Medicinal Chemistry, Pharmacology and Potential Therapeutic Benefits of Cannabinoid CB_2 Receptor Agonists

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1. INTRODUCTION

The endocannabinoid system is represented by a group of neuromodulatory lipids and their receptors that are involved in a variety of physiological and pathological conditions. The main endocannabinoids, or endogenous cannabis-like substances, are small molecules derived from arachidonic acid, N-arachidonoyl ethanolamine (anandamide, AEA) and 2-arachidonoyl-glycerol (2-AG).[1] Each endocannabinoid belongs to a larger class of lipids termed N-acyl-ethanolamines (NAEs) and monoacylglycerols (MAGs), respectively, where the members differ in the length and unsaturation degree of their acyl chains.[2] In particular, AEA and 2-AG represent polyunsaturated derivatives with potential biological activities as, for example, in the nervous system where they act as retrograde messengers.[3] These endocannabinoids are not stored in vesicles and mediate intercellular signals from postsynaptic neurons back to presynaptic terminals where they inhibit the neurotransmitter release.[4] Their levels are controlled by selective deactivating enzymes such as fatty acid amide hydrolase (FAAH) for AEA, and monoacylglycerol lipase (MGL) for 2-AG, that primarily influence the endocannabinoid tone.[5]

Two different types of G protein-coupled receptors (GPCRs) have been discovered and named as central cannabinoid (CB_1) and peripheral cannabinoid (CB_2) receptors. The CB_1 and CB_2 receptors are negatively coupled to adenylyl cyclase, exert an inhibitory effect on cyclic AMP (cAMP) production, and modulate the mitogen-activated protein kinase (MAPK) pathway.[6] In addition, CB_1 receptors inhibit presynaptic N- and P/Q-type calcium channels, activate inwardly potassium channels, reduce neuronal excitability and neurotransmitter release.[4] CB_1 receptors are primarily distributed in different areas of the brain related to motor control, in sites associated with pain processing, cognition, emotional responses, motivated behaviour and homeostasis.[7]

CB_2 receptors were originally believed to be present in periphery, primarily to immune cells, but nowadays it has been reported that these receptors are localized neuronally in different species. They are expressed in all hematopoietic cells such as lymphocytes, natural killer cells, macrophages

and neutrophils, and represent an attractive target for the treatment of the inflammatory status.[8] These receptors also mediate a significant protection in brain microglia against neurotoxicity by modulating the release of anti- or pro-inflammatory cytokines.[9]

CB receptors can also be directly modulated by exogenous ligands, for example, by the main psychotropic constituent found in *cannabis sativa*, named Δ^9-tetrahydrocannabinol (Δ^9-THC). It is well reported that nearly 400 distinct chemical entities have been isolated from this plant, and that it contains approximately 60 separate centrally active or psychoactive components.[5] Experimental evidence has reported the role of CB receptors in the pathophysiology, and novel developments in the medicinal chemistry of these receptor subtypes have tried to identify potential therapeutic areas for drug research.[10] In vitro and in vivo studies of the specific effects of selective CB ligands have determined important progress on the role of CB receptors in various pathologies. Interestingly, selective agonists, antagonists, inverse agonists and novel allosteric modulators interacting with CB_1 or CB_2 receptors have been developed to inhibit or augment their basal tone.[11]

Over the past decades, the discovery of CB receptors has been followed by intensive research related to identifying key proteins involved in their modulation and to investigating their interaction with endocannabinoid ligands.[12] In addition, the search related to novel CB_2 agonists has been greatly developed and a large number of ligands has been synthesized and tested with the aim to identify novel therapeutic drugs.[13]

From the pharmacological point of view, CB_2 agonists show different ways of interacting with the receptor site, suggesting various binding and functional properties well represented by pharmacodynamic concepts of full, partial and inverse agonism.[14–16] In recent years, pharmaceutical companies and academic research laboratories have attempted to identify novel CB_2 agonists without CB_1 central effects by focusing on the discovery of high affinity, selective agonists for CB_2 receptors with a therapeutic potential in the treatment of different pathologies such as

neurodegenerative diseases, pain transduction and perception, ischemic stroke, severe inflammation, autoimmune diseases, osteoporosis and cancers.[9,11]

The purpose of this review is to analyze the molecular characterization, distribution and signal transduction pathways of CB_2 receptors together with their pharmacological role in some of the most relevant biological systems. The structure-activity relationships of several interesting CB_2 agonists, including synthetic schemes in order to clarify the chemistry involved in this field, are reported. Moreover, the corresponding pharmacological and beneficial potentials of selected CB_2 compounds have been focused as well as the patent literature and the identification of promising therapeutic candidates.

2. MOLECULAR CHARACTERIZATION OF CB_2 RECEPTORS

The distinction between CB_1 and CB_2 receptors is primarily based on differences in their predicted amino acid sequence, signaling mechanisms, and tissue distribution. Cloning of the neuronal CB receptor initiated several years ago with a molecular approach to the study of cannabinoid actions.[17] Since then, rapid progress has been made, which includes the cloning of a second CB receptor from cells of immune origin, the identification of two endogenous CB ligands and specific elucidation of their metabolic pathways.[18,19] Briefly, CB_1 receptors have been cloned in different vertebrates including mammals, amphibians, birds and fish, exhibiting 97% to 99% amino acid sequence identity across species.[20] CB_1 mRNA and protein are primarily found in brain and neuronal tissues encoding protein residues of 473 and 472 amino acids in rat and human, respectively. The human and rat CB_1 receptors differ in only 13 residues, and the mouse CB_1 protein displays 99% amino acids identity compared to rats.[21]

The rat CB_2 receptors consist of 410 amino acids among transmembrane domain, and exhibit 48% homology with CB_1 receptors, less than the 70-80% usually seen between different types of GPCRs.[22] The mouse CB_2 receptor has been cloned and reveals a high degree of homology with rat or human CB_2 receptor except in the carboxy terminus where rat CB_2 is 50 and 63 residues longer

than hCB_2 and mCB_2, respectively.[23] The importance of the carboxy terminus in regulating CB_2 receptor desensitization and internalization is well established and the differences identified in this specific region between species suggest caution when extrapolating experimental results from non human models to humans.[24] The differences between CB_1 and CB_2 receptors have been associated to their sequences with a high concentration in N-terminal and other extracellular regions.[25] Amino acids amenable to potential post-translational modifications include some serines/threonine residues that are phosphoacceptor sites and specific sites for asparagines N-linked glycosylation.[22] These mutations generally give the same results in the ligand-receptor interaction even if they could play a key role in transmitting the binding signal to the G proteins.[26]

A novel human CB_2A isoform has been discovered by sequencing RT-PCR fragments amplified from human brain cDNA that differentially encodes tissue specific CB_2 gene expression.[27] The CB_2A isoform is predominantly expressed in human testis, and the promoter of CB_2A is located upstream of CB_2B isoform promoter, that is primarily present in spleen.[28] The quantitative RT-PCR revealed that CB_2A mRNA is expressed in different human brain regions with similar levels found in muscle, spleen, intestine, leukocytes and kidney.[29] In contrast, CB_2B mRNA is mostly present in spleen and to a lesser extent in leukocytes, muscle, intestine, liver and heart.[27] The human CB_2A and CB_2B isoforms contain different 5′ untranslated regions and are alternatively spliced to the major protein coding exon of the CB_2 gene.[30] The discrepancies on CB_2 mRNA sizes in the literature indicate incomplete structure of CB_2 gene in different species, or polymorphism in the same species. Moreover, species comparison has revealed that the CB_2 gene of human, rat and mouse genomes deviate in their gene structures and isoform expression patterns. The presence of tissue specific isoforms of CB_2 gene may provide tissue specific targeting of CBs in inflammatory and neuropathic pain disorders associated with CB_2 receptors in the brain and peripheral tissues.[31]

There is a large body of experimental evidence that is compatible with the presence of heterodimers and/or homodimers of the major subclasses of GPCRs with different functional

properties. In addition, allosteric interactions associated to the receptors, or across the receptor-receptor interface, could modulate the binding properties and thereby change their pharmacology.[32] It has been reported that CB_1 receptors can exist as homodimers or heterodimers with one or more other classes of GPCRs, such as dopamine D_2, opioid and orexin receptors. Resulting cross talk between CB_1 and other receptors may involve the sequestration of Gαi/o subunit mediating an alteration in the cellular response.[33,34] The allosteric modulation found for CB_1 receptors suggests a new approach to the manipulation of the endocannabinoid system for therapeutic benefits based on the pharmacological effects of direct full or partial agonists and/or competitive antagonists.[35,36] At present, no pharmacological or chemical details are present in the literature regarding homodimers, heterodimers or the allosteric modulation of the CB_2 receptors, even if, given the co-localization of CB receptors and of various GPCRs, the existence of physical interactions between these receptor subtypes that may affect CB pharmacology could be hypothesized.

3. DISTRIBUTION OF CB_2 RECEPTORS

The discovery of CB receptors was followed by the observation that CBs affect every organ system, suggesting that CB expression is present throughout the body.[6] Further evidence has established that some of the most relevant molecular and functional responses in the central nervous system (CNS) are primarily associated to CB_1 receptors, and those in the periphery to CB_2 receptors. Briefly, CB_1 receptors are found in high concentrations within CNS and their concentration is considerably less in peripheral tissues.[10,11]

It has been well established that CB_2 receptors are present in immune tissue, spleen, thymus, tonsils, bone marrow, pancreas, lung, splenic macrophage/monocyte preparations, mast cells and peripheral blood leukocytes (Table 1).

Table 1- Distribution and known functions in different cells or tissues of the CB_2 receptors

Cell type/ Tissue	Species	Functions
Macrophages[a]	Human	Decrease release of NO, IL-12, IL-23, TNF-α; increase IL-10 secretion; reduce ROS production and CCL2 (chemokine C-C motif ligand 2)
B and T lymphocytes[b]	Human	Affect B and T cells differentiationTNF and the balance of pro-inflammatory cytokines; immunosuppressive effect
Stomach (enteric nerves)[c]	Human	Inhibit cholinergic and noradrenergic transmission
Liver[d]	Human	Inhibit fibrosis, reduce inflammation and injury
Myocardium[e]	Human	Protect in ischemia/reperfusion injury
Endothelial cells[f]	Human	Slow the progression of atherosclerosis
Intimal macrophages[g]	Human	Inhibit cytokine production and leukocyte migration; reduce macrophage activation and increase apoptosis
Lung and airways[h]	Human	Antitussive effect and prevent bronchoconstriction
Osteoclast[i]	Human	Decrease osteoclast precursor proliferation and osteoclast activity
Osteoblast[j]	Human	Increase osteoblast stimulation
Whole brain[k]	Rat, mouse, human	Sleepiness, alterations in cognition and memory, increase analgesia; orexigenic effect, antiemetic effect, decrease intraocular pression; decrease inflammation and cause immunosuppression
Brainstem[l]	Rat, mouse	Modulate immunoreactivity, inhibit emesis
Cerebellum[m]	Rat, mouse, human	Inhibit T cells and adenylate cyclase, neuroprotective effect, prevent neuron loss
Cortex[n]	Rat, mouse, human	Mediate anti-hyperalgesia
Hippocampus[o]	Rat, mouse, human	Reduce food consumption
Other brain regions[p]	Rat	Modulate neuron responses in nociceptive transmission
Periphereal neurons/ spinal cord[q]	Rat, mouse, guinea pig, human	Suppress nociceptive transmission
Microglia[r]	Human	Decrease IL-β and IL-6 levels and TNF-α release; p38MAPK and NADPH oxidase activation; interfere with CCR2 (chemokine C-C motif receptor 2) and iNOS expression; inhibit migration and inflammation
Glia[s]	Human	Enhance release of IL-4 and IL-10

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Moreover, they have been found in a variety of cultured immune cell models, including the myeloid cell line, U937, and granulocyte-like or macrophage-like cells.[37] The distribution pattern of CB_2 mRNA displays major variation in human blood cell populations, with a rank order of lymphocytes type B, natural killer cells, monocytes, neutrophils and lymphocytes.[38] CB_2 receptors have been localized in osteocytes, osteoblasts and osteoclasts where they modulate bone formation and turnover.[39] CB_2 receptors are also expressed in myofibroblasts of cirrhotic liver but their presence in normal liver is low.[40] Several studies have reported that CB_2 receptors in the enteric nervous system modulate ileum contractility.[41] In rodents, CB_2 mRNA has been identified in cerebellum, cortex, brainstem and in glial cells, whilst in humans these receptors are expressed in microglial cells and in dorsal root ganglion.[42] CB_2 receptors are implicated in microglial migration and infiltration into brain areas with active inflammation and degeneration, although the healthy brain microglia do not express these receptor subtypes.[43] In accordance with this evidence, CB_2 receptors are expressed in the brain only under certain conditions where inflammation is present at high levels.[44] In addition, it cannot be assumed that immune responses are solely regulated by CB_2 receptors because CB_1 receptor transcripts have also been found in immune cells and tissues such as Jurkat T cell line, human lymphoblastoid and macrophage like cells.[45]

4. INTRACELLULAR PATHWAYS OF CB_2 RECEPTORS

Several reports present evidence that CB receptor molecular structure is similar to the GPCRs conformation and constituted of seven transmembrane domains with biochemical and cellular determinations of G proteins signal transduction.[10,20,22] Both CB receptors are primarily coupled to Gi/Go proteins and inhibit adenylyl cyclase activity and cyclic adenosine monophosphate (cAMP) production.[30] CB_1 receptors are positively coupled to the potassium channels and decrease N-type and P/Q-type calcium channel conductance playing a key role in the suppression of neuronal excitability and neurotransmitter release.[46]

The signaling pathways used by CB_2 receptors (Figure 1) depend on the type of cells and tissues where the receptor is localized, the specific G protein involved and the kinases present in the cells.[28] In particular, CB_2 receptor signaling mechanisms involve inhibition of adenylyl cyclase, mitogen-activated protein kinase (MAPK) activation and a transient increase in intracellular free calcium levels via a phospholipase C (PLC) modulation.[42] CB_2 receptors are linked to three main components of MAPK pathway that have been identified as extracellular receptor kinase (ERK), Jun N-terminal kinase (JNK) and phospho-p38.[47] It is well reported that ERK–MAPK pathways are essential in mitogenesis and also associated to the cell proliferative stages of cancer. Interestingly, CB_2 receptors regulate ERK–MAPKs, depending on the cell type and experimental conditions employed.[48]

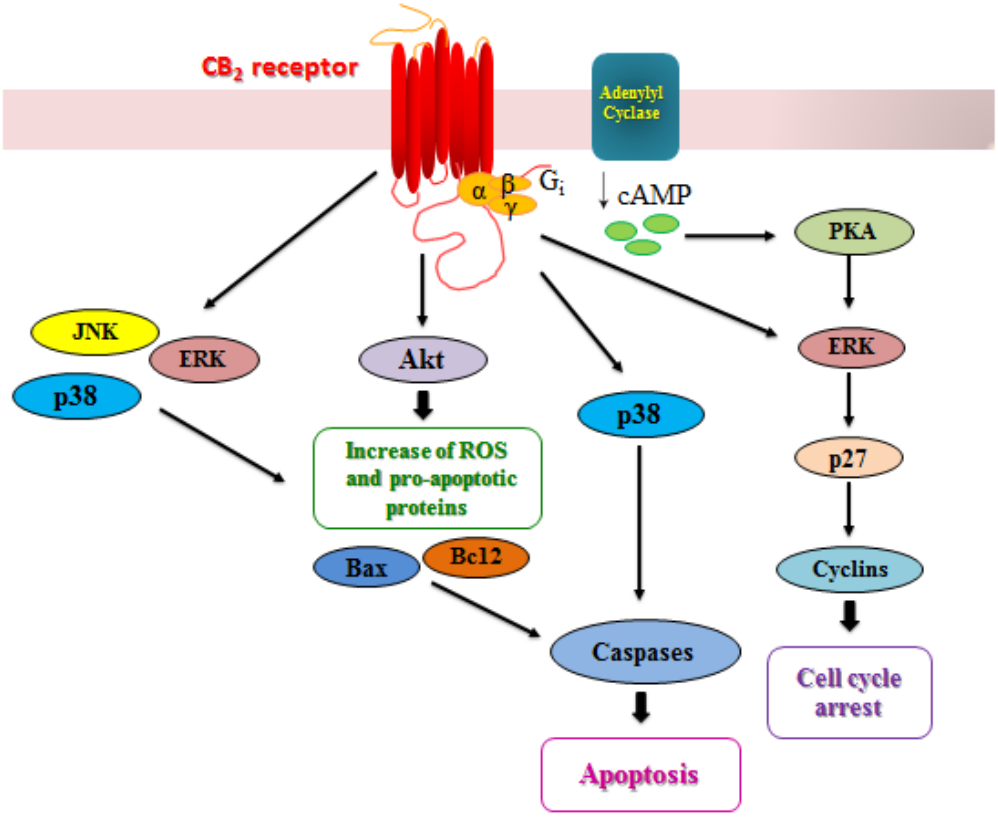


Figure 1 – Scheme of the main signaling pathways mediated by CB_2 receptors.

cAMP, cyclic adenosine monophosphate; Gi, inhibitory class of G protein; PKA, cAMP dependent protein chinase; JNK, jun N-terminal kinase; p38, p38 mitogen-activated protein kinase; ERK, extracellular receptor kinase; Akt, prtein kinase B; Bax, apoptosis regulator Bcl2 like protein 4; p27, cyclin dependent kinase; ROS, reactive oxygen species.

CB_2 receptors are also involved in the inhibition of the intercellular communications through a mitogen activated protein kinase kinase (MEK)-dependent pathway and the ERK family activation involving different cellular responses like proliferation, apoptosis and differentiation.[49] It has been reported that CB_2 receptor stimulation counteracts the ability of morphine to upregulate protein kinase B or Akt and ERK1/2 activation induced by lipopolysaccharide (LPS), thus reducing nitric oxide (NO) and proinflammatory cytokine release.[50] The ability to modulate microglia and MAPK could be very interesting because their activation in the CNS and peripheral system contributes to morphine tolerance and dependence.[51] It has also been demonstrated that CB_2 receptor stimulation activates the MAPK pathway, inhibits pro-inflammatory LPS-induced production of NO and the attenuation of the inducible nitric oxide synthase (iNOS).[52]

Moreover, CB_2 receptor modulation promotes anti-inflammatory therapeutic responses in activated microglia, suggesting a regulatory role for CB_2 receptors in preventing excessive microglial cell response to injury.[53] Different studies have demonstrated that the presence of ceramide has been associated with decreased proliferation and apoptosis in glioma cells closely associated with CB_2 receptor stimulation via serine palmitoyl transferase, Raf-1 activation and MAPK (p42/44) activation.[54] It has been observed that CB receptors enhance the antinociceptive properties of opioids, and adolescent exposure to CB agonists blocks opiate dependence.[55] Opioid and CB receptors are coupled to similar intracellular signaling mechanisms leading to a decrease in cAMP production through the activation of Gi proteins. CB receptors also interfere with the tolerance and dependence effects induced by opioids because the administration of low-dose combinations of CB and opioid drugs seems to be an alternative regimen that reduces the need to escalate opioid dose, while increasing opioid potency.[56]

It is well reported that CB_2 agonists produce pain relief in a variety of animal models by acting on glia and neurons, inhibiting the release of pro-inflammatory cytokines such as interleukin (IL) family and tumor necrosis factor-α (TNF-α). CB_2 agonists are able to prevent the stress-induced

increase in proinflammatory cytokines, nuclear factor-kB (NF-kB), cyclooxygenase-2 (COX-2) and the cellular oxidative and nitrosative damage such as lipid peroxidation.[57] CB_2 agonists inhibit IL-1β-induced proliferation of the fibroblasts in rheumatoid arthritis (RA), as well as the production of metalloproteinases (MMP) such as MMP-3 and MMP-13 in a dose-dependent manner.[58] However, CB_2 receptor activation decreases liver fibrosis by reducing IL-17 production through Th17 lymphocytes via a STAT5-dependent pathway, and inhibits the proinflammatory effects of IL-17 by preserving IL-22 production.[59] The spinal administration of the anti-inflammatory cytokine, IL-10, abolishes pathological pain and suppresses IL-1β and TNF-α release, similarly to CB_2 agonists which behave like anti-inflammatory drugs.[60] A novel mechanism of CB_2 receptor activation has been proposed and associated to an increase in β-endorphin release and to MAPK signaling pathway involvement, suggesting the potential role of CB_2 agonists as peripheral analgesic agents.[61]

5. THE ENDOCANNABINOID SYSTEM

The endocannabinoid system is modulated by several structurally related lipid mediators belonging to either the amide or ester families which are involved in the modulation of CB receptors.[62] Among those, the most potent endogenous CBs are biologically active fatty acid ligands, namely, AEA and 2-AG.[63] AEA is formed by a family of membrane phospholipids such as N-arachidonoyl-phosphatidylethanolamine through multiple pathways. The production of AEA could be due to the action of a specific phosphodiesterase Ca^{2+} dependent that catalyzes the hydrolysis of the phospholipid, or to the sequential action of a hydrolase with a phosphodiesterase that converts the glycerophosphoanandamide.[64] A biosynthetic precursor, 1-acyl-2-arachidonoyl-glycerols (DAGs), produced from the hydrolysis of phosphatidylinositols, could be directly converted into 2-AG through the action of two Ca^{2+} sensitive lipases.[65]

The catabolic enzymes for these endocannabinoids are also different, suggesting that their regulation is independent of each other even if they are broken down inside the cell by intracellular enzymes.[66] Different hydrolytic enzymes, such as the fatty acid amide hydrolase (FAAH), N-

acylethanolamine hydrolyzing acid amidase (NAAA) and monoacylglycerol lipase (MAGL),[67] have been identified as forming complex enzymatic cascades that regulate endocannabinoid production and inactivation. In particular, NAAA is a cysteine hydrolase belonging to the N-terminal nucleophile hydrolase superfamily that preferentially hydrolyzes palmitoylethanolamide and NAPE-selective phospholipase D catalyzes the synthesis of AEA, and the FAAH is involved in AEA degradation into arachidonate and ethanolamine.[68] The selective diacylglycerol lipases (DAGL) α and β are thought to catalyze the formation of 2-AG, and MAGL degrades this compound into arachidonate and glycerol.[69] They have relatively short half-lives that could hamper pharmacological characterization by using *in vivo* selected models, which highlights the need to identify more stable analogues.[70]

Endocannabinoids are released on demand following specific and appropriate stimuli, and their lipophilic nature allows them to diffuse passively through the cell membrane.[71] In addition, these hydrophobic compounds are able to cross the cell membrane by using a putative membrane transporter that facilitates their uptake between aqueous intercellular space and other intracellular carrier proteins known as fatty acid binding proteins (FABPs).[72] Multiple lines of evidence suggest the potential use of selective inhibitors involved in enzymatic degradation of endocannabinoids that could provide selected therapeutic opportunities.[73]

From a pharmacological point of view AEA is a partial CB_1 agonist and a weak partial CB_2 agonist, whilst 2-AG acts as a typical full CB_2 agonist.[65] Apart from their capability to bind CB receptors, endocannabinoids are able to interact with other receptors like the vanilloid receptor 1 (VR1), the transient potential vanilloid type 1 (TRPV-1) channels and other specific GPCRs such as opioid or GPR55 and GPR35 receptors.[74] The capability of the endocannabinoids to interact with different receptors allows a high number of biological and pharmacological actions. As a consequence, endocannabinoids do not regulate only the activity of CB receptors, but also control cell homeostasis through coordinated and fine interactions with different receptor sites. For

example, in several cancer types, CB_1, CB_2 and TRPV-1 receptors appear to be increased if compared with the corresponding non tumoral cells, and their activation counteracts cancer growth and cell invasiveness.[75] Moreover, AEA activating other GPCRs suggests the possibility of a multiple mechanism acting on the regulation of synaptic plasticity that could be very important in the disorders based on neuroinflammation, stress, emotionality and cognition.[75,76]

Interestingly, alternative molecular targets for endocannabinoids have been identified, such as the inhibition of T-type Ca^{2+} channels, the modulation of nicotinic acetylcholine receptors and the allosteric enhancement of glycine receptors. Moreover, 2-AG has been reported as able to potentiate GABA-A receptors through a direct modulation with neurosteroids on the subunit located extra synaptically. An inhibitory effect of AEA and 2-AG has been shown on several types of K^+ channels. A similar direct effect of these endocannabinoids has been verified on peroxisome proliferator activated receptor- γ (PPAR-γ) related to its pro-adipogenic function in adipocytes.[77] The discovery that the endocannabinoids interact with different receptor sites has contributed to the identification of this signaling system as a versatile tool to control various functions in the cells and tissues of the organism.

6. PHARMACOLOGICAL ROLE OF CB_2 RECEPTORS

Increasing evidence has been published implicating a role for CB_2 receptors in a variety of disease models involving central or peripheral nervous systems (Figure 2). It is well recognized that the psychoactive effects of CBs are associated with CB_1 and the CB_2 receptors which mainly mediate anti-inflammatory and immunomodulatory actions.[78] Briefly, CB_1 agonists possess neuroprotective properties primarily via diminishing excitotoxicity in postsynaptic neurons, enhancing vasodilation in vascular smooth muscle and inhibition of endothelin-1.[79] The therapeutic limitations of CB_1 agonists are related to the delicate balance between their beneficial actions and psychoactive effects.[80]

Therefore, selective CB_2 agonists could represent an attractive target for the development of neuroprotective therapies and, above all provide to anti-inflammatory effects without psychoactive activities.[81,82]

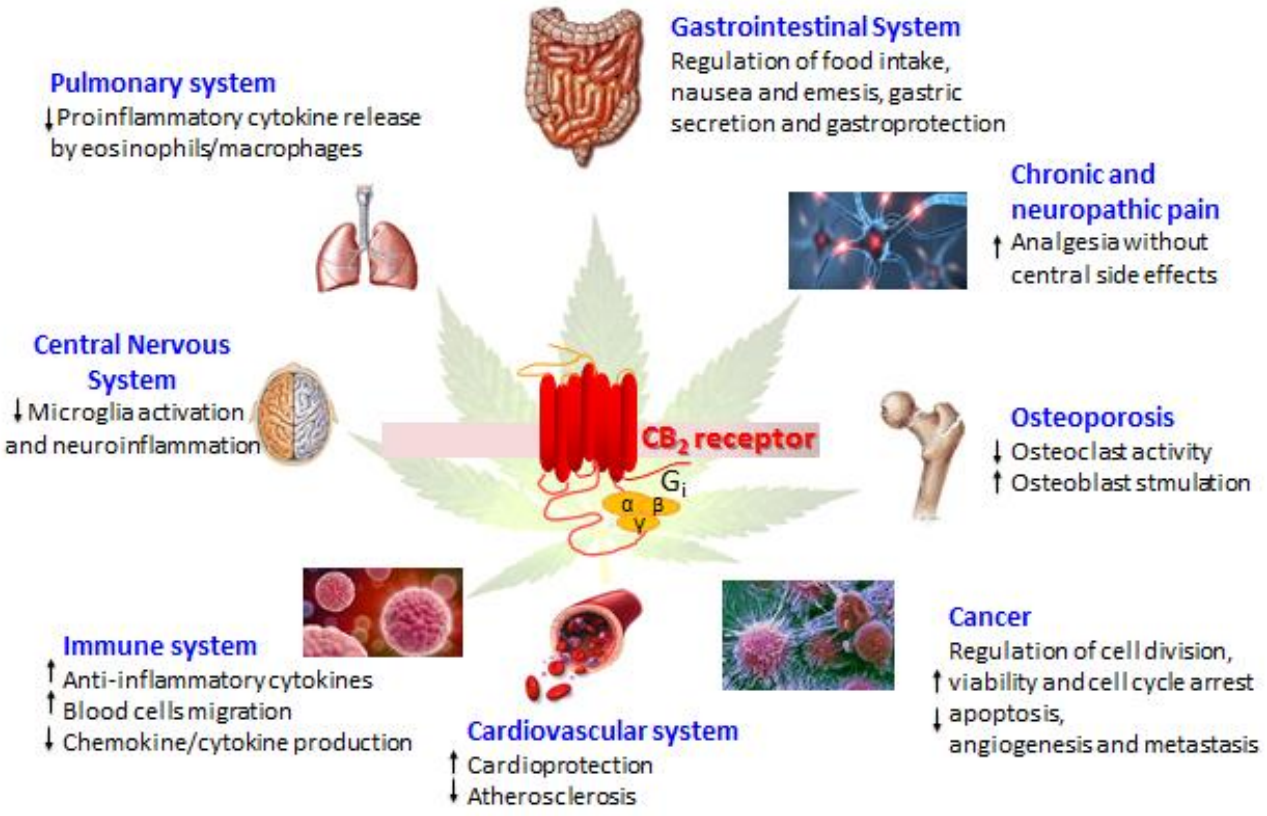


Figure 2 – Scheme for the functional role of CB_2 receptors.

