Published in final edited form as:
Future Med Chem. 2012 February ; 4(2): 187–204. doi:10.4155/fmc.11.179.

Latest advances in novel cannabinoid CB_2 ligands for drug abuse and their therapeutic potential

Peng Yang[1,2,3], **Lirong Wang**[1,2,3], and **Xiang-Qun Xie**[1,2,3,4,*]

[1]Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15260, USA

[2]Drug Discovery Institute, University of Pittsburgh, Pittsburgh, PA 15260, USA

[3]Pittsburgh Chemical Methods and Library Development (CMLD) Center, University of Pittsburgh, Pittsburgh, PA 15260, USA

[4]Departments of Computational Biology & Structural Biology, University of Pittsburgh, Pittsburgh, PA 15260, USA

Abstract

The field of cannabinoid (CB) drug research is experiencing a challenge as the CB_1 antagonist Rimonabant, launched in 2006 as an anorectic/anti-obesity drug, was withdrawn from the European market due to the complications of suicide and depression as side effects. There is interest in developing CB_2 drugs without CB_1 psychotropic side effects for drug-abuse treatment and therapeutic medication. The CB_1 receptor was discovered predominantly in the brain, whereas the CB_2 is mainly expressed in peripheral cells and tissues, and is involved in immune signal transduction. Conversely, the CB_2 receptor was recently detected in the CNS, for example, in the microglial cells and the neurons. While the CB_2 neurons activity remains controversial, the CB_2 receptor is an attractive therapeutic target for neuropathic pain, immune system, cancer and osteoporosis without psychoactivity. This review addresses CB drug abuse and therapeutic potential with a focus on the most recent advances on new CB_2 ligands from the literature as well as patents.

Cannabinoid drug abuse & the endocannabinoid system

Drug abuse is a concerning issue worldwide and often carries with it criminal penalties and negative physical, social and psychological effects. Drugs that are used and abused by humans for nonmedical purposes can be grouped into several major categories that include marijuana or cannabis (cannabinoids [CBs]), alcohol (ethanol), nicotine and tobacco, depressants (barbiturates and benzodiazepines), stimulants (amphetamines, cocaine), opioids (morphine, heroin and methadone), psychedelics (LSD, mescaline and ecstasy), inhalants (glue and nitrous oxide) and phencyclidine. CBs remain the most widespread drugs in use worldwide. The term 'cannabinoid' was first used to describe the tricyclic natural compounds from *Cannabis sativa* L [1]. Marijuana is the most used illicit drug in the USA,

*Author for correspondence: Tel.: +1 412 383 5276, Fax: +1 412 383 7436, xix15@pitt.edu.

Financial & competing interests disclosure
The authors would like to acknowledge the financial support for their laboratory from the NIH R01DA025612. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.
No writing assistance was utilized in the production of this manuscript.

and is very often ingested with other drugs of abuse. National Institute on Drug Abuse 2009 reported that 16.4 million Americans aged 12 or older used marijuana at least once in the month prior to being surveyed [201]. Marijuana abuse and toxicities are a serious threat to human health in the USA and worldwide. It is now known that Δ^9-tetrahydrocannabinol (Δ^9-THC), the main psychoactive ingredient of marijuana, activates the mesocorticolimbic system, the same system responsible for the reinforcing properties of all drugs of abuse [2–4]. Δ^9-THC acts primarily through the **endocannabinoid system** in the brain. This system modulates diverse physiologic functions including motor function, memory, motivation, drive, pain and emotion [5–7].

Effective treatments for the abuse of marijuana and other drugs of abuse remain elusive as evident by high rates of unpleasant withdrawal symptoms and relapse. Hence, there is a tremendous medical need for new rationally designed medications to treat drug abuse and associated diseases, an advance that likely requires the development of new research strategies and resources. There is ample evidence that most of the centrally mediated effects of many drugs of abuse, including CBs, opioids, alcohol and nicotine, occur through the endocannabinoid system [8]. The studies show that release of endocannabinoids in the ventral tegmental area can modulate the reward-related effects of dopamine and might, therefore, be an important neurobiological mechanism underlying drug addiction. There is strong evidence that the endocannabinoid system is involved in drug-seeking behavior (especially behavior that is reinforced by drugrelated cues), as well as in the mechanisms that underlie relapse to drug use [8]. Therefore, the endocannabinoid system represents a promising target for development of new treatments for drug addiction.

CB receptors & ligands

To date, at least two CB receptors have been cloned and characterized: CB_1 and CB_2, which share 48% identity at the amino acid level [9,10]. CB receptors contain an N-terminal extracellular domain that possesses glycosylation sites, a C-terminal intracellular domain coupled to a G protein complex and seven hydrophobic transmembrane segments connected by alternating extracellular and intracellular loops. Three dimensional models of the helix bundle arrangement of human, rat and mouse CB_1 and CB_2 receptors have been constructed and compared [11–13]. Both signal through activation of pertussis toxin-sensitive G proteins to inhibit adenylate cyclase, and both are positively coupled to the activation of MAPK [14].

It was initially believed that the CB_1 receptor was expressed predominantly in the brain (central receptor for CBs) [9], whereas the CB_2 receptor in peripheral cells and tissues was derived from the immune system (peripheral receptor for CBs) [10]. The CB_1 receptor was recently also found in a number of peripheral tissues, for example, the cardiovascular and reproductive systems and in the GI tract [15–17]. In addition, recent studies have indicated that the CB_2 receptor may also exist in the CNS, for example, in microglial cells as well as neurons [18–20]. Thus, CB_2 receptor biology may in the future be used to develop nonpsychotropic (or non-CB_1-mediated) approaches to manipulate endocannabinoid levels localized in the brain, offering therapeutic promise for treating CNS disorders. However, the CB_2 CNS neural activities still need to be investigated further and evaluated in greater detail.

CB_1 receptor, ligands & drug abuse

The CB_1 receptor is primarily, but not exclusively, expressed in the CNS, in particular in the hippocampus, some olfactory regions, caudate, putamen, accumbens nucleus (ventral striatum), the substantia nigra pars reticulata, globus pallidus and the horizontal limb of the diagonal band [20,21]. CB_1 mRNA is found in a lesser extent in peripheral tissues, such as the adrenal gland, heart, lung, prostate, testis, bone marrow, thymus and spleen [14]. The

binding of CBs to the CB_1 receptor, which triggers the activation of this receptor, is responsible for the psychoactive effects associated with CBs, such as euphoria, drowsiness, memory lapses, disruption of motor skills, lack of concentration and disorientation [22].

Extensive evidence exists that drugs of abuse exert their reinforcing and rewarding properties through the dopaminergic mesocortical and mesolimbic associative processing and motivational pathways in the brain involving the prefrontal cortex, ventral tegmentum, amygdala and their projections to the striatum [23]. As reported, endocannabinoids are capable of indirectly enhancing dopamine outflow in the nucleus accumbens. The actions of CBs are thought to be mediated via CB_1 receptors located presynaptically on the glutamatergic fibres. In agreement with the neuroanatomical localization and function of CB receptors in reward and motivational pathways, CB_1 receptor antagonists show an ability to attenuate self-administration and/or relapse involving a variety of drugs of abuse, including nicotine [24]. Rimonabant (SR141716A) was the first selective CB_1 receptor antagonist developed [25]. Several reports indicate Rimonabant (SR141716, also inverse agonist for CB_1) can facilitate abstinence from tobacco in tobacco users and these reports have helped propel this interest in the potential applications of CB_1 antagonists as treatments for drug abuse disorders [23]. All these findings indicate that CB_1 antagonists can interfere with brain systems responsible for the expression of the acute reinforcing and motivational properties of drugs of abuse, including marijuana, cocaine and ethanol [23]. However, CB_1 agonists that penetrate the CNS result in catalepsy, sedation and undesirable psychotropic effects, which have also limited the therapeutic utility of nonselective, brain-permeable CB agonists [26]. Thus, more research is needed to confirm if the ubiquitous distribution of the CB_1 receptor in the CNS is the real reason for the adverse psychiatric effects.

CB_2 receptor, ligands & drug abuse

The CB_2 receptor was initially discovered to be widely distributed in peripheral tissues and particularly in immune tissues. Expression of the CB_2 receptor gene transcripts was found in the spleen, tonsils, thymus, mast cells and blood cells [10,27–29]. Interestingly, the CB_2 receptor was also recently detected in the CNS, for example, in the microglial cells as well as the neurons [20]. Abundant CB_2 immunoreactivity in neuronal and glial processes was detected but at a much lower level than as reported in CB_1 receptors [30]. The expression level of the *CB1* gene using RT-PCR analysis was 100-times that of the *CB2* gene expression level in the brain stem [31]. A review of the distribution of CB_1 and CB_2 receptors in the mammalian nervous system was summarized by Svizenska [20]. The most prominent staining of the CB_2 receptor was observed in the anterior olfactory nucleus, in the neurons of the piriform, orbital, visual, motor and auditory cortex, where bodies and apical dendrites of pyramidal neurons in the layers III and V were heavily stained. Moderate density of CB_2 immunopositive cell bodies was found in the periaqueductal gray, substantia nigra pars reticulata and other nuclear structures of the brain stem [20]. Data obtained *in vitro* and from animal models demonstrated the inducible nature of CB_2 receptors under neuroinflammatory conditions and suggests that the upregulation of CB_2 receptors is a common pattern of response against different types of chronic human brain neuropathology [32]. Mounting evidence also shows that CB_2 and its gene variants may play possible roles in neuroinflammation occurring in multiple sclerosis (MS), traumatic brain injury, HIV-induced encephalitis, Alzheimer's, Parkinson's and Huntington's diseases [32]. These multifocal distributions and the presence of the CNS CB_2 receptor suggest that the CB_2 receptor may play an important role in neurotransmission.

CB_2 and their gene transcripts are expressed in the brains of naive mice and are modulated following exposure to stressors and administration of abused drugs. Onaivi found that mice preferring alcohol had reduced *CB2* gene expression in the ventral midbrain; whereas, the *CB2* gene expression was unaltered in the ventral midbrain region of mice with little or no

preference for alcohol. Treatment of mice with the CB_2 agonist JWH-015 enhanced alcohol consumption in mice subjected to chronic mild stress and treatment with the CB_2 antagonist AM630 reduced the stress-induced increase in alcohol consumption. This CB_2 agonist or antagonist effect was absent in normal mice that were not subjected to chronic mild stress. Researchers also found that animals treated with cocaine or heroin showed increased CB_2 gene transcripts in comparison to controls, indicating the presence of CB_2 gene transcripts in the brain that are influenced by abused substances [33–35]. Onaivi utilized behavioral and molecular methods to study and determine whether there was a link between depression in drug/alcohol addiction and the CNS CB_2 receptor. Their studies provided the first evidence for the CNS effects of CB_2 and its possible involvement in drug addiction and neuropsychiatric disorders [33–36].

Very recently, Xi *et al.* found that systemic, intranasal or intra-accumbens local administration of JWH133, a selective CB_2 receptor agonist, dose-dependently inhibited intravenous cocaine self-administration, cocaine-enhanced locomotion and cocaine-enhanced accumbens extracellular dopamine in wild-type and CB_1 receptor knockout ($CB_1^{-/-}$, also known as $Cnr1^{-/-}$) mice, but not in $CB_2^{-/-}$ ($Cnr2^{-/-}$) mice [37]. The result also indicated that JWH133-induced reduction in cocaine self-administration resulted from a reduction in cocaine's rewarding efficacy, and intranasal JWH133-induced pharmacological effects are mediated by activating brain rather than peripheral CB_2 receptors. Furthermore, their findings suggested that JWH133 has no cocaine-like reinforcing or aversive effects in mice. This finding not only challenges current views that CB_2 receptors are absent from the CNS and that CB_2 receptor ligands lack CNS effects, but also suggests that brain CB_2 receptors may be a target for the pharmacotherapy of drug abuse and addiction [37].

While efforts have been devoted to develop CB_2-selective ligands for therapeutic immune intervention, emerging research and current data demonstrate that the functional expression of CB_2 receptors in brain may provide novel targets for the effects of cannabinoids in depression and drug-abuse disorders beyond neuro-immunocannabinoid activity. In addition, selective activation of CB2 receptor would not be expected to elicit undesired psychotropic effects [22]. Thus, more detailed discussions are presented below.

