formation phases

One of the most common bone disorders is osteoporosis, which means “porous bone”; this is a metabolic disorder characterised by an imbalance in the activity of osteoblast and osteoclast. This means that osteoporosis occurs when the formation of new bone does not keep up with of old bone

resorption; as a result, there is a deterioration of bone mass and enhanced bone fragility and fracture risk.

Several key components of the EC system have been identified in bone. Anandamide and 2-AG (**Fig. 4**) are present in bone at levels similar to those found in the brain [284]. In addition, both ligands are produced by osteoblasts and osteoclasts in culture. In a similar way, both CB1 and CB2 cannabinoid receptors are also present in the skeleton [285].

Studies of the role of CB1 have reported different effects that are age- and strain-dependent [286]. In this review, we focus on the role of CB2 receptor. Regarding CB2, several studies have indicated that the effect of CB2 in the skeletal is age- and strain-dependent but not gender-dependent. However, the effect is different in trabecular and cortical bond. In fact, the role of the CB2 receptor was not clear until recent years due to the contradictory results of published works. Different reasons could be responsible for these experimental inconsistencies, such as the species differences, off-target effects of CB2 receptor ligands at different concentrations, the activity of endogenous cannabinoids and the interactions of ligands with other receptors such as GPR55 [287].

Ofek *et al*. showed that CB2-deficient mice had developed an accelerated age-related trabecular bone loss and cortical expansion, which also characterises human osteoporosis. The CB2-/- phenotype is characterised by the increased activity of trabecular osteoblasts (bone-forming cells), increased osteoclast (the bone-resorbing cell) number and a decreased number of osteoblast precursors [288].

In vitro and *in vivo* studies showed that the agonist cannabinoid HU-308 (**Fig. 2**) and its enantiomer HU-433 are anti-osteoporotic, both *in vitro* and *in vivo* [114, 287]. Both agonists stimulate osteoblast proliferation, exhibiting a dose-response biphasic effect, with their effect being blocked by the CB2 receptor antagonist SR144528. Regarding *in vivo* experiments, the treatment of an ovariectomy (OVX)-induced bone loss mouse model with the agonist HU-433 showed a completely reversed OVX-induced decrease in bone volume density associated with the increased trabecular thickness and a higher trabecular number. Suggesting that CB2 signalling contributes to the maintenance of bone mass by two mechanisms: (*i*) the direct stimulation of stromal cells/osteoblasts; and (*ii*) the inhibition of monocytes/osteoclasts, both directly and by the inhibition of osteoblast/stromal cell RANKL expression [287].

Other potential therapeutic applications of the CB2 receptor agonist is in relation to bone cancer. JWH015, a CB2 agonist, treatment significantly reduced serum markers of bone degradation in cancer animals and increases the markers of the serum bone formation marker, osteocalcin.

7. Conclusions

The eCS is a complex signalling system which has been implicated in a wide diversity of biological processes, in both the central and peripheral nervous systems. The CB1 cannabinoid receptors are the most abundant G protein-coupled receptors in the central neural system, expressed in both neurons and glial cells. Moreover, CB1 receptors are also found in peripheral tissues, such as the cardiovascular and reproductive systems as well as the gastrointestinal tract. CB2 receptors are mainly expressed in

peripheral tissue and widely localised in cells of the immune system, as well as in other peripheral organs, such as the muscle, liver, intestine and testis. Likewise, in the nervous system, CB2 receptors are mainly located in microglia and in some neurons.

Therefore both receptors play significant functions in many biological processes, in normal and pathological conditions that affect the physiology and pathology in the central and peripheral nervous systems. Furthermore, the cannabinoid receptors may act as CB1-CB2 receptor heteromers in the brain and form heteromeric complexes with other G protein-coupled receptors [43-45].

All of these facts extremely complicate the development of novel drugs as a consequence of cross-correlation between different biological systems.

The study of the function of homodimers and/or heterodimers for cannabinoid receptors is essential for discover novel drugs. Moreover, the possible cross-talk between endocannabinoid system and other endogenous systems is crucial to establish the potential therapeutics of cannabinoids.

In spite of numerous efforts to develop effective therapies to cure AD or significantly inhibit the progression of AD symptoms, the studies have been unsuccessful. At present, many of the most promising current clinical trials for AD aimed at modifying disease progression are focused on Aβ, the hyper-phosphorylation of tau and energy metabolism. The clinical trial studies include acetylcholinesterase inhibitors, β-secretase (BACE) or γ-secretase inhibitors, agonists and antagonists of neurotransmitter receptors (glutaminergic and serotonin receptors), vaccines or antibodies, as well as anti-inflammatory compounds.