The mechanisms of CB receptor regulation highlight the pathophysiology of diseases and are of interest from a therapeutical point of view. Recently, it has been reported that during cell or tissue injury CB_2 receptors are upregulated, and their activation has an important protective role, suggesting the potential use of selective agonists in different pathologies.[83] Although the physiological role of endocannabinoids and CB receptors in the regulation of cellular signaling has been well studied, relatively little is known about the involvement of CB receptors in human disease

processes. To better investigate the functional contribution of the CB_2 receptor to disease regulation, it may be of interest to evaluate the genetic variations in the human CB_2 gene causing an alteration in the CB_2 receptor function.[84,85] Receptor function could be closely associated with the regulation of gene expression and/or with the presence on the cell surface of the receptors affected by the cell trafficking, receptor dimerization and desensitization/downregulation as well as an altered ligand binding and G protein coupling.[31] It is also reported that the capability of the compounds to modulate pharmacological effects is closely associated to the drug-receptor interaction.[86] Thermodynamic analysis of the binding equilibrium permits us to evaluate the standard enthalpy and entropy that are responsible for the drug-receptor recognition phenomenon. Enthalpy is related to hydrogen bonding and multipolar interactions, whilst entropy is associated to the solvent reorganization.[86] In particular, CB_1 or CB_2 agonist binding is always totally entropy driven while antagonist binding is enthalpy and entropy driven, suggesting that CB_1 and CB_2 receptors are thermodynamically discriminated.[87] The thermodynamic discrimination of the CB ligands confirms the data obtained for other compounds interacting with GPCRs, such as adenosine, opioid, dopamine D_2 and adrenergic receptors.[87–90] Experimental studies performed on several membrane receptors have demonstrated the presence of the enthalpy-entropy compensation also for ligand-gated ion channels like serotonin $5HT_3$, glycine, GABA-A and nicotinic receptors.[86] Characterization of the enthalpy and entropy contribution of CB receptor binding could provide important information regarding the ligand-receptor interaction with fundamental implications for the decision-making process in drug development of novel CB ligands.

6.1. Immune system and inflammation

Most of the effects of CBs within the immune system have been attributed to CB_2 receptors, expressed in different types of leukocytes, that are able to mediate anti-inflammatory effects and immunomodulation.[91] CBs modulate immune responses during inflammatory processes and their immunomodulatory effects have been studied in many disease models such as RA, multiple

sclerosis (MS), diabetes and septic shock.[58,92] CBs exert their immunomodulatory properties through the induction of apoptosis, suppression of cell proliferation, inhibition of proinflammatory cytokine/chemokine production, increase in anti-inflammatory cytokines, and induction of regulatory T cells.[93]

Interestingly, the levels of CB_2 receptors can be upregulated by inflammatory cytokines such as IL-1β, IL-6 and TNF-α.[94] It has also been shown that CB_2 receptor activation inhibits the proliferation of human lymphocytes and the proliferative response of T and B cells to mitogens through induction of apoptosis.[95] Direct measurements of oxidative stress have revealed that CBs prevent serum-deprived cell death by antioxidation, confirming their ability to antagonize oxidative stress and consequent cell death.[96] Stimulation of CB_2 receptors triggers apoptosis in immune cells, suggesting the possibility of novel pharmacological approaches to the treatment of inflammatory and autoimmune diseases.[97] CB_2 receptors also regulate migration of blood cells such as lymphocytes, neutrophils, monocytes and macrophages which are influenced by CB in a dose dependent manner and by receptor stimulation.[98] In particular, CB_2 receptor expression and function is upregulated in immature B cells, fundamental in their retention and subsequent development of these cells into the bone marrow. CB_2 receptor stimulation increases the activity of the α4β1 integrins and vascular adhesion molecule (VCAM-1), suggesting a selective cooperation into cell migration.[99]

Pre-clinical studies have demonstrated that CB drugs have therapeutic potential in different inflammatory diseases, including RA and osteoarthritis (OA).[100,101] CB_1 and CB_2 protein and RNA are present in the synovia of RA and OA patients. CB receptor stimulation of synovial fibroblast-like cells from RA and OA patients produce a time-dependent phosphorylation of ERK-1 and ERK-2 determining a key role of the endocannabinoid signalling system.[102] Selective activation of CB_2 receptors modulate chemotaxis of human monocytes, and phosphatidylinositol 3-kinase/Akt and ERK1/2 showing an important role in the pathophysiology of RA.[103]

CB_2 receptors appear to be involved in allergic dermatitis where their modulation mediates an inflammatory response elicited by antigen-specific effector T cells upon repeated allergen contact.[104] Contrasting preclinical studies are reported regarding the involvement of CB_2 receptors and allergic dermatitis, suggesting the potential use of both CB_2 agonists or inverse agonists/antagonists in the pharmacological therapy.[105]

The additional role of CB_2 agonists has been studied in experimental autoimmune uveoretinitis, an animal model for human posterior uveitis induced by immunization with retinal proteins or their peptide fragments characterized by retinal infiltration in autoreactive T cells. The efficacy of the pharmacological treatment has been impressive with clinical scores positively correlated with the number of infiltrating cells.[106]

6.2. Central nervous system

For several years CB_2 receptors were thought to be present only in the periphery, whilst CB_1 receptors were the predominant form within the brain. Recently, it has become apparent that CB_2 receptors are present in the CNS, even if in lesser quantities than CB_1 receptors. In particular, the neuroprotective effects of CB_2 agonists involve the microglia and are associated with suppression of microglia activation via inhibition of the release of neurotoxic factors and by decreasing neuronal damage in cellular or tissue culture models.[107,108]

Increased expression of CB_2 receptors under neuroinflammatory conditions has been found in human brain.[109] Prominent CB_2 upregulation has been reported in brain tissues affected by MS and amyotrophic lateral sclerosis.[110] In Alzheimer's disease, a potential use of CB_2 specific radiotracer as an imaging biomarker of neuroinflammation in the early stages of this disease has been reported.[82] In Parkinson's disease (PD), an upregulation of CB_2 receptors in glial elements in post mortem tissues of PD patients has been studied together with marked anti-inflammatory effects.[111,112]

CB_2 agonists prevent neuronal injury during neuroinflammation via upregulation of MAPK that results in ERK1/2 inhibition.[108] CB_2 receptor stimulation specifically reduces iNOS production via inhibition of ERK1/2 phosphorylation in microglia during CNS inflammation.[53] These findings have relevance to anti-inflammatory effects of CB_2 stimulation in brain and the regulation of neuroinflammation, that have been confirmed in different animal models for neurodegenerative or inflammatory diseases.[78] However, CBs can be considered neuroprotective via their immunomodulatory properties, which have mainly been attributed to CB_2 receptors involving the inhibition of different pro-inflammatory events such as chemotaxis of immune cells, activation of endothelium and leukocyte infiltration into tissues and injury of endothelial barriers.[113] CB_2 receptors appear to play a major role in leukocyte migration where their stimulation has shown a decrease in migration and in chemokine response via down-regulation of their receptors and inhibition of interferon γ (IFNγ)-induced intercellular adhesion molecule-1 (ICAM-1) expression.[114]

CBs have been reported to inhibit chemokine-induced chemotaxis of various cell types including blood cells and microglia where endocannabinoids drive the regulation of microglial activation in normal and pathological conditions.[103,115] It has been shown that CB_2 agonists affect dendritic cell migration in vitro and in vivo, primarily through the inhibition of MMP-9 expression.[116] Anti-inflammatory properties of CB_2 agonists may be related to their actions on the endothelium. CB_2 receptors have been found in brain endothelium and in endothelial cells from other organs and are able to reduce TNFα-induced activation of human coronary artery endothelial cells, the secretion of monocyte chemoattractant protein 1 (MCP-1) and attenuated monocyte migration.[117]

CBs have been investigated as potential candidates for MS, a chronic inflammatory demyelinating disorder of the CNS characterized by autoreactive myelin-specific T cells and infiltrating macrophages that cause inflammation resulting in demyelinated plaques and axonal loss.[118] An important role in the pathogenesis of MS could be played by the resident microglial cells and by CB_2 receptor stimulation that suppresses microglial cell activation.[119] The activation of CB_2

receptors reduces IL-12 and IL-23 cytokines in human, and murine microglial cells.[120] The chronic treatment of CB_2 agonists decreases plasma and cerebrospinal fluid viral load and tissue inflammation, significantly reducing morbidity and mortality in an immunodeficiency virus-infected animal model.[121] CB_2 receptor-mediated control of bone marrow-derived myeloid progenitor cell trafficking to the inflamed spinal cord has been proposed.[122] While CB_2 agonists have been reported as having a protective effect in cerebral ischemia because they are related to improvements in vascular reperfusion through inhibition of leukocyte rolling and adhesion to cerebral microvessels, CB_1 receptor activation was found to be deleterious.[123]

The effects of CB_2 receptors on the CNS are controversial as far as their role in depression and/or substance abuse is concerned. Behavioral and molecular methods have been performed to study the presence of a link between CB_2 receptors and drug/alcohol addiction.[124,125] A direct association has been suggested with the polymorphism *of Cnr2* gene, encoding for CB_2 receptors, in alcoholism and neuropsychiatric disorders.[126] Mice preferring alcohol had reduced *Cnr2* gene expression in the ventral midbrain, whereas it was unaltered in mice with little or no preference for alcohol.[127] Animals treated with cocaine or heroin showed increased *Cnr2* gene transcripts in comparison with controls, indicating that this gene alteration in the brain could be influenced by substance abuse.[128] CB_2 receptors are also involved in the endocannabinoid signaling mechanisms associated with the regulation of food intake and in eating disorders, including anorexia and bulimia nervosa, that occur most commonly in young women and much less frequently in men.[129] It has been observed that the involvement of CB_2 receptors in eating disorders and food consumption may be due to a dysregulation in the immune system and to the presence of these receptors in hypothalamic nuclei linked to feeding behavior.[130] A high association between R63Q polymorphism of the *Cnr2* gene and eating disorders with a significant suppression of food intake in a time dependent manner has been reported.[131]

6.3. Cardiovascular system

In the literature, several papers have established a positive role of CB_1 receptors in the cardiodepression associated with various forms of stroke and heart failure, even if modulation of these receptors is associated with adverse psychiatric effects.[132] On the other hand, the protective effect of CB_2 receptors on ischemia reperfusion (IR) injury, a pathophysiologic process due to inadequate blood supply and to hypoxic tissue damage, has also been investigated.[133,134] CB_2 receptor knockout mice develop an increase in the IR-induced hepatic tissue damage, accompanied by elevated levels of proinflammatory markers such as TNF-α, or macrophage inflammatory proteins such as MIP-2 and MIP-1α or ICAM-1 as an example of chemokine secretion.[135] The activation of CB_2 receptors mediates a suppression of proinflammatory cytokines and a reduction in tissue damage, low levels of lactate dehydrogenase enzymes, neutrophil infiltration, tissue lipid peroxidation and DNA fragmentation.[136] CB_2 agonists significantly reduce cerebral infarction in different animal models, suggesting a significant protective effect of CB_2 receptors.[137] A cardioprotective mechanism of CB_2 receptor is associated with the modulation of inflammatory response and myocardial remodeling during cardiac adaptation to pressure overload in a murine model of transverse aortic constriction.[138] The relevance of the endocannabinoid-CB_2 receptor axis has been underlined by the findings that CB_2 receptors are upregulated in ischemic wild type cardiomyocytes and that CBs levels are transiently increased during IR.[139] A protective role for CB_2 receptors on the cardiac myocytes from hypoxic damage and an increase in NO production by the stimulation of iNOS has been provided. The ventricular arrhythmias induced by coronary occlusion are reduced after administration of CB_2 agonists, an effect blocked by the use of selective CB_2 antagonists.[140]

6.4. Atherosclerosis

Atherosclerosis is a chronic inflammatory disease of the vascular wall that involves a complex interplay between vascular endothelial and smooth muscle cells, mononuclear cells, growth factors,

and cytokines.[141] Selective CB_2 agonists significantly reduce the progression of atherosclerosis, which implies a protective role for CB_2 receptors that inhibit the TNF-α activation of endothelial cells and the subsequent transendothelial migration of monocytes.[142] Infiltrating macrophages as well as T cells in the atherosclerotic plaques and lesions are found to express CB_2 receptors which modulate apoptosis associated with the development and progression of atherosclerosis.[143]

CB_2 receptor deficiency reduces the susceptibility of macrophages to the apoptosis induced by 7-ketocholesterol, the major oxysterol present in oxidized low-density lipoprotein (oxLDL) due to suppressed activity of caspase-3. In addition, a selective activation of CB_2 receptors reduces the oxLDL and the expression of CD36 scavenger receptor.[144] Moreover, CB_2 receptor activation inhibits the ICAM-1 and VCAM-1 expression in the aorta and adhesion of monocytes to aortic vascular endothelium. CB_2 agonists also attenuate cell proliferation of human vascular smooth muscle and modulate different signaling molecules such as Ras, p38 MAPK, ERK-1, ERK-2 and Akt.[145]

CB_2 receptors attenuate many of the principal steps involved in the development and progression of atherosclerosis, and their activation modulates anti-inflammatory and protective actions within atherosclerotic vessels in both atherosclerosis human and animal models.[146] As a consequence, the basic and preclinical research data suggest that medications activating CB_2 function in the peripheral target organs might be a promising approach against atherogenesis.

6.5. Gastrointestinal system and liver

Cannabinoids have been used for the treatment of many gastrointestinal (GI) disorders, including anorexia, emesis, abdominal pain, diarrhea, even if their use is limited owing to the psychotropic side effects. Several cannabinoid receptors, which include CB_2 receptors, have been described in the esophagus, stomach, ileum and gut, that is an organ of host defence and has an extensive immune system.[147] The most abundant source of these receptors is expressed in the longitudinal muscle with the adherent myenteric plexus.[148] These receptors may play a role in the regulation of food intake,

nausea and emesis, gastric secretion and gastroprotection, GI motility, ion transport, visceral sensation, intestinal inflammation, and cell proliferation in the gut.[147]

The expression of CB_2 receptors is markedly upregulated in different types of liver injury and diseases. Immunoreactivity of these receptors has been localized on myofibroblasts, in fibrotic septa, hepatocytes, biliary epithelial cells derived from active cirrhosis, steatosis and hepatocellular carcinoma.[149,150] Molecules stimulating CB_2 receptors are promising pharmacological tools considering that liver cirrhosis and related complications represent important clinical application in the gastrointestinal system.[151]

Different reports support a functional role of CB_2 receptors in the control of stomach and intestinal motility.[152] It has been demonstrated that, through the involvement of CB_2 receptors, CBs stimulate the release of vasoactive intestinal peptide from enteric synaptosomal preparations.[153] Moreover, the interaction between the immune and nervous system serves to maintain a barrier that protects the gut which, in particular conditions, may become chronically inflamed.[154]

CB_2 receptor stimulation significantly inhibits an experimental model of colitis in mice induced by oil of mustard and dextran sodium sulfate and, in an *in vitro* model represented by HT-29 cells, suppresses the IL-8 production TNF-α induced.[155] Immunohistochemical analysis has revealed the presence of CB_2 receptor expression in human colonic tissue on plasma cells and macrophages in the lamina propria in inflammatory bowel diseases, ulcerative colitis and Crohn's disease.[156,157] In particular, increased expression of CB_2 receptor has been reported in the epithelium, implying a role for CB_2 receptor in colonic inflammation.[158] These data further confirm that CB_2 receptor activation protects against experimental colitis, suggesting that these receptors may be a novel therapeutic target in this pathology.[159]

6.6. Pulmonary system

Literature data have highlighted the potential importance of CB receptors on the regulation of the airways functions and cough reflex. CB_2 receptor activation prevents bronchoconstriction and

airway edema in a guinea-pig model of gastro-esophageal reflux.[160] Coughing is a very common clinical symptom, and current therapies are largely ineffective, indicating a major pharmacological need. CB_2 agonists appear to be protective in the cough reflex and this effect is blocked by the presence of CB_2 antagonists suggesting the direct involvement of this receptor.[161]

Stimulation of the CB_2 receptor signalling pathway diminishes cAMP accumulation and IL-8 release induced by TNF-α, suggesting that CBs could exert anti-inflammatory properties in airways by modulating cytokine release.[162] It has also been reported that the activation of β2-adrenoceptor and CB_2 receptors can inhibit sensory nerves and prevent coughing.[163] CB_2 agonists, together with TRPV-1 or $P2X_3$ antagonists, could represent the most promising drugs in the treatment of coughs, since they are involved in nociception, acting as MAP38 kinase inhibitors, and the reduction of the inflammatory status.[164]

Moreover, melilotus extracts, that have been used to treat inflammatory infectious diseases, have significantly reduced lung inflammation in an animal model of acute lung injury, since they induce a significative up-regulation of CB_2 receptor expression.[165] As a consequence CB_2 receptor activation mediates immunosuppressive effects, which limit inflammation and associated tissue injury in various pathological conditions, suggesting that the protective actions of CB_2 receptors with endocannabinoids or synthetic agonists could be implied in the biological mechanisms involved in these diseases.[166]

6.7. Osteoporosis

CB_2 signaling contributes to the maintenance of bone mass through direct stimulation of stromal cells/osteoblast, inhibition of monocytes/osteoclasts and the receptor activator of nuclear factor kB ligand (RANKL) expression.[39] It has been reported that CB_2 receptors are well expressed in osteocytes or osteoblasts or bone forming cells, and osteoclasts or bone resorbing cells.[167]

CB_2 agonists are able to mitigate osteoclastogenesis by inhibiting the proliferation of osteoclast precursors and mediating a mitogenic effect on osteoblasts.[85] In these cells, CB_2 and TRPV1

receptors are both expressed together and, in culture, oppositely modulate human osteoblast activity in such a way that the CB_2 receptor stimulation improves the osteogenesis, whereas TRPV1 receptor stimulation inhibits it.[168]

CB_2 receptor knockout mice have accelerated age-related trabecular bone loss and cortical expansion, that also characterize human osteoporosis. Given the low bone mass phenotype in this mice model, it is plausible that the endocannabinoid system could play an essential role in maintaining bone mass, suggesting that CB_2 receptors could be a potential drug target for osteoporosis therapy.[169] Accordingly, CB_2 receptor activation might be an endogenous protective mechanism against the inflammation and the age-related bone loss, osteo-protective, especially with regard to the aging skeleton.[170]

A molecular-modeling analysis has revealed that CB_2 receptor enantiomers have two different binding conformations able to discriminate the affinity or the potency of the orientation-targeted ligands, which could have a promising potential for the pharmacological activation of distinct processes.[171] In addition, CB_2-selective compounds show a good efficacy in leukocyte recruitment models when added with low doses of selected anti-inflammatory agents, consistent with their biological functions and indicative of their potential therapeutic utility. [172]

6.8. Cancer

A great interest in CBs as potential anticancer agents has been reported since they inhibit tumor growth in different animal models.[173] Significant upregulation of CB_2 receptors has been shown in prostate cancer cell lines if compared with normal prostate cells, and in lymphoma and breast cancer tissues.[174] The degree of increased expression of the CB_2 receptors correlates with tumor aggression and progression.[175] It is well known that CB agonists affect a range of pathways that regulate cell division and viability and a wide range of markers associated with the invasion and metastasis of cancers, including markers of migration, adhesion, invasion and metastasis.[176] Endocannabinoids regulate the synthesis of ceramides, lipid-based components of the cell

membrane that perform both structural and signaling functions associated with the control of apoptosis, growth arrest, differentiation, cell migration and adhesion.[177] CB_2 receptor activation is closely linked to an increase in ceramide levels leading to the involvement of ERK and p38MAPK activation.[178] It has been reported that adjuvant cannabinoid use in the treatment of adverse side effects from chemotherapy, such as neuropathic pain, loss of appetite, nausea and vomiting, is the most studied potential therapeutic application for these compounds.[179] Endocannabinoids are recognised for their role in the regulation of the key processes involved in the development of cancer.[180] For example, the endocannabinoid system is reported to induce apoptosis, cell cycle arrest and the inhibition of angiogenesis and metastasis in animal models and in cell lines.[181–183] The variability of endocannabinoid effects in different tumor models is highly incongruous and most likely derives from the differential expression of CB receptors, playing a determining role in the progression and/or inhibition of malignancy. High levels of CB_2 mRNA have been detected by *in situ* hybridization in well differentiated human hepatocellular carcinoma and in cirrhotic liver samples, while a low expression of these receptors has been revealed in poorly differentiated hepatocellular carcinoma.[150]

In addition, increased expression of CB_1 and/or CB_2 receptors has been noted in human lymphoma, breast cancer, acute myeloid leukaemia, hepatocellular carcinoma and prostate cancer cell lines.[184–186] In general, a relationship between CB receptor expression and the outcome of cancer has been documented. In astrocytoma cells, 70% of cells expressing CB_1 and/or CB_2 receptors directly correlates with the degree of tumor malignancy, whilst in gliomas a higher expression of CB_2 compared to CB_1 receptors is found which is closely related to the tumor grade.[187] Moreover, tumor-associated endothelial cells of human glioblastoma multiforme indicate the high presence of CB_2 receptors that are able to alter blood vessel morphology.[188] As a consequence, CBs may produce a dual attack on the development of tumor blood vessels through the inhibition of pro-angiogenic regulators, such as vascular endothelial growth factor (VEGF), and

through a direct effect on endothelial cells.[189] It has been found that the CB_2 receptor stimulation decreases the release of NO and other pro-inflammatory mediators in macrophage cell lines and NF-kB-dependent apoptosis in immune cells.[190] The local administration of CB_2 agonist reduces chronic granuloma formation induced by carrageenan-soaked rat implant associated with angiogenesis by inhibiting NF-kB activation.[191] Similarly, increased expression of both CB_1 and CB_2 receptors has been documented in non-Hodgkin lymphoma when compared to reactive lymphonodes.[184]

CB_2 receptor stimulation, which has been investigated in skin melanoma, reduces growth, proliferation, angiogenesis and metastasis.[192] Activation of these receptors decreases melanoma cell proliferation via inhibition of Akt, a key element of a major prosurvival pathway that is deregulated in many types of tumors including melanoma.[193] A reduced expression of CB_1, but not of CB_2, compared with the normal adjacent mucosa has been noted in colon cancer [194] Of interest is the importance of CB_2 receptor in mediating protective effects by inhibition of colorectal cancer cell proliferation.[195] It seems that CBs might regulate the tissue response to gut inflammation via CB_2 receptor activation by involving the smooth-muscle response to pro-inflammatory mediators that affect gastrointestinal transit time, and by causing the direct suppression of pro-inflammatory mediator production.[196]

In breast carcinoma, a relationship between CB_2 expression, the histological grade of the cancer, and other markers of prognostic and predictive value, such as HER-2 oncogene, oestrogen, and progesterone receptors, has been reported.[197] CB_2 receptors have been found in different breast carcinoma cell lines, such as MCF-7, T-47D, MDA-MB-231, MDA-MB-468, EVSA-T and SkBr3 and in human breast tissues using RT-PCR, immunofluorescence and/or Western blot assays.[180]

The activation of CB_2 receptors in prostate cancer, the second most frequently diagnosed malignancy in men, where the cancer cells may metastasize to other body parts, mediates

significative anti-proliferative and pro-apoptotic properties.[198] As a consequence, endocannabinoids may be a beneficial option for the treatment of prostate cancer.[199]

These studies imply a role for CB_2 receptors and their expression in relation to disease prognosis and outcome, closely associated with the specific cancer, and could help to translate this knowledge into the clinical setting to develop new pharmacological therapies.

6.9. Chronic and neuropathic pain

Available therapies often provide incomplete pain relief, and treatment-related side effects are common in chronic and neuropathic pain. Preclinical neuropathic pain models have facilitated identification of several promising targets, which have progressed to human clinical phases of evaluation.[200] Some of the most important drugs that could be used are summarized: calcium channel antagonists, vanilloid receptor antagonists, NMDA antagonists, opioid receptor agonists, histamine H_3 receptor antagonists, serotonin modulators, acetylcholine and adrenoreceptor agonists, nitric oxide synthase inhibitors, orexin antagonists, apoptosis inhibitors, fatty acid amide hydrolase inhibitors and CB agonists.[200,201] From these it is widely accepted that neurotrophin, CB_1 and CB_2, and thermo-transient receptor potential such as TRPs, TRPV1, TRPA1 and TRPM8 receptors could play a pivotal role in this pathology.[202,203] In particular, endocannabinoids appear to be a first line of defence against pain, while neurotrophins and thermoTRPs are the major generators of painful signals.[203] Recently, it has been reported in a clinical review on 28 randomized clinical trials of cannabinoids as pharmacotherapy that medical marijuana is used to treat a host of indications among them the treatment of chronic and neuropathic pain.[204] Randomized clinical trials of cannabinoid drugs reported their potential use to treat disease or alleviate symptoms such as nausea and vomiting due to chemotherapy, depression, anxiety and sleep disorder, psychosis, treatment of chronic pain and spasticity.[205] Although the diversity of pharmacological mechanisms of interest emphasise the complexity of neuropathic pain transmission, the considerable number of agents under development reflect a continued enthusiasm in drug development for neuropathic pain.[206]

The relevance of the therapeutic effects mediated by CB_2 agonists is well established for the treatment of inflammatory and neuropathic pain and neurodegenerative disorders.[207] Commercially available CBs are subject to psychotomimetic and addictive adverse effects, largely through activation of the CB_1 receptors. Regional and peripherally restricted CBs could reduce the side effects of CBs. Spinal CBs may increase the therapeutic index by limiting the dose necessary for response and minimize drugs exposure to supraspinal sites where CB side effects originate. CB bifunctional ligands or the combination of a CB_2 agonist with a TRPV-1 antagonist may improve the therapeutic index of the CB_2 agonist. Selective enzyme inhibitors plus TRPV-1 blockers could be further explored to limit CB hydrolyzing enzyme inhibitors.[208]

Recently, it has been reported that the systemic administration of a CB_2 agonist produces robust analgesia in different pain models, such as writhing and formalin assays, streptozotocin (STZ)-induced diabetic neuropathy and bone cancer pain, providing an interesting approach to analgesic therapy in inflammatory and chronic pain without CB_1 mediated central side effects.[209] These data have also been confirmed by the use of other CB_2 agonists in neuropathic pain of STZ-induced diabetic mice, suggesting that these compounds might have an ability to resolve diabetic neuropathic pain.[210] The combined administration of subthreshold doses of a COX inhibitor and a selective FAAH inhibitor increases CBs levels and decreases prostaglandin levels in whole brain. These data confirm that the dual blockade of FAAH and COX represents a potential therapeutic strategy for the treatment of neuropathic and inflammatory pain states.[211] It has been also reported that the prolonged use of CB_2 agonists for managing chemotherapy-induced allodynia mediates a favorable therapeutic ratio marked by sustained efficacy and absence of tolerance, physical withdrawal, or CB_1-mediated side effects decreasing mRNA levels of TNF-α and MCP-1 in mice spinal cord.[212]

Obtaining detailed information on the internalization and trafficking of the human CB_2 receptor in response to an agonist could have a significant impact on drug discovery. The internalized CB_2

receptors are co-localized with the early endosome probe and are recycled to the cell surface after the removal of the agonist, but treatment with a specific cell-permeable proteasome inhibitor reduces the recycling of internalized CB_2 receptor, suggesting that the proteasome-mediated degradation pathway may be involved in CB_2 internalization.[213] A novel CB_2 agonist has also been identified with high affinity versus CB_2 receptors, which failed to promote CB_2 internalization, suggesting a potential starting point for further investigations of CB_2 pharmacology and pain treatment.[214]

The ability of CBs to produce signs of analgesia in chronic and neuropathic pain reveal important clinical implications. A major point in the clinic is the need to identify strategies that could reduce or abolish the adverse central effects of CBs without attenuating their clinical effects. One possibility could be to focus on CBs that induce analgesia by acting on biological targets, for example, through the use of selective CB_2 agonists. Another strategy could be activate the endocannabinoid system indirectly by inhibiting the tissue uptake or metabolism of endogenous CBs to increase their levels to CB_2 receptors. Moreover, the synergistic interactions between CBs and opioid or TRPV1 ligands for antinociception could be explored. The potential use of dualistic drugs would obtain therapeutic advantage in comparison with administering combinate therapy with the primary aim to limit the unwanted side effects. A summary of the involvement of CB_2 receptors in different systems is reported in Table 2 to correlate their main pharmacological role with the potential therapeutic implications.

Table 2. Potential involvement of CB_2 receptors and their pharmacological role in various diseases.