Therapeutic potential of CB CB_2 ligands

Nonselective CB ligands display a wide range of physiological effects including analgesic, antiinflammatory, anticonvulsive and immunosuppressive activities. Since its discovery in 1993, the CB_2 receptor has been an appealing therapeutic target for novel immunomodulators. It is now known that the CB_2 receptor is expressed in most organ systems including the cells of the brain, heart, liver, cardiovascular and gastrointestinal systems. In many cases, CB_2 receptor expression is regulated by injury or disease. Recent advances in the chemical synthesis of CB_2 receptor ligands, prompted by their potential therapeutic indications for the treatment of pain and other conditions, have led to the rapid expansion of tools for the exploration of this receptor system [38,101].

CB_2 ligands & the immune system

Most of the effects of CBs within the immune system have been attributed to the CB_2 receptor. CB_2 ligands have been demonstrated to attenuate aberrant immune responses in autoimmune disorders and, in some cases, to provide protection to the tissue that is being inappropriately targeted by the immune system. Such cases include:

- MS: an autoimmune disorder that results in the demyelination of neurons in the CNS. CB_2-selective agonist HU-308 markedly reduces the recruitment of immature

myeloid and T cells, microglial and infiltrating myeloid cell proliferation, and axonal loss in the experimental autoimmune encephalomyelitis model [39];

- Allergy: CB_2 ligands were found useful in the treatment of allergic reactions. Topical administration of the CB_1/CB_2 agonist HU-210 reduces these histamine-induced responses in human skin [40]. In contrast, injection of the CB_2 receptor antagonist SR144528 exacerbates this inflammation and pruritis [41];
- Conditions associated with inflammation: CB_2 agonists have been demonstrated to attenuate inflammation in the CNS.

Administration of CB_2 agonists prevents the activation of microglia in rodent models of Alzheimer's disease [42]. Likewise, administration of CB_2 agonists reduces the volume of infarcts by 30% in a rodent occlusion model of stroke [43].

CB_2 ligands & pain

The analgesic properties of CBs have been recognized for many years and the ability of CBs to affect pain perception has supraspinal, spinal and peripheral components [44,45]. Besides the role of CB_1 in mediating these analgesic effects, CB_2 also plays a role in mediating the analgesic effects of CBs. It is not known how CB_2 receptor- selective agonists inhibit pain. However, it is more widely accepted that CB_2 receptors could modulate pain through an indirect mechanism involving circulating cells of the immune system [46]. For example, systemic delivery of the CB_2- selective agonist AM1241 suppresses hyperalgesia induced in the carrageenan, capsaicin and formalin models of inflammatory pain in rodents [47]. Another CB_2-selective agonist GW405833 administered systemically significantly reverses hypersensitivity to mechanical stimuli in rats following the ligation of spinal nerves [48]. As for the CB_2-selective agonist O-3223, it reduced nociceptive behavior in both phases of the formalin test, reduced thermal hyperalgesia in the chronic constriction injury of the sciatic nerve model and reduced edema and thermal hyperalgesia elicited by intraplantar injection of lipopolysaccharide without affecting basal nociception or eliciting overt behavioral effects [49]. It is now clear that the CB_2 receptor plays a critical role in nociception and has been shown to modulate acute pain, chronic inflammatory pain, postsurgical pain, cancer pain and pain associated with nerve injury [46,49].

CB_2 ligands & cancer

CBs and modulators of the endocannabinoid system have recently been shown to produce antitumor actions. Guindon *et al.* summarized the different mechanisms and signaling pathways that CBs/CBs receptors impact proliferation, migration and apoptosis cancer cells [50]. The endocannabinoid system may be targeted to suppress the evolution and progression of the breast, prostate and bone cancer. And activation of the endocannabinoid signaling system also produces anticancer effects in other types of cancer, including skin, brain and lung [50]. For example, the CB_2 agonist JWH-133 showed good ability to decrease size and number of tumors, reduce the number and size of lung metastases, inhibit cell proliferation and decrease angiogenesis in mice injected with different breast cancer cell lines [51,52].

CB_2 ligands & osteoporosis

There is accumulating evidence to suggest that CBs and their receptors play important roles in bone metabolism by regulating bone mass, bone loss and bone cell function [53]. Osteoblasts, osteoclasts and osteocytes express CB_2 receptors at significantly higher levels than that reported for CB_1 [54–56]. Recent studies reported that bone cells also express GPR55 and TRPV1, which are known to be targeted by endocannabinoids and synthetic CB ligands [53]. The CB_2 agonist HU-308 enhances endocortical osteoblast numbers and

activity while simultaneously inhibiting proliferation of osteoclast precursors in bone marrow-derived osteoblasts/stromal cells *in vitro*, and attenuates ovariectomy-induced bone loss and stimulates cortical thickness by stimulating endocortical bone formation and suppressing osteoclast number *in vivo* [54].

CB_2 ligands & other potential therapeutic uses

Endocannabinoids are also involved in the pathophysiology of acute and chronic liver disease and gastrointestinal disease [57]. Munoz-Luque *et al.* used the carbon tetrachloride model to induce fibrosis of the liver and treated rats chronically with the selective CB_2 receptor agonist JHW- 133. JWH-133 improved many indices of damage and markedly improved the extent of liver fibrosis leading to reduced portal pressures [58].

CB_2 receptor agonist JWH015 significantly protects retinal pigment epithelial (RPE) cells [59]. While RPE cells provide trophic support to photoreceptor cells in the eye, RPE cell death has been demonstrated to be a major contributor to age-related macular degeneration. Therefore, CB_2-selective agonists also have potential therapeutic use in preventing the onset or progression of vision loss associated with age-related macular degeneration.

CB_2 antagonists inhibit the proliferation of cultured neural stem cells and the proliferation of progenitor cells in the subventricular zone of young animals; whereas, CB_2-selective agonists stimulate progenitor cell proliferation *in vivo*, with this effect being more pronounced in older animals [60]. So agonists of CB_2 are useful in regenerative medicine, for example to promote the expansion of progenitor cells for the replacement of neurons lost during injury or disease, such as Alzheimer's disease, stroke-induced damage, dementia, amyotrophic lateral sclerosis and Parkinson's disease [101].

Novel CB_2-selective ligands

Due to the unwanted psychotropic effects resulting from activation of the CB_1 receptor, there exists much controversy surrounding the use of medicinal marijuana and the potential for abuse. CB_2-selective ligands with significantly low CB_1 affinity would not be expected to elicit undesired psychotropic effects. Increasing evidence shows that the CB_2 receptor is an attractive therapeutic target. Thus, research is currently focused on the development of CB_2-selective ligands. This review summarizes the literature, particularly patent documents, on CB_2 receptor-selective ligands developed recently and classifies them based on their chemical scaffolds as below. Readers can read other previously published articles about CB_2 ligands elsewhere [61–63].

Five-membered rings as scaffolds for CB_2 ligands

Pyrazole & pyrrolidine derivatives—Abbott Laboratories synthesized a series of selective compounds for the CB_2 receptor, among which 133 compounds were bound to CB_1 receptors, with K_i values of approximately 10–500-fold higher than that for CB_2 receptors. In a 2010 patent, pyrazole 1 (**1**; Figure 1) was a representative example with a very high CB_2 receptor affinity (human CB_2: K_i = 0.7 nM; rat CB_2: K_i = 1.3 nM) [102]. The *in vivo* activities were also tested in an incisional model of postoperative pain, a capsaicin-induced secondary mechanical hypersensitivity model and a monosodium iodoacetate-induced knee joint osteoarthritic pain model. The data indicated that certain tested compounds showed a statistical change at less than approximately 300 μM/ kg and certain measured compounds showed efficacy at less than approximately 50 μM/kg.

Over 300 exemplified compounds were claimed as CB_2 agonists in a patent application from Boehringer Ingelheim International GmbH. Among these substituted pyrrolidine derivatives,

compound **2** (Figure 1) is representative and is quoted as having an EC_{50} value of 0.015 nM against CB_2 cAMP in the binding assay [103].

Thiazole & isothiazole derivatives—Frost *et al.* for Abbott Laboratories disclosed a series of thiazole compounds as CB receptor ligands in 2010. The compounds were evaluated *in vitro* and *in vivo* and 38 compounds tested exhibited approximately 10–1000-times weaker binding affinity for CB_1 receptors than for CB_2. The similar result was obtained in the cyclase assays. These results showed that these compounds preferably bind to CB_2 receptors and, therefore, are selective ligands for the CB_2 receptor. From these assays, example compound **3** (Figure 1) was found to display the following values: K_i = 3.1 nM in human CB_2 binding; K_i = 1.1 nM in rat CB_2 binding, and EC_{50} = 0.07 nM for rat CB_2 cyclase [104].

In the same year, Wang *et al.* at Abbott Laboratories published two patents of thiazole compounds with an azolylidenebenzamides group. In the first patent, 25 compounds tested bound to CB_2 receptors with K_i values of less than approximately 1000 mM but bound to CB_1 receptors with K_i values 10–1000-times higher than that for CB_2. The example compound **4** (Figure 1) had a high CB_2 affinity (human CB_2 binding: K_i = 1.63 nM; rat CB_2 binding: K_i = 0.80 nM) [105]. In the other patent, another six compounds were claimed as selective CB_2 ligands. The binding value of the representative compound **5** (Figure 1) was K_i = 46.37 nM in human CB_2 binding and K_i = 29.09 nM in rat CB_2 binding [106].

Abbott Laboratories are continuing their interest in this area and have disclosed 267 isothiazole derivatives. Compounds tested are approximately 100-fold to approximately >10,000-fold more potent at activating rat CB_2 versus rat CB_1 receptors in the cyclase assays. Compound **6** (Figure 1) is a representative example that was found to display the following values: human CB_2 binding: K_i = 16 nM; rat CB_2 binding: K_i = 1.5 nM; rat CB_2 cyclase: EC_{50} = 0.72 nM [107]. The *in vivo* activities were also tested in the incisional model of postoperative pain, the spinal nerve ligation model of neuropathic pain, the capsaicin-induced secondary mechanical hypersensitivity model and the monosodium iodoacetate-induced knee joint osteoarthritic pain model. The data indicated that certain compounds tested showed a statistical change at less than approximately 300 μM/kg and certain compounds measured showed efficacy at less than approximately 50 μM/kg.

Imidazole derivatives—Beckett *et al.* at Cara Therapeutics, Inc. published over 600 substituted imidazoheterocycles compounds and tested their EC_{50} against human CB_2, rat CB_2 and human CB_1 receptors. These compounds were separated as agonist and inverse agonist. The binding affinity data (EC_{50}) were showed as four levels from 0.1 nM to 10 μM [108]. Many of these compounds have high affinity and good selectivity to CB_2 receptor. Compound **7** (Figure 1) is a representative compound with the following values: human CB_2 binding: EC_{50} < 0.1–10 nM; rat CB_2 binding: EC_{50} < 0.1–10 nM; human CB_1 binding: EC_{50} >10 μM [108]. The *in vivo* activities, such as antihyperalgesia and acute inflammation were also tested in the inflammatory pain model, the carrageenan model of acute inflammation and the spinal nerve ligation model. The results indicated that some compounds showed a statistical effect. No side effects were observed during the course of the experiment.

In 2010, Lange and coworkers produced a SAR study of imidazole. They found a novel imidazole compound **8** (Figure 1), which exhibited the highest CB_2 receptor affinity (K_i = 1.03 nM) in this series, as well as the highest CB_2/CB_1 subtype selectivity (>9708- fold) [64]. This represents a novel chemotype of potent and selective CB_2 receptor antagonists/ inverse agonists.

Six-membered rings as scaffolds for CB_2 ligands

Pyridine & pyrazine derivatives—Bartolozzi *et al.* prepared over 150 pyridinebased compounds, of which 126 compounds are preferred CB_2 agonists. One exemplified compound **9** (Figure 2) is said to have an EC_{50} value at the CB_2 receptor of 0.093 nM [109]. They also claimed these compounds are useful for treating inflammation or pain.

Chu and co-workers replaced the phenyl ring with a pyridine ring when they further explored carboxamide CB ligands, and they found a potent and selective CB_2 agonist compound **10** (Figure 2), which displayed good affinity at the CB_2 receptor (Ki = 24 nM), 160-fold selectivity versus CB_1 (CB_1: K_i = 3800 nM) and moderate metabolic stability in rat and human liver microsomes. Importantly, compound **10** exhibited *in vivo* efficacy after oral administration in a rat model of neuropathic pain [65].