Different studies have demonstrated the effect of cannabinoid agonists on Aß, tau hyper-phosphorylation and inflammation. Cannabinoid agonists reduced Aβ-induced memory impairment, and attenuated neuroinflammatory responses; therefore, the use of CB2 receptor agonists offers an interesting, novel and promising therapeutic approach for AD. In this sense, with the current state of the art methods, it is difficult to understand why a CB2 cannabinoid receptor agonist has not already been studied in clinic phases.

There are some reasons that could explain the difficulty of translating research to the development of efficacious drugs in the clinic.

a) The complexity of eCS makes it extremely difficult to accurately predict the action of drugs. In fact, minor modifications of substituents in different scaffolds determined a functionality switch from agonist to antagonists/inverse agonists and of course in the selectivity of CB1/CB2.

b) CB1 receptor agonists are psychotropics and therefore it is necessary to remove that effect.

c) Most of the knowledge acquired about *in vivo* cannabinoid receptor pharmacology was obtained from the study of the mechanisms of action of a very small number of cannabinoids as mixed CB1/CB2 phytocannabinoids (Δ9-THC, CBD), mixed CB1/CB2 synthetic cannabinoids (WIN55,212-2, HU-210) and selective CB2 agonist (JWH-133) cannabinoids.

d) The promiscuity shown of several cannabinoid agonists with other receptors (AChE, BuChE, BACE-1, etc.) means that the extrapolation of results between different cannabinoids cannot be performed.

e) The development of new effective drugs targeting the CNS generally is an enormously complicated and difficult task, which is often accompanied by a very high failure rate. In fact, since 2003, no new drugs have been approved by the FDA for the treatment of AD and during this time over two hundred compounds have reached Phase II clinical trials, and some even Phase III.

In relation to alternative strategies, it should be mentioned that several genomics studies have shown redundancy in proteinaceous drug targets suggesting that a more promiscuous approach through the application of multi-targets drugs can have advantages. This topic is very attractive both in academia and in industry, and is an approach for the treatment of neurological disorders.

Throughout the review, we have been able to confirm that we are still far from having cannabinoid compounds approved for the treatment of these diseases.

Regarding AD, it is evident that there is an urgent necessity for new and improved treatment options; therefore, additional efforts to develop better drugs need to be a priority. It seems particularly necessary to explore new targets as cannabinoid receptors and new strategies as "multi-target–directed ligands".

As for the rest of the disorders mentioned, in general, in our opinion, it is necessary to perform experimental studies both *in vitro* and *in vivo* with new characterised pure agonist and antagonist CB2 compounds.

Author Contributions

Páez J. A. and Campillo, N. E. contributed equally to this work.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Abbreviations

2-AG:2-arachidonoylglycerol;
5-HT2aR: 5-hydroxytryptamine receptor 2a
A2aR: adenosine A2a receptor;
AChE: acetylcholinesterase;
Aβ: β-amyloid protein;
AChE: acetylcholinesterase;
AD: Alzheimer's disease;
AEA: anandamide (N-arachidonoylethanolamine);
BuChE: butyrylcholinesterase;
CBD: cannabidiol;
eCS: endocannabinoid system;
FAAH: Fatty acid amide hydrolase;
GPR18: G protein-coupled receptor 55
hERG: human Ether-à-go-go-Related Gene;
HI: hypoxia-ischemia;
IFNγ: interferon gamma;
IL-1β: interleukin-1beta;
iNOS: inducible nitric oxide synthase;
I/R: ischaemic/reperfusion;
LADCA: left anterior descending coronary artery;
MAGL: monoacylglycerol lipase;
MCAO: middle cerebral artery occlusion;
MAPK: Mitogen-activated protein kinase;
NADA: N-arachidonoyldopamine;
NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells;
PPAR-γ: peroxisome proliferator activated receptor gamma;
SOD1 and SOD2: superoxide dismutase 1 and 2;
THC: (−)-Δ9-trans-tetrahydrocannabinol;
TNF-α: tumor necrosis factor-alpha;
TRVP: transient receptor potential vanilloid

Tables

Table 1. Binding affinity and selectivity values of selected cannabinoid receptor agonists from CB1 and CB2 binding sites[a]