System/ Disease	Proposed Role	Potential use in therapy	Ref
Immune and metabolic system	Inhibition of inflammation	RA, OA, allergic dermatitis, uveoretinitis, septic shock	91,92,215,216
Central nervous system	Inhibition of inflammation	MS, ALS, AD, PD, cerebral ischemia, neuropsychiatric diseases	109,122,123,217
Chronic and neuropathic pain	Inhibition of inflammation	Chronic pain associated to different disease state	206,211,218,219
Cardiovascular system	Decrease plaque progression	Stroke, heart failure, ischemia reperfusion, atherosclerosis	132,137,139,220
Gastrointestinal system and liver	Inhibition of inflammation	Colitis, diabetes	148–150,221
Pulmonary system	Reduce bronchoconstriction and edema	Coughs, lung injury	160–163
Bone system	Increase osteoblast activity	Osteoporosis	169,222–224
Cancer	Apoptosis and cell cycle regulation	Prostate, breast and colon cancer, hepatocellular carcinoma, myeloid leukemia, astrocytoma, glioblastoma	173,174,176,177

RA, rheumatoid arthritis; OA, osteoarthritis, MS, multiple sclerosis, ALS, amyotrophic lateral sclerosis, AD, Alzheimer disease, PD, Parkinson disease

7. CHEMISTRY OF CB_2 AGONISTS

The discovery of CB_1 and CB_2 receptors was followed by rigorous research efforts to investigate this interesting biochemical system and identify the key proteins implicated in its modulation. At the same time, the pharmaceutical industries and academic laboratories produced numerous new and structurally related compounds with very potent biological activities.

In the past years, the discovery of tricyclic and bicyclic analogs of tetrahydrocannabinol as well as the aminoalkylindole derivatives increased evidence to support the potential utility of selective CB_2 agonists for the treatment of pain. Compounds such as JWH133,[225–230] HU308[231,232] and AM1241[233–235] have been extensively used in preclinical research and have demonstrated efficacy in preclinical models of inflammatory, moderate to severe post-operative and neuropathic pain.

The development of CB_2 selective ligands represents an important advance in cannabinoid therapeutics. In literature, it is well reported that clinical trials with published results showed the

presence of inactive drugs without efficacy. The reasons for this contrast may be linked to the localization of the CB_2 receptors that appeared to be present only peripherally, and to the use of unspecific antibodies that did not permit an exact mapping of these receptors. Another problem was that CB_2 knockout mice had only a small portion of the CB_2 gene which was partially translated into CB_2 proteins able to interact with other receptors.

Moreover, compounds which have significant selectivity and affinity for the CB_2 receptor belong to different structural groups. These include the traditional CB_2 ligands and the recently discovered CB_2 agonists, commonly characterized by lipophilic molecules, such as aromatic heterocycles, linked to bulky alkyl or aryl moieties.

The chemical section of the present review will provide an update of the progress made in structure-activity relationships in the field with a particular focus on the CB_2 agonists developed from 2008 onwards. We have included some synthetic schemes in order to show the chemistry involved in this field. Finally, a review of patent publications collects the most important cores of the CB_2 agonist molecules.

7.1. Pyridine, Pyridazine

Following the discovery of pyrimidine-5-carboxamide derivative **1** (GW842166X, Figure 3) in GlaxoSmithKline Research Laboratories as a clinical candidate, a program was begun to reach a CB_2 agonist back-up candidate with improved aqueous solubility over **1**.[236] The replacement of the central pyrimidine core of compound GW842166X with a pyridine nucleus led to the pyridine-3-carboxamide CB_2 agonists.[237] An extended SAR of the new pyridine-3-carboxamide template was concluded in the identification of analogue 6-(2,4-dichlorophenylamino)-4-cyclopropyl-N-(tetrahydro-2*H*-pyran-4-yl)methyl)pyridine-3-carboxamide **2** (Figure 3, Scheme 1), which demonstrated efficacy in an in vivo model of inflammatory pain, despite its low aqueous solubility. Boehringer Ingelheim also discovered a series of 2-aminopyridines bearing chiral morpholine ring

(compound **3**) which was reported potent and selective.(CB_2 EC_{50} = 50 nM, efficacy (Emax) = 100%; CB_1 EC_{50}/CB_2 EC_{50} > 2000).[238]

2-Amino-5-aryl-pyridines had been identified as a synthetically tractable series of CB_2 agonists from a high-throughput screen of the GlaxoSmithKline compound collection. Replacement of the pyridine core of the initial lead **4** (CB_2 EC_{50} = 79 nM)[239] with a pyridazine (compound **5**) brought about the discovery of a number of analogues with increased in vitro metabolic stability. Introduction of the *cis*-2,5-dimethylpyrrolidine moiety was found to significantly increase CB_2 activity. Combining this group with a number of bicyclic heterocycles led to the identification of compound **6** (Figure 1, Scheme 2), which showed high potency in a model of inflammatory pain.[240]

The CB_2 ligands based on the 3-carbamoyl-2-pyridone derivatives was also discovered. The structure-activity relationship around this template by adjusting the size of side chains at 1-, 5- and 6-positions led to the identification of **7** (S-777469, Figure 3) as a selective CB_2 receptor agonist. This compound exhibited moderate potency for CB_2 receptor (hCB_2 K_i = 36 nM) and good selectivity for CB_1 receptor (>120).[241]

…..Merck Research Laboratories disclosed the 1-(4-(pyridin-2-yl)benzyl)imidazolidine-2,4-dione series as a novel chemotype cannabinoid CB_2 receptor agonist. The optimization of pharmacokinetic properties and human ether-a-go-go-related gene (hERG) affinity of this novel class of CB_2 agonists by systematically modulating physicochemical properties led to the synthesis of **8** (LU-101, Figure 3) as an orally bioavailable CB_2 agonist. It was shown in vivo activity in a spinal nerve ligation model of neuropathic pain.[242]

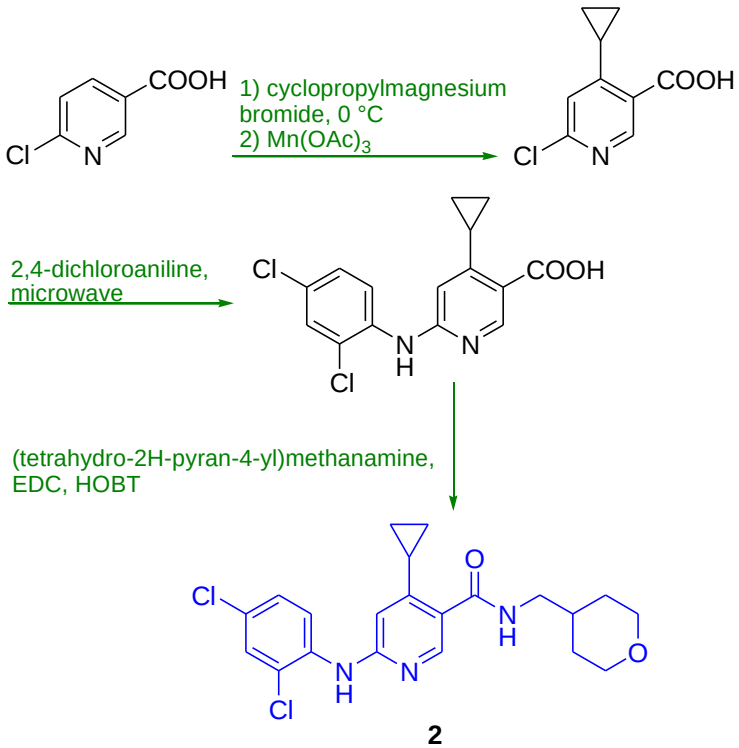
…..Adolor Corporation discovered a novel chemical series of pyridine-based potent and selective CB_2 agonists by replacement of the phenyl ring in their previous (morpholinomethyl)aniline carboxamide cannabinoid receptor ligands with a pyridine ring. Compound **9** (Figure 3), that is 2,2-dimethyl-N-(5-methyl-4-(morpholinomethyl)pyridin-2-yl)butanamide showed good affinity at the CB_2 receptor (K_i = 24 nM), 160-fold selectivity versus CB_1 and moderate metabolic stability in rat

and human liver microsomes. This compound displayed antiallodynic activity after oral administration in a rat model of neuropathic pain.[243]

Hoffmann-La Roche disclosed in a patent publication the pyridine-oxadiazoles as CB_2 agonists with EC_{50} below 0.05 μM and selectivity versus CB_1 in the corresponding assay of at least 500 fold. within a large number of compounds the most representive are the 4-cyclopropylmethoxypyridines exemplified by cyclopropyl-4-(cyclopropylmethoxy)pyridin-2-yl]-3-methyl-l,2,4-oxadiazole **10** (Figure 3) that showed EC_{50} value of 25 nM for the CB_2 receptor.[244]

Preparation of 6-(2,4-dichlorophenylamino)-4-cyclopropyl-N-(tetrahydro-2H-pyran-4-yl)methyl)pyridine-3-carboxamide (2).[237] The addition of cyclopropylmagnesium bromide to the 6-chloronicotinic acid, followed by reaction of this with 2,4-dichloroaniline by standard amide formation gave the desired final compound 2 (Scheme 1).

Scheme 1. Synthesis of pyridine derivative 2.



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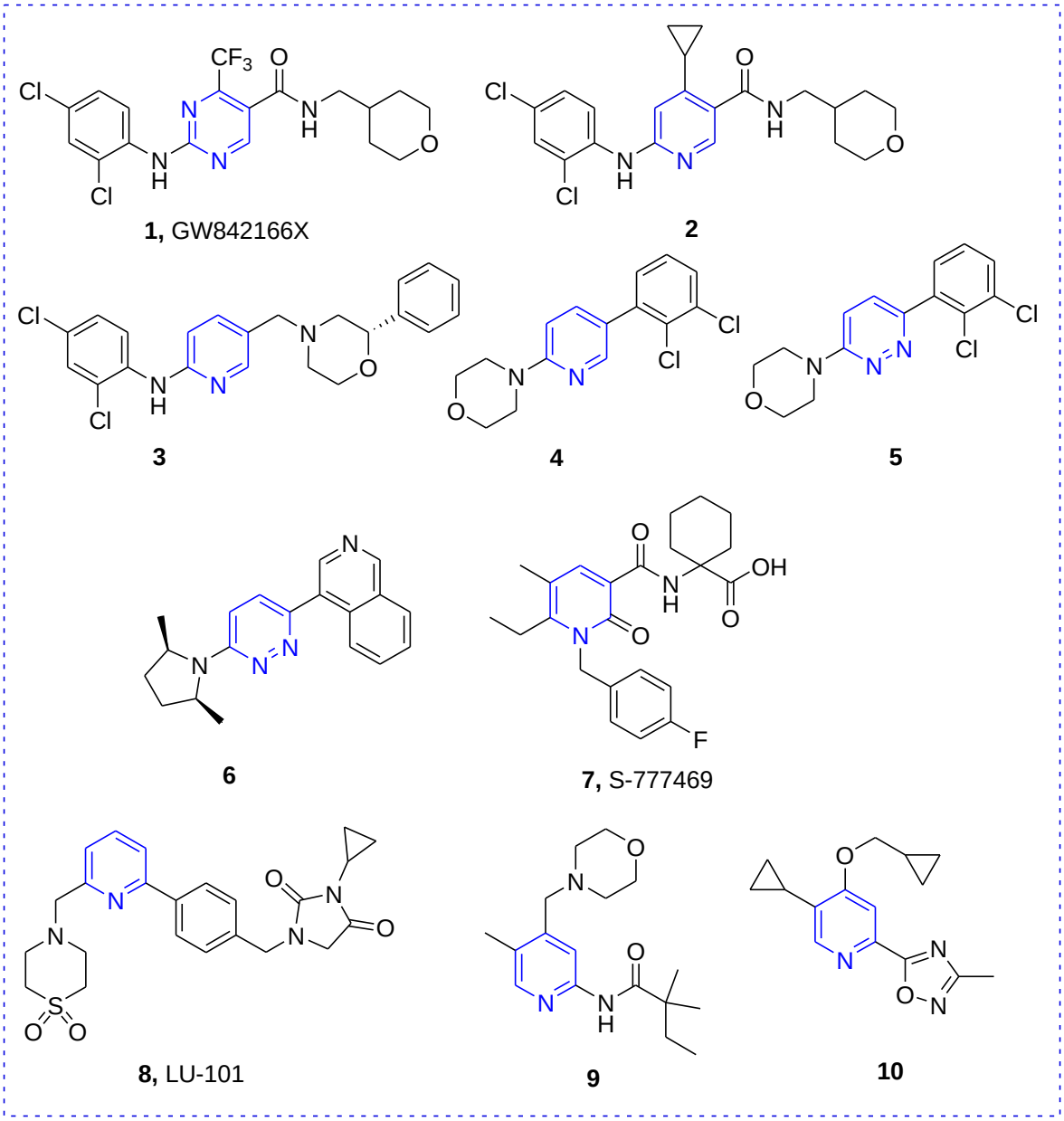
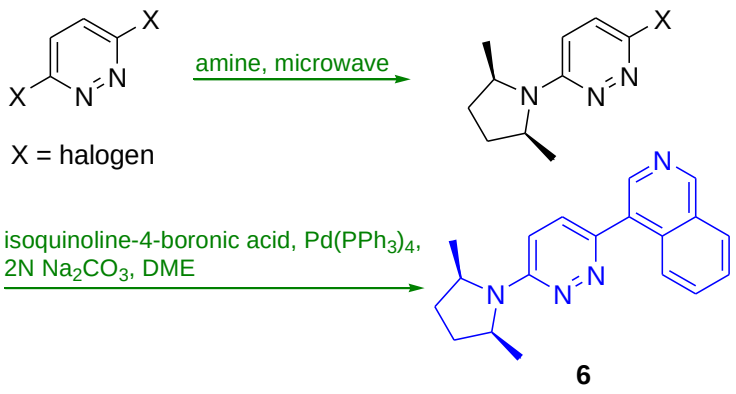


Figure 3. Pyridine and pyridazine derivatives.

Preparation of 4-(6-((2R,5S)-2,5-dimethylpyrrolidin-1-yl)pyridazin-3-yl)isoquinoline (6).[240] Pyridazine analogue 6 was synthesized from the commercially available 3,6-dihalogen pyridazine according to Scheme 2. Synthesis proceeded via a palladium catalyzed cross coupling combined with an aromatic halide displacement. Halide displacement with the amine was carried out at high temperature in a microwave reactor.

Scheme 2. Synthesis of pyridazine 6.



6

7.2. Sulfamoyl Benzamide

Starting from a screening hit **11** (Figure 4) with modest affinity for the CB_2 receptor; Adolor Corporation identified sulfamoyl benzamides as a novel series of CB ligands. Several benzamides were prepared by introducing large lipophilic amide substituents, and increased selectivity for the CB_2 receptor was achieved by the introduction of an *S*-fenchyl residue such as in compound **12** (Figure 4). This had a 120-fold functional selectivity for the CB_2 receptor and produced robust antiallodynic activity in rodent models of postoperative pain and neuropathic pain without the habitual cannabinergic side effects. Small changes in the sulfonamide part of the molecule produced a switch from full agonist to inverse agonist.[245]

Further SAR studies and the optimization of the amide linkage led to the reverse amide derivative **13** with superior selectivity over the CB_1 receptor while retaining good affinity for CB_2. The best compound of this study, the tetramethylcyclopropy derivative **14 (**Figure 4, EC_{50} = 11 nM, Emax = 84%), displayed poor metabolic stability in rat pain model.[246] This compound exhibited robust antiallodynic activity in a rodent pain model when administered intraperitoneally. Efficacy after oral administration was observed only when pretreated with aminobenzotriazole (ABT), a cytochrome P450 suicide inhibitor, before administration. The isosteric replacement of the amide functionality

of **11** with various heterocycles (thiazoles, oxadiazoles, pyrazoles, imidazoles, triazoles and tetrazoles) did not significantly improve affinity or selectivity for the CB_2 receptor in comparison to the lead compound **11**. Further efforts to improve the in vitro metabolic stability profile in the sulfonamide series led to the identification of *N*-[3,4-dimethyl-5-(morpholin-4-ylsulfonyl)phenyl]-2,2-dimethyl butanamide **15** (Figure 4, Scheme 3) that displayed the best overall in vitro profile and demonstrated robust efficacy in an animal model of post-operative pain.[247]

Preparation of N-[3,4-dimethyl-5-(morpholin-4-ylsulfonyl)phenyl]-2,2-dimethyl butanamide (15).[247] Compound 15 was prepared according to Scheme 3. Chlorosulfonylation of commercially available substituted nitrobenzene was achieved using chlorosulfonic acid. Condensation of the corresponding sulfonyl chlorides with morpholine followed by reduction of the nitro group yielded the substituted aniline derivative. Classical amide formation led to the target compound 15.

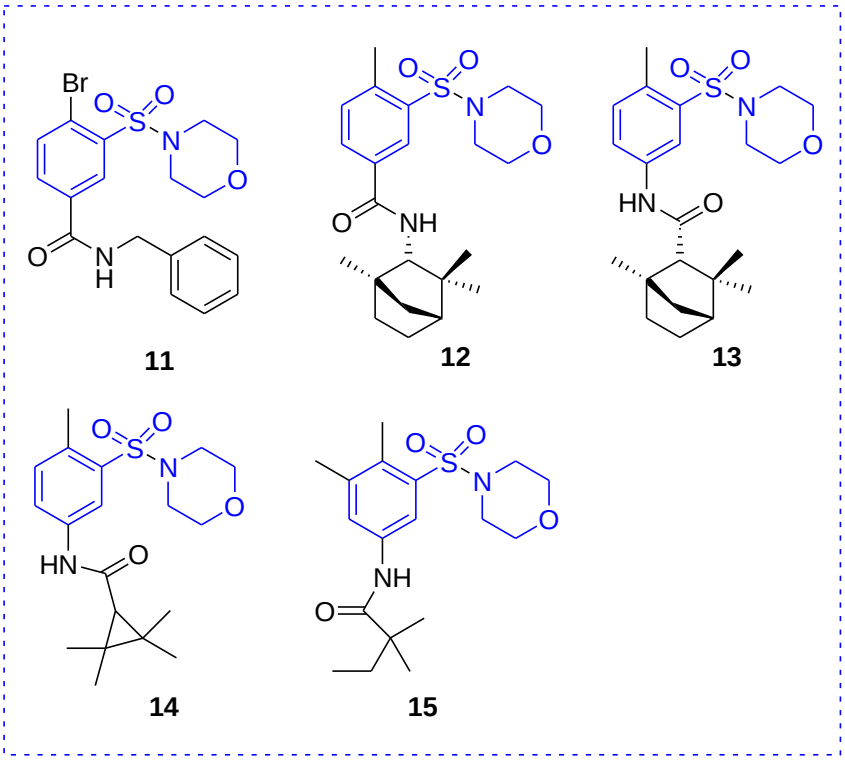
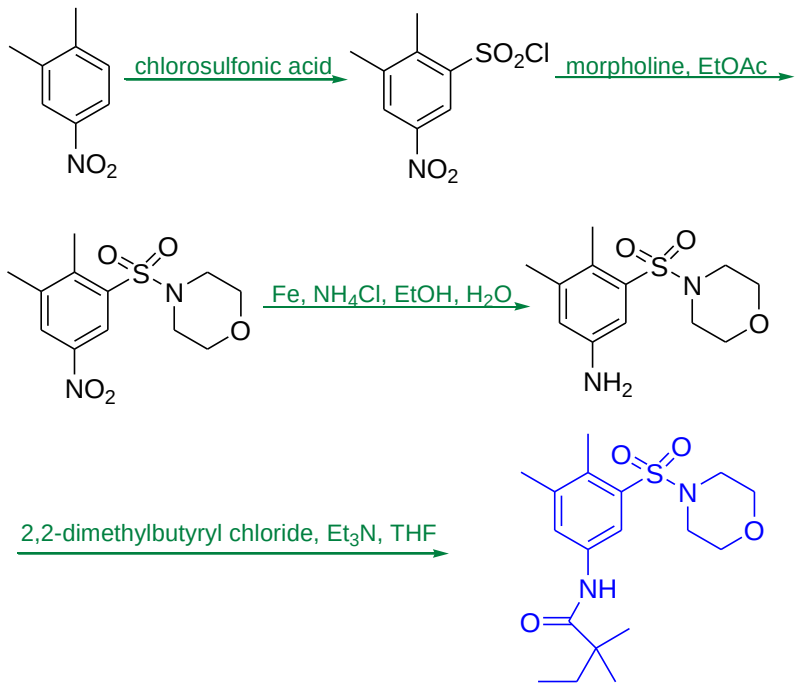


Figure 4. Sulfamoyl benzamide derivatives.

Scheme 3. Synthesis of Sulfamoyl benzamide 15.



15

7.3. Heterocyclic Ylidene

Taisho Pharmaceuticals published the five-memebred heterocyclic cores as CB_2 selective agonists. The synthesis and SARs of thiazole derivatives as potent CB_2 agonist showed that the functional groups at 3- and 5-position in the thiazole ring and the aromatic group in the amide deeply influence the affinity and selectivity for the CB_2 receptor. Thiazolylidene compounds such as **16** (Figure 5) with a bulky functional group like trifluoromethyl exhibited much higher CB_2 affinities (hCB_2 IC_{50} = 13 nM, 270 fold selectivity)[248]

Modifying the heteroring of **16** to improve binding affinity and selectivity for the CB_2 receptor led to the preparation of five-membered heterocyclic derivatives **17-21** (Figure 5). Thiadiazole **17** exhibited lower affinity for the CB_2 receptor than the thiazole derivative. Oxazolylidene **18**, isothiazol-ylidene **19**, and isoxazolylidene **20** had good affinity for the CB_2 receptor (**18**: IC_{50} = 24 nM, **19**: IC_{50} = 9.5 nM, **20**: IC_{50} = 46 nM). Incorporation of the pyrazole reduced the overall

lipophilicity of this chemical series and led to an improvement in aqueous solubility. These studies led to compound **21** (IC_{50} = 51 nM) and to the N-(3-tert-butyl-1-(cyclopropylmethyl)-1,2-dihydro-2-methylpyrazol-5-ylidene)-2-fluoro-3-(trifluoromethyl)benzamide **22** (CBS0550, Figure 5, Scheme 4), which exhibited particularly high affinity and selectivity for the CB_2 receptor (IC_{50} = 2.9 nM, Emax = 85%, SI =1400). This compound had good solubility in water, exhibited good metabolic stability in human and rat liver microsomes. A dose dependent (10 and 30 mg/kg) compound **22** significantly reversed mechanical hyperalgesia in the Randall–Selitto model of inflammatory pain in rats.[249]

Preparation of (14E)-N-(3-tert-butyl-1-(cyclopropylmethyl)-1,2-dihydro-2-methylpyrazol-5-ylidene)-2-fluoro-3-(trifluoromethyl)benzamide (22)[249] According to Scheme 4, cyclopropylmethyl alcohol was treated with methanesulfonyl chloride (MsCl) and triethylamine (Et_3N) to yield mesylate intermediate. This was transformed into alkylhydrazine by treatment with hydrazine. Cyclization of 1-(cyclopropylmethyl)hydrazine by treatment with pivaloylacetonitrile yielded the 5-aminopyrazole. The latter was treated with trifluoroacetic anhydride and pyridine to obtain *N*-(3-*tert*-butyl-1-(cyclopropylmethyl)-1*H*-pyrazol-5-yl)-2,2,2-trifluoroacetamide. The 1-position of the pyrazole ring was alkylated, followed by deprotection under basic conditions, producing the imine intermediate which was easily transformed into pyrazole final compound 22 in one step by treatment with the corresponding aryl carbonyl chloride and Et_3N.

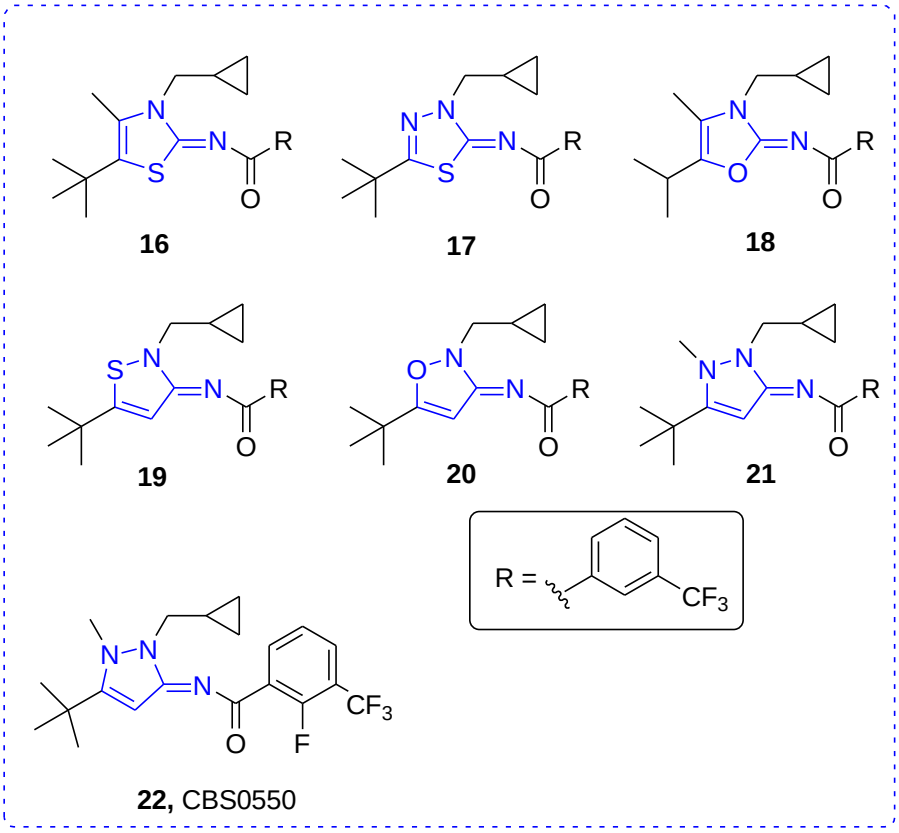
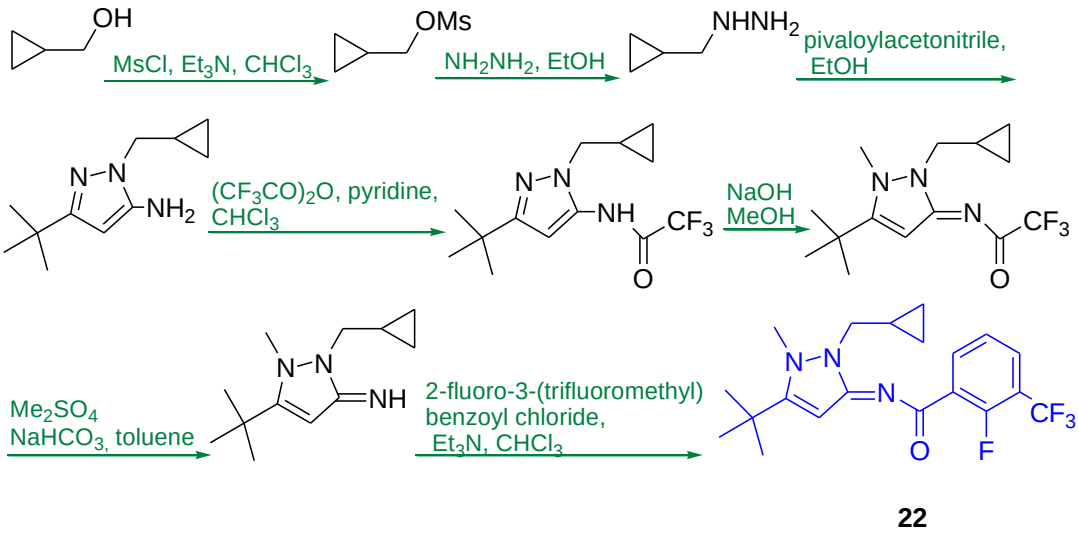


Figure 5. Heterocyclic ylidene derivatives.

Scheme 4. Synthesis of Pyrazolylidene 22.