A series of pyrazine-2-carboxamides compounds has been claimed as CB_2 receptor ligands by F Hoffmann-La Roche AG. Three compounds are selective for the CB_2 receptor, with affinities K_i = 43–63 nM, and all of them exhibit at least tenfold selectivity against the CB_1 receptor. Compound **11** (Figure 2) has the highest affinity with K_i = 43 nM [110].

Pyridazine derivatives—Chen *et al.* have published a series of pyridazine derivative as therapeutic CB_2 receptor agonists. Compound **12** (Figure 2) shows high CB_2 affinity and good selectivity against CB_1 (CB_1 binding: IC_{50} = 1028 nM; CB_2 binding: IC_{50} = 5.4 nM; CB_2 selectivity = 189) [111].

Morpholine derivatives—By both reducing the entropy of the molecule, and incorporating a linker between the aryl rings to reduce potential for unproductive protein binding, Zindell *et al.* found two compounds **13** and 14 (**13**: CB_2 cAMP EC_{50} = 6 nM; CB_1/ CB_2: EC_{50} = 960; **14**: CB_2 cAMP EC_{50} = 10 nM, CB_1/ CB_2 EC_{50} >2000; Figure 2) [66]. Each of the compounds is a very potent CB_2 agonist based on the functional data with very good selectivity over CB_1.

Seven-membered rings as scaffolds for CB_2 ligands

Diazepane derivatives—A high-throughput screening campaign identified aryl 1,4-diazepane compounds as potent and selective CB_2 agonists as compared with CB_1. Cirillo *et al.* have synthesized 279 diazepane compounds as CB_2 receptor modulators for treating inflammation, pain and disease. Among these, over a quarter compounds showed high affinity to CB_2 receptor and exhibited agonistic activity. The representative compound **15** (Figure 3) has an EC_{50} (concentration at which 50% of forskolin-stimulated cAMP synthesis was inhibited) of 0.7 nM or an agonist efficacy of 95% in a human CB_2 receptor binding assay [112].

Cirillo *et al.* reported another series of diazepane compounds as CB_2 receptor modulators and claimed that these compounds were useful for treating inflammation and pain. Fifty three compounds were preferred CB_2 agonists. As a representative sample, compound **16** (Figure 3) exhibited the EC_{50} value of 0.017 nM [113].

However, many compounds of this class suffered from poor drug-like parameters as well as low microsomal stability and poor solubility. In 2011, Zindell *et al.* further described the SARs with a focus on improving the drug-like parameters, resulting in compounds with improved solubility and permeability. They found incorporation of heteroalkyl offered little change to the CB_2 potency but greatly enhanced the selectivity profile, while significantly improving the aqueous solubility of the molecule. The representative compound **17** (Figure

3) showed high CB_2 affinity and selectivity with good drug-like properties (CB_2 cAMP EC_{50} = 1 nM, CB_1 EC_{50}/ CB_2 EC_{50} = 1770; solubility > 96 μg/ml; clog p = 2.23) [67].

Bicyclic scaffolds for CB_2 ligands

Imidazopyridine derivatives—Scientists at Acadia Pharmaceuticals Inc. recently reported the synthesis of new class ligands with high affinity to native CB_2 receptors. All these compounds have an imidazopyridine structure and the pKi value range is from 4.9 to 8.6. Among 120 analogues, **18** (Figure 4) is a representative compound, with a pK_i of 4.9 [114]. In the same year, they described another 136 imidazopyridine compounds and claimed these compounds had high affinity to native CB_2 receptors. The pK_i value range is from 4.9 to 8.6 and the representative sample is compound **19** (Figure 4), with a pK_i of 5.2 [115].

In 2009, scientists at Merck & Co., Inc. reported 12 imidazopyridine compounds, with two substituents at the 7- and 9-position, of which three compounds are quaternary ammonium salts. In the cAMP assay, these compounds have IC_{50} value ranging from 1 to >17000 nM. The representative compound **20** (Figure 4) has IC_{50} = 17 nM [116].

In 2011, Trotter *et al.* described a new series of imidazopyridine CB_2 agonists. They found a directly attached morpholine substituent displayed improved CB_2/CB_1 selectivity. Hydroxymethyl-containing amide 21 (Figure 4) was a potent CB_2 agonist that displayed no CB_1 agonism *in vitro* (hCB_2 cAMP IC_{50} = 33 nM, hCB_1 cAMP IC_{50} > 17000; Rat CB_2 cAMP IC_{50} = 58 nM, Rat CB_1 cAMP IC_{50} > 17000 nM) [68].

Indole & Azaindole derivatives—Liu *et al.* at Bristol-Myers Squibb Company synthesized 11 indanyl indole amide compounds as CB_2 agonists. All compounds were tested in filtration binding assays and/or GTPgS binding assays and have shown activity as an agonist of CB_2. For example, exemplified compound **22** (Figure 4) had a K_i value of 3 nM in the CB_2 binding assay and an EC_{50} value of 2.4 ± 0.55 nM in the CB_2 GTPgS binding assay [117].

Srivastava *et al.* reported 69 indolecarboxylic acid trimethylbicycloheptylamides as CB_2 receptor modulators. The representative compound **23** (Figure 4) showed an IC_{50} value of 0.027 nM in an *in vitro* cAMP assay [118]. Meanwhile, the *in vivo* activities of this compound were also tested in an CFA-induced hyperalgsia model, a chronic constriction injury of the sciatic nerve-induced neuropathic pain model and a formalin-induced nociception model. The results indicated that this compound showed a statistical effect.

In 2009, Giblin *et al.* published a series of novel anaindole CB_2 agonists. The representative compound **24** (Figure 4) is a highly potent CB_2 agonist with over 1200-fold selectivity for the human CB_1 receptor (CB_2: EC_{50} = 5 nM; CB_1: EC_{50} = 6300 nM) [69]. Furthermore, compound **24** is a potent full agonist at the human CB_2 receptor expressed in Chinese hamster ovary cells using a forskolin-induced cAMP readout (EC_{50} = 8 nM, efficacy 100%).

Benzo-fused heterocyclic derivatives—Gahman *et al.* synthesized 345 aminoquinazoline CB receptor modulators [119]. All the invention compounds were evaluated for their CB receptor modulatory activity. The CB_2 ligand-binding data were given as EC_{50} <1 μM or ≥1 μM and the selectivity of CB_2 versus CB_1 was given as >tenfold or ≤tenfold. Compound **25** (Figure 5) is an example.

Newcom *et al.* synthesized 110 benzo-fused heterocycles analogues and tested their EC_{50} against human CB_2, rat CB_2 and human CB_1 receptors. These compounds were separated as

agonist and inverse agonist. The binding EC_{50} data were showed as five ranges from 0.1 nM to 10 μM. Compound **26** (Figure 5) is an example [120].

In 2010, a series of 3-substituted oxindole derivatives as CB_2 agonists were synthesized by Dollings *et al.* [121]. Among 490 analogues, compound **27** (Figure 5) was the representative compound with high CB_2 binding affinity (CB_2: K_i = 1 nM; EC_{50} = 0.002 nM). Then, another series of substituted oxindole CB_2 agonists (695 analogues) was synthesized by Zhang *et al.* for Wyeth LLC. The representative compound **28** (Figure 5) showed high CB_2 binding affinity (CB_2: K_i = 1 nM; EC_{50} = 0.002 nM) [122].

Pasquini *et al.* recently synthesized a series of quinolone-3-carboxamides and described the SAR study. Except for six compounds exhibiting K_i >100 nM, all the quinolone-3-carboxamides proved to be high-affinity CB_2 ligands, with K_i values ranging from 73.2 to 0.7 nM and selectivity (CB_1/CB_2) varying from >14,285 to 1.9. Compound **29** (Figure 5) in particular has very high CB_2 receptor affinity (K_i = 0.7 nM) and good selectivity of 14,285-fold for this receptor [70]. Recently, in their continuing effort to explore SAR for quinolones binding at CB receptors, they discovered the 8-methoxy derivative 30 (Figure 5) endowed with the higher affinity and selectivity (CB_2: K_i = 0.6 nM; CB_1: K_i >10,000 nM; selectivity >16,666), which behaved as an inverse agonist [71].

Pyrazole-fused heterocyclic derivatives—In 2009, Xia *et al.* reported a series of new structure CB receptor ligands. All these compounds have a cyclooctanopyrazole core structure. Compound **31** (Figure 5) is a representative compound with high CB_2 affinity and selectivity (CB_2: IC_{50} = 0.1 nM; 400-fold CB_1/CB_2 selectivity). But the binding to CB_1 receptor of this compound is also very high (CB_1: IC_{50} = 40 nM) [123].

Recently, Jones *et al.* in Arena Pharmaceuticals, Inc. synthesized a series of pyrazole-fused heterocyclic analogues (931 compounds) and tested their binding activity against CB_1/CB_2 receptors. Parts of the binding data were given, and some compounds have high affinity to CB_1 and CB_2 receptor. For example, compound **32** (Figure 5) has high affinity to both CB_1 receptor and CB_2 receptor (EC_{50}: hCB_1 = 1.1 nM; EC_{50}: hCB_2 = 0.17 nM), while some other analogues such as compound **33** (Figure 5), show high affinity against CB_2 receptor and good selectivity (EC_{50}: hCB_1 = no response; EC_{50}: hCB_2 = 6.28 nM) [101]. In the meanwhile, the *in vivo* activities of some compounds were also tested in eight models, such as the osteoarthritis pain model, the skin-incision model, the Freund's complete adjuvant (FCA)-induced hyperalgesia model, the paclitaxel-induced allodynia model and so on. The compounds tested exhibited therapeutic efficacy in these models.

THC derivatives

By a concise and efficient procedure for converting a phenol to the corresponding aryl bromide, Huffman *et al.* modified a series of traditional THC ligands and got corresponding bromo CB analogues. All of these compounds showed selectivity for the CB_2 receptor and one of them, compound **34** (Figure 6), exhibits 52-fold selectivity for CB_2 receptor with good affinity (CB_1: K_i = 1444 nM; CB_2: K_i = 28 nM) [72].

Burdick and colleagues published the SAR study of substitutions at the C-1 position of Δ^9-THC. They focused on conversion of the phenol of Δ^9-THC to other functionality and found two analogues with sub-100 nM affinity for the CB_1 and CB_2 receptors, of which the representative compound **35** (CB_1: Ki = 67.8 nM; CB_2: Ki = 5.3 nM; Figure 6) shows a 13-fold selectivity for CB_2 over the CB_1 receptor, representing a significant improvement over Δ^9-THC [73].

Sulfone & sulfonamide derivatives

In 2008, Berry *et al.* found 192 sulfonyl carboxamide compounds and all the invention compounds were evaluated for their CB_2 receptor modulatory activity. Over 80 compounds showed good CB_2 agonist activity and the representative compound **36** (Figure 7) showed high CB_2 binding affinity (CB_2: EC_{50} = 0.04 nM) [124].

Regan *et al.* published a series heterocyclic sulfone compounds as modulators of the CB_2 receptor for treating inflammation, pain and disease. Many compounds are preferred CB_2 agonists and the exemplified compound **37** (Figure 7) showed K_i of 25 nM in CB_2 binding assay [125].

Triaryl bis-sulfone is a core structure of a series of selective CB_2 ligands. Gilbert *et al.* modified this core structure by converting the aryl A-ring to a piperidine ring, and further replaced the piperidine ring with a spirocyclopropyl piperidine, then got a new selective CB_2 ligand 38 (CB_2: K_i = 0.9 nM; ratio CB_1/ CB_2 >1000; Figure 7) [74]. The further SAR studies on triaryl bis-sulfone CB_2 receptor ligands by Tong *et al.* led to another potent and selective compound **39** (CB_2: K_i = 0.4 nM; ratio CB_1/CB_2 = 3500; Figure 7) [75].