	CB1 K_i (nM)	***CB2 K_i (nM)***	**CB1/CB2**	Ref.
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Anandamide	89 ± 10	371 ± 102		[143]
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Δ^9-THC	53.3	75.3		[61]
Δ^8-THC	44 ± 12	44 ± 17	1.0	[107]
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Δ^9-THCV	75.4	62.8	1.2	[95]
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HU-308	>10000 [b]	22.7 ± 3.9 [b]	>440	[109]
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3 (CBS055)	4000[c]	2.9 [c]	1379	[115]
4	2550 [d]	5 [d]	510	[116]
5 (ALIAE809)	> 1000	9.0 ± 0.6	> 111	[117]
6	>3000	60.8 ± 6.9	> 49	[117]
7	3500[c]	13 [c]	269	[118,119]
9	220[e]	1.7[e]	129	[120]
10	1430[e]	7[e]	204	[121]
11	>30000[f]	3.7[f]	> 8108	[122]
Six-member heterocyles (Fig. **10, 11**)				
Piridine				
12	>30000	79	> 380	[125]
13	>2000 [f]	10 [f]	> 2000	[126]
14 (LEI-101)	>10000	32	> 312	[127]
8 (S-777469)	4627	36	129	[128]
15	>3000	20±3	> 150	[129]
Piridazine (Fig. **11**)				
GW-842166X	>30000 [f]	63 [f]	> 476	[123,124]

16	>30000 [f]	79,4 [f]	> 378	[131]
17	8000 [f]	10 [f]	800	[131]
18	>10000	3.665 ± 0.553	> 2729	[132]
19	>5000	9	>550	[133]
20	2472 [d]	0.60 [d]	4120	[136]
[6+5] heterocycles				
Indole (Fig. 6)				
WIN55212-2	1.9 ± 0.1	0.3 ± 0.2	6.8	[143]
JWH-015	164 ± 22	13.8 ± 4.6	11.9	[107]
JWH-120	1054 ± 31	6.1 ± 0.7	172.8	[108]
JWH-151	>10000	30 ± 1.1	>333	[108]
L768242 (GW405833)	1917 ± 381	12 ± 0.2	159.8	[144]
AM1241(R)	5000 ± 300	15.1 ± 4.18	>330	[146]
AM1241(S)	>10000	658 ± 44.2	>15	[146]
21	4000	8	500	[149]
A-796260	845	4.37	193	[150]
Benzo[d]imidazole (Fig. 12)				
22	-	0.3 [f]	4266 [f]	[152]
23	>5000	4.1	1220	[153]
24	7500 [d]	5.1 [d]	1471	[154]
27	>10000 [f]	11 [f]	> 909	[155]
26	>10000 [f]	8.2 [f]	> 1220	[156]
27	17000 [f]	6.3 [f]	2698	[157]
28 (RQ-00202730)	>25000 [f]	19 [f]	>1316	[158]
Pyrazolo[3,4-b]pyridine (Fig. 13)				
29	4752	11.6	432	[159]
Imidazo[4,5-c]pyridine (Fig. 13)				
30	17000 [g]	58 [g]	293	[160]
31	1068 [g]	11 [g]	97	[160]
Purine				
32		37.5 ± 4.8	655	[162]
33		40.3 ± 6.9	4975	[162]
Isoxazolo[5,4-b]pyridine				
34 (MDA7)	>10000	422	24	[163]
[6+6] heterocycles (Fig. 14)				

Quinoline				
35	>1000	3.3 ± 0.4	> 330	[164]
36	1925 ± 179	13.4 ± 1.2	143.7	[165]
37	1220	6.3	1220	[166]
38	>17000 [g]	6.1 [g]	2787	[167]
Naphthyridine				
39 (CB13)	>1000	50 ± 4	>20	[164]
40 (cis)	1519	5.8	262	[170]
41	1011 ± 46.5	1.36 ± 0.053	743	[171]
Tricyclyc heterocycles (Fig. 15)				
Pyrido[4,3-b]indole				
42	120	9	13.3	[172]
Pyrazolo[4,3-c]quinoline				
43	>3000	0.39	>77	[173]
Benzo[b]quinolizine				
44 (Sch35966)	2633 ± 829	6.8 ± 2.3	387	[174]
Oxazino[2,3,4-ij]quinoline				
45 (MT178)	>10000	8.12	1231	[175]

[a] Affinity of compounds was evaluated using membranes from HEK-293 cells transfected and [^{3}H]CP-55,940. [b] Experiments were performed with [^{3}H]HU243. [c] Binding affinity IC_{50} ([^{3}H]CP 55,940 binding to rat spleen membranes). [d] Binding affinity EC_{50} ([^{3}H]CP 55,940 binding to CHO cell membranes). [e] EC50 [35S]GTPγS binding assay. [f] EC_{50} values based on inhibition of forskolin-stimulated cAMP production in CHO cells expressing CB2 or CB1 receptor. [g] IC_{50} (CHO-K1 cells,AMP detection).