7.4. Oxadiazole, α-Amidosulfone

The five-membered oxadiazole core as a novel class of potent and selective CB_2 agonists by a targeted library screening and subsequent hit assessment was identified.[250] The SARs investigation into this group of compounds showed that the oxadiazole core was fundamental, and substitutions at the 2- and 4- positions of the phenyl ring joined to the oxadiazole were important for desired CB_2 activity. The amino quinoline derivative **23** (Figure 6, Scheme 5) was a potent and selective CB_2 agonist (EC_{50} = 10 nM, Emax = 104%), which displayed an excellent pharmacokinetic profile with oral bioavailability in rats.[250] Structural modifications in the central portion of the *N*-arylamide oxadiazole scaffold led to the identification of *N*-arylpiperidine oxadiazoles as conformationally constrained analogs, such as compound **24** (Figure 6), that offered improved stability with comparable potency and selectivity (EC_{50} = 80 nM, Emax = 101%).[251] The strictly correlated α-amidosulfones were found to be CB_2 potent and selective agonists. The effect of the amide substituent on CB_2 activity and selectivity was explored. 3,4-Disubstituted phenyl ring, such as in 2-(4-chlorophenylsulfonyl)-*N*-(4-chloro-3-(trifluoromethyl)phenyl)-2-methylpropanamide **25** (Figure 4), enhanced the potency and selectivity for CB_2 receptor (EC_{50} = 20 nM, Emax = 102%). For the five-membered heterocycles it was found that rings with *tert*-butyl group induced activity and selectivity. This series is represented by 2-(4-chlorophenylsulfonyl)-N-(3-*tert*-butyl-1-methyl-1*H*-pyrazol-5-yl)-2-methylpropanamide **26** (Figure 6) (EC_{50} = 20 nM, Emax = 104%). α-Amidosulfones behaved as selective CB_2 full agonists and showed high functional and cellular activity on CB_2 receptors.[252]

Preparation of 6 3-(4-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)quinoline (24).[251] Condensation of 2-chloro-4-fluorobenzonitrile and hydroxylamine afforded N-hydroxybenzamidine, which was condensed with 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid to afford the 4-(3-(2-chloro-4-fluorophenyl)-1,2,4-oxadiazol-5-yl)piperidine. The piperidine

intermediate was coupled with 3-bromoquinoline using a Buchwald-Hartwig reaction to afford 24. (Scheme 5)

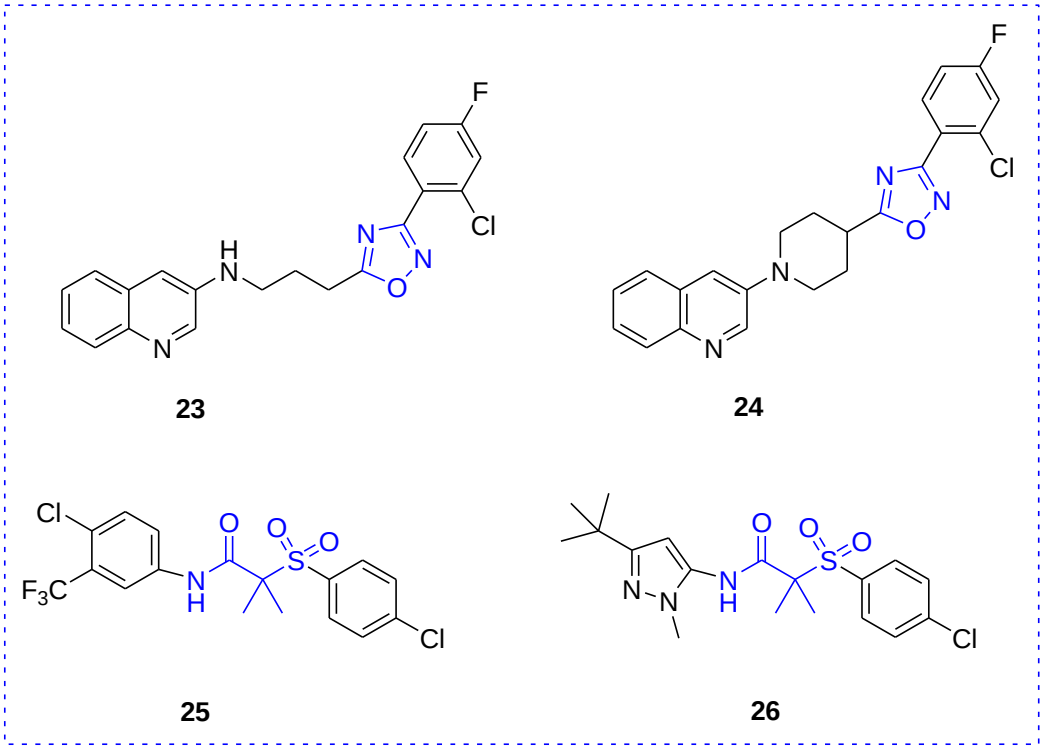
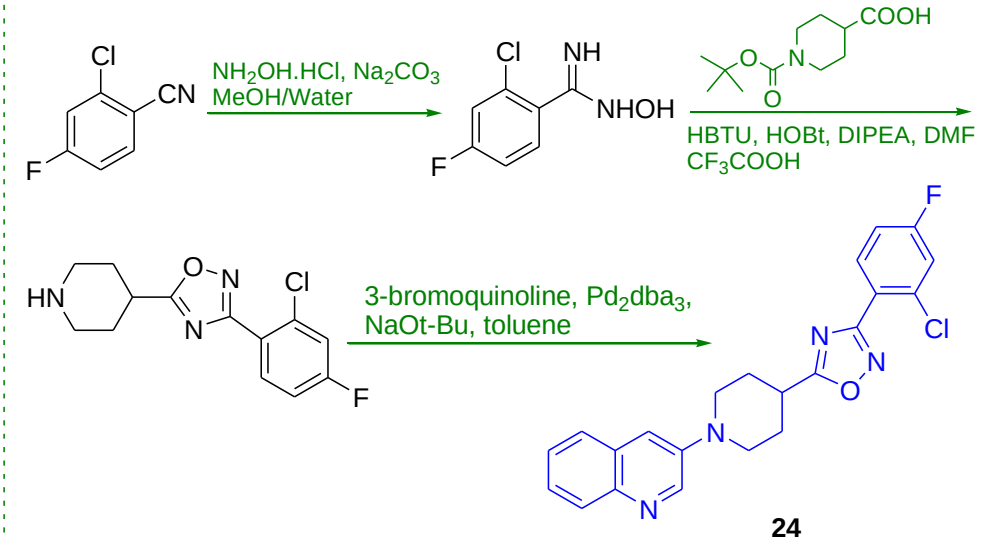


Figure 6. Oxadiazole and α-Amidosulfone derivatives.

Scheme 5. Synthesis of N-arylpiperidine oxadiazole 24.



7.5. Imidazole

Merck Research Laboratory published 2,4-diphenyl-1*H*-imidazole analogues as CB_2 receptor ligands. Compound **27** (Figure 7, Scheme 6), one of the most active compounds in this series (hCB_2 EC_{50} = 8 nM) displayed high selectivity over hCB_1, but did not possess high plasma exposure in rats. The replacement of morpholine side chain with the 4-fluoro or 4,4-di-fluoro piperidinyl moiety yielded compounds **28** and **29**, respectively, as potent CB_2 full agonists (**28**: hCB_2 EC_{50} = 9 nM, **29**: 5 nM). Replacement of the 3-trifluoromethyl group of phenyl ring with 2,4-dichlorine atoms (**30**: hCB_2 EC_{50} = 10 nM) did not affect the potency compared to that of **28**.[253]

Preparation of 4-(3-(5-(3-(trifluoromethyl)phenyl)-1H-imidazol-2-yl)benzyl)morpholine (27).[253]

The synthetic route to the 2,4-diphenyl-1*H*-imidazole 27 is summarized in Scheme 6. The commercially available 1-(3-(trifluoromethyl)phenyl)ethanone was brominated and then cyclized with formamide to form 4-phenylimidazole. Consequent N-protection with 2-(trimethylsilyl)ethoxymethyl (SEM) chloride and iodination using iodine were achieved under basic conditions to afford 1-((2-(trimethylsilyl)ethoxy)methyl)-4-(3-(trifluoromethyl)phenyl)-2-iodo-1*H*-imidazole. The iodo displacement with 3-phenylaldehyde was conducted under Suzuki coupling condition using $Pd(dppf)_2Cl_2$ as the catalyst. Reductive amination and final removal of the SEM yielded the target compounds 27.

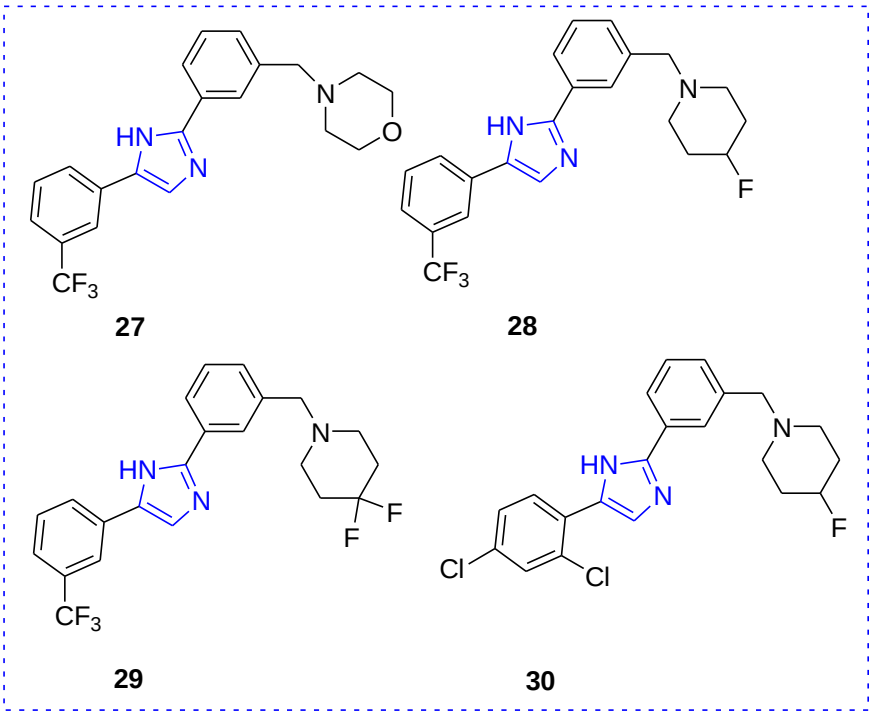
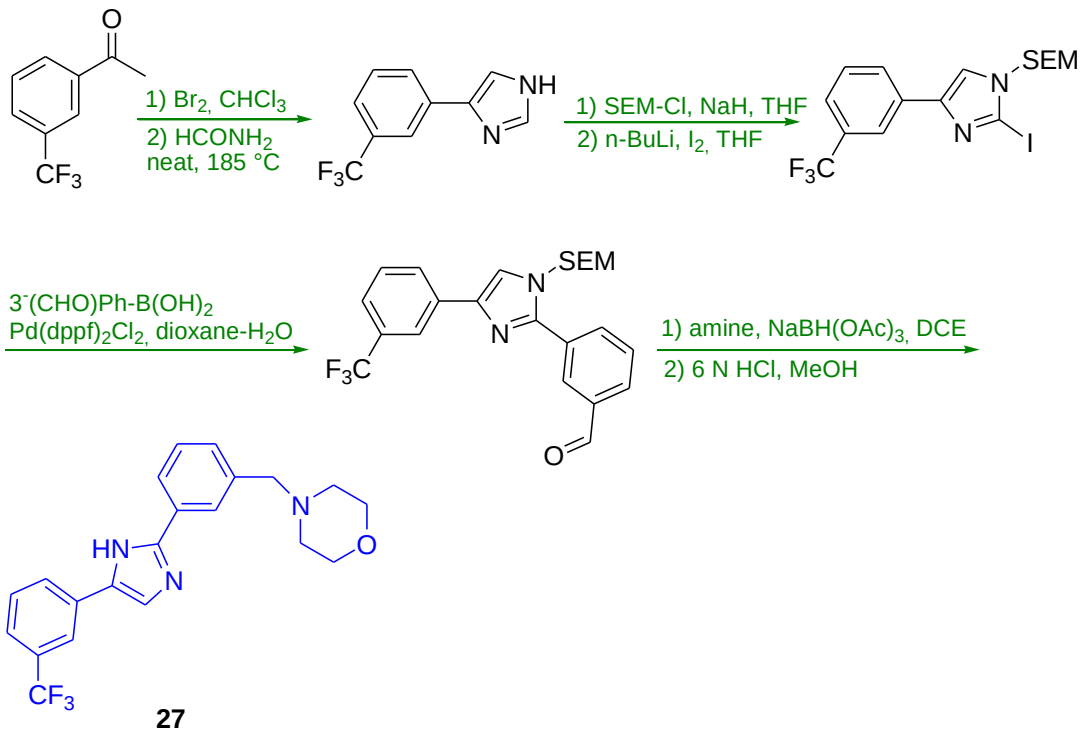


Figure 7. Imidazole derivatives.

Scheme 6. Synthesis of Imidazole 27.



7.6. Benzoimidazole

CB_2 agonists based on benzimidazole template have been reported by Page et al. from AstraZeneca. One of the first, discovered as part of an HTS campaign, was 2-(4-ethoxybenzyl)-1-(2-(dimethylamino)ethyl)-*N*,*N*-diethyl-1*H*-benzo[*d*]imidazole-5-carboxamide **31** (Figure 8). SAR studies around compound **31** revealed that on the N-1 position various alkyl and aromatic groups were tolerated. In this series, with N-cyclopropylmethyl (**32:** hCB_2 K_i = 4.1, hCB_1 K_i = > 5000 nM) and N-isopentyl (**33:** hCB_2 K_i = 4.5 hCB_1 K_i = > 5000 nM) an optimal potency and selectivity was obtained.[254] The diethyl amide modifications were all well tolerated demonstrating probably the occurrence of a large binding pocket at the receptor site. Reducing the size of the group to a secondary amide showed about a 10-fold decrease in CB_2 binding affinity, having only a primary amide on the left-hand side resulted in a total loss of activity.[254] Further studies by Pfizer on the benzimidazole scaffold led to the preparation of a large number of sulfonyl derivatives with CB_2 receptor agonistic activity. Incorporation of neopentyl chain into the 2-position together with introduction of a sulfonyl group into the 5-position led to one of the best compound of series 1-(1-(cyclopropylmethyl)-2-neopentyl-1*H*-benzo[*d*]imidazol-5-ylsulfonyl)cyclopropanecarboxamide (**34**, Figure 8), a selective and potent full agonist of CB_2 receptor. From lead compound **34**, a structurally similar series based on saturation of the benzene ring was designed. The alkyl, carbamate, urea, amide and sulfonamide derivatives of the tetrahydro-1*H*-imidazo[4,5-c]pyridin-5-ium scaffold were prepared. Lipophilic Efficiency (LipE) was introduced as a parameter that combines both potency and lipophilicity as a helpful instrument for design of potent and metabolically stable CB_2 agonists. The sulfonamide derivatives such proved to be better than the other functionalities being the most potent full agonist at the hCB_2 receptor. Among this series, the isoxazole **35** (Figure 8) displayed agonist activity at 10 μM on the hCB_1 receptor (EC_{50} **35** = 7.5 μM).[255] In a related effort, RaQualia Pharma claimed N-substituted saturated heterocyclic sulfone compounds with CB_2 agonistic activity. One of the representative derivative is 3-(1-(2-

(dimethylamino)ethyl)-2-neopentyl-1*H*-benzo[*d*]imidazol-5-ylsulfonyl)azetidine-carboxamide **36** **(**Figure 8).[256]

Subsequently, the structural modifications to improve Human Liver Microsomes (HLM) stability and membrane permeability produced compound **37** (RQ-00202730, Figure 8), which bears a hydroxyethyl group as a side chain on the azetidine ring. RQ-00202730 demonstrated the best overall profile in terms of potency, selectivity (EC_{50} = 19 nM, SI >1300-fold) over the CB_1 receptor showing a dose dependent analgesic effect on TNBS-induced visceral hypersensitivity in rats by oral administration.[257] Following investigations focused on the benzoimidazole scaffold improved the pharmacokinetic profile of compound **38** by removing polar functionality at the 5-position to improve CNS penetration. Optimization of the benzimidazole substituents of **38** led to the identification of compound **39 (**Figure 8), a potent CB_2 full agonist (EC_{50} = 2.7 nM) with excellent selectivity over the CB_1 receptor (>3000 fold). Compound **39** demonstrated good CNS penetration in rat. Further optimization led to the discovery of the fully CNS penetrant CB_2 agonist **40 (**Figure 8) with reduced human *Ether-à-go-go*-Related Gene (hERG) activity.[258]

The benzimidazole CB_2 receptor agonists were synthesized and developed by Janssen Pharmaceuticals and the structure–activity relationship was explored. The size of the substituent on the 2-position determined the level of agonism, ranging from inverse to partial or full agonism, which was more pronounced for the rat CB_2 receptor. The benzoimidazole cannabinoid agonists bearing a substituted aryl group, in particular, the pyridyl sulfone, such as compound 2-*tert*-butyl-5-(2-ethoxypyridin-4-sulfonyl)-1-tetrahydropyran-4-ylmethyl)-1*H*-benzimidazole **41 (**Figure 8), had excellent binding affinity and selectivity for CB_2 (h EC_{50} = 0.3 nM, hCB_1 EC_{50} = 1280 nM).[259] Pyridyl-sulfone **40** was reported to be 43% orally bioavailable in rat and to have a low brain to plasma ratio.[260] Further optimization of this series led to formation of peripherally acting CB_2 agonists, such as **42** (Figure 8, Scheme 7) and **43** (Figure 8). While both compounds were not active in acute pain models, the less selective compound **41** (hCB_2 EC_{50} = 8.4 nM, hCB_1 EC_{50} >10000

nM) displayed activity in a chronic model of neuropathic pain.[259] In a related effort, *N*-{1-(cyclohexylmethyl)-2-[(5-ethoxypyridin-2-yl)methyl]-1*H*-benzimidazol-5-yl}-*N*-methylthiophene-2-sulfonamide (**44**, AZ-11713908, Figure 8) was produced by AstraZeneca as a potent CB_1 agonist and as a partial CB_2 agonist. It displayed poor blood-brain barrier penetration and was consequently a peripherally selective compound exhibiting analgesic effects in inflammatory and neuropathic pain models.[261] Subsequently, researchers in Merck designed a series of benzimidazole CB_2 agonists from imidazopyridine scaffold.[262] Evaluation of the SAR revealed that introduction of the amide group at the 2-position, as well as the N-alkyl imidazoles, provided more potent CB_2 agonism when compared to N-unsubstituted benzimidazoles. The lipophilic adamantyl amide gave a very potent CB_2 agonist, but its selectivity over CB_1 was poor. The most potent compound in this series was obtainad by introduction of an aliphatic amide bearing hydroxyl group (compound **45:** hCB_2 EC_{50} = 6.3 nM, hCB_1 EC_{50} >17,000, Emax 75%, Figure 8). Although the new structural class provided CB_2-selective compounds, the pharmacokinetic profiles were moderate.[263]

Preparation of 1-(4-((2-tert-butyl-5-(ethylsulfonyl)-1H-benzo[d]imidazol-1-yl)methyl)piperidin-1-yl)ethanone (42).[259] Starting from 5-chloro-2-nitro-aniline, 4-methoxybenzylthiol (PMBSH) was introduced as a protecting group via nucleophilic aromatic substitution and subsequent reaction with pivaloylchloride gave N-(5-(4-methoxybenzylthio)-2-nitrophenyl)pivalamide. Reduction of the nitro group was followed by reductive amination with piperidine-4-carbaldehyde and subsequent condensation under acidic conditions resulted in debocylation, which allowed for subsequent introduction of acetyl group. Deprotection of the *p*-methoxybenzyl group with trifluoroacetic acid (TFA), ethyl introduction via nucleophilic substitution, and finally, the oxidization with *m*-chloroperoxybenzoic acid yielded the sulfone 42. (Scheme 7)

Preparation of 1-ethyl-N-(2,3-dihydroxypropyl)-4-phenyl-1H-benzo[d]imidazole-2-carboxamide 45.[263] Phenyl boronic acid was coupled to 3-chloro-2-nitroaniline which was then reduced with tin(II)chloride to give *o*-diamine intermediate. Treatment with methyl-2,2,2-trichloroacetimidate,

and ethanolysis gave benzimidazole-2-ethyl ester. Alkylation by iodoethane produced exclusively one alkylated product, which was then hydrolyzed and subsequent peptide coupling conditions yielded the desired amide 45. (Scheme 8)

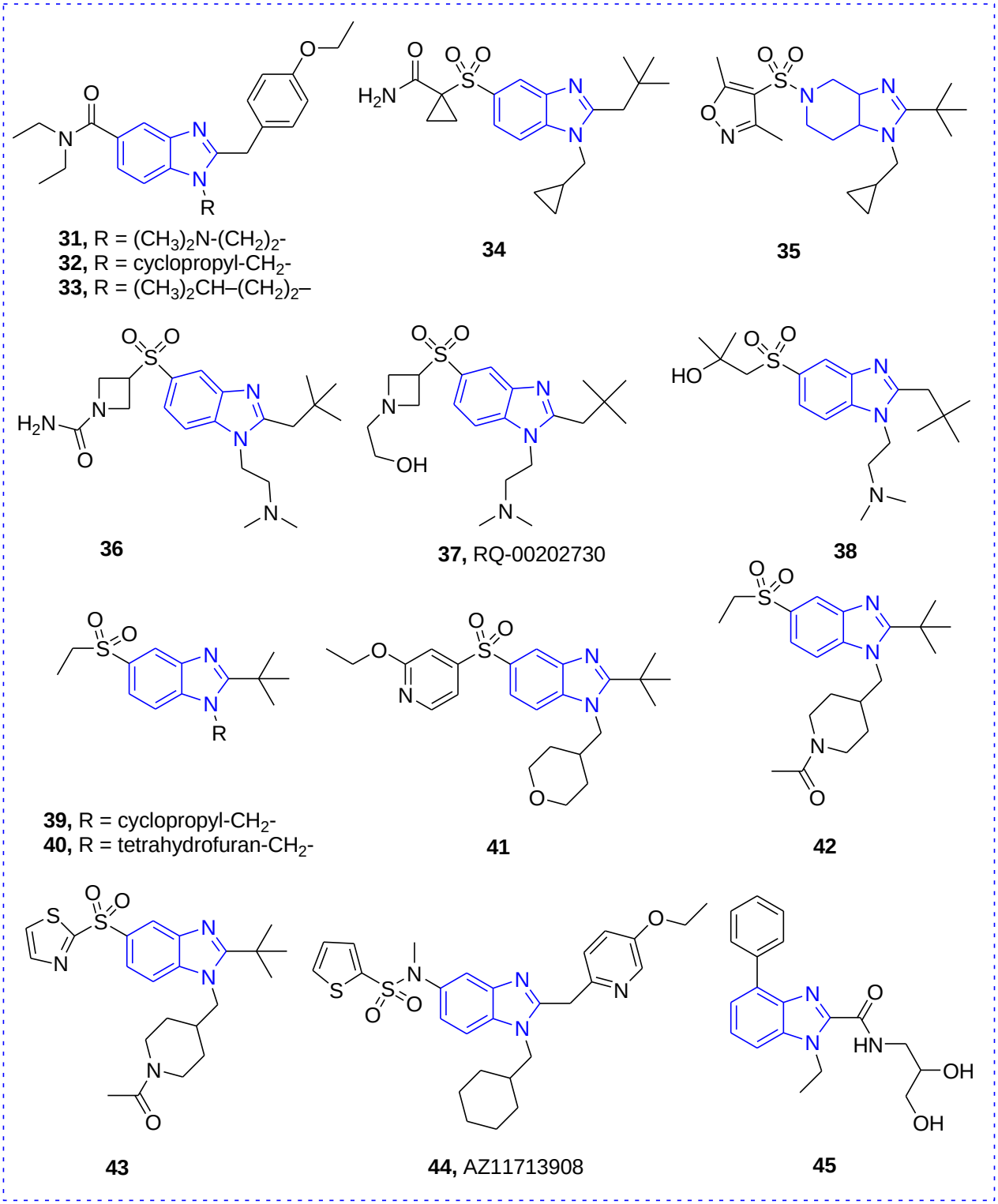
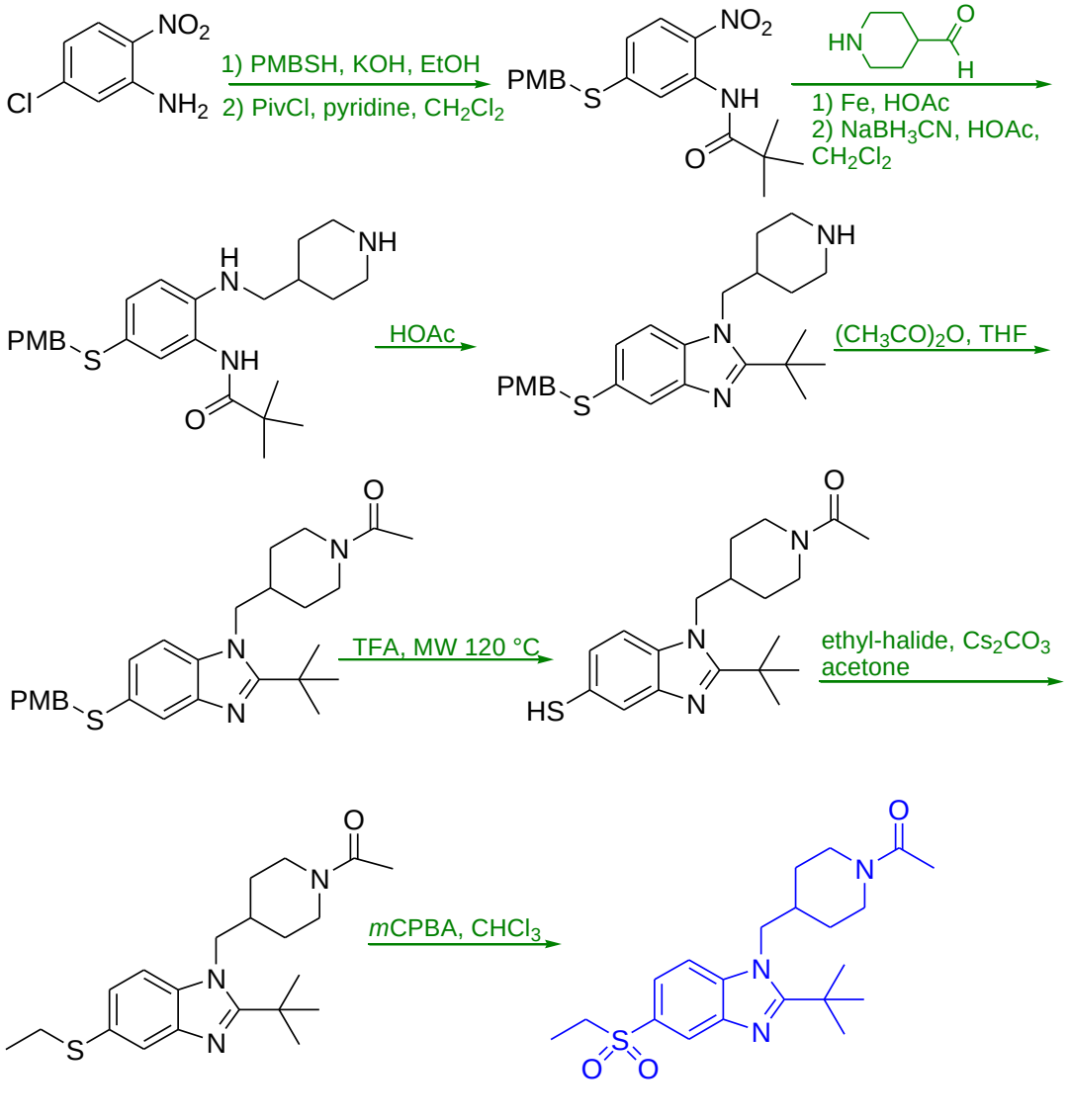


Figure 8. Benzomidazole derivatives.

Scheme 7. Synthesis of 5-Ethylsulfonyl benzoimidazole 42.



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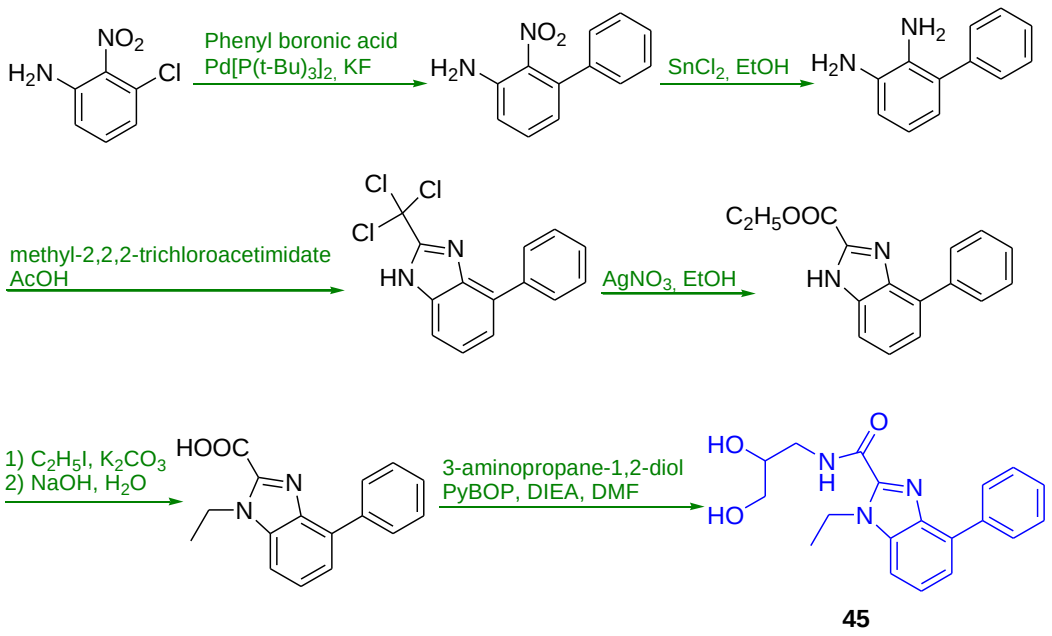
Scheme 8. Synthesis pf 4-Phenyl benzoimidazole 45.