Sulfonamide derivatives—After sulfamoyl benzamide was identified by high-throughput screening as novel CB receptor ligands, Goodman *et al.* further explored the SAR around the sylfonamide core and found compound **40** (Figure 8) with high CB_2 affinity and selectivity (CB_1: K_i = 3400 nM; CB_2: K_i = 23 nM; ratio CB_1/CB_2 = 147) [76]. This compound exhibited robust antiallodynic activity in a rodent pain model when administered intraperitoneally. However, this compound displayed poor metabolic stability in rat and human liver microsomes. To improve the metabolic stability and retain potent affinity and selectivity, a novel sulfamoyl benzamide **41** (CB_1: K_i = 2500 nM; CB_2: K_i = 17 nM; ratio CB_1/CB_2 = 150; rat liver microsomes = 28%; human livermicrosomes = 17%; Figure 8) as selective CB_2 agonists with improved *in vitro* metabolic stability was reported by Sellitto and colleagues [77].

Yacovan *et al.* synthesized a series of sulfonamide analogues. Among 110 compounds, the representative compound **42** (Figure 8) exhibited K_i values of 21,020 and 933 nM against CB_1 and CB_2, respectively [126].

Two patents have been published by Boehringer Ingelheim International GmbH and claimed many compounds are preferred CB_2 agonists. In the first patent, Cirillo *et al.* synthesized a series of amine and ether compounds which modulate the CB_2 receptor. Of these compounds, over 40 compounds showed good CB_2 agonist activity and the representative compound **43** (Figure 8) with sulfone structure showed high CB_2 binding affinity (CB_2: EC_{50} = 0.13 nM) [127]. Compound **44** (Figure 8) is the representative example of the second patent with high CB_2 EC_{50} values of 1.3 nM [128]. And both of them have sulfonamide core structures.

Miscellaneous scaffolds for CB_2 ligands

Due to the diversity of chemical structures, its always a challenge to assign a structural class of molecules to one of the categories above. The following series discussed in this section display various chemical scaffolds and bioactivities.

Through 3D-quantitative SAR studies of arylpyrazole antagonists CB receptors [78] and 3D pharmacophore database *in silico* screening [79], Chen *et al.* disclosed a novel class of CB ligands with an amidine amide core structure [129]. The representative compound **45**

(Figure 9) has high CB_2 receptor affinity (K_i = 31.7 nM) and good selectivity of 132-fold over CB_1 receptor (CB_1: K_i = 4185 nM).

Recently, sulfamoyl benzamides were identified as a novel series of CB receptor ligands, Worm *et al.* replaced the sulfonamide functionality and reversed the original carboxamide bond and discovered compound **46** (K_i = 2.7; CB_1/CB_2 = 190; Figure 8) as a potent and selective CB_2 agonist, which displayed robust activity in a rodent model of postoperative pain [80].

In 2010, two patents of different amide CB ligands were published by the scientists at Boehringer Ingelheim International GmbH. In the first patent, all the compounds have the 2-azetidinecarboxamide core structure and were tested for the binding activity to CB_1/CB_2 receptor. Many of them are preferred CB_2 agonists, and compound **47** (Figure 9) is a representative compound with high CB_2 affinity (CB_2: EC_{50} = 0.02 nM) [130]. They claimed these compounds are useful for treating inflammation or pain. As for the second patent, 91 compounds with *N*-azolyl α-aminoalkanamide core structure were synthesized and tested for their binding activity to CB_1/CB_2 receptor. Many of them are preferred CB_2 agonists, and **48** (Figure 9) is a representative compound with high CB_2 affinity and better selectivity (CB_2: EC_{50} = 0.02 nM; CB_1: EC_{50} > 50000 nM) [131].

Gertsch and colleagues published a novel class of CB ligands, namely dodeca-*2E*,4*E*diene amides [81]. Among these analogues, **49** (Figure 9) is a representative compound with high CB_2 affinity and selectivity (CB_2: K_i = 60 ± 7 nM; CB_1: K_i = 1940 ± 213 nM) [132]. The results also indicated the claimed dodeca-2*E*,4*E*diene amides inhibit AEA re-uptake and some compounds of the invention also inhibit fatty acid amide hydrolase.

Bab *et al.* disclosed a series of phenyl substituted pinenes compounds. Compared with the traditional selective CB_2 ligand HU-308 (CB_1: K_i >10 μM; CB_2: K_i = 22.7 nM), the representative compound **50** (HU-433; Figure 9) was found to be significantly more potent (CB_1: K_i >20 μM; CB_2: K_i = 12.2 nM) [133]. The comparative skeletal activities of HU-433 and HU-308 were also tested. The data indicated that HU-433 was a 1000-fold more active compared with HU-308 *in vitro*. The *in vivo* skeletal activity of these two compounds was analyzed in an ovariectomy (removal of ovaries) mouse model: the most widely used animal model for osteoporosis. The result showed that HU-433 was at least 100-fold more active than HU-308. They also claimed the effect of HU-433 was substantially greater than the reversal of bone volumetric density by parathyroid hormone, the only clinically approved bone anabolic agent.

In 2011, Mechoulam published a series of novel arylated camphene compounds. The representative compound **51** (HU-910; Figure 9) has high CB_2 affinity (CB_2 EC_{50} = 26.4 nM) [134]. The *in vivo* data indicated HU-910 displayed a significantly greater recovery than the control group in the closed head injury model.

Conclusion

Many new selective CB_2 ligands have been emerging in the literature over the last 5 years. It is estimated from SciFinder that there are 1419 journal articles and 387 patents reported about CB_2 research and new CB_2 ligands. The recently available Web-interfaced CB molecular information database repository constructed by the Xie laboratory has over 8500 records of CB ligands [202]. As discussed here, these compounds represent a variety of different chemical classes that are distinct from chemotypes typified by the endogenous CBs. The binding affinity and selectivity of reviewed CB_2 ligands were summarized in **T**able **1**. Among these compounds, many of them have notably high CB_2 binding affinity,

for example the binding affinities of compounds **1**, **2**, **9** and **15** are less than 1 nM. Other compounds show good selectivity, such as compounds **8**, **14**, **17** and **24** with >1000. Here, the authors suggest that caution should be taken in using these data because the receptor binding data may vary as different laboratories may use different approaches. Even with the use of the same protocols, different cell lines may produce dissimilar sets of binding affinity data. Overall, this article reports on the recent advances by providing an overview of novel classes of CB_2 ligands reported in research articles and patents. The structural and bioactivity data of these novel CB_2 ligands will be valuable for scientists in industrial and academic chemistry, pharmacology and computational chemistry laboratories conducting CB_2 lead optimization/modification and SAR medicinal chemistry synthesis, pharmacological/biochemical studies and computer- aided drug design research for novel CB_2 drug-design discovery.

Future perspective

New advances in CB drug research are reviewed with a focus on the most recent development of CB_2 ligands reported in literature and patents. Overall, CBs represent an important family of large structurally diverse molecules with promising therapeutic potential. In particular, research studies involving CB_2-targeted ligands have been steadily proliferating. The quantity and the quality of this special class of molecules are expected to grow at a much faster rate in the future. These new generations of CB_2 receptor-selective compounds will overcome many of the hurdles that plague currently available pharmacological studies, including poor selectivity, low potency and/or efficacy and unsatisfactory pharmacokinetic properties. As indicated above, while such a physiological role of CB_2 receptors remains to be fully defined, several intriguing preclinical studies suggest that CB_2 ligands may be clinically useful and possible medications for chronic pain, autoimmune MS, osteoporosis and atherosclerotic lesions. Nevertheless, with all exciting points about CB_2 ligands, however, several fundamental questions still remain to be further explored in detail, in order to better understanding of physiological roles of the CB_2 receptor in immune responses. Also needed is a thorough assessment of the pharmacological properties and the relevant signaling pathways of the discovered CB_2 agonists and antagonists. As such, the newly developed CB ligands and their bioactivities will help researchers to better understand the role of CB_2 receptor played in both physiological and pathophysiological processes. With great efforts being devoted towards CB_2 drug research, we expect that highly potent and selective druggable CB_2 agents will be discovered, which will ultimately be translated in the clinic in to new CB_2 drugs that possess great therapeutic values without causing psychotropic side effects in humans.

Key Term

Endocannabinoid system	Endocannabinoids are found in the nervous and immune systems of animals and activate cannabinoid receptors. The endocannabinoid system represents a neuromodulator system consisting of endogenous ligands, enzymes and cannabinoid receptors (subtypes CB_1 and CB_2) that are involved in a variety of physiological processes including appetite, pain sensation, mood and memory; it mediates the psychoactive effects of cannabis.

References

1. Raitio KH, Salo OM, Nevalainen T, Poso A, Jarvinen T. Targeting the cannabinoid CB2 receptor: mutations, modeling and development of CB2 selective ligands. Curr Med Chem. 2005; 12(10): 1217–1237. [PubMed: 15892633]

2. Nordstrom BR, Levin FR. Treatment of cannabis use disorders: a review of the literature. Am J Addict. 2007; 16(5):331–342. [PubMed: 17882603]

3. Tanda G, Goldberg SR. Cannabinoids: reward, dependence, and underlying neurochemical mechanisms – a review of recent preclinical data. Psychopharmacology. 2003; 169(2):115–134. [PubMed: 12827346]

4. Tanda G, Pontieri FE, Di Chiara G. Cannabinoid and heroin activation of mesolimbic dopamine transmission by a common mu1 opioid receptor mechanism. Science. 1997; 276(5321):2048–2050. [PubMed: 9197269]

5. Clapper JR, Mangieri RA, Piomelli D. The endocannabinoid system as a target for the treatment of cannabis dependence. Neuropharmacology. 2009; 56(Suppl 1):235–243. [PubMed: 18691603]

6. Piomelli D. The molecular logic of endocannabinoid signalling. Nat Rev Neurosci. 2003; 4(11): 873–884. [PubMed: 14595399]

7. Xie S, Furjanic MA, Ferrara JJ, et al. The endocannabinoid system and rimonabant: a new drug with a novel mechanism of action involving cannabinoid CB1 receptor antagonism – or inverse agonism – as potential obesity treatment and other therapeutic use. J Clin Pharm Ther. 2007; 32(3):209–231. [PubMed: 17489873]

8. Justinova Z, Panlilio LV, Goldberg SR. Drug addiction. Curr Top Behav Neurosci. 2009; 1:309–346. [PubMed: 21104390]

9. Matsuda LA, Lolait SJ, Brownstein MJ, Young AC, Bonner TI. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature. 1990; 346(6284):561–564. [PubMed: 2165569]

10. Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature. 1993; 365(6441):61–65. [PubMed: 7689702]

11. Bramblett RD, Panu AM, Ballesteros JA, Reggio PH. Construction of a 3D model of the cannabinoid CB1 receptor: determination of helix ends and helix orientation. Life Sci. 1995; 56(23–24):1971–1982. [PubMed: 7776821]

12. Onaivi ES, Chakrabarti A, Chaudhuri G. Cannabinoid receptor genes. Prog Neurobiol. 1996; 48(4–5):275–305. [PubMed: 8804112]

13. Xie XQ, Chen JZ, Billings EM. 3D structural model of the G-protein-coupled cannabinoid CB2 receptor. Protein. 2003; 53(2):307–319.

