Recent Advances in the Development of Selective Ligands for the Cannabinoid CB_2 Receptor

Karla-Sue C. Marriott[a] and John W. Huffman[b,*]

[a]*Department of Natural Sciences and Mathematics, Savannah State University, Savannah, GA 31404, USA and* [b]*Howard L. Hunter Laboratory, Clemson University, Clemson, SC 29634-0973, USA*

Abstract: Two subtypes of the mammalian cannabinoid receptor have been identified and successfully cloned since 1990. The CB_1 receptor is primarily located in the central nervous system and the CB_2 receptor is almost exclusively expressed in cells of the immune system. The CB_1 and CB_2 receptors are both G-protein coupled receptors and are involved in the inhibition of adenylate cyclase. The CB_2 receptor is of particular importance due to its involvement in signal transduction in the immune system, making it a potential target for therapeutic immune intervention. A number of these selective ligands are derivatives of traditional dibenzopyran based cannabinoids. These include the very recently synthesized (2'*R*)-1-methoxy-3-(2'-methylbutyl)-Δ^8-THC (JWH-359) which has a 224 fold selectivity for the CB_2 receptor, readily comparable to the well known 1-deoxy-3-(1',1'-dimethylbutyl)-Δ^8-THC (JWH-133) which has 200 fold selectivity for the CB_2 receptor. Several 9-hydroxyhexahydrocannabinols have also been synthesized and are found to be selective high affinity ligands for the CB_2 receptor. These are 1-deoxy-9β-hydroxy-dimethylhexylhexahydrocannabinol (JWH-361, K_i = 2.7 nM) and 1-deoxy-9β-hydroxy-dimethylpentylhexahydrocannabinol (JWH-300, K_i = 5.3 nM). CB_2 selective cannabimimetic indoles include 1-(2,3-dichlorobenzoyl)-2-methyl-3-(2-[1-morpholine]ethyl)-5-methoxyindole (L768242), (*R*)-3-(2-Iodo-5-nitrobenzoyl)-1-(1-methyl-2-piperidinylmethyl)-1H-indole (AM1241) and 1-propyl-2-methyl-3-(1-naphthoyl) indole (JWH-015), which exhibit significant selectivity for the CB_2 receptor coupled with weak affinity for the CB_1 receptor. Bristol-Meyer Squibb has produced a phenylalanine derived cannabimimetic indole which possesses high CB_2 affinity (K_i = 8 nM) and very low affinity for the CB_1 receptor (K_i = 4000 nM). This review will discuss the current advances and recent results in the structure-activity relationships (SAR) of selective ligands for the cannabinoid CB_2 receptor.

Key Words: Cannabinoids, structure-activity relationships, CB_2 receptor, CB_2 selective ligands, aminoalkylindoles.

INTRODUCTION

The hemp plant, *Cannabis sativa* L., better known as marijuana has been used for centuries as a therapeutic agent and recreational drug [1]. The active components of marijuana and their derivatives are classified as cannabinoids. The major psychotropic component in *Cannabis sativa* is Δ^9-tetrahydrocannabinol [Δ^9-THC, **1a**, Fig. (**1**)], the structure of which was elucidated in 1964 by Gaoni and Mechoulam, an achievement that led to renewed interest in cannabinoid chemistry [2]. THC exhibits several biological properties, such as anti-inflammatory, anti-emetic, analgesic, anti-cancer and anti-convulsive effects. For many years very little was known regarding the mechanism of action of cannabinoids in humans and animals, however structure-activity relationships for compounds related to THC were developed [3]. In 1988 with the use of a potent synthetic cannabinoid, tritium labeled CP-55,940 [**2**, Fig. (**1**)] a cannabinoid binding site in the rat brain was described and identified [4]. A human cannabinoid receptor designated the CB_1 receptor was subsequently identified and cloned, which has 97% homology to that of the rat [5, 6]. The CB_1 receptor is primarily expressed in the central nervous system; CB_1 mRNA is found mainly in neural tissue and to a lesser extent in peripheral tissues such as adrenal gland, heart, lung, prostate, testis, bone marrow, thymus, tonsils and spleen [7]. The binding of cannabinoids to the CB_1 receptor, which triggers the activation of this receptor is responsible for the psychoactive effects associated with cannabinoids, such as euphoria, drowsiness, memory lapses, disruption of motor skills, lack of concentration and disorientation.