45

7.7. Imidazopyridine

Imidazopyridine CB_2 agonists have been described as closely related scaffolds of indole based CB_2-selective ligands. Exploration of SAR in this chemical series by variation of substituents on both carbon positions of the imidazole ring of the core produced CB_2 agonists. Compounds N-((1-(hydroxymethyl)cyclopentyl)methyl)-3-morpholino*H*-imidazo[1,5-*a*]pyridine-1-carboxamide **46** (Figure 9) (hCB_2 IC_{50} = 33 nM, hCB_1 IC_{50} > 17000 nM, Emax 99%) and 2,3-dichlorophenyl(1-(morpholinomethyl)*H*-imidazo[1,5-*a*]pyridin-3-yl)methanone **47** (hCB_2 IC_{50} = 5 nM, Emax 94%; hCB_1 IC_{50} = 2565 nM, Emax 66%) (Figure 9, Scheme 9), which were completely selective for CB_2 versus CB_1, are representatives of this series. In vivo evaluation of these compounds indicates a significant impact of the degree of selectivity for CB_2 on analgesic effects.[264] A related series of 3-arylimidazopyridine analogues was reported, wherein 3-(3-(trifluoromethyl)phenyl)-1-(4,4-difluoropiperidin-1-yl)methyl)*H*-imidazo[1,5-*a*]pyridine **48** (Figure 9) was the most potent CB_2 agonist disclosed.[265]

Preparation of (2,3-dichlorophenyl)(1-(morpholinomethyl)H-imidazo[1,5-a]pyridin-3-yl)methanone (47).[266]As depicted in Scheme 9, condensation of 2-aminomethyl pyridine with ethyl oxalyl chloride provided the ester intermediate. Formylation with the Vilsmeier reaction gave imidazopyridine aldehyde which, by means of reductive amination, yielded the ethyl 1-(morpholinomethyl)*H*-imidazo[1,5-*a*]pyridine-3-carboxylate, that was then transformed into Weinreb amide intermediate. The hydrolysis and Grignard reaction yielded the final compound 47.

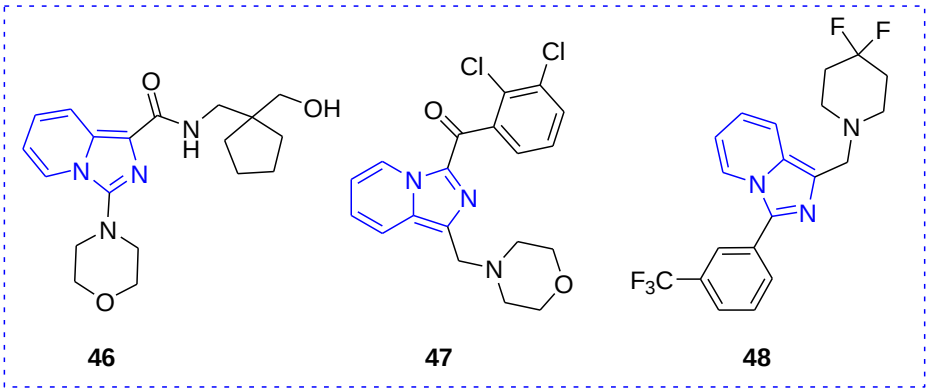
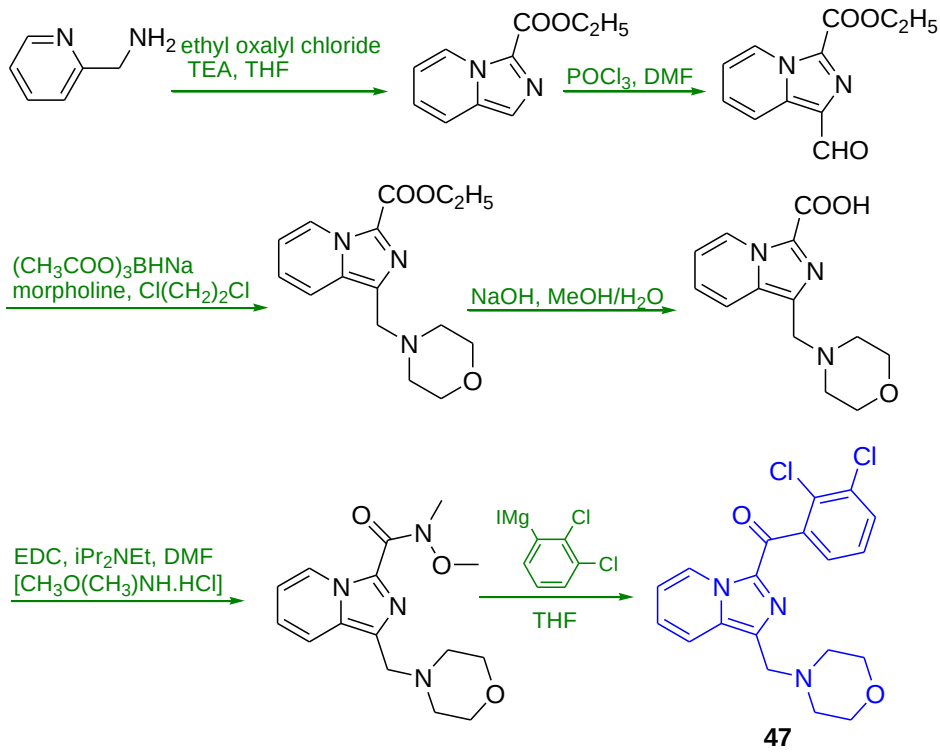


Figure 9. Imidazopyridine derivatives.

Scheme 9. Synthesis of Imidazopyridine 47.



7.8. Purine

Lilly Research Laboratories discovered the purine derivatives as CB_2 receptor agonists based on the thieno[2,3-*d*]pyrimidine scaffold **49** (hCB_2 EC_{50} = 25 nM, SI = 104, Figure 10) with moderate selectivity and poor in vitro metabolic profile. The selectivity for CB_2 and metabolic profile were further optimized by varying the substituent at the 9-position of the purine core yielding a number of potent CB_2 agonists including compounds (*R*)-8-(2-chlorophenyl)-9-((*S*)-tetrahydrofuran-3-yl)-2-methyl-6-(4-methylpiperazin-1-yl)-9*H*-purine **50** (hCB_2 EC_{50} = 8.8 nM, SI > 1130, Figure 10) and the 9-tetrahydro-2*H*-pyran derivative **51** (Figure 10, Scheme 10), which possessed good biopharmaceutical properties and potent oral activity in a preclinical model of joint pain.[267,268]

Preparation of 8-(2-chlorophenyl)-9-(tetrahydro-2H-pyran-4-yl)-2-methyl-6-(4-methylpiperazin-1-yl)-9H-purine hydrochloride (51).[268] The starting material 5-amino-4,6-dichloro-2-methyl pyrimidine was reacted with tetrahydro-2*H*-pyran-4-amine to afford the monoamino intermediate (Scheme 10). Subsequent reaction with 2-chlorobenzaldehyde was followed by cyclization by means of $FeCl_3$–SiO_2. The subsequent oxidation by 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) ensured the complete conversion to the desired purine compound. Successive reaction with 1-methylpiperazine gave the desired product which was then converted into the corresponding HCl salt 51.

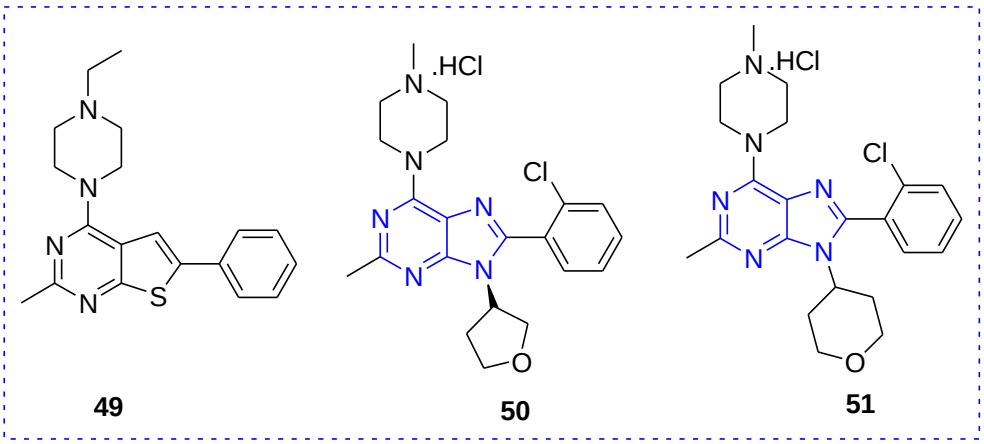
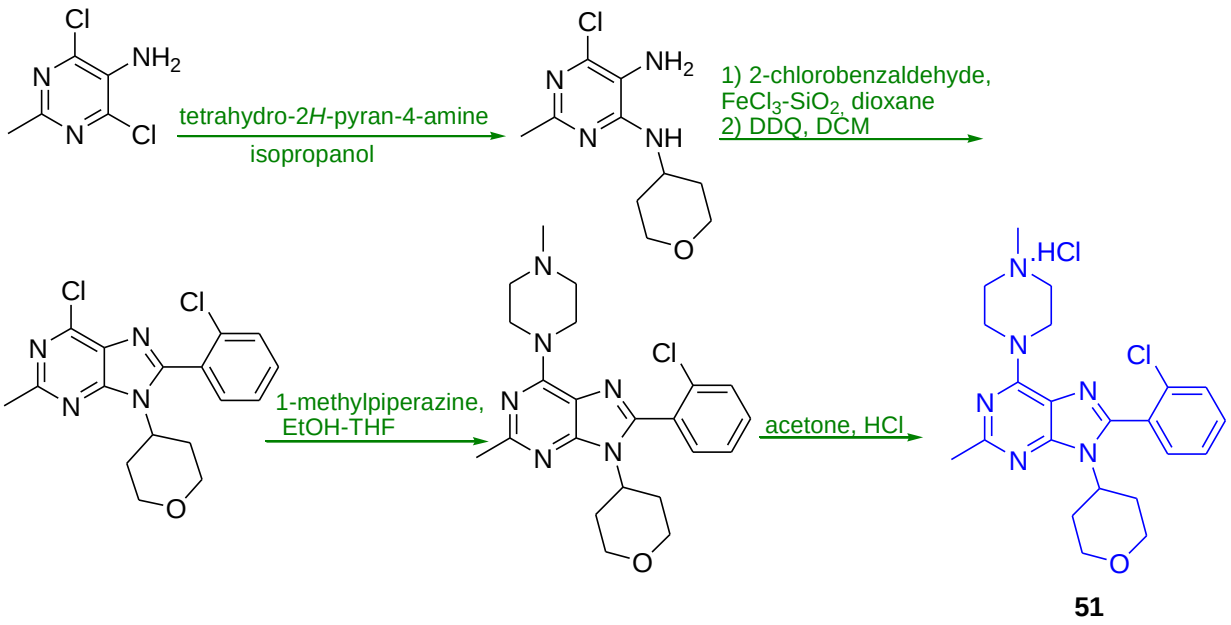


Figure 10. Purine derivatives.

Scheme 10. Synthesis of Purine 51.



7.9. Quinolone, Naphthyridine

The CB_2 receptor agonists also include 4-oxo-1,8-naphthyridine-3-carboxamide and 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives endowed with high affinity and selectivity toward CB_2 receptors.[269,270] Some of these analogues were demonstrated to act as agonists or inverse agonists in functional activity assays, depending on the nature of the substituents on the different positions of the heterocyclic scaffold. A closely related series of 1,8-naphthyridin-2(1*H*)-on-3-carboxamide analogues were described in subsequent publications.[271] In the series, the *cis*-4-methylcyclohexylamido analogue **52** (Figure 11) exhibited the highest affinity, with K_i values of 0.7 nM. Very recently, a number of additional derivatives in which the same central scaffold was variously functionalized in position 1 or 6, have been reported.[272] The best results in terms of both CB_2 affinity and selectivity were obtained with the 6-methoxyphenyl analogue **53** (Figure 11, Scheme 11) showing high CB_2 affinity and selectivity (CB_2 K_i = 1.47 nM, CB_1 K_i > 10000 nM).

Within this series, the functional activity of compounds is controlled by the presence of the substituents at position C-6 of the naphthyridine scaffold; introduction of substituents in this position determined a functionality switch from agonist to antagonists/inverse agonists.[272]

For the new 1,8-naphthyridin-2(1*H*)-one-3-carboxamide derivatives, docking studies have shown that both the antagonists/inverse agonists and the agonists bind in the TMH2-3-6-7 regions of CB_2 receptor, suggesting that the difference between the pharmacology of these ligands depends on their ability/inability to block the Toggle Switch W6.48 (χ1 g+ → trans) transition.[272]

The naphthyridine CB_2 agonist **54** (CB13, Figure 11) was shown to induce apoptosis through ceramide de novo synthesis in colon cancer cells.[273] The closely related 4-quinolone-3-carboxamides CB_2 chemotype have also been described. The nature of substituents at positions N-1, C-6 or C-8 were studied, and one of the most potent CB_2 agonist was **55 (**hCB_2 K_i = 6.3 nM, hCB_1 K_i = 1220 nM, Figure 11), which demonstrated significant analgesic effects in a mouse formalin test of acute peripheral and inflammatory pain.[274]

Introduction of 2-furyl in position 6 (analogue **56**, Figure 11) produced a significantly improved CB_2/CB_1 selectivity profile relative to **55**. It behaved as a potent CB_2 neutral antagonist in the formalin test of acute and inflammatory pain.[275] The 8-substituted-4-quinolone-3-carboxamide derivatives were also investigated, the 8-methoxy derivative **57 (**hCB_2 K_i = 0.6 nM, hCB_1 K_i > 10000 nM) (Figure 11) was assayed in vivo in the formalin test of analgesia in mice, behaved as an inverse agonist and showed antihyperalgesic effects.[276]

Preparation of 1,2,3,4-tetrahydro-6-(4-methoxyphenyl)-N-(4-methylcyclohexyl)-1-(2-morpholinoethyl)-2,4-dioxo-1,8-naphthyridine-3-carboxamide (53).[272] The synthetic route to obtain 1,8-naphthyridin-2(1*H*)-one-3-carboxamide 53 is outlined in Scheme 11. Aminonicotinaldehyde was treated with bromine in glacial acetic acid to obtain the corresponding 6-bromo derivative, which was refluxed with diethyl malonate and in the presence of piperidine in ethanol to afford ethyl 6-bromo-1,8-naphthyridin-2(1*H*)-one-3- carboxylate intermediate. The reaction of ethyl ester

with a *cis*/*trans* diastereoisomeric mixture of 4-methylcyclohexylamine at 150 °C provided the carboxamide. N-Alkylation with 4-(2-chloroethyl)morpholine afforded the desired 1,8-naphthyridin-2-one derivative. Suzuki reaction by generating in situ $Pd(PPh_3)_4$, in aqueous Na_2CO_3 and boronic acid yielded the final compound.

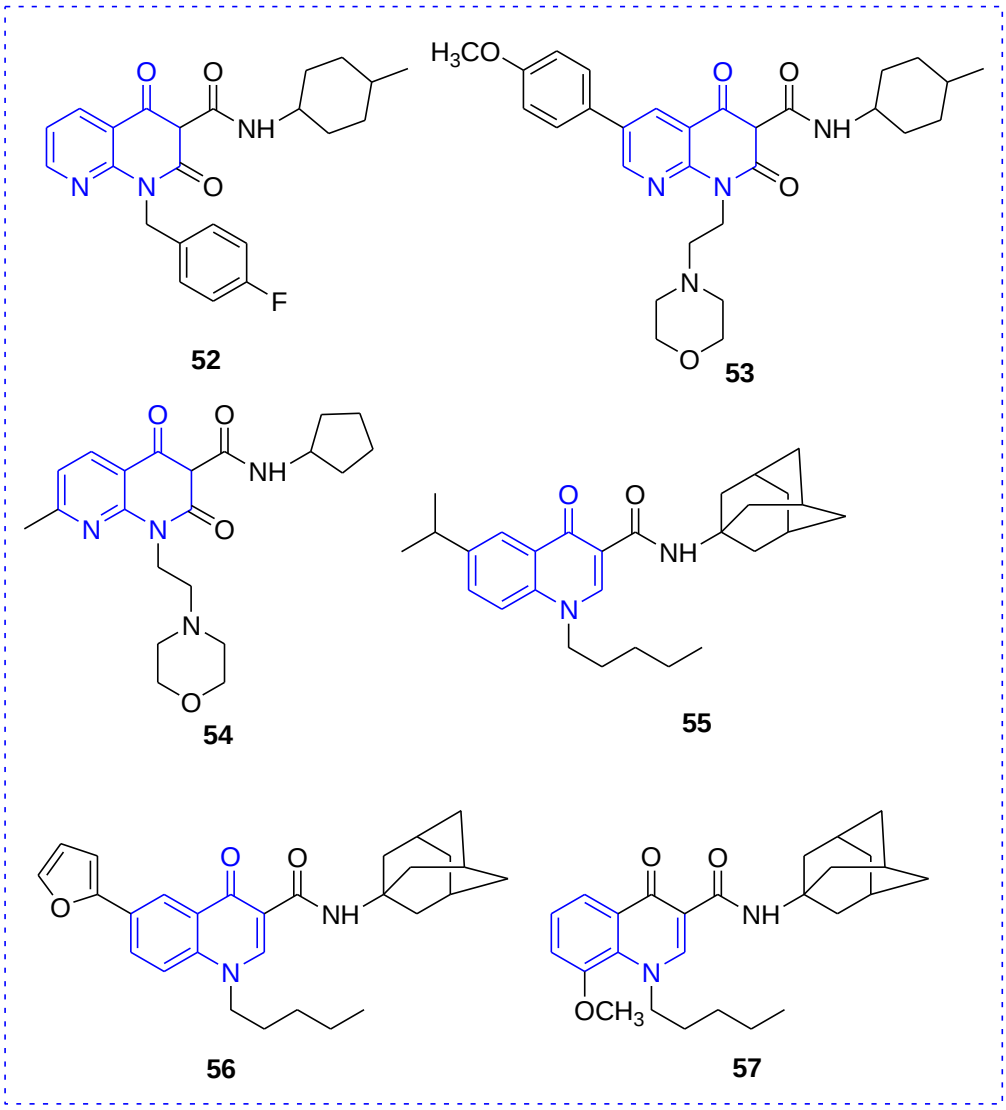
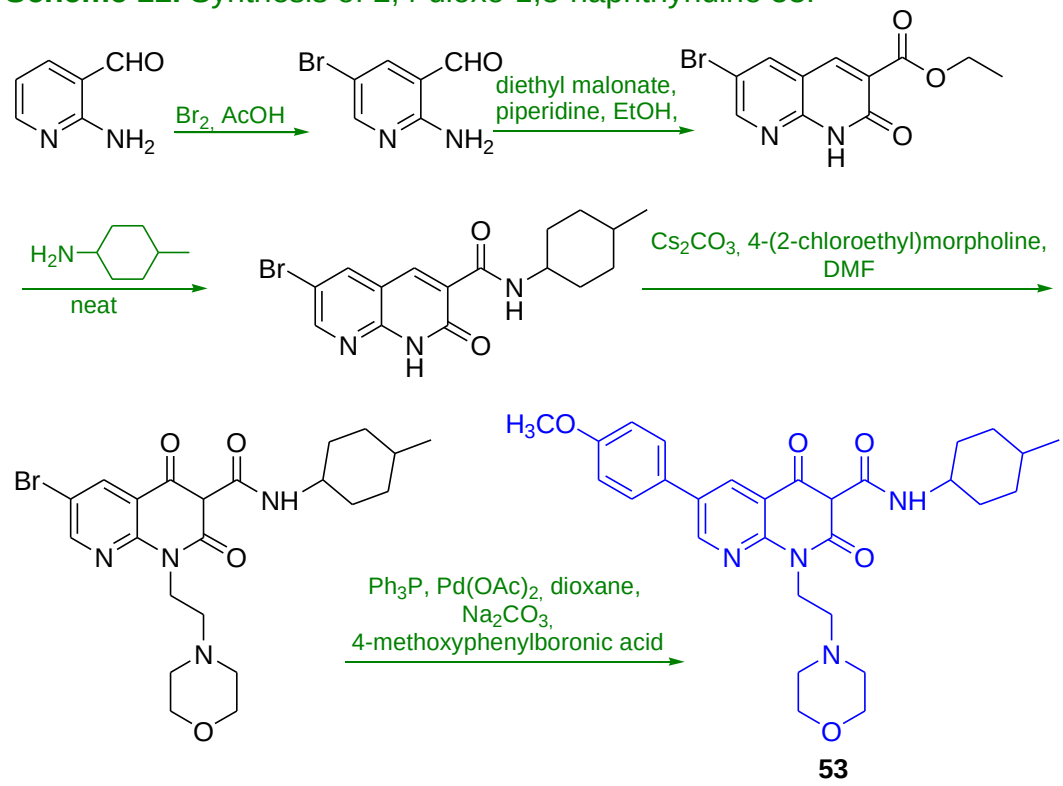


Figure 11. Quinolone and Naphthyridine derivatives.

Scheme 11. Synthesis of 2,4-dioxo-1,8-naphthyridine 53.



53

7.10. 4-Oxo-1,4-dihydropyridine

4-Oxo-1,4-dihydropyridines, as exemplified by the compound **58** (Figure 12, Scheme 12), has been described as potent and selective CB_2 receptor agonist (hCB_2 K_i = 20 nM, hCB_1 K_i > 3000 nM, Emax 148%).[277] Affinity and functionality of this group of compounds was shown to be dependent on the nature of substituents around the heterocycle and the C-6 substituent was crucial in controlling the functionality of this series of compounds . Using a β2-adrenergic receptor-based CB_2 receptor model, the authors suggested that the phenyl at C-6 confers the inverse agonist profile by blocking the χ1 torsion of Trp6.48 side chain in its inactive conformation. The substituents at N-1 and C-3 position were important for affinity but not for functionality. Further optimization efforts focused on the introduction of substituent at C-5, such as a pyridine heterocycle (**59**, Figure 12), was well tolerated with a K_i of 36 nM. Compound **58** failed to act on TNF-α and Il-1β levels, had

drastically decreased macroscopic scores, histological damage, and Myeloperoxidase (MPO) activity, and had a protective effect against colitis.[278]

Very recently, the 2*H*-pyrazolo[4,3-*c*]quinolin-3(5*H*)-one scaffold was developed as constrained analogues of 4-oxo-1,4-dihydroquinoline-3-carboxamide series with improved affinity for the hCB_2 and selectivity over the hCB_1 receptors. The lead of this series, (**60**: hCB_2 K_i = 0.39 nM, hCB_1 K_i > 3000 nM, Figure 12) was found to protect mice against experimental colitis after oral administration.[279]

Preparation of N3-(1-Adamantyl)-6-methyl-1-pentyl-4-oxo-1,4-dihydropyridine-3-carboxamide (58).[277] The synthesis of compound 58 is outlined in Scheme 12. The commercially available 4-hydroxy-6-methyl-2-pyrone was reacted with N,N-dimethylformamide dimethyl acetal to give (3*Z*)-3-((dimethylamino)methylene)-3,4-dihydro-4-hydroxy-6-methylpyran-2-one which, treated with *n*-pentylamine, under alkaline conditions gave the carboxylic acid derivative. Under peptide coupling conditions with 1-aminoadamantane hydrochloride the target amide was obtained.

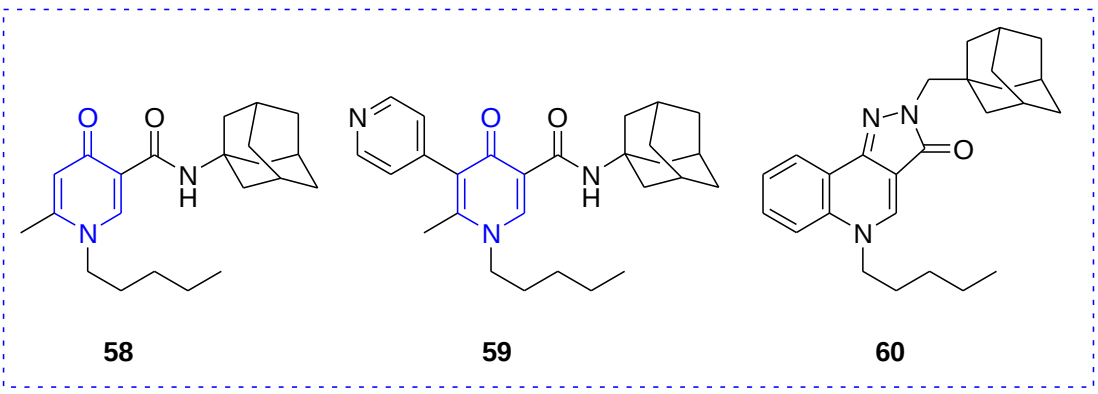
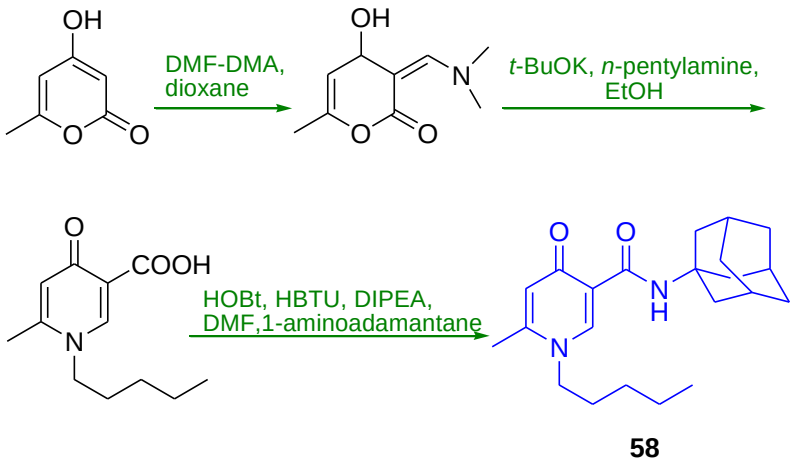


Figure 12. 4-Oxo-1,4-dihydropyridine derivatives

Scheme 12. Synthesis of 4-oxo-1,4-dihydropyridine 58.