14. Howlett AC, Barth F, Bonner TI, et al. International Union of Pharmacology. XXVII Classification of cannabinoid receptors. Pharmacol Rev. 2002; 54(2):161–202. [PubMed: 12037135]

15. Croci T, Manara L, Aureggi G, et al. *In vitro* functional evidence of neuronal cannabinoid CB1 receptors in human ileum. Br J Pharmacol. 1998; 125(7):1393–1395. [PubMed: 9884065]

16. Pertwee RG. Cannabinoid receptors and pain. Prog Neurobiol. 2001; 63(5):569–611. [PubMed: 11164622]

17. Szabo B, Nordheim U, Niederhoffer N. Effects of cannabinoids on sympathetic and parasympathetic neuroeffector transmission in the rabbit heart. J Pharmacol Exp Ther. 2001; 297(2):819–826. [PubMed: 11303075]

18. Sanchez C, De Ceballos ML, Gomez Del Pulgar T, et al. Inhibition of glioma growth *in vivo* by selective activation of the CB(2) cannabinoid receptor. Cancer Res. 2001; 61(15):5784–5789. [PubMed: 11479216]

19. Fernandez-Ruiz J, Romero J, Velasco G, Tolon RM, Ramos JA, Guzman M. Cannabinoid CB2 receptor: a new target for controlling neural cell survival? Trends Pharmacol Sci. 2007; 28(1):39–45. [PubMed: 17141334]

20. Svizenska I, Dubovy P, Sulcova A. Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures: a short review. Pharmacol Biochem Behav. 2008; 90(4):501–511. [PubMed: 18584858]

21. Wilson RI, Nicoll RA. Endocannabinoid signaling in the brain. Science. 2002; 296(5568):678–682. [PubMed: 11976437]

22. Marriott KS, Huffman JW. Recent advances in the development of selective ligands for the cannabinoid CB(2) receptor. Curr Top Med Chem. 2008; 8(3):187–204. [PubMed: 18289088]

23. Beardsley PM, Thomas BF, Mcmahon LR. cannabinoid CB1 receptor antagonists as potential pharmacotherapies for drug abuse disorders. Int Rev Psychiatry. 2009; 21(2):134–142. [PubMed: 19367507]
24. Beardsley PM, Thomas BF. Current evidence supporting a role of cannabinoid CB1 receptor (CB1R) antagonists as potential pharmacotherapies for drug abuse disorders. Behav Pharmacol. 2005; 16(5–6):275–296. [PubMed: 16148435]
25. Rinaldi-Carmona M, Barth F, Heaulme M, et al. SR141716A: a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 1994; 350(2–3):240–244. [PubMed: 8070571]
26. Valenzano KJ, Tafesse L, Lee G, et al. Pharmacological and pharmacokinetic characterization of the cannabinoid receptor 2 agonist, GW405833, utilizing rodent models of acute and chronic pain, anxiety, ataxia and catalepsy. Neuropharmacology. 2005; 48(5):658–672. [PubMed: 15814101]
27. Berdyshev EV. Cannabinoid receptors and the regulation of immune response. Chem Phys Lipids. 2000; 108(1–2):169–190. [PubMed: 11106790]
28. Sugiura T, Kondo S, Kishimoto S, et al. Evidence that 2-arachidonoylglycerol but not N-palmitoylethanolamine or anandamide is the physiological ligand for the cannabinoid CB2 receptor. Comparison of the agonistic activities of various cannabinoid receptor ligands in HL-60 cells. J Biol Chem. 2000; 275(1):605–612. [PubMed: 10617657]
29. Wilson RI, Nicoll RA. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature. 2001; 410(6828):588–592. [PubMed: 11279497]
30. Beltramo M, Bernardini N, Bertorelli R, et al. CB2 receptor-mediated antihyperalgesia: possible direct involvement of neural mechanisms. Eur J Neurosci. 2006; 23(6):1530–1538. [PubMed: 16553616]
31. Onaivi Emmanuel S, Ishiguro H, Gong J-P, et al. Brain neuronal CB2 cannabinoid receptors in drug abuse and depression: from mice to human subjects. PLoS One. 2008; 3(2):e1640. [PubMed: 18286196]
32. Benito C, Tolon RM, Pazos MR, Nunez E, Castillo AI, Romero J. Cannabinoid CB2 receptors in human brain inflammation. Br J Pharmacol. 2008; 153(2):277–285. [PubMed: 17934510]
33. Onaivi ES. Neuropsychobiological evidence for the functional presence and expression of cannabinoid CB2 receptors in the brain. Neuropsychobiology. 2006; 54(4):231–246. [PubMed: 17356307]
34. Ishiguro H, Iwasaki S, Teasenfitz L, et al. Involvement of cannabinoid CB2 receptor in alcohol preference in mice and alcoholism in humans. Pharmacogenomics J. 2007; 7(6):380–385. [PubMed: 17189959]
35. Onaivi ES, Ishiguro H, Gong JP, et al. Functional expression of brain neuronal CB2 cannabinoid receptors are involved in the effects of drugs of abuse and in depression. Ann NY Acad Sci. 2008; 1139:434–449. [PubMed: 18991891]
36. Uhl GR, Ishiguro H, Onaivi ES, et al. Molecular neurobiological methods in marijuana-cannabinoid research. Methods Mol Med. 2006; 123:1–17. [PubMed: 16506399]
37. Xi Z-X, Peng X-Q, Li X, et al. Brain cannabinoid CB2 receptors modulate cocaine's actions in mice. Nat Neurosci. 2011; 14:1160–1166. [PubMed: 21785434]
38. Patel KD, Davison JS, Pittman QJ, Sharkey KA. Cannabinoid CB(2) receptors in health and disease. Curr Med Chem. 2010; 17(14):1393–1410. [PubMed: 20166925]
39. Palazuelos J, Davoust N, Julien B, et al. The CB(2) cannabinoid receptor controls myeloid progenitor trafficking: involvement in the pathogenesis of an animal model of multiple sclerosis. J Biol Chem. 2008; 283(19):13320–13329. [PubMed: 18334483]
40. Dvorak M, Watkinson A, Mcglone F, Rukwied R. Histamine induced responses are attenuated by a cannabinoid receptor agonist in human skin. Inflamm Res. 2003; 52(6):238–245. [PubMed: 12835895]
41. Karsak M, Gaffal E, Date R, et al. Attenuation of allergic contact dermatitis through the endocannabinoid system. Science. 2007; 316(5830):1494–1497. [PubMed: 17556587]
42. Ashton JC, Glass M. The cannabinoid CB2 receptor as a target for inflammationdependent neurodegeneration. Curr Neuropharmacol. 2007; 5(2):73–80. [PubMed: 18615177]

43. Zhang M, Martin BR, Adler MW, Razdan RK, Jallo JI, Tuma RF. Cannabinoid CB(2) receptor activation decreases cerebral infarction in a mouse focal ischemia/ reperfusion model. J Cereb Blood Flow Metab. 2007; 27(7):1387–1396. [PubMed: 17245417]
44. Hohmann AG. Spinal and peripheral mechanisms of cannabinoid antinociception: behavioral, neurophysiological and neuroanatomical perspectives. Chem Phys Lipids. 2002; 121(1–2):173–190. [PubMed: 12505699]
45. Walker JM, Hohmann AG. Cannabinoid mechanisms of pain suppression. Handb Exp Pharmacol. 2005; (168):509–554. [PubMed: 16596786]
46. Whiteside GT, Lee GP, Valenzano KJ. The role of the cannabinoid CB2 receptor in pain transmission and therapeutic potential of small molecule CB2 receptor agonists. Curr Med Chem. 2007; 14(8):917–936. [PubMed: 17430144]
47. Guindon J, Hohmann AG. Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain. Br J Pharmacol. 2008; 153(2):319–334. [PubMed: 17994113]
48. Hu B, Doods H, Treede RD, Ceci A. Depression-like behaviour in rats with mononeuropathy is reduced by the CB2- selective agonist GW405833. Pain. 2009; 143(3):206–212. [PubMed: 19345493]
49. Kinsey SG, Mahadevan A, Zhao B, et al. The CB(2) cannabinoid receptor-selective agonist O-3223 reduces pain and inflammation without apparent cannabinoid behavioral effects. Neuropharmacology. 2011; 60(2–3):244–251. [PubMed: 20849866]
50. Guindon J, Hohmann AG. The endocannabinoid system and cancer: therapeutic implication. Br J Pharmacol. 2011; 163(7):1447–1463. [PubMed: 21410463]
51. Qamri Z, Preet A, Nasser MW, et al. Synthetic cannabinoid receptor agonists inhibit tumor growth and metastasis of breast cancer. Mol Cancer Ther. 2009; 8(11):3117– 3129. [PubMed: 19887554]
52. Caffarel MM, Andradas C, Mira E, et al. CBs reduce ErbB2-driven breast cancer progression through Akt inhibition. Mol Cancer. 2010; 9:196. [PubMed: 20649976]
53. Idris AI, Ralston SH. CBs and bone: friend or foe? Calcif Tissue Int. 2010; 87(4):285–297. [PubMed: 20532878]
54. Ofek O, Karsak M, Leclerc N, et al. Peripheral cannabinoid receptor, CB2, regulates bone mass. Proc Natl Acad Sci USA. 2006; 103(3):696–701. [PubMed: 16407142]
55. Idris AI, Van 'T Hof RJ, Greig IR, et al. Regulation of bone mass, bone loss and osteoclast activity by cannabinoid receptors. Nat Med. 2005; 11(7):774–779. [PubMed: 15908955]
56. Rossi F, Siniscalco D, Luongo L, et al. The endovanilloid/endocannabinoid system in human osteoclasts: possible involvement in bone formation and resorption. Bone. 2009; 44(3):476–484. [PubMed: 19059369]
57. Izzo AA, Camilleri M. Emerging role of CBs in gastrointestinal and liver diseases: basic and clinical aspects. Gut. 2008; 57(8):1140–1155. [PubMed: 18397936]
58. Munoz-Luque J, Ros J, Fernandez-Varo G, et al. Regression of fibrosis after chronic stimulation of cannabinoid CB2 receptor in cirrhotic rats. J Pharmacol Exp Ther. 2008; 324(2):475–483. [PubMed: 18029545]
59. Wei Y, Wang X, Wang L. Presence and regulation of cannabinoid receptors in human retinal pigment epithelial cells. Mol Vis. 2009; 15:1243–1251. [PubMed: 19547718]
60. Goncalves MB, Suetterlin P, Yip P, et al. A diacylglycerol lipase-CB2 cannabinoid pathway regulates adult subventricular zone neurogenesis in an age-dependent manner. Mol Cell Neurosci. 2008; 38(4):526–536. [PubMed: 18562209]
61. Marriott K-SC, Huffman JW. Recent advances in the development of selective ligands for the cannabinoid CB2 receptor. Curr Top Med Chem. 2008; 8(3):187–204. [PubMed: 18289088]
62. Reggio PH. Cannabinoid receptors and their ligands: ligand–ligand and ligand–receptor modeling approaches. Handb Exp Pharmacol. 2005; 168(CBs):247–281. [PubMed: 16596777]
63. Padgett LW. Recent developments in cannabinoid ligands. Life Sci. 2005; 77(14):1767–1798. [PubMed: 15993427]
64. Lange JHM, Van DNMaW, Wals HC, et al. Synthesis and SAR of novel imidazoles as potent and selective cannabinoid CB2 receptor antagonists with high binding efficiencies. Bioorg Med Chem Lett. 2010; 20:1084–1089. [PubMed: 20031412]

65. Chu G-H, Saeui CT, Worm K, et al. Novel pyridine derivatives as potent and selective CB2 cannabinoid receptor agonists. Bioorg Med Chem Lett. 2009; 19:5931–5935. [PubMed: 19736007]

66. Zindell R, Riether D, Bosanac T, et al. Morpholine containing CB2 selective agonists. Bioorg Med Chem Lett. 2009; 19:1604–1609. [PubMed: 19243942]

67. Zindell R, Walker ER, Scott J, et al. Aryl 1,4-diazepane compounds as potent and selective CB2 agonists: optimization of drug-like properties and target independent parameters. Bioorg Med Chem Lett. 2011; 21:4276–4280. [PubMed: 21689933]

68. Trotter BW, Nanda KK, Burgey CS, et al. Imidazopyridine CB2 agonists: optimization of CB2/CB1 selectivity and implications for *in vivo* analgesic efficacy. Bioorg Med Chem Lett. 2011; 21:2354–2358. [PubMed: 21420860]

69. Giblin GMP, Billinton A, Briggs M, et al. Discovery of 1-[4-(3-chlorophenylamino)-1- methyl-1H-pyrrolo[3,2-c]pyridin-7-yl]-1- morpholin-4-ylmethanone (GSK554418A), a brain penetrant 5-azaindole CB2 agonist for the treatment of chronic pain. J Med Chem. 2009; 52:5785–5788. [PubMed: 19743867]

70. Pasquini S, Ligresti A, Mugnaini C, et al. Investigations on the 4-quinolone-3- carboxylic acid motif. 3 Synthesis, structure– affinity relationships and pharmacological characterization of 6-substituted 4-quinolone- 3-carboxamides as highly selective CB-2 receptor ligands. J Med Chem. 2010; 53:5915–5928. [PubMed: 20718492]

71. Pasquini S, De RM, Pedani V, et al. Investigations on the 4-quinolone-3- carboxylic acid motif. 4 Identification of new potent and selective ligands for the cannabinoid type 2 receptor with diverse substitution patterns and antihyperalgesic effects in mice. J Med Chem. 2011; 54:5444–5453. [PubMed: 21702498]