Table **2**. Cannabinoid receptor agonists studied using *in vivo* models of Alzheimer's disease

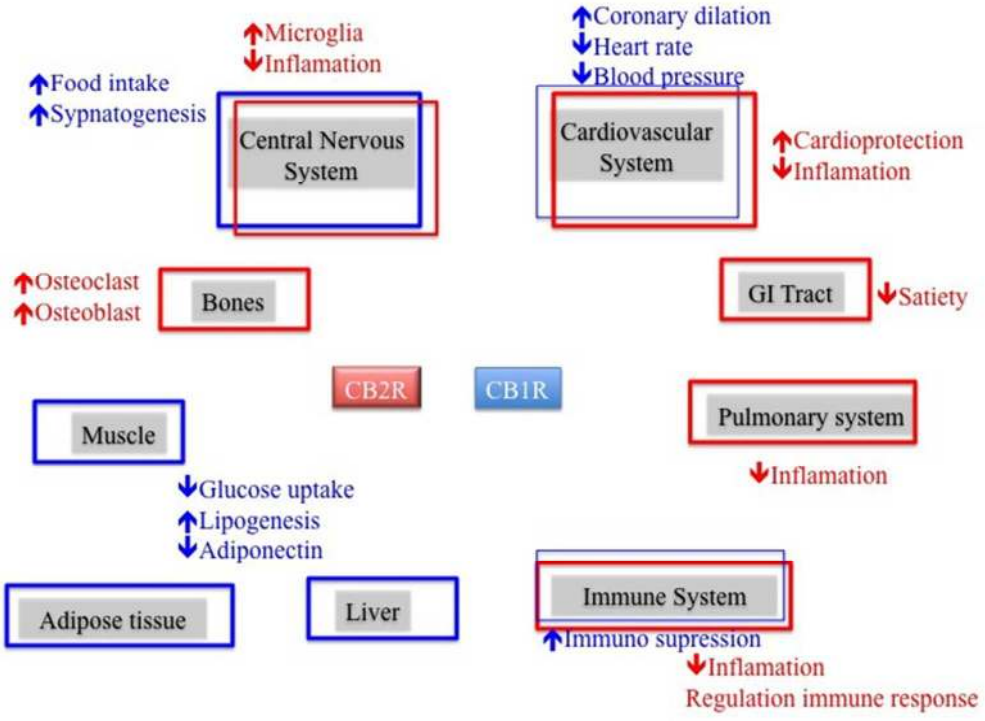
Animal Model	**Drugs**	**Ref.**
Male Wistar rats and Swiss mice	AEA, noladin ether	[231]
5XFAD APP mice	Δ^9-THC	[232]
AβPP/PS1 mice	Δ^9-THC, CBD	[233]
PK−/− /TauVLW	Δ^9-THC, CBD	[234]
C57BL/6J mice	CBD	[235]
Tg APP	CBD; WIN55,212-2	[221]
Male Wistar rats	WIN55,212-2	[201]

Male Wistar rats	WIN55,212-2	[222]
Tg APP	JWH-133; WIN55,212-	[236]
Tg APP	JWH-133; WIN55,212-	[237]
AβPP/PS1	JWH-133	[238]
APP23/PS45 mice	HU-210	[239]
APP/PS1 mice	ß-caryophyllene	[240]
Male Sprague–Dawley rats	MDA7	[241]
Tg APP, 5xFAD	NP137, NP43	[186]