Another cannabinoid receptor designated the CB_2 receptor, which is located almost exclusively in tissues of the immune system, spleen, tonsils and lymph nodes was identified and cloned in 1993, [8]. CB_2 mRNA is found in spleen, tonsils, thymus, pancreas, bone marrow, splenic macrophage/monocyte preparations, mast cells, peripheral blood leukocytes and in a variety of cultured immune cell models, including the myeloid cell line U937 and undifferentiated and differentiated granulocyte-like or macrophage-like human promyelocytic HL-60 cells [7-14]. CB_2 receptor distribution is highly suggestive of a CB_2 receptor mediated immunomodulatory effect. The CB_2 receptor exhibits only 44% homology (68% in the helical regions) with the CB_1 receptor [8].

The identification of both CB_1 and CB_2 receptors has led to renewed interest in the medicinal chemistry and pharmacology of cannabinoids, and has resulted in significant advances in understanding the mechanism of action of cannabinoids and their interaction with biological systems. However, at the molecular level there remain many unanswered questions concerning the manner in which cannabinoid receptor ligands interact with the receptors [7, 15]. THC is an activating substrate for both the CB_1 and CB_2 receptors. There exists much controversy surrounding the use of

*Address correspondence to this author at the H. L. Hunter Chemistry Laboratory, Clemson University, Clemson, South Carolina 29634-0973, USA; E-mail: huffman@clemson.edu

1568-0266/08 $55.00+.00

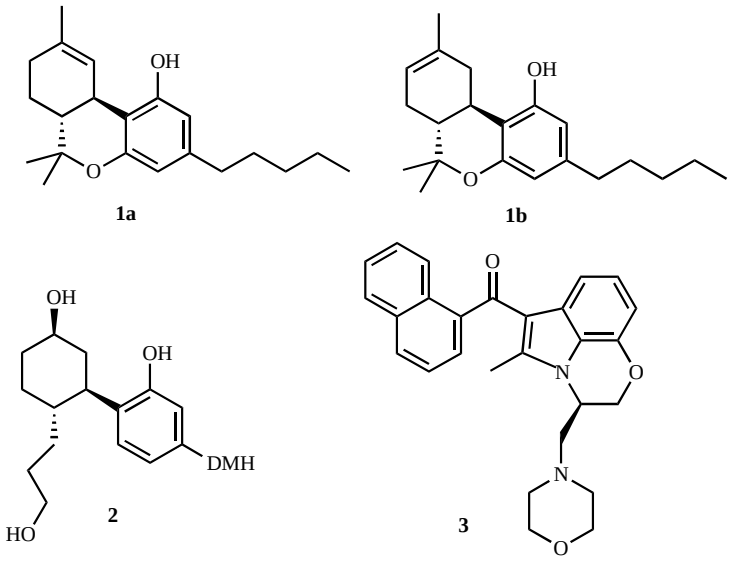


Fig. (1). Structures of Δ^9-THC, Δ^8-THC, CP-55,940 and WIN-55,212-2. DMH= 1,1-dimethylheptyl.

medicinal marijuana and the potential for abuse due to the unwanted psychotropic effects resulting from activation of the CB_1 receptor. The CB_2 receptor is potentially a target for therapeutic immune intervention and as such, research is currently focused on the development of CB_2 selective ligands. CB_2 selective ligands with significantly low CB_1 affinity would not be expected to elicit undesired psychotropic effects.

WIN-55,212-2 [**3**, Fig. (**1**)] was the first CB_2 selective ligand to be reported and has been thoroughly investigated. As a result there are several different documented values for the binding affinity of WIN-55,212-2 at both receptors [16]. WIN-55,212-2 exhibits significant affinity for the CB_2 receptor over the CB_1 receptor with K_i = 1.9±0.1 nM at CB_1