72. Huffman JW, Hepburn SA, Lyutenko N, et al. 1-bromo-3-(1′,1′-dimethylalkyl)-1-deoxy-Δ8-tetrahydrocannabinols: new selective ligands for the cannabinoid CB2 receptor. Bioorg Med Chem. 2010; 18:7809–7815. [PubMed: 20943404]

73. Burdick D, De OR, Guzzo P, et al. Synthesis and structure–activity relationship of substitutions at the C-1 position of Δ9- tetrahydrocannabinol. Bioorg Med Chem Lett. 2010; 20:1424–1426. [PubMed: 20079638]

74. Gilbert EJ, Zhou G, Wong MKC, et al. Nonaromatic A-ring replacement in the triaryl bis-sulfone CB2 receptor inhibitors. Bioorg Med Chem Lett. 2010; 20:608–611. [PubMed: 20005710]

75. Tong L, Shankar BB, Chen L, et al. Expansion of SAR studies on triaryl bis sulfone cannabinoid CB2 receptor ligands. Bioorg Med Chem Lett. 2010; 20:6785–6789. [PubMed: 20850969]

76. Goodman AJ, Ajello CW, Worm K, et al. CB2 selective sulfamoyl benzamides: optimization of the amide functionality. Bioorg Med Chem Lett. 2009; 19:309–313. [PubMed: 19091565]

77. Sellitto I, Le BB, Worm K, et al. Novel sulfamoyl benzamides as selective CB_2 agonists with improved *in vitro* metabolic stability. Bioorg Med Chem Lett. 2010; 20:387–391. [PubMed: 19919895]

78. Chen J-Z, Han X-W, Liu Q, Makriyannis A, Wang J, Xie X-Q. 3D-QSAR Studies of arylpyrazole antagonists of cannabinoid receptor subtypes CB_1 and CB_2 A combined NMR and CoMFA approach. J Med Chem. 2006; 49(2):625–636. [PubMed: 16420048]

79. Chen JZ, Xie XQ. GPCR structure-based virtual screening approach for the CB_2 antagonist search. J Comput Info Modeling. 2007; 47:1626–1637.

80. Worm K, Weaver DG, Green RC, et al. Discovery of *N*-(3-(morpholinomethyl)- phenyl)-amides as potent and selective CB_2 agonists. Bioorg Med Chem Lett. 2009; 19:5004–5008. [PubMed: 19646869]

81. Gertsch J, Leonti M, Raduner S, et al. β-caryophyllene is a dietary CB. Proc Natl Acad Sci USA. 2008; 105(26):9099–9104. [PubMed: 18574142]

Patents

101. Arena Pharmaceuticals, Inc. WO2011025541A1. 2011.

102. Abbott Laboratories. WO2010111572A1. 2010.

103. Boehringer Ingelheim International GmbH. WO2010077836A2. 2010.

104. Abbott Laboratories. WO2010071783A1. 2010.
105. Abbott Laboratories. WO2010111573A1. 2010.
106. Abbott Laboratories. WO2010111574A1. 2010.
107. Abbott Laboratories. US20100234345A1. 2010.
108. Cara Therapeutics, Inc. WO2008157751A2. 2008.
109. Boehringer Ingelheim International GmbH. WO2010096371A2. 2010.
110. F Hoffmann-La Roche AG. WO2008040649A2. 2008.
111. Allergan, Inc. WO2011097553A1. 2011.
112. Boehringer Ingelheim International GmbH. WO 2008064054A2. 2008.
113. Boehringer Ingelheim International GmbH. WO 2009055357A1. 2009.
114. Acadia Pharmaceuticals Inc. WO2008141239A1. 2008.
115. Acadia Pharmaceuticals Inc. WO2008141249A1. 2008.
116. Merck & Co., Inc. WO2009025785A2. 2009.
117. Bristol-Myers Squibb Company. WO2009015169A1. 2009.
118. Cadila Healthcare Ltd. WO2009063495A2. 2009.
119. Kalypsys, Inc. WO2008157500A1. 2008.
120. Cara Therapeutics, Inc. WO2009035997A2. 2009.
121. Wyeth, John, and Brother Ltd. WO2010077839A1. 2010.
122. Wyeth LLC. WO2010090680A1. 2010.
123. Janssen Pharmaceutica N.V. WO2009023292A1. 2009.
124. Boehringer Ingelheim International GmbH. WO2008039645A1. 2008.
125. Boehringer Ingelheim International GmbH. WO2010005782A1. 2010.
126. Pharmos Corporation. WO2008075353A1. 2008.
127. Boehringer Ingelheim International GmbH. WO2009105509A1. 2009.
128. Boehringer Ingelheim International GmbH. WO2009061652A1. 2009.
129. University Of Pittsburgh. WO2009058377A1. 2009.
130. Boehringer Ingelheim International GmbH. WO2010147791A1. 2010.
131. Boehringer Ingelheim International GmbH. WO2010147792A2. 2010.
132. Dr. August Wolff GmbH & Co. Kg Arzneimittel. WO2010136221A1. 2010.
133. Yissum Research Development Company Of The Hebrew University Of Jerusalem, Ltd. WO2010041253A1. 2010.
134. Yissum Research Development Company Of The Hebrew University Of Jerusalem, Ltd. WO2011061744A2. 2011.

Websites

201. NIDA InfoFacts: Marijuana. www.drugabuse.gov/infofacts/marijuana.html
202. Cannabinoid Ligand Database. www.CBLigand.org

Executive summary

- Marijuana or cannabinoids (CBs) drug abuse and toxicities are a serious threat to human health in the USA and the world. It contains a complex mixture of compounds, including tetrahydrocannabinol, the major psychoactive constituent.
- There are no effective treatments for the abuse of marijuana today as most treatments are still relatively ineffective and have a high failure rate due to drug-addict relapse and withdrawal symptoms.
- Two CB receptors have been cloned and characterized: CB_1 and CB_2.
- Studies show that release of endocannabinoids in the ventral tegmental area can modulate the reward-related effects of dopamine and might, therefore, be an important neurobiological mechanism underlying drug addiction.
- Both CB_1 ligands and CB_2 ligands showed some good treatment effects in drug abuse. The undesirable psychotropic effects of CB_1 ligands has limited the therapeutic utility, while CB_2 ligands would not be expected to elicit such side effects.
- Selective CB_2 receptor ligands developed recently are summarized and classified in to seven types based on their chemical scaffolds.
- CB_2 ligands have therapeutic potentials, such as treatment in immune disorders, pain, cancers and osteoporosis.
- Future research will focus on finding selective and efficacious CB_2 compounds with good drug-like properties but no undesired psychotropic effects.

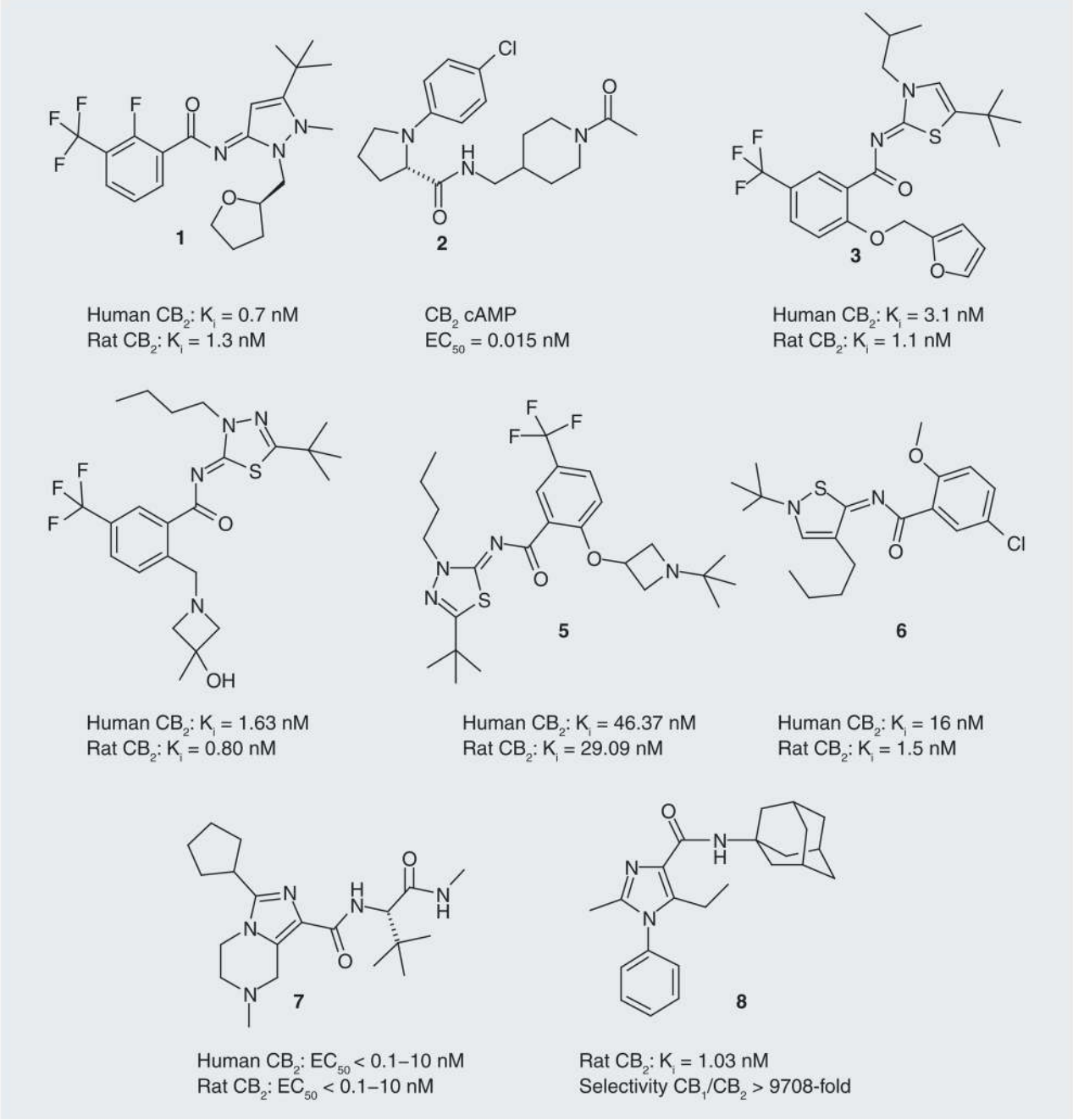

Figure 1.
Representative structures of pyrazole, pyrrolidine, thiazole, isothiazole and imidazole derivatives.

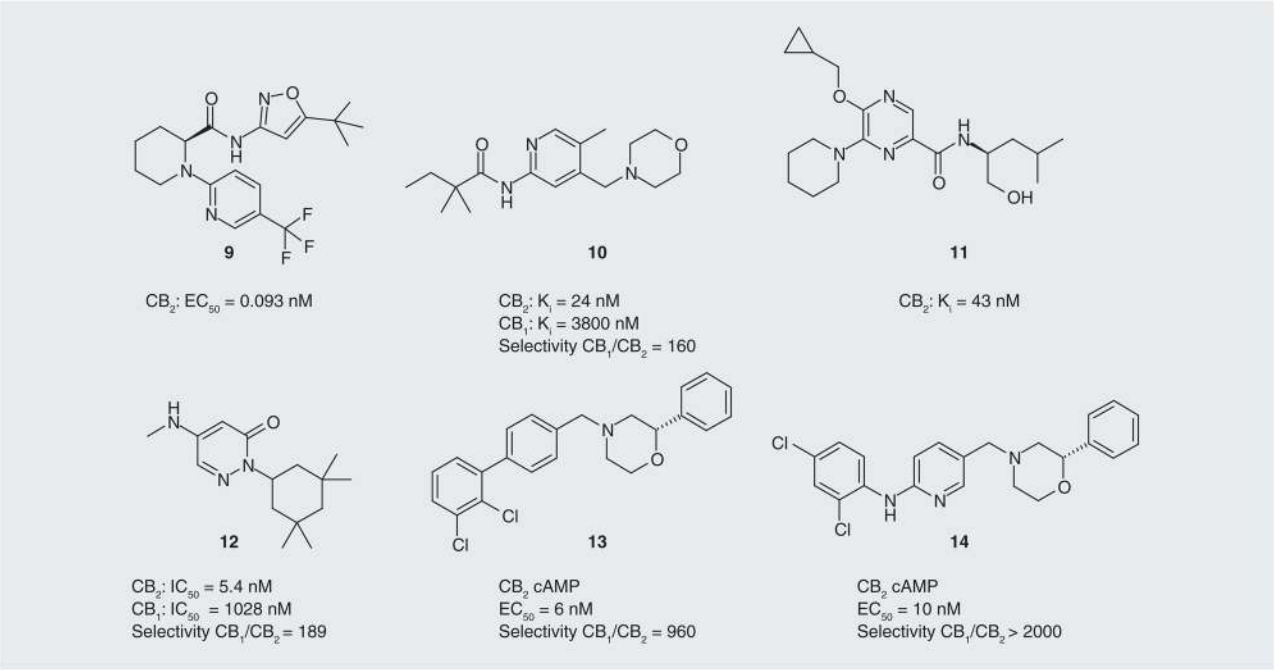

Figure 2.
Representative structures of pyridine, pyrazine, pyridazine and morpholine derivatives.