58

7.11. Decahydroquinoline amide

Decahydroquinoline CB_2 agonists were reported by the Merck Research Laboratories. Optimization of the amide substituent led to improvements in CB_2 selectivity over CB_1 receptors as well as physicochemical properties. Two compounds were examined in a rat model of acute inflammatory pain where the quite selective CB_2 agonist **61** (ratCB_2 EC_{50} = 6.3 nM, ratCB_1 EC_{50} = 1887 nM) (Figure 13, Scheme 13) displayed a dose-dependent analgesic effect. The CB_2 agonist (**62**, Figure 13), (ratCB_2 EC_{50} = 81 nM, ratCB_1 EC_{50} > 17000 nM) lacking functional CB_1 activity was inactive in this model even with high in vivo exposure both peripherally and centrally.[280]

Preparation of ((4R,4aS,8aR)-octahydro-4-hydroxy-4-phenylquinolin-1(2H)-yl)(6-methylpyridin-3-yl)methanone (61).[280] 1-Acetylcyclohexene was reacted with paraformaldehyde and dimethylamine hydrochloride under Mannich conditions to give the aminoketone, which was treated with concentrated aqueous ammonia under pressure to yield decahydroquinolinone intermediate. Acylatation with benzyloxycarbonyl chloride gave a primarily *trans* mixture of (4a*R*,8a*R*)-benzyloctahydro-4-oxoquinoline-1(2*H*)-carboxylate. Isomerization of the center adjacent to the

ketone was achieved by treatment with K_2CO_3 in MeOH to give *cis*-isomer in 30%. Addition of phenylmagnesium bromide at low temperature gave alcohol intermediate. Deprotection by hydrogenolysis gave racemic (4*R*,4a*S*,8a*R*)-decahydro-4-phenylquinoline which was acylated in standard peptide coupling conditions. (Scheme 13)

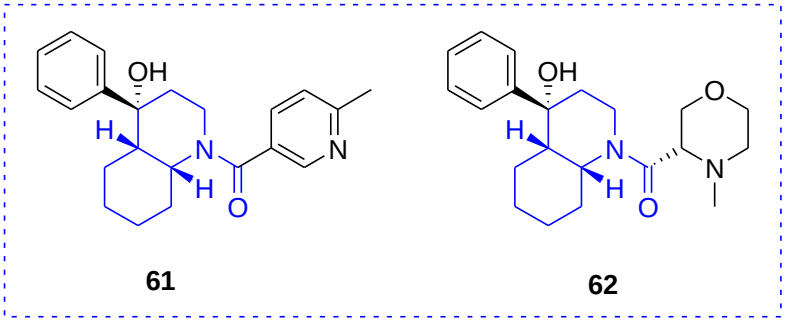
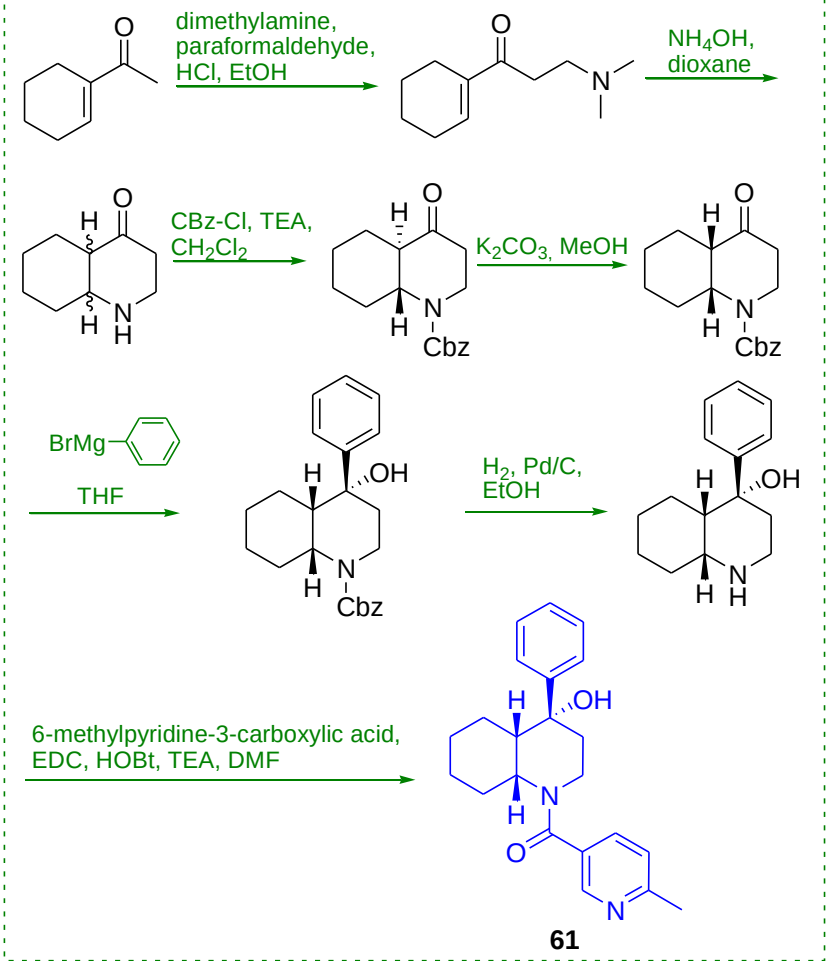


Figure 13. Decahydroquinoline amide derivatives.

Scheme 13. Synthesis of Decahydroquinoline 61.



7.12. Carboline

The γ-carboline series was identified by scaffold-hopping from the bicyclic benzimidazole core as mixed CB_1 and CB_2 agonists.[281] This new structural class of cannabinoid agonists showed good physicochemical properties and low CNS penetration. The SAR of the tetrahydro-1*H*-pyrido[4,3-*b*]indole motif of compound **63** (Figure 14) was extensively examined and the cyclopentyl and 4-tetrahydropyran analogs **64** and **65** (Figure 14) showed a >10 fold increase in agonist activity and perserved reasonable metabolic stability. Optimization of physicochemical properties was achieved by the ethylsulfonyl analogue, compound **66** (Figure 14). This molecule was found to be a potent agonist on hCB_1 (EC_{50} = 49 nM, Emax 120%) and in rat brain tissue ($ratCB_1$ EC_{50} = 85 nM, Emax 156%) exhibiting significant anti-hyperalgesia in a rat inflammatory pain model with low levels of CNS penetration.

Preparation of 5-(ethylsulfonyl)-8-[(4-methylpiperidin-1-yl)carbonyl]-2-(tetrahydro-2H-pyran-4-yl)-2,3,4,5-tetrahydro-1H-pyrido[4,3-b]indole (66).[281] 4-Hydrazinobenzoic acid was heated with 4-piperidinone in dioxane and hydrochloric acid, then treated with BOC-anhydride to give 2-(*tert*-butoxycarbonyl)-2,3,4,5-tetrahydro-1*H*-pyrido[4,3-*b*]indole-8-carboxylic acid. Coupling reaction with methylpiperidine furnished the amide intermediate, which was treated with ethylsulfonyl chloride and sodium hydride to yield the 5-ethylsulfonyl compound. Subsequent debocylation with trifluroacetic acid gave the free amine which was transformed to the target compound by means of 4-tetrahydropyranone and sodium triacetoxyborohydride in dichloromethane. (Scheme 14)

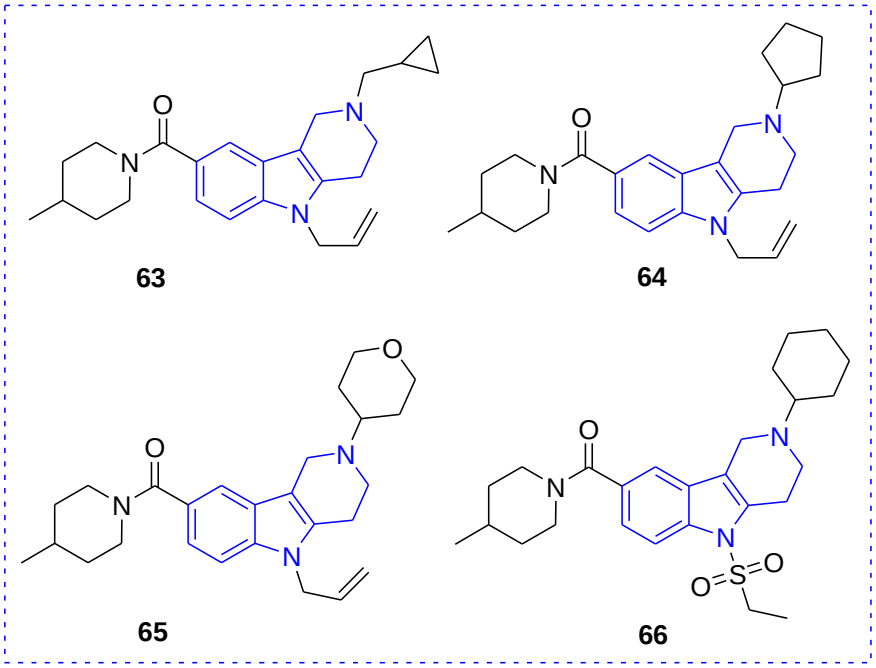
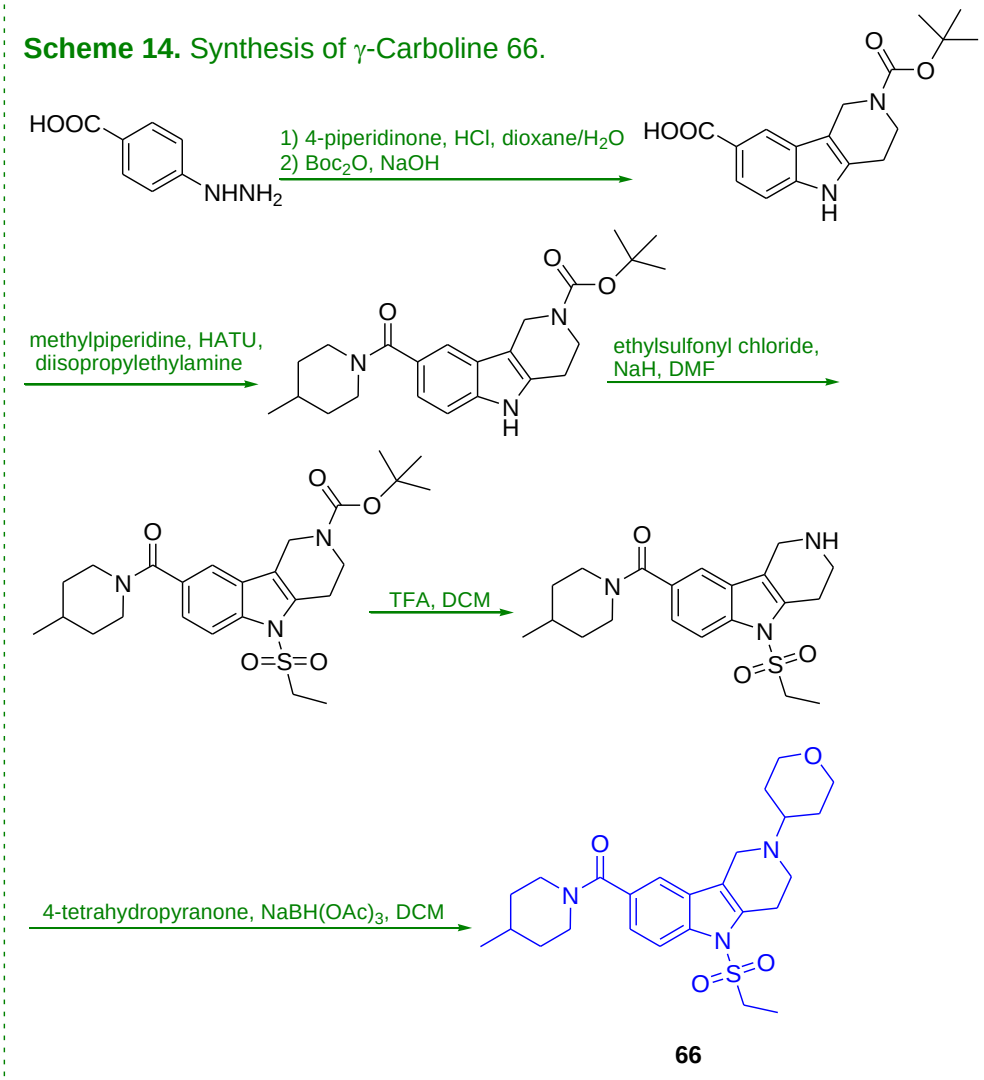


Figure 14. γ-Carboline derivatives.

Scheme 14. Synthesis of γ-Carboline 66.



7.13. Heteroarylpyridine, Heteroarylpyrimidine

The heteroaryl-4-oxopyridine/pyrimidine derivatives have been described.[15] In this family, the core structure of molecules defined the type of activity to be seen, while the substituents around the core structure were used to modulate the potency. Compound **67** (Figure 15, Scheme 15), a representative analog of the heteroarylpyridine series, displayed high affinity and selectivity for CB_2 receptor (hCB_2 K_i = 11.4 nM, hCB_1 K_i = 4568 nM). This compound was found to act as partial agonist (Emax = 14%).

In this study, further CB_2 ligands were synthesized by replacing the pyrazolo ring with different heterocycles that were found to be potent CB_2 receptor ligands. Replacement of the pyrazolo ring of the parent nucleus with differently substituted 5-membered heterocycles allowed further SAR studies. The isoxazolopyridines, such as compound **68** (Figure 15), behaved as full agonists and the thienopyridines (for example **69**, Figure 15) acted as inverse agonists. The inverse agonists thieno[2,3-*b*]pyridine derivatives showed high affinity at the CB receptors (hCB_2 K_i values from 1.12 to 12.3 nM) with an apparent decrease in selectivity. The calculation of log P indicated the absence of a significant correlation between the lipophilicity and biological activities within this series.

A major focus on the optimization effort was to increase selectivity of the previous series of heteroarylpyridine/pyrimidine derivatives. The 7-oxopyrazolo[1,5-*a*]pyrimidine-6-carboxamides, structural isomers of the previously pyrazolo[3,4-*b*]pyridines were synthesized, exemplified by structures such as **70 (**hCB_2 K_i = 2.5 nM, hCB_1 K_i > 10000 nM), and **71** (hCB_2 K_i = 4.9 nM, hCB_1 K_i > 10000 nM) (Figure 15), which showed stimulatory effects on forskolin-induced cAMP production acting as inverse agonists.[16]

Preparation of trans-4,7-dihydro-1,3-dimethyl-N-(4-methylcyclohexyl)-4-oxo-7-pentyl-1H-pyrazolo[3,4-b]pyridine-5-carboxamide (67).[16] The synthetic route to obtain the target compound 67 is outlined in Scheme 15. Replacement of the ethoxy group of diethyl ethoxymethylenemalonate

by the 5-amino function of the pyrazole afforded the intermediate diethyl 5-pyrazolylaminomethylenemalonate, which was cyclized giving the ethyl pyrazolo[3,4-*b*]pyridine-5-carboxylate. Hydrolysis yielded the corresponding 5-carboxylic acid intermediate which by coupling reaction afforded the amide (The separation of *cis* and *trans* isomers was obtained by flash chromatography on silica gel). Alkylation at N-7 in the presence of potassium carbonate with pentybromide was performed to give the target compound 67.

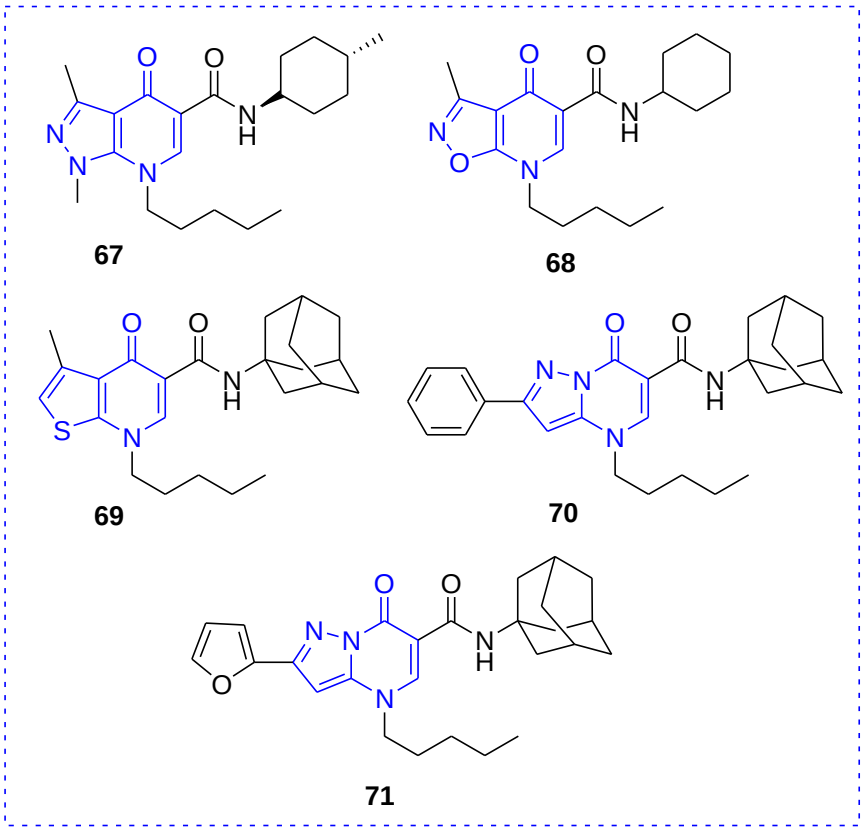
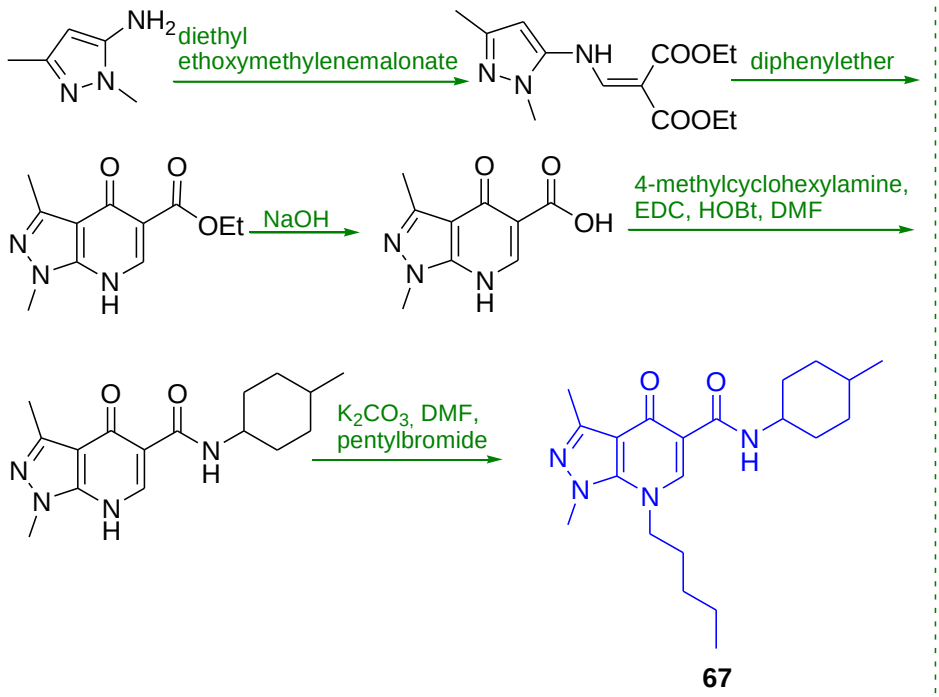


Figure 15. Heteroarylpyridone, Heteroarylpyrimidone derivatives.

Scheme 15. Synthesis of pyrazolopyridine 67.

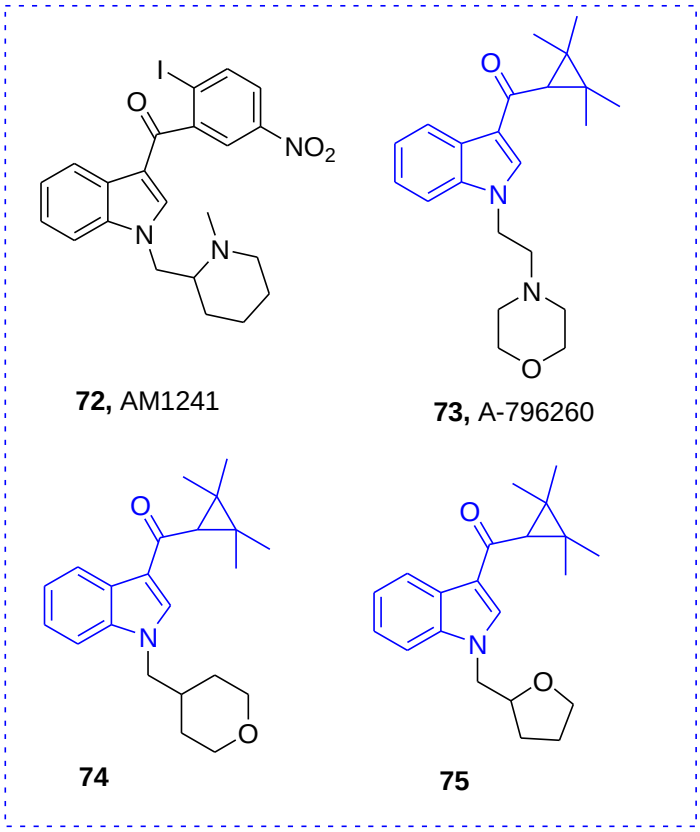


7.14. Indol-3-ylcycloalkyl ketone

Huffman and co-workers have widely investigated indole cannabinoid ligands and were the first to illustrate that N-1 alkyl side chains are tolerated in place of the aminoalkyl side chains that were previously thought to be necessary for activity at the cannabinoid receptors.[282,283] They also found that the size of the N-1 alkyl side chain had a significant impact on selectivity toward CB_2 receptors. Makriyannis's group also discovered the aminoalkylindoles, including the CB_2 selective ligand **72** (AM1241, Figure 16). Compound **72** was efficacious in a range of preclinical inflammatory and neuropathic pain models.[234,233] While many of the indole derivatives have commonly favored 3-naphthoyl or 3-aroyl groups, a diverse chemical series comprising a tetramethyl cycloalkyl ketone at this position (compound **73**, A-796260, Figure 16, Scheme 16) was discovered.[284] Compound **73** was found to possess receptor affinity and selectivity, having a CB_2 K_i of 4.6 nM versus 945 nM at CB_1 receptor. It has potent analgesic and anti-inflammatory actions in models of neuropathic pain without producing cannabis-like side effects.[285] The

tetrahydropyranylmethyl analogue **74** (Figure 16), one of the most potent CB_2 agonists in this series, did not exhibit good selectivity for the CB_2 receptor versus the CB_1 receptor (CB_2 K_i = 0.2 nM, CB_1 K_i = 12 nM).[284] A stereochemical effect was noted on binding selectivity with the tetrahydrofuranylmethyl **75** (Figure 16), the *R*-enantiomer exhibited more selectivity for the CB_2 receptor than the *S*-enantiomer.

Preparation of (2,2,3,3-tetramethylcyclopropyl)(1-(2-morpholinoethyl)-1H-indol-3-yl)methanone (73).[284] As depicted in Scheme 16, the unsubstituted indole core was acylated with 2,2,3,3-tetramethylcyclopropanecarbonyl using ethylmagnesium bromide and zinc(II) chloride ($ZnCl_2$). The C-3 acylated product then underwent N-alkylation with 2-morpholin-4- ylethyl methanesulfonate in DMF by means of sodium hydride.



72, AM1241

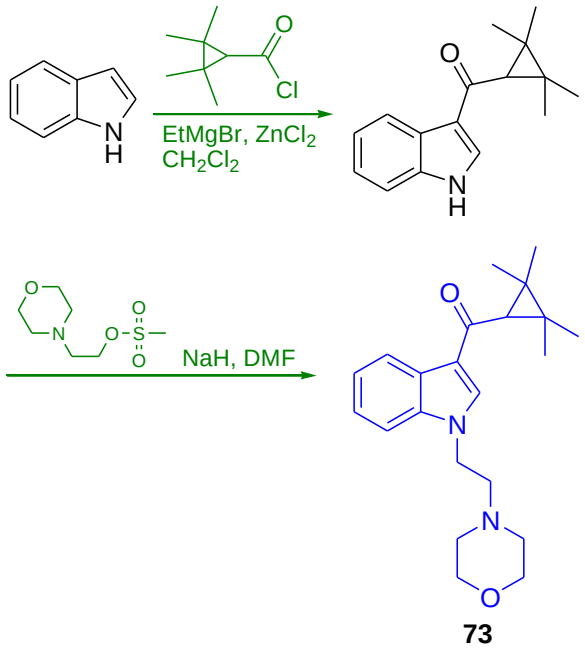
73, A-796260

74

75

Figure 16. Indol-3-ylcycloalkyl ketone derivatives.

Scheme 16. Synthesis of Indol-3-ketone 73.



73

7.15. Oxazinoquinoline

7-Oxo-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamide chemotype is reported as a novel cannabinoid ligand possessing high CB_2 receptor affinity.[14] This scaffold was designed as a hybrid chemical structure that incorporated the structural features of known cannabinoid ligands. The design of the new oxazinoquinolines was based on the quinolone compounds and the cannabimimetic indole derivative WIN55,212-2.[286]

The N-cycloheptyl-3,7-dihydro-7-oxo-3-phenyl-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxamide (**76**, Figure 17) exhibited high affinity and selectivity for the CB_2 receptor (hCB_2 K_i = 0.8 nM, hCB_1 K_i = 310 nM). Compound **77** (MT178, Figure 17, Scheme 17), in which a pyrrolidine moiety was introduced in position 10, showed high affinity and selectivity at the CB_2 receptor (hCB_2 K_i = 8.1 nM, SI > 1231). MT178 produced a robust analgesia in inflammatory and chronic pain models via CB_2 receptors without CB_1-mediated central side effects.[209] The SAR studies were extended by replacement of the C-3 phenyl ring with a series of aliphatic moieties. The effect of a chiral center

on the biological activity was also investigated, and it was found that the *R*-enantiomers exhibited greater affinity at the CB_2 receptor than the *S*-enantiomers. Compound (*R*)-**78** (Figure 17), the enantiopure derivative bearing an isobutyl moiety at C-3, was found to bind to the CB_2 receptor with high affinity and excellent selectivity (hCB_2 K_i = 9.2 nM, SI > 1082). In 3,5- cyclic adenosine monophosphate assays, the novel series behaved as full agonists, exhibiting functional activity at the human CB_2 receptor.[14]

Preparation of N-Adamant-1-yl-3,7-dihydro-3-ethyl-7-oxo-10-pyrrolidin-1-yl-2H-[1,4]oxazino [2,3,4-ij]quinoline-6-carboxamide (77).[14] The target oxazinoquinoline 77 was prepared following the synthetic route depicted in Scheme 17. Ethyl 3-(2,3,4-trifluorophenyl)-3-oxopropanoate was prepared from the corresponding benzoic acids by reaction with carbonyldiimidazole (CDI) in THF to afford the imidazolide, followed by condensation with the magnesium salt of monoethyl malonate. Subsequent condensation with triethyl orthoformate in refluxing acetic anhydride produced the ethyl 2-(ethoxymethylene)propionate. This intermediate was reacted with racemic 2-aminobutanol in an addition−elimination sequence to afford the ethyl trifluorobenzoyl acrylate. Cyclization using potassium carbonate in dimethylformamide (DMF) at high temperature and then saponification afforded the carboxylic acid that was coupled with 1-adamantanamine to yield the amide derivative. The target compound 77 was obtained by displacement of the fluorine substituent by treating the amide with nucleophile pyrrolidine in alkaline conditions.

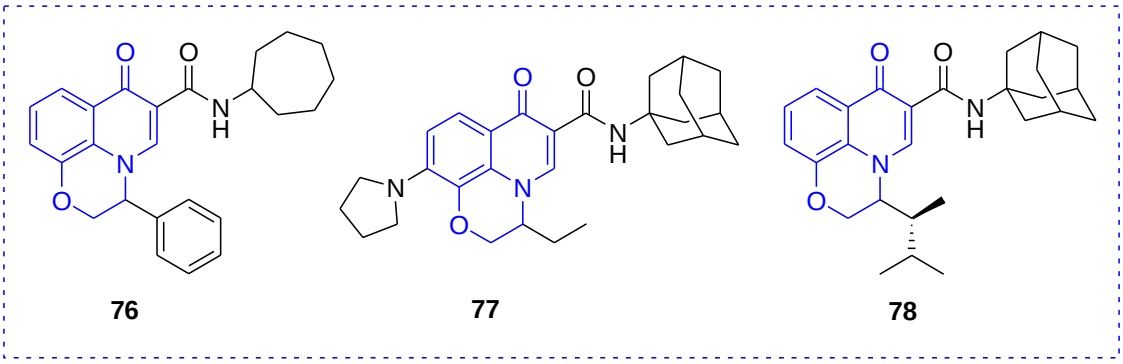
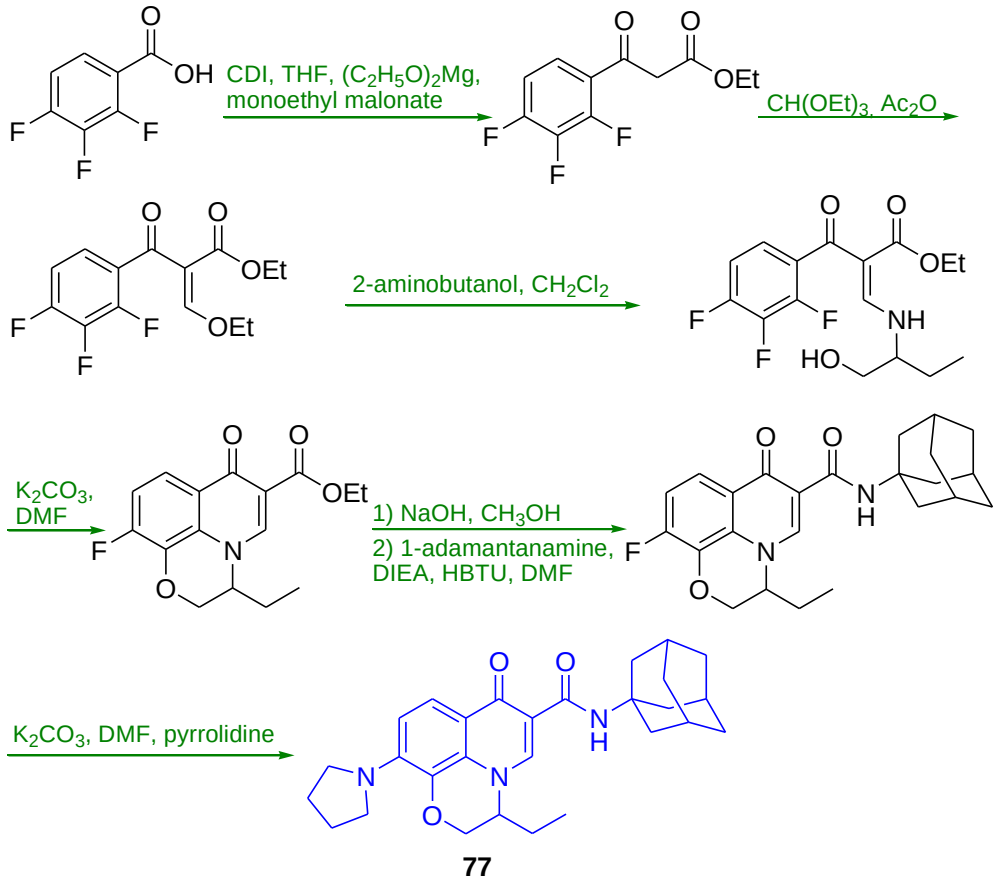


Figure 17. Oxazinoquinoline derivatives.