Table 3. Effects of CB2 agonists in animal models of cardiovascular disorders

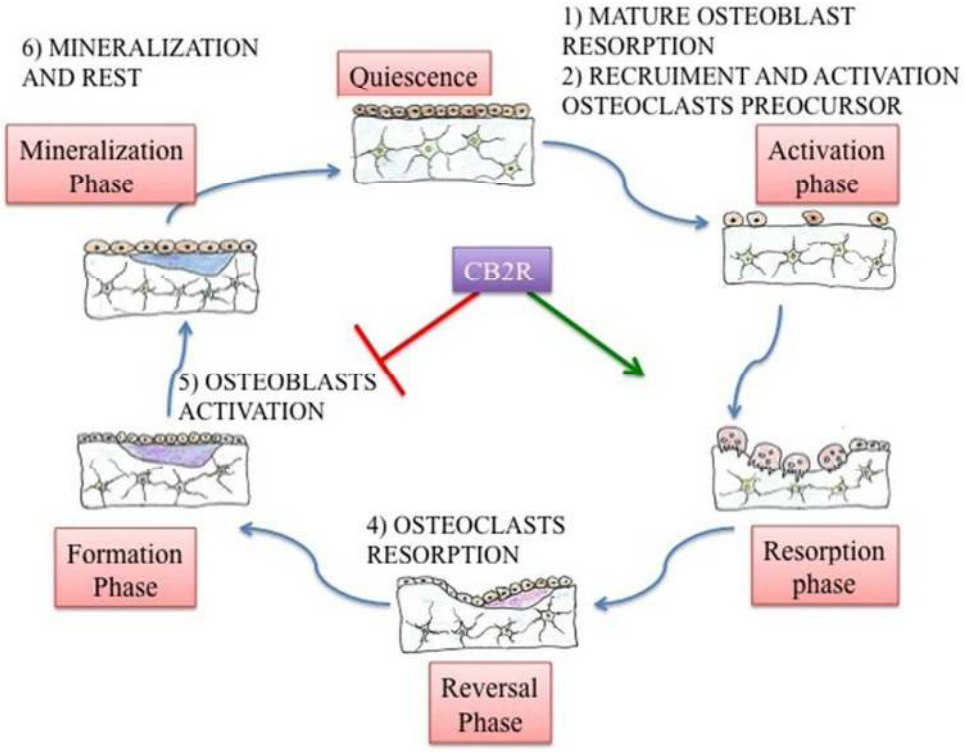
Disease/ in vivo model	*CB2 agonist (Treatment)*	*CB2 antagonist (Block effect)*	*Results*	*Ref*
Atherosclerosis				
Male ApoE$^{-/-}$ mice	THC	SR144528	⇓Atherosclerotic lesions ⇓ Macrophage inflitration ⇓ Leukocyte adhesion	[254]
Male ApoE$^{-/-}$ mice	WIN55212-2	AM630	⇓Atherosclerotic lesions ⇓ Macrophage inflitration ⇓TNF-α, IL-6 (inflammatory markers)	[256]
Myocardial I/R				
AMI induced by ligation of LAD artery and I/R	HU308	AM630	⇓ Infarct size ⇓ TNF-α ⇓ ROS	[259]
Oclusion LADCA+reperfusion rat model	WIN55212-2	AM630	⇓ Myeloperoxidase ⇓ IL-1βband IL-8 ⇓ Infarct size	[265]
Oclusion LADCA+reperfusion Mouse model	JWH-015	SR144528	⇓ Infarct size	[261]
Oclusion LADCA+reperfusion Mouse model	JWH-133	AM630	⇓ Infarct size ⇓ Oxidative stress ⇓ Neutrophil inflitration	[266]
Oclusion LADCA+reperfusion Mouse model and I/R CB2R$^{-/-}$	JWH-133	AM630	⇓ Infarct size (in I/R-WT) No effect (in I/R CB2R$^{-/-}$)	[267]
Oclusion LADCA+reperfusion rat model	JWH-133	AM630	⇓ Infarct size ⇓ Apoptosis during ischemia/reperfusion through inhibition of the pathway PI3K/Akt signal	[268]
AMI induced by ligation of LAD artery	AM1241	-	⇓ Fibrosis ⇓ TNF-α and IL-6 ⇓ MDA (ROS)	[269]
Oclusion LADCA+reperfusion	HU210	SR144528	⇓ Infarct size	[270]

Mouse model			⇓ Incidence ventricular arrhythmias	
Stroke I/R				
MCAO Mouse model	0-3853 / 0-1966	-	⇓ Cerebral infarction size ⇑ Motor function ⇓ Endothelial/leukocyte interactions	[271,272]
MCAO Mouse model	0-1966 + SR141716 (antagonist CB1)	SR144528	⇓⇓Cerebral infarction size ⇑⇑ Motor function	[272]
MCAO WT, $CB2^{-/-}$model and $CB1^{-/-}$ model	JWH-133	-	⇓ Cerebral infarction size (WT, $CB1^{-/-}$) ⇓ Neutrophil recruiment No effect in $CB2^{-/-}$	[273]
MCAO Mouse model	JWH-133	-	⇓Microglia and macrophage infiltration ⇓IL-6, MCP-1, MIP-1a, CCL-5 and TNF-a ⇓iNOS	[274]
MCAO Mouse model	WIN55212-2	-	⇓ Cerebral infarction size ⇓ Microglial cells accumulation and proliferation ⇓ Citokine expression	[275]



Schematic representation of the functional role of CB1 and CB2 receptors

253x191mm (72 x 72 DPI)



Bone formation phases

228x173mm (72 x 72 DPI)

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