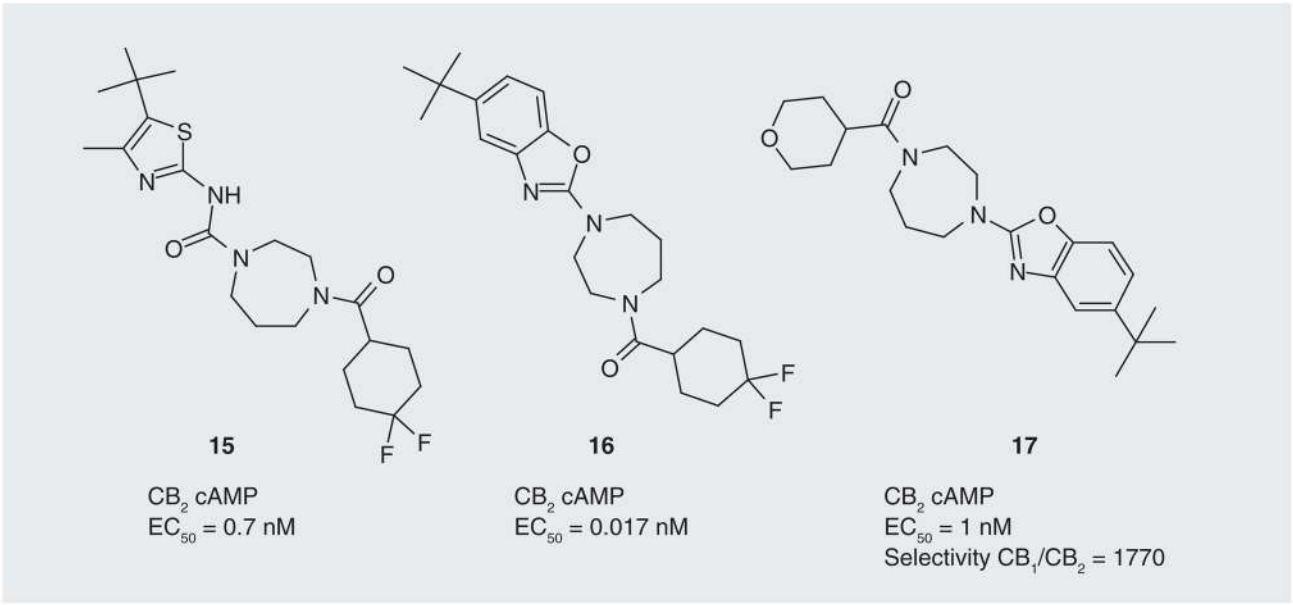

Figure 3.
Representative structures of diazepane derivatives.

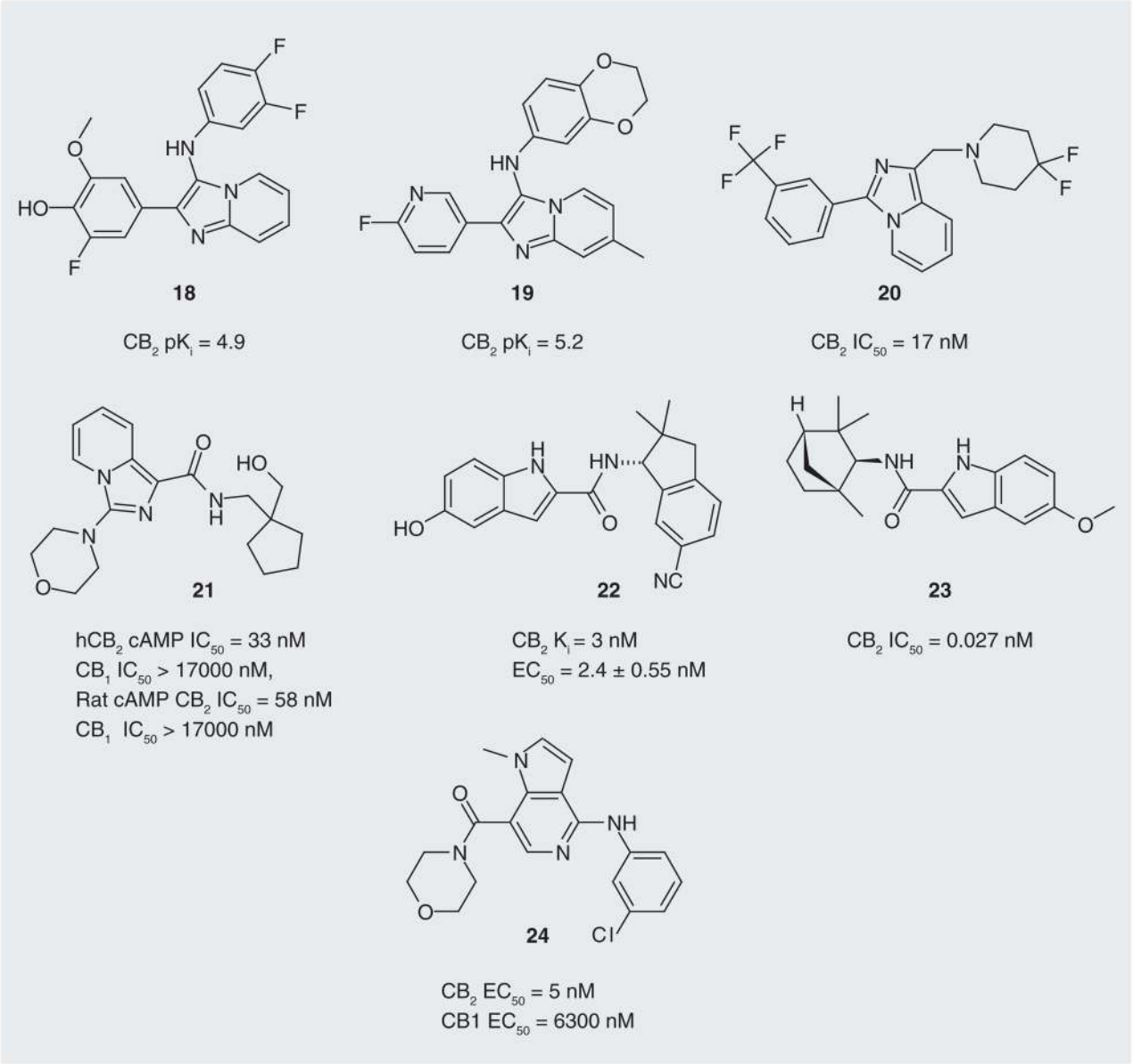

Figure 4.
Representative structures of imidazopyridine, indole and azaindole derivatives.

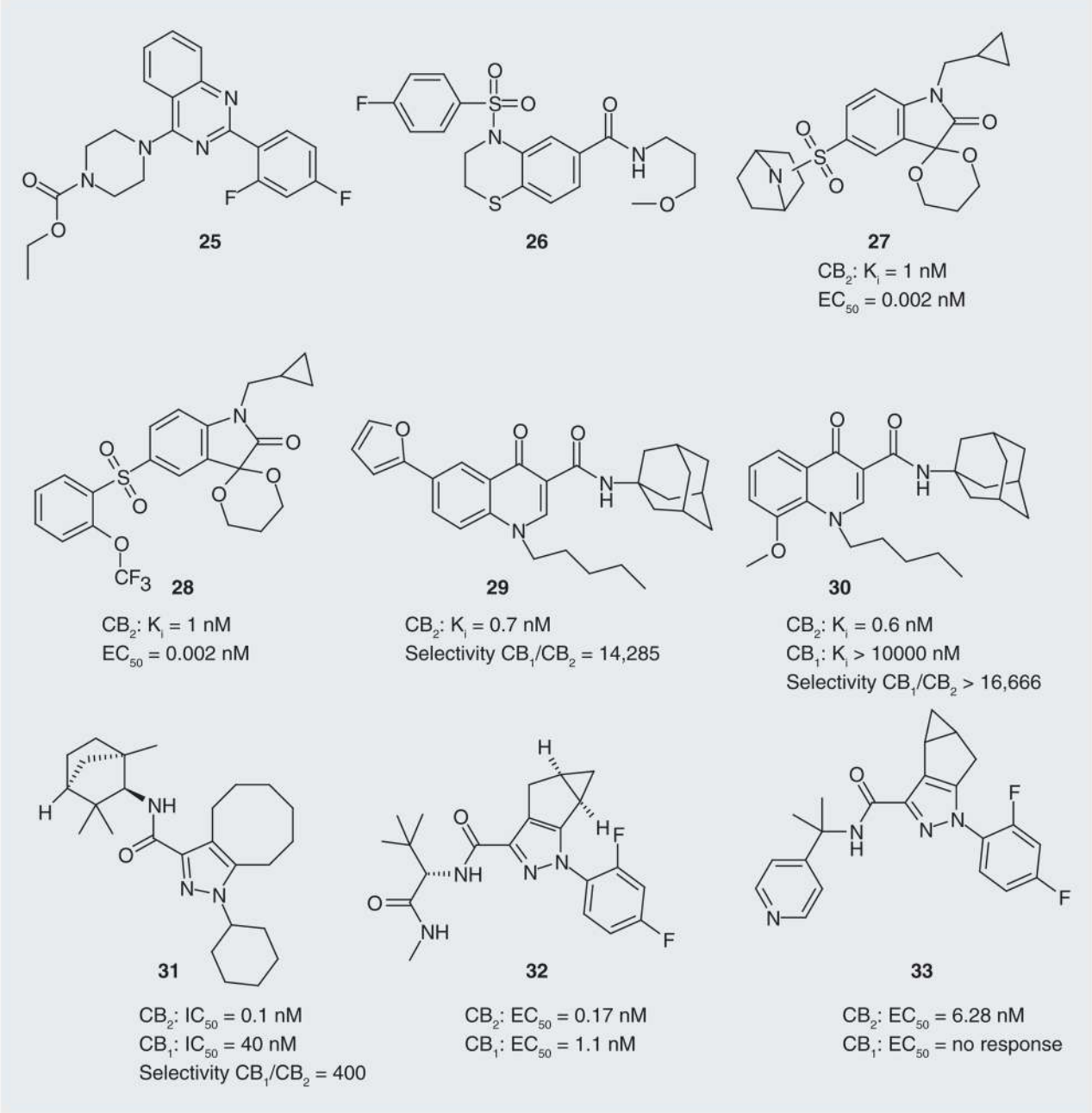

Figure 5.
Representative structures of benzo-fused and pyrazole-fused heterocyclic derivatives.