Scheme 17. Synthesis of Oxazinoquinoline 77.



77

7.16. 1,4-Diazepane

From a high-throughput screen to identify CB_2 agonists, the 1,4-diazepane hit compound **79** (EC_{50} = 136 nM, Emax 67%) was identified (Figure 18, Scheme18).[287] It was revealed to be a partial agonist and did not activate the CB_1 receptor up to 20 µM, but suffered from low metabolic stability. The optimization effort on the diazepane scaffold, achieved by decreasing lipophilicicty, generated molecules which improved in vitro clearance as compared to the hit **79**. The pharmacokinetic profile of compound **80** (EC_{50} = 67 nM) bearing an isoxazole (Figure 18), more polar substituent in concomitance with the tetrahydropyran fragment in various in vitro assays were more favorable than those of the phenyl urea **79**. Expanding upon the tetrahydropyran substituent, analog **81** (Figure 18) was synthesized and greatly enhanced CB_2 potency (EC_{50} = 1 nM), selectivity and improved the aqueous solubility.[288]

Preparation of 4-(3-cyclopentylpropanoyl)-N-(4-butylphenyl)-1,4-diazepane-1-carboxamide (79).[287] The commercially available *tert*-butyl homopiperazine-1-carboxylate was coupled with 3-cyclopentylpropanoic acid employing either amide coupling conditions or via acid chloride, followed by BOC deprotection using HCl. The urea coupling was performed using the 1-butyl-4-isocyanatobenzene which was generated in situ using triphosgene. (Scheme 18)

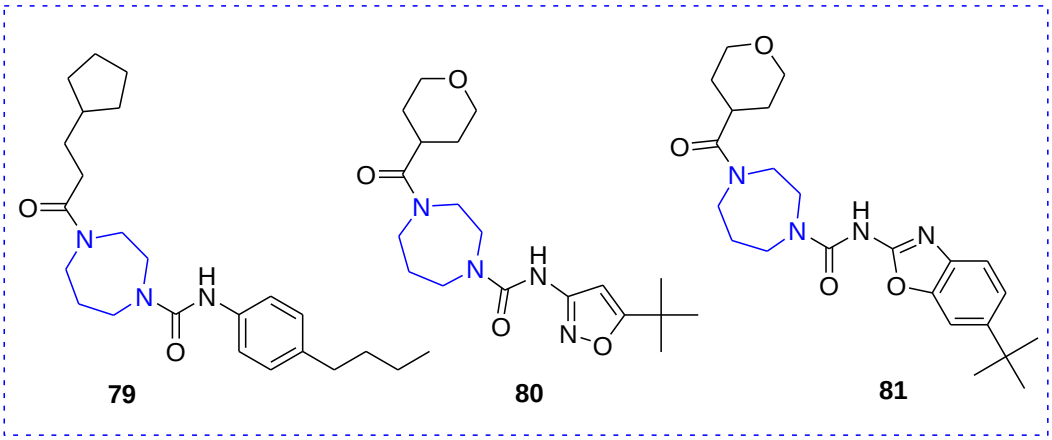
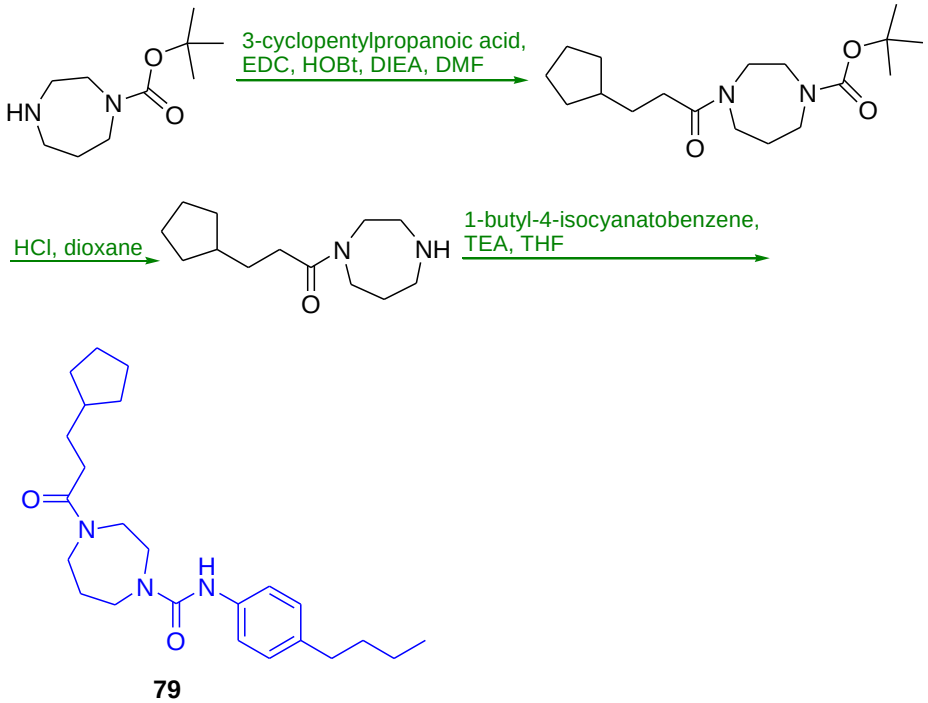


Figure 18. 1,4-Diazepane derivatives.

Scheme 18. Synthesis of 1,4-diazepane-1-carboxamide 79.



7.17. (*S*)Proline, (*S*)Pieperidine

Boehringer Ingelheim Pharmaceuticals analyzed the diazepane and the β-sulfonylacetamide compounds, through computational and experimental approaches in search of the bioactive conformation common to these chemical series. Additionally, computer-aided drug design (CADD) strategies were adopted toward the identification of new cores to replace the diazepane scaffold. The parallels in SAR between the two series led to the synthesis of the proline-, and piperidine-based series as CB_2 full agonists (compounds **82** and **83**, respectively, Figure 19). Analogs containing the new proline scaffold exhibited picomolar CB_2 activity, and high selectivity over CB_1 receptors (**82:** CB_2 EC_{50} = 0.07 nM, CB_1 EC_{50} = 53 nM; **83:** CB_2 EC_{50} = 0.09 nM, CB_1 EC_{50} = 250 nM).[289] Further optimization within the series led to oxo-proline compound **84** (Figure 19, Scheme 19), which showed a favorable pharmacokinetic profile of which the antinociceptive effect of this CB_2 selective agonist in a diabetic neuropathy model was evaluated.[290] The correlated CB_2 agonist based on a (*S*)-piperidine is represented by compound **85** (Figure 19) that displayed selectivity over CB_1 receptor and suitable drug like properties (hCB_2 EC_{50} = 10 nM, hCB_1 EC_{50} > 20000 nM).[291] In rats, compound **85** demonstrated a favorable pharmacokinetic profile and efficacy in a Streptozotocin-induced diabetic neuropathy model, with full reversal of mechanical hyperalgesia.

Preparation of (S)-N-(3-tert-butylisoxazol-5-yl)-1-(4-(trifluoromethyl)phenyl)-5-oxopyrrolidine-2-carboxamide (84).[290] As shown in Scheme 19, (*L*)-pyroglutamic acid and 4-trifluoromethyl phenylboronic acid are converted in the presence of catalyst di-μ-hydroxo-bis[(*N*,*N*,*N*′.*N*′-tetramethylethylenediamine)copper(II)] chloride (CuTMEDA) to the N-aryl substituted intermediate. Typical peptide coupling conditions were applied to introduce 3-*tert*-butylisoxazol-5-amine to provide the final compound.

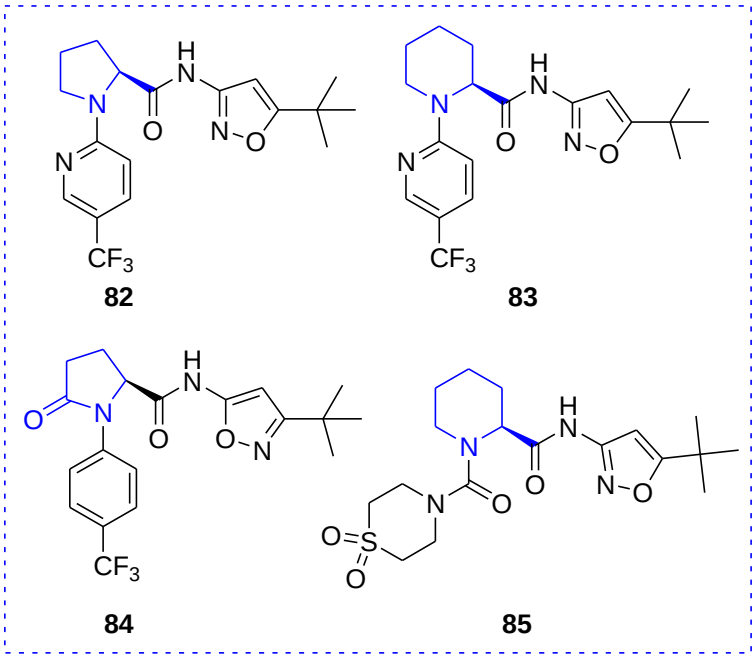
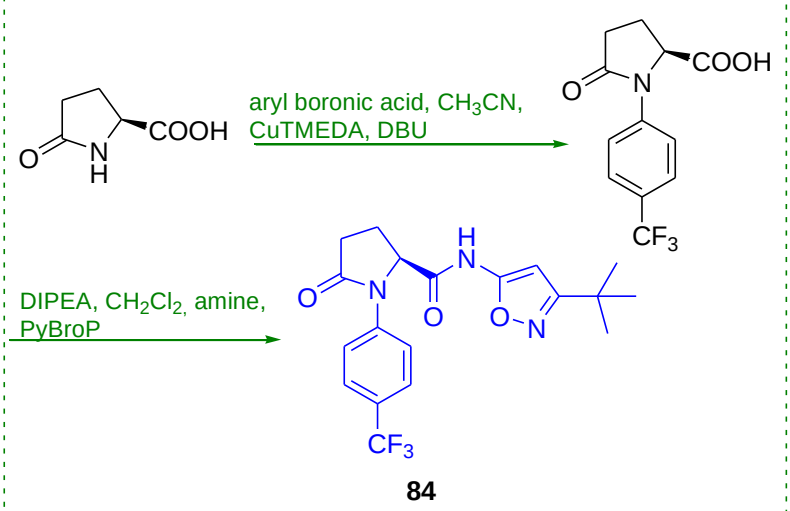


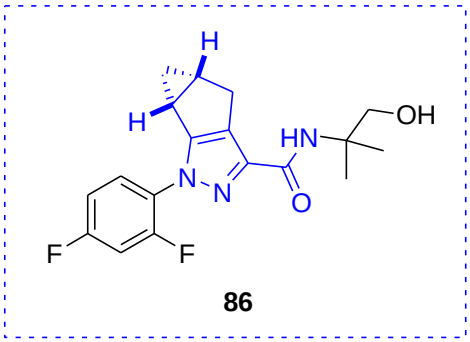
Figure 19. Proline, Piperidine derivatives.

Scheme 19. Synthesis of Proline 84 .



7.18. Pyrazole-3-carboxamide

Arena Pharmaceuticals designed tricyclo pyrazole 3- carboxamides that displayed agonism at both CB receptors, and also suffered from poor in vitro and in vivo properties. Starting from this series, investigation on N-1 pyrazole substituents, through introduction of a variety of alkyl and aryl moieties, led to improvements in CB_2 potency and microsomal stability. SAR studies led to the finding of the favored compound (*R*,*R*)-**86** (Figure 20) which had good activity at CB_2 (hEC_{50} = 3.7 nM) and was selective against CB_1 (SI > 8100). This compound exhibited an overall favorable ADME, and was efficacious at reversing inflammatory pain after oral administration in the rat.[292]



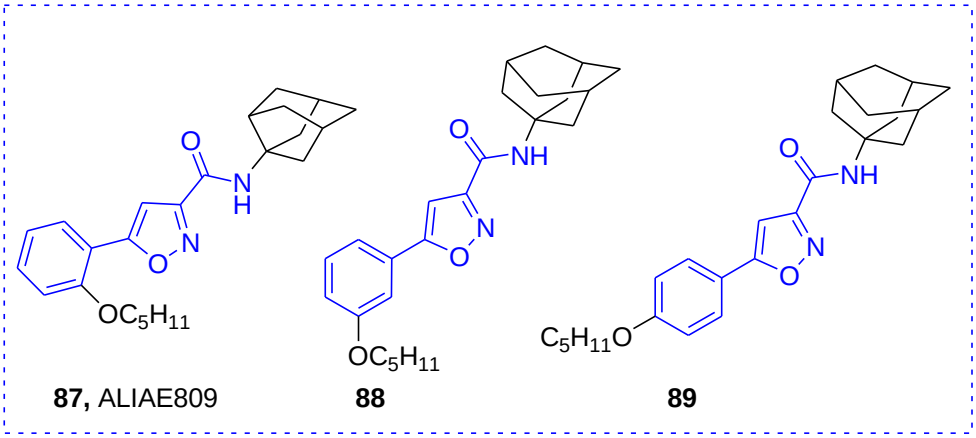
86

Figure 20. Pyrazole-3-carboxamide derivative.

7.19. 3-Carboxamido-5-aryl-isoxazole

The 3-carboxamido-5-aryl-isoxazole is reported as a good scaffold to design selective CB_2 agonists and FAAH inhibitors. In this family, the substituents on the heterocycle define the activity on CB_2 receptor or on FAAH enzyme. The synthesis and SARs of isoxzole derivatives show that the presence and position of the alkoxy chain deeply influence the biological activity of the molecule. Compounds such as **87** (ALIAE809, Figure 21) with a 2-substituted phenyl group at position 5 and a bulky aliphatic group on the carboxamide function have a K_i value of of 9.0 nM on hCB_2 receptors. Some of the isoxazole compounds have shown, in vivo, anti-inflammatory activities in a colitis mouse model. [293] When the alkoxy chain is missing, or is at positions 3 or 4 (compounds **88**

and **89,** Figure 21), the molecules show potent FAAH inhibitor activity. Compounds **88** and **89** inhibit the development of dextran sulfate sodium (DSS)-induced acute colitis in mice.[294]



87, ALIAE809 **88** **89**

Figure 21. 3-Carboxamido-5-aryl-isoxazole

7.20. 1,3,5-Triazine

2,4,6-Trisubstituted 1,3,5-triazines were identified as potent CB_2 agonists by 3D ligand-based virtual screening. Whitin this series, several CB_1 receptor antagonists/ inverse agonists and CB_2 receptor agonists, of which *N*-cyclopentyl-4-ethoxy-6-(4-methylpiperidin-1-yl)-1,3,5-triazin-2-amine (**90**) (Figure 22, Scheme 20) was selected for further development. SAR study of the analogs exposed that replacing of the cyclopentyl substituent by a bulkier *N*-adamantan-1-yl chain was useful, such as in compound **91** of this series. It possessed the best potency and selectivity with only a weak CB_1 agonist activity (CB_2 EC_{50} = 3.2 nM).[295] Subsequentely, an additional series of more polar analogues, which were found to possess high CB_2 agonist activity with enhanced water solubility, were described. The most potent compounds in the series were N-(adamantan-1-yl)-4-ethoxy-6-(4-(2-fluoroethyl)piperazin-1-yl)-1,3,5-triazin-2-amine (**92**, Figure 22) and N-(adamantan-1-yl)-4-ethoxy-6-(4-methylpiperazin-1- yl)-1,3,5-triazin-2-amine (**93**, Figure 22) with EC_{50} value of 0.60 and 1.6 nM, respectively.[296] Compound **93** induced cell viability decreased in prostate and leukemia cell lines, and diminished proliferation of C8161 melanoma cells.

Preparation of N-cyclopentyl-4-ethoxy-6-(4-methylpiperidin-1-yl)-1,3,5-triazin-2-amine (87).[295] As shown in Scheme 20, displacement of chlorine atoms in cyanuric chloride with different substituents in basic conditions and tetrahydrofuran as solvent led to the preparation of target compound.

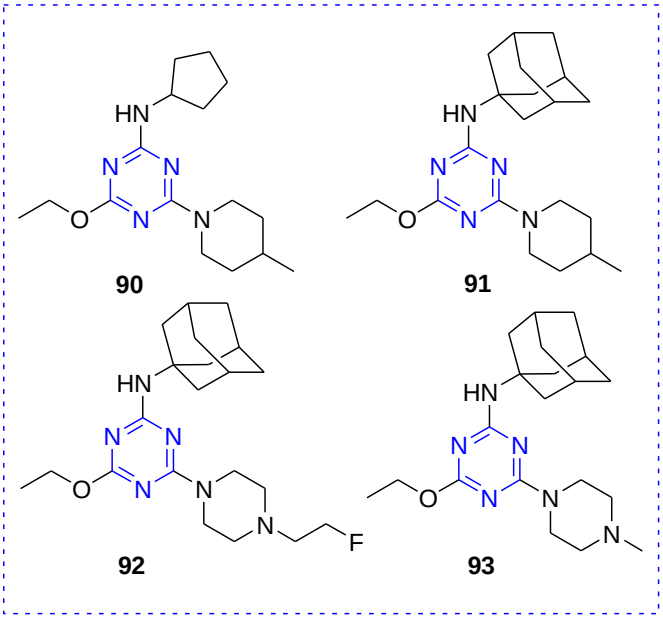
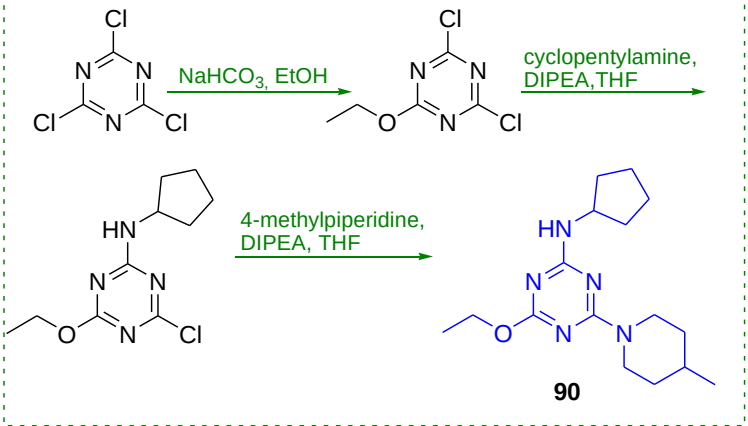


Figure 22. Triazine derivatives.

Scheme 20. Synthesis of Triazine 90.



7.21. Analogs of HU-308

The discovery of CB_2-selective ligand HU-308 (hCB_2 K_i = 22 nM, rCB_1 K_i > 10000)[231] (**94**, Figure 23) supported the evidence of analgesic effects induced by the selective activation of CB_2 receptors .[232] More recently, a structurally similar compound labeled HU-910 (**95**, Figure 23) is reported as potent CB_2 agonist (hCB_2 K_i = 6 nM, hCB_1 K_i = 1400 nM), which may exert protective effects in several diseases related to inflammation and tissue injury.[297] The enantiomer of HU-308 labelled as HU-433 (**96**, Figure 23) was also synthesized with higher potency than HU-308 in mouse models such as the release of ovariectomy-induced bone loss and ear inflammation. In addition, HU-433 has a lower affinity in comparison with HU-308 showing an inverse relationship between binding affinity and biological potency of the two enantiomers with two different binding conformations responsible for the affinity difference.[298]

Preparation of ((1R,4R,5S)-4-(2,6-dimethoxy-4-(2-methyloctan-2-yl)phenyl)-6,6 dimethylbicyclo [3.1.1]hept-2-en-2-yl)methanol (96). As shown in Scheme 21, (1*R*)-myrtenol was treated with pivaloyl chloride to give mytenyl pivalate which was trasformed to the 4-oxo-myrtenyl pivalate by means of chromium trioxide and *tert*-butyl hydroperoxide. The reduction with $NaBH_4$ yielded the 4-hydroxy-6,6-dimethylbicyclo intermediate which was reacted with 5-(1,1-dimethylheptyl)resorcinol in presence of *p*-toluenesulfonic acid to give 2-(3-myrtenyl pivalate)-5-(1,1-dimethylheptyl) resorcinol. O-alkylation with methyl iodides and successive reduction reaction with lithium aluminium hydride gave the desired product.

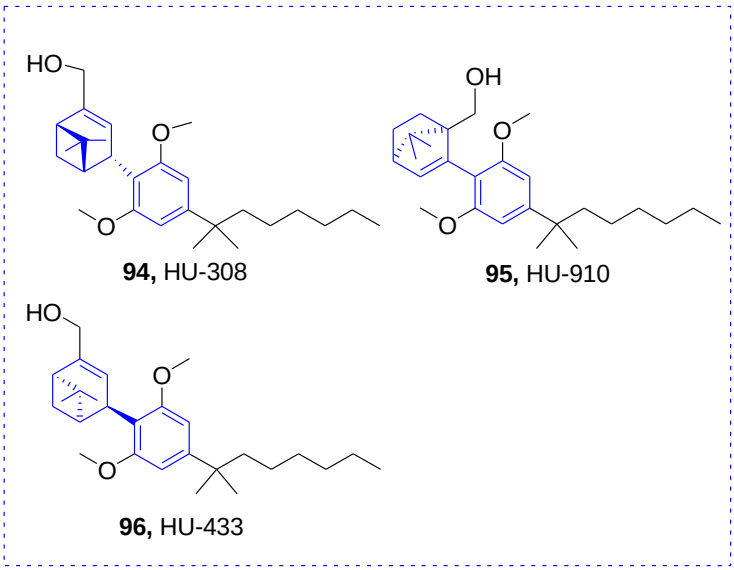
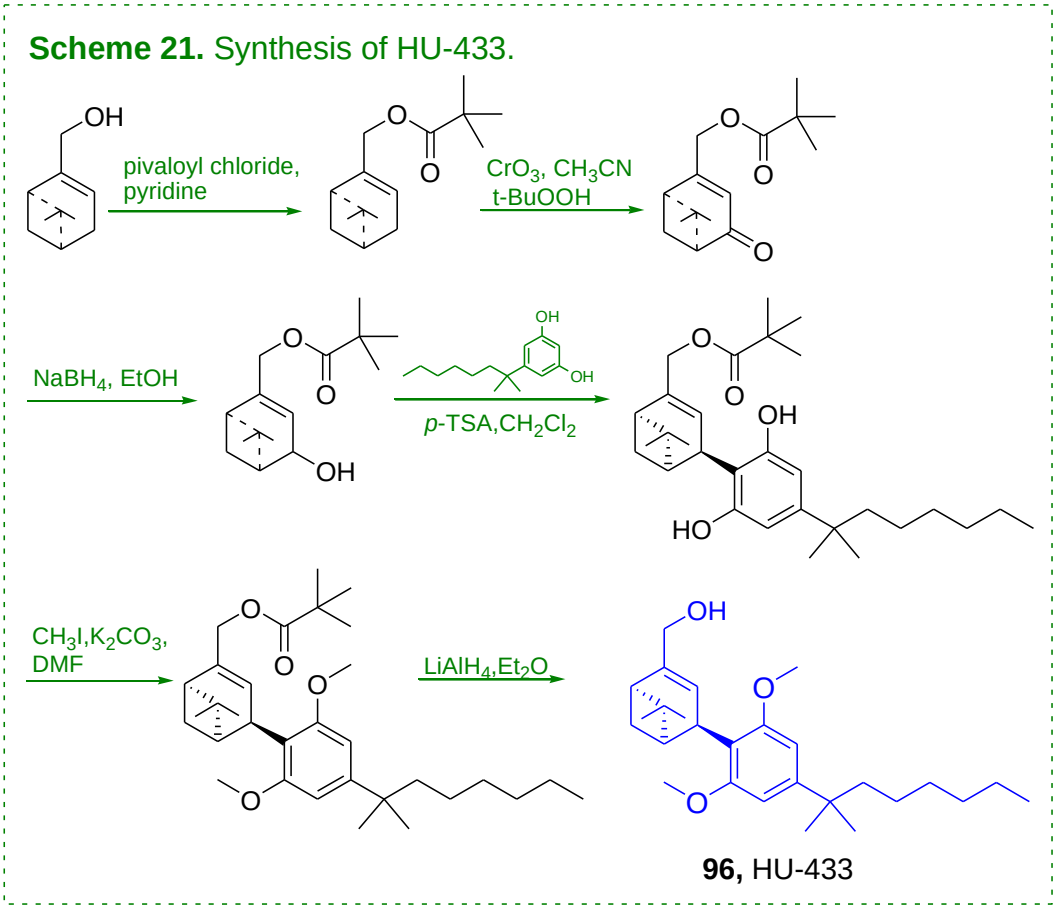


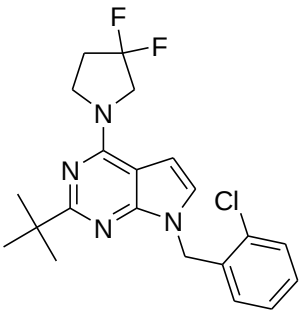
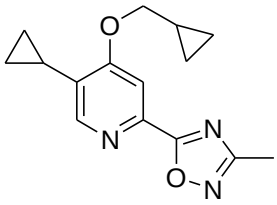
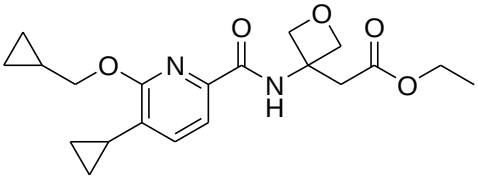
Figure 23. Analogs of HU-308.

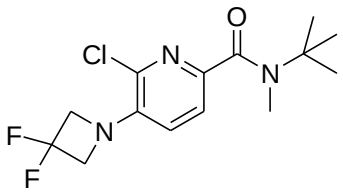
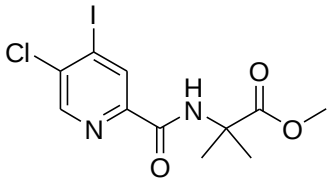
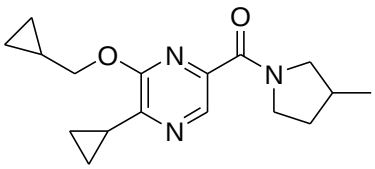
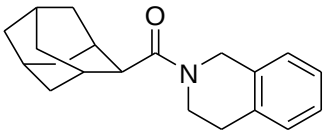
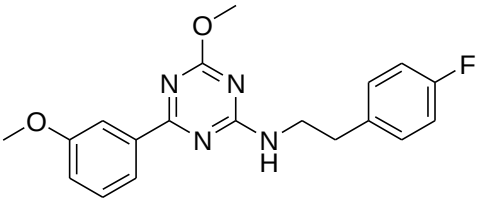


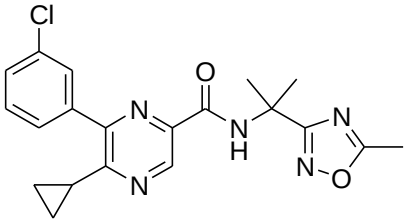
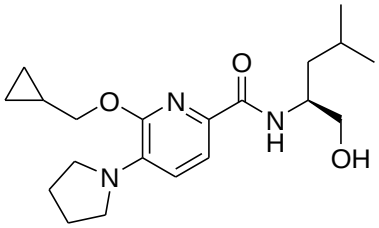
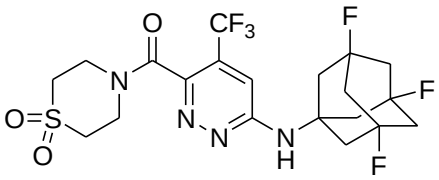
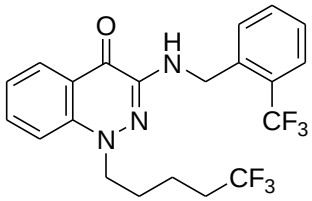
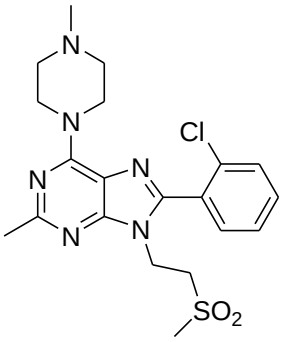
8. PATENTS

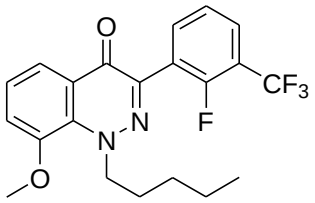
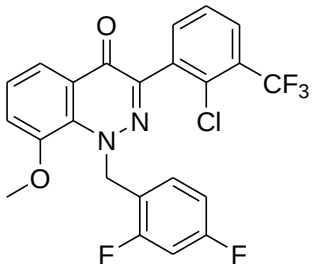
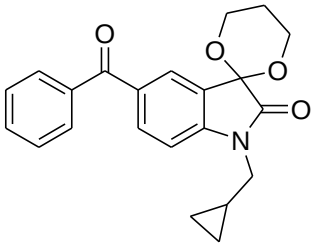
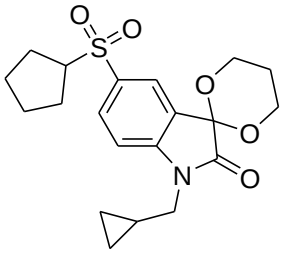
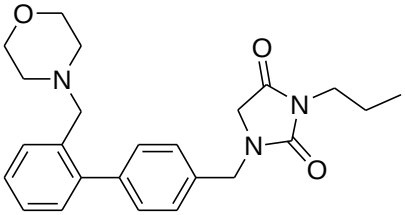
This section reports the patent literature from 2008 until today on novel molecules targeting CB_2 receptor that are currently studied in clinical trials or are candidates for future clinical evaluation in the management of diseases. The purpose is to show selected scenarios of chemical structures adopted in this field of research (Table 3).

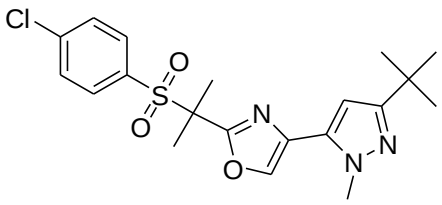
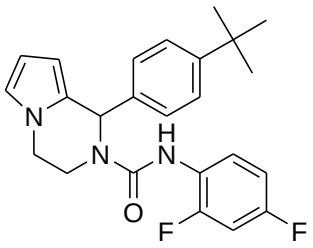
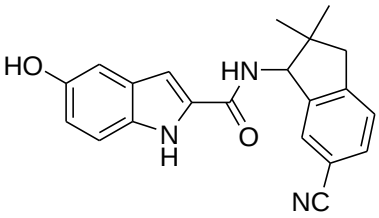
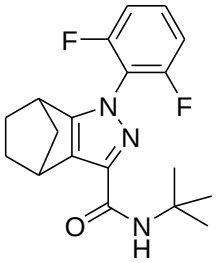
Table 3. Scaffold-based CB_2 agonists in patent literature.

Scaffold	*Representative Compound*	*Biological Activity*	*Company name*
Pyrrolo[2,3*d*]pyrimidine[a]		EC_{50} = 10 nM	*Hoffmann-La Roche*
Pyridine[b]		EC_{50} = 26 nM	*Hoffmann-La Roche*
Pyridine[c]		K_i < 1 µM	*Hoffmann-La Roche*

Pyridine[d]		EC_{50} = 5 nM	*Hoffmann-La Roche*
Pyridine[e]		EC_{50} = 36 nM	*Hoffmann-La Roche*
Pyrazinecarboxamide[f]		EC_{50} = 36 nM	*Hoffmann-La Roche*
Adamantane[g]		EC_{50} = 7 nM	*Hoffmann-La Roche*
1,3,5-Triazine-2-amine[h]		IC_{50}< 500 nM	*Sanofi*

Pyrazine[i]		IC_{50} < 3 µM	*Hoffmann-La Roche*
Pyridine[j]		EC_{50} = 3 nM	*Hoffmann-La Roche*
Pyridazin-3-amine[k]		No data	*Astellas Pharma*
3-aminocinnolin-4(1*H*)-one[l]		IC_{50}< 3 nM	*Sanofi-Aventis*
Purine[m]		No data	*Eli Lilly and Company*

1-alkylcinnolin-4(1*H*)-one[n]		IC_{50} < 500 nM	*Sanofi-Aventis*
1-benzylcinnolin-4(1*H*)-one[o]		No data	*Sanofi-Aventis*
Oxindole[p]		IC_{50} ranging from 0.062 to 20 µM	*Wyeth LLC*
Oxindole[q]		IC_{50} = 54 nM	*Wyeth LLC*
1-(biphenyl-4-ylmethyl)imidazolidine-2,4-dione[r]		EC_{50} < 0.4 nM	*N.V. Organon*

Sulfone[s]		No data	*Boehringer Ingelheim International G.m.b.H.*
Diazepine[t]		EC_{50} < 1 µM	*Kalypsys, Inc.*
Indaylindole[u]		EC_{50} = 2.4 nM	*Bristol-Myers Squibb Company*
Methanoindazole[v]		No data	*Glenmark Pharmaceuticals, S.A.*

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9. POTENTIAL THERAPEUTIC APPLICATIONS OF CB_2 AGONISTS

The therapeutic value of CB_2 agonists has been well investigated for several years and medicinal chemistry research has identified a principal role of these compounds in the pharmacological treatment of pain and inflammation.[11,13,105] Clinical applications of novel compounds have been complicated by several problems such as the presence of CB_2 receptors, that is very low in healthy but which significantly increases in the disease. It is, therefore, difficult to compare the effect of CB_2 agonists in animals with injury, illness or inflammatory conditions. Another problem is the CB_2 selectivity because, in the past, the older agonists at high doses were able to bind CB_1 receptors that are much more abundant compared to CB_2 receptors. As a consequence, an analysis of the CB_2 effects could be confused with CB_1 functional response, suggesting a possible explanation for the failure of the clinical trial. Moreover, it cannot be excluded that CB_2 agonists might interact with other membrane receptors involved in different diseases, like cancer or chronic pain.

In spite of all CB_2 agonists have progressed to various clinical trials by several pharmaceutical companies including PRS-211375 (Figure 24) from Pharmos Corporation; GW842166 (compound 1, Figure 3) and GW833972A (Figure 24) from GlaxoSmithKline; GRC10693 (Figure 24) from Glenmark Pharmaceuticals; JBT101 (Figure 24) from JB Therapeutics, Inc.; KHK6188 (structure not disclosed) from Kyowa Hakko Kirin; ABT521 (structure not disclosed) from Abbott Laboratories; LY2828360 (Figure 24) from Eli Lilly and Company; AZD1940 (Figure 24) from

Astra Zeneca; CR701 (structure not disclosed) from Cara Therapeutics, Inc.; APD 371 (structure not disclosed) from Arena Pharmaceuticals, Inc. and S-777469 (compound 7, Figure 3) from Shionogi & Company (Table 4).

A CB_2 selective cannabinoid library from Pharmos Corporation has shown promise in animal models for autoimmune inflammatory disorders, such as MS and inflammatory bowel disease. These compounds also have a good efficacy in animal models of neuropathic, inflammatory and chronic nociceptive pain. In selected preclinical models, these ligands have an analgesic activity equivalent better than Gabapentin. These CB_2-selective synthetic compounds belong to nonclassical CBs and display fewer of the undesired psychotropic and cardiovascular side-effects seen with some natural CBs. These compounds have been used in preclinical testing for neuropathic pain and the specific pharmacological properties have highlighted a wide range of beneficial therapeutic indications in common which could be useful, for example, as analgesic, neuroprotective, immunomodulatory and anti-inflammatory agents. As a consequence, CB_2 agonists could be important treatments in humans for analgesic activities and autoimmune diseases, such as MS and RA. Cannabinor (PRS-211375) was the first lead candidate to emerge from the chemical Pharmos technology used to experimental models of pain even if it failed in the inhibition of capsaicin-induced pain in Phase IIa trials.[299] The effects of cannabinor were investigated in rats with partial urethral obstruction, treated daily for 14 days, and showed that the ability to empty the bladder was preserved and contraction frequency was low, if compared to those in controls, with a significant reduction in bladder weight.[300] Detrusor preparations from cannabinor treated rats showed a higher response to nerve stimulation than those from controls, suggesting that cannabinor may be a novel principle to enable improved bladder function after partial urethral obstruction.[301] An additional study in Phase IIa trials was organized to evaluate CB_2 agonists used intravenously in a third molar dental extraction. Unfortunately, the results of this trial were confounding because this compound in the lowest dose showed significant effects, whereas higher doses failed to achieve significance.[12]

GlaxoSmithKline completed clinical trials by using selective CB_2 receptor agonists for dental pain, third molar tooth extraction and OA pain. Selective investigations of the structure activity relationships suggested an identification of several potent and selective agonists. These compounds showed antihyperalgesic effects in models of inflammatory and neuropathic pain, indicating a potential role in the treatment of chronic pain. A clinical program to evaluate the analgesic efficacy of GW842166 in acute dental pain failed to demonstrate either pharmacological and/or statistical significance.[302] In particular, the phase IIa study was conducted as a multi-centre study to evaluate the analgesic efficacy of pre-emptive doses of GW842166 following dental surgery after 3rd molar tooth extraction.[303] Safety and tolerability were evaluated by adverse event monitoring, cardiovascular assessments and clinical laboratory tests such as haematology, clinical chemistry and urinalysis. Ibuprofen was significantly more effective than placebo across all endpoints and single doses of GW842166 (100 and 800 mg) failed to demonstrate clinically analgesia in the setting of acute dental pain. Moreover, GW833972A was able to inhibit Fos expression and reduces the number of trigeminal brainstem neurones activated by electrical tooth pulp stimulation caudally, but not rostrally, in animal models, suggesting an analgesic efficacy in dental pain. In addition, this compound inhibited capsaicin-induced depolarization in human and guinea-pig and prostaglandin E_2 (PGE_2) and hypertonic saline-induced depolarization of the guinea-pig isolated vagus nerve.[161] Of interest, GW 833972A decreased citric acid-induced cough, but not plasma extravasation, in the guinea-pig, this effect being blocked by a CB_2 antagonist. This study highlights the role of CB_2 receptors in the modulation of sensory nerve activity elicited both by the exogenous ligands, capsaicin and hypertonic saline, but also by endogenous modulators, such as PGE_2 and low pH stimuli, suggesting that these receptors could be an interesting target for the treatment of chronic cough.[161]

Glenmark Pharmaceuticals reported successful completion of phase I trial in Europe with their candidate, GRC10693 (Tedalinab), demonstrating both safety and efficacy.[304] GRC10693, a potent

and selective CB_2 agonist, is a drug developed for the treatment of OA and neuropathic pain. This compound has shown promise in the treatment of OA in phase I clinical trials. GRC 10693 was also investigated in neuropathic pain as primary indication in phase IIb clinical trials following oral route of administration.[304]

JB Therapeutics is a clinical stage biopharmaceutical company which has developed the first new class of analgesic agents in two decades. Their lead compound, JBT-101, is a potent CB agonist with demonstrated efficacy in several animal models of pain and inflammation. It has also completed a Phase II clinical study in chronic refractory neuropathic pain. JBT-101 has been developed as a potential disease modifying OA treatment. It has been demonstrated that the endocannabinoid system is up-regulated in pathologic fibrosis and that the modulation of CB receptors might limit the progression of uncontrolled fibrogenesis associated to scleroderma.[305] JBT-101 also represents a platform for combination with other anti-inflammatory and analgesic agents as well as various drug delivery strategies including ointment and gels, topical patches, and timed release formulations.

A phase II Study of KHK6188 from Kyowa Hakko Kirin, a placebo-controlled trial, double blind in postherpetic neuralgia was carried out to evaluate the efficacy and safety of this orally administered compound.[306] It is well reported that CBs could suppress neuropathic nociception in animal models of postherpetic neuralgia, and clinical studies largely affirm that neuropathic pain patients derive benefits from CB treatment.[307]

Abbott Company is investigating, in phase I clinical trials, the CB_2 agonist ABT-521 (possibly A-796260 or a similar compound with indole structure) for pain disorders.[12] Another CB_2 selective ligand A-836339 (Figure 24) was found to possess high hCB_2 affinity with a 425-fold selectivity versus hCB_1 but did not display significant affinity to other GPCRs.[308] However, A-836339 exhibited selective analgesic, anti-inflammatory and anti-hyperalgesic effects even if typical cannabis-like effects appeared at higher doses despite its low binding affinity for CB_1 mediating a

significant decrease in spontaneous motor activity.[309,310] It has been also reported that this compound could be a suitable target for the development of positron emission tomography (PET) radiotracers that could serve as imaging biomarkers in Aβ-induced neuroinflammation.[311] The specificity of the PET signal was confirmed by the use of a specific CB_2 antagonist, AM630. The data collected in this study indicate that Aβ amyloidosis without concomitant tau pathology is sufficient to activate CB_2 receptors that are suitable as an imaging biomarker of neuroinflammation.[82]

Eli Lilly and Company has carried out, in phase II clinical trials, the safety, efficacy and pharmacokinetics of a single daily oral dose of LY2828360 in OA knee pain.[312] It is well reported that OA is a degenerative joint disease associated with articular cartilage degradation. The major clinical outcome of OA is a complex pain state that includes both nociceptive and neuropathic mechanisms. Currently, the therapeutic approaches for OA are limited to controlling disease progression and the analgesic treatment has restricted efficacy. Increasing evidence from preclinical studies supports the interest of the endocannabinoid system as an emerging therapeutic target for OA pain.[105] Indeed, pharmacological studies have shown the anti-nociceptive effects of CBs in different rodent models of OA, and compelling evidence suggests an active participation of these compounds in the disease pathophysiology. The ubiquitous distribution of CB receptors, together with the physiological role of the endocannabinoid system in the regulation of pain, inflammation and even joint function further support the therapeutic interest of CBs for OA.[312]

From AstraZeneca derived a study that investigated the effects of AZD1940, a novel peripherally acting CB agonist, on capsaicin-induced pain and hyperalgesia, as well as on biomarkers of CNS effects.[313] This study was a randomized, double-blind, placebo-controlled, four-sequence, two-period, cross-over study in healthy male volunteers for postoperative pain. A phase II clinical trial showed the effects of two single oral doses of AZD1940 (400 and 800 μg) that were compared with placebo. Pain intensity after intradermal capsaicin injections in the forearm was assessed on a

continuous visual analogue scale. Primary and secondary hyperalgesia induced by application of capsaicin cream on the calf were assessed by measuring heat pain thresholds and the area of mechanical allodynia, respectively. The CNS effects were assessed at baseline and up to 24 h after dosing using a visual analogue mood scale for various feeling. AZD1940 did not significantly attenuate ongoing pain or primary or secondary hyperalgesia compared with placebo. As part of the preclinical evaluation of AZD1940, a microdosing study with PET was conducted to assess brain exposure and the regional distribution of radioactivity within brain that was homogenous and low in comparison to peripheral organ exposure.[314] Treatment with AZD1940 mediated mild CNS effects while dose-dependent adverse events related to both CNS and gastrointestinal were reported. No evidence of analgesic efficacy for a peripherally acting CB agonist in human capsaicin pain model was found.[105]

Cara Therapeutics Inc., a clinical-stage biopharmaceutical company, analyzed CR701 in a preclinical trial in order to treat neuropathic and inflammatory pain, and CR845, that has completed phase II clinical trials for the treatment of moderate-to-severe acute chronic pain. [105,315]

APD371 from Arena Pharmaceutical Inc., an orally available agonist of CB_2 receptors, is a drug candidate intended for the treatment of pain.[105] Currently available CB agonists have been limited in utility by the psychotropic effects associated with the activation of CB_1, but not CB_2 receptors. Several novel, potent, CB_2-selective, lead compounds have been developed to retain the analgesic activity while avoiding the psychotropic side effects. There is a need for non-opioid (non-narcotic), non-steroidal anti-inflammatory drugs (NSAIDs) for novel approaches to acute or chronic pain. Narcotics involve problems of tolerance, addiction and abuse potential, whereas long term use of NSAIDs may be associated with stomach injury, bleeding, death, heart attacks, hypertension, and kidney injury.

Shionogi & Company investigated several novel CB_2 agonists and among them, the compound S-777469 or raclopride as a CB_2 selective agonist for clinical development. This compound is

considered to be a promising candidate for an orally active antipruritic effect since it significantly inhibited histamine-induced peripheral nerve firing in mice, suggesting that S-777469 produces its antipruritic effects by inhibiting itch signal transmission through CB_2 agonism.[280,281] The metabolism and pharmacokinetics of S-777469 were investigated after a single oral administration of [^{14}C]-S-777469 to healthy human subjects and showed a total radioactivity which was rapid and well absorbed in humans.[318]

It is well reported that CB_2 receptors are abundant in splenocytes and leukocytes and responsible for many of the CB immunomodulatory and anti-inflammatory effects. CB_2 selective agonists are very attractive therapeutic agents without CB_1 mediated psychoactive effects because of their predominant peripheral distribution. Quantitative analysis of administered small molecule drugs such as S-777469 in biological tissues must be carried out through use of imaging mass spectrometry in order to understand the mechanisms underlying its therapeutic efficacy or toxicity.[319] Moreover, safety assessments of S-777469 have included adverse event monitoring, vital sign measurements, physical examination measurements, electrocardiograms assessments, and standard clinical laboratory safety tests such as hematology, blood chemistry, and urinalysis. Pharmacokinetic endpoints for each dose level of S-777469 based on the sampling schedule have been evaluated and suggest that this compound is rapidly and well absorbed with bioavailability values ranging from 50 to 70% in rats and dogs. Time required to achieve steady state and serum protein binding was determined for each dose level.[320] Plasma and urine concentrations of S-777469 were determined for single dose or multiple doses in all examined cohorts. In vivo metabolism in rats and dogs showed good qualitative agreement with in vitro metabolism. Therefore, rats and dogs were thought to be appropriate species for non-clinical toxicity studies.[321] These data could be useful for the characterization of the pharmacokinetic properties of S-777469 and the estimation of its pharmacokinetic fate in humans. To assess the potential anti-pruritic and anti-inflammatory efficacy of S-777469 after single-dose administration and twice daily administration in subjects

with mild-to-moderate atopic dermatitis was investigated. The efficacy endpoints primarily included the physician's overall assessment and patient assessment of pruritus and indicated that the CB_2 selective agonists could have significant antipruritic effects.[241]

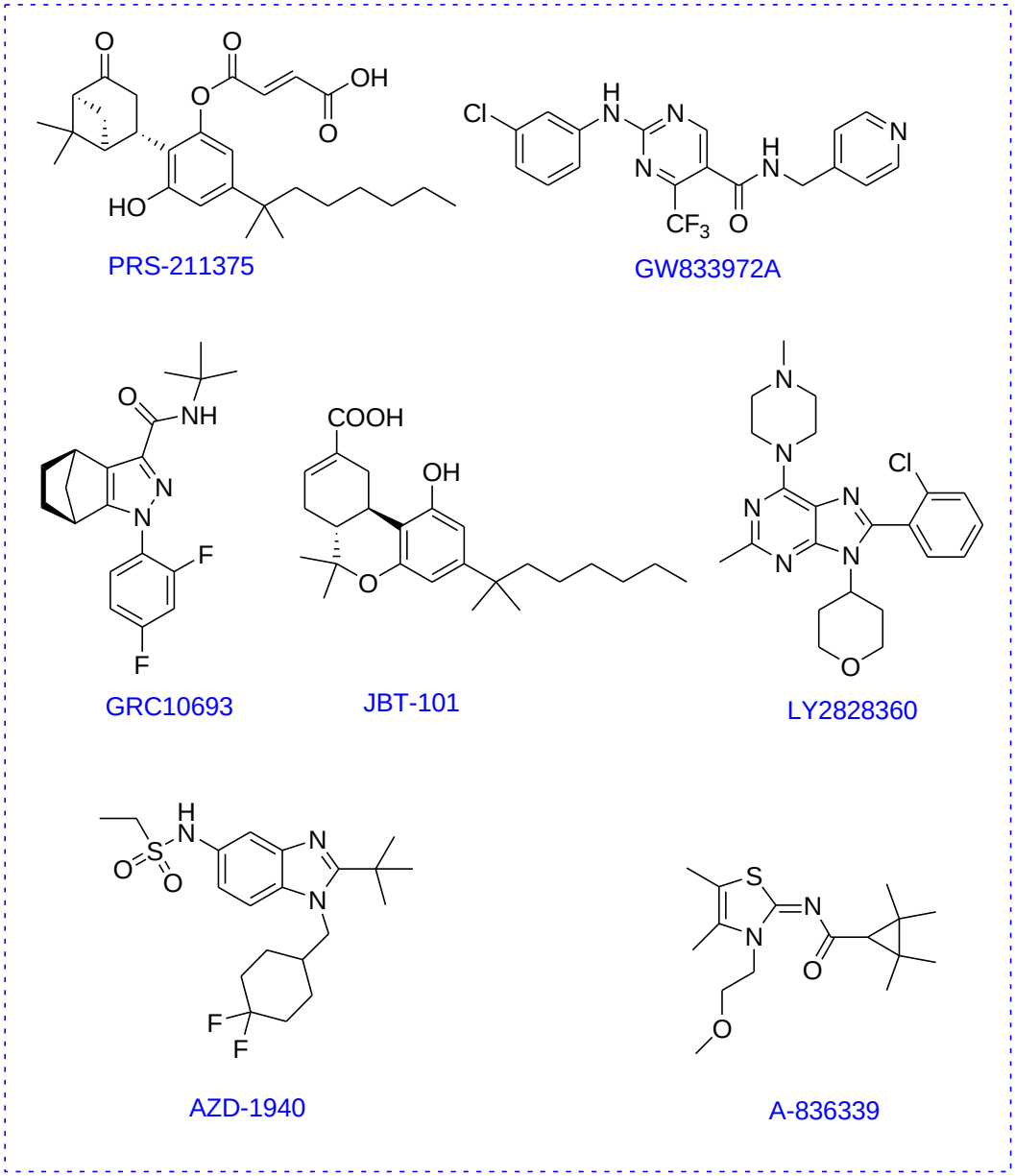

Figure 24. Selected CB_2 agonists in clinical development.

Table 4. Clinical studies of some of the most important CB_2 agonists.

Compound	*Therapeutic Implications*	*Clinical Status*	*Company*	*Source*
PRS-211375 (Cannabinor)	Chronic pain	Phase 2a completed	Pharmos Corporation	WO 2006043260
GW842166	Osteoartritis pain	Phase 2 completed	GlaxoSmithKline	NCT00447486
GRC10693 (Tedalinab)	Neuropathic pain, Osteoartritis	Phase 1 Completed	Glenmark Pharmaceuticals	http://www.pipelinereview.com/
JBT101	Diffuse cutaneous systemic sclerosis	Phase 2	JB Therapeutics, CorbusPharmaceuticals	NCT02465437
KHK6188	Postherpetic neuralgia	Phase 2 completed	Kyowa Hakko Kirin	NCT01544296
ABT521	Pain	Phase 1	Abbott Laboratories	http://media.corporateir.net
LY 2828360	Knee pain, Osteoartritis	Phase 2	Eli Lilli	NCT01319929
AZD1940	Pain	Phase 1	AstraZeneca	NCT00689780
CR701	Pain	Preclinical	Cara Therapeutics	http://www.caratherapeutics.com
CR845	Osteoarthritis	Phase 2	Cara Therapeutics	NCT02524197
APD 371	Pain	Phase 1	Arena Pharmaceuticals	http://www.arenapharma.com
S-777469	Atopic Dermatitis	Phase 2	Shionogi & Company	NCT00697710

10. FUTURE PERSPECTIVES AND CONCLUSIONS

The knowledge gained from several studies indicates the presence of CB_2 receptors in the brain and raises many questions about the possible roles that CB_2 receptors may play in the CNS. These results therefore extend previous evidence that CB_2 receptors play an important role in the immune function to other putative neuronal functions through their apparent presence in neuronal processes. As neuroinflammation is known to be associated with a number of autoimmune and neurological disorders, the close association of the immune system with CB_2 receptors and their functional expression in neurons requires a new evaluation of CB_2 receptors in mental disorders. Both CB_1 and CB_2 receptors seem likely to work both independently or in opposite directions and/or cooperatively in differing neuronal and/or glial cell populations to regulate important physiological activities in the CNS. Thus, more studies are required to determine the exact role of CB_2 receptors and the

nature of their interactions with other receptors or with CB_1 receptors in the brain, which may then determine the therapeutic utility of CB_2 ligands.

Taken together, the studies published in the literature point to potential therapeutic benefits of CB stimulation in the treatment of inflammatory status. Despite some differences in preclinical models and in the signaling of human and rodent CB_2 receptors, the development of selective CB_2 agonists may open new possibilities in therapeutic intervention. Such interventions would aim at reducing the release of pro-inflammatory mediators, particularly in chronic neuropathologic conditions, and delineate the therapeutic effects in neuropathic and chronic pain. Other potential applications for CB_2 agonists could be their use in atherosclerosis, allergic dermatitis, uveoretinitis, cough, gastrointestinal diseases and osteoporosis. A great interest appears to be the use of CB_2 agonists as anticancer agents in skin melanoma, colon cancer, breast and prostate tumor, scleroderma and different grades of lymphoma. Moreover, additional effects of CBs in cancer patients include inhibition of nausea and emesis associated to chemio- or radiotherapy, appetite stimulation, pain relief, mood elevation and insomnia reduction, all of which suggest meaningful clinical outcomes.

In conclusion, some issues remain to be clarified: a) the development of novel CB_2 agonists without CB_1 central side effects and their pharmacological and clinical characterization to specific pathological disorders; b) clinical and functional implication of neuronal CB_2 receptors in the brain and their contribution and interaction with CB_1 receptors in neuropsychiatry and in the neuroimmune activity; c) the mechanism and the presence of an allosteric site and the identification of allosteric modulator enhancers versus CB_2 receptors with the aim to limit the adverse side effects and to potentiate the effect of endocannabinoids; d) the characterization of homodimers and/or heterodimers for CB_2 receptors to better investigate drug-receptor interaction and the possible cross-talk between endocannabinoid system and other endogenous systems; e) identification of selected inhibitors on the endocannabinoid transport and enzyme inhibitors to augment the activation of CB_2 receptors from the endogenous CBs and highlight their autoprotective role in several disease states.

Several pharmaceutical companies and academic institutes have shown important scientific interest towards the chemical and pharmacological development of novel, potent and selective CB_2 agonists, in spite of the difficulties involved in marketing any new drug, which is expensive and full of regulatory and commercial obstacles. The relevant scientific and clinical advances illustrated in this review provide hope that CB_2 agonists will become manageable in some of the most common chronic diseases with the aim to improve human health.

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The authors declare no competing financial interest

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Pier Giovanni Baraldi received his degree in Chemistry in 1974 from the University of Ferrara where he is currently Full Professor of Medicinal Chemistry. He has published more than 400 scientific papers including about 50 patents focusing his research activity on the design and synthesis of DNA minor groove alkylating agents, combretastatin analogs, and ligands for ARs and TRP channel modulators. He has presented more than 90 lectures as speaker in international congresses. He was founder with Prof. Pier Andrea Borea of the spin off Pharmeste involved in the discovery of TRPV1 compounds.

Pier Andrea Borea received his degree in Chemistry from the University of Ferrara in 1967. He is currently President of the Evaluation Board of the University of Ferrara. He has published 400 publications in international journals and contributed about 20 chapters to International Books. He has participated in more than 200 congresses, presenting main, oral and poster communications. He is also named as inventor in about 20 Patents. He has been invited to give about 50 Plenary Lectures at International Symposia and at several Universities. His main field of interest is represented by the study of drug-receptor interactions at molecular level.

Katia Varani received her Doctor degree in Biology in 1988 and her Ph.D. degree in Cellular and Molecular Pharmacology in 1995 from the University of Ferrara. She is currently Associate Professor of Pharmacology at the Medical Sciences Department of the University of Ferrara. She is author/co-author of several scientific publications involving various topics such as the following: pharmacological, biochemical and molecular study of adenosine receptors; interactions of G-protein coupled receptors and human neurodegenerative diseases; chronic and neuropathic pain, cardiovascular disorders; inflammatory diseases and cancer.

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