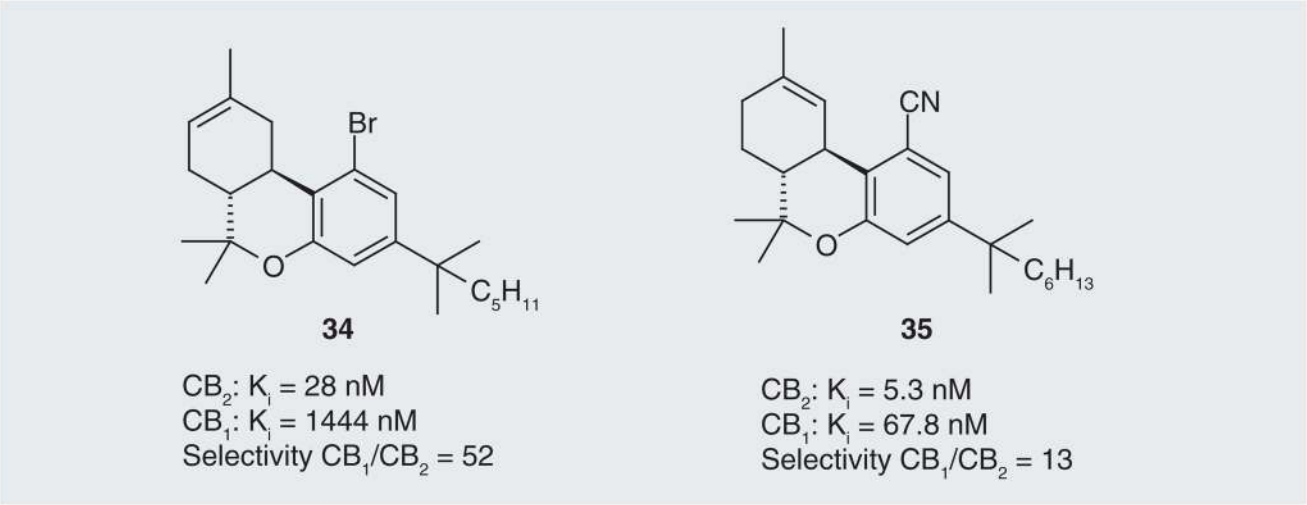

Figure 6.
Representative structures of tetrahydrocannabinol derivatives.

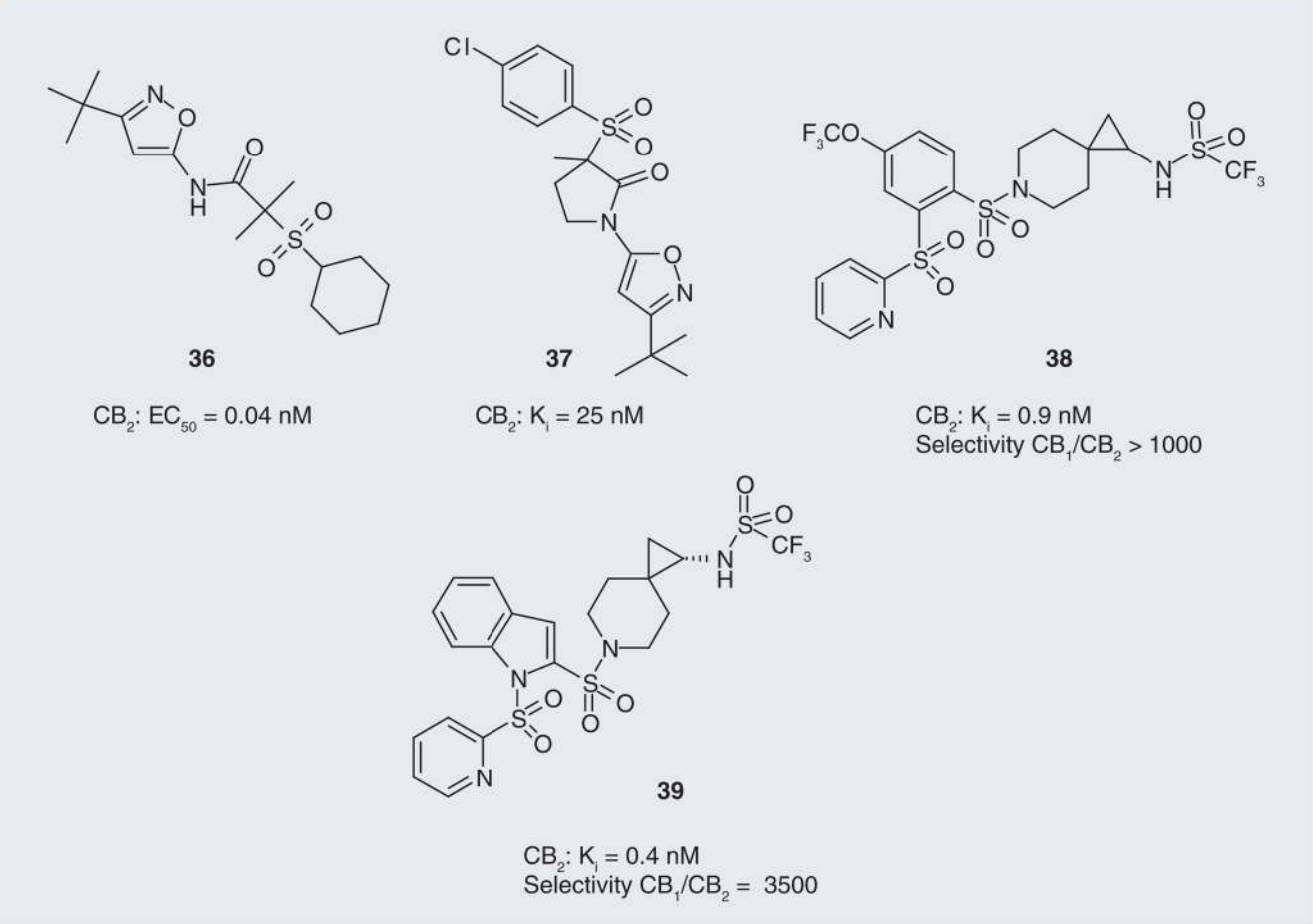

Figure 7.
Representative structures of sulfone derivatives.

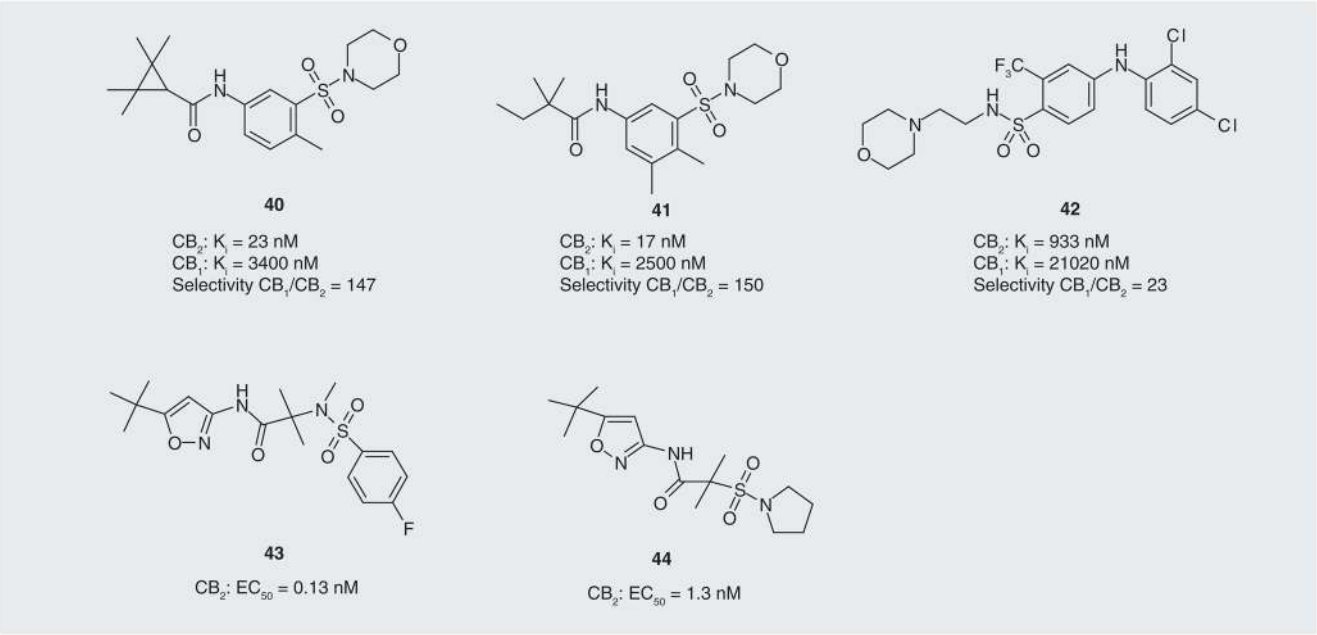

Figure 8.
Representative structures of sulfonamide derivatives.

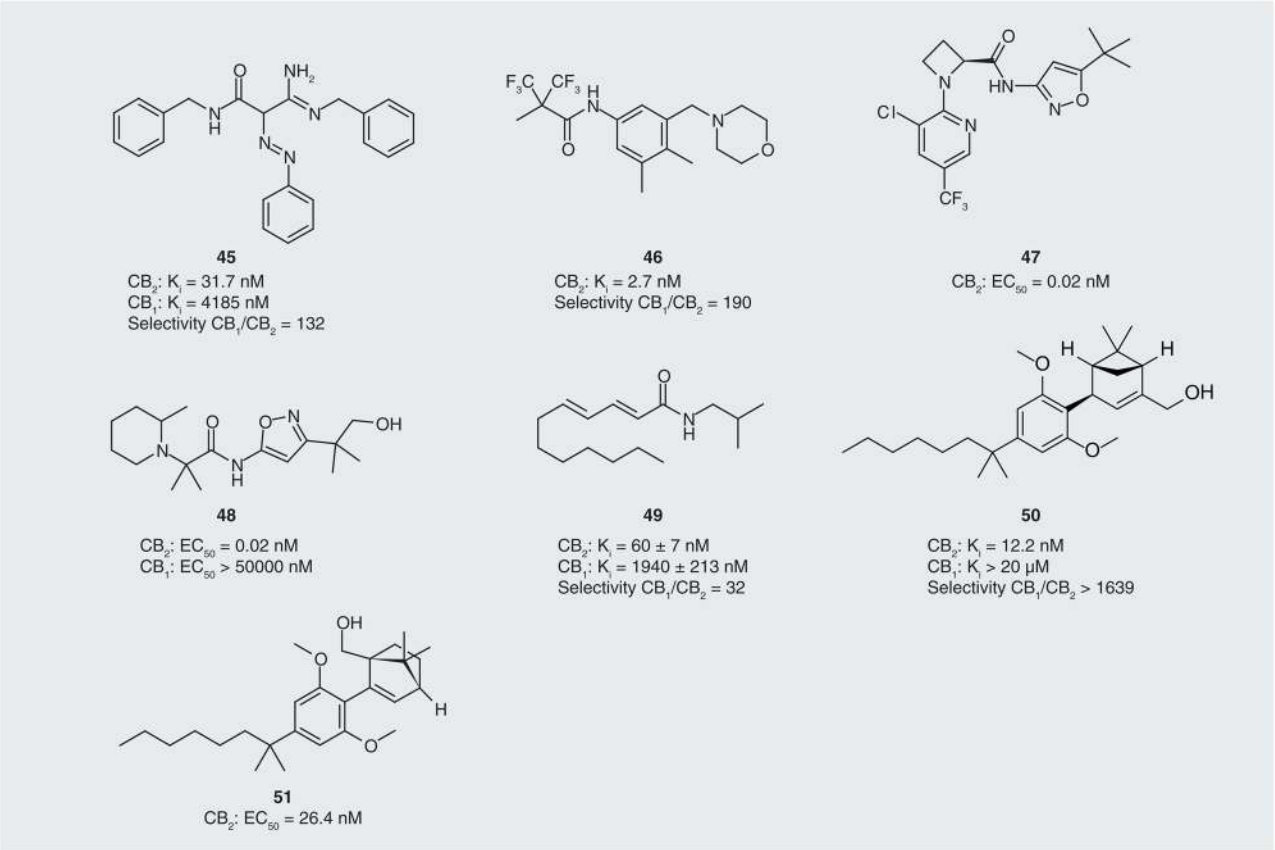

Figure 9.
Representative structures of miscellaneous scaffolds of CB_2 derivatives.

Table 1

Summary of binding affinity and selectivity of the CB_2 ligands reviewed.

Affinity K_i (CB_2, nM)	**Compound**	**CB_1/CB_2 selectivity**	**Compound**
≤1	**1, 2, 9, 15, 16, 17, 23, 27, 28, 29, 30, 31, 32, 36, 38, 39, 43, 47, 48**	>1000	**8, 14, 17, 24, 29, 30, 33, 38, 39, 48, 50**
01–10	**3, 4, 8, 12, 13, 14, 18, 19, 22, 24, 33, 35, 44, 46**	100–1000	**10, 12, 13, 21, 31, 40, 41, 45, 46**
10–100	**5, 6, 10, 11, 20, 21, 34, 37, 40, 41, 45, 49, 50, 51**	10–100	**34, 35, 42, 49**
>100	**42**	1–10	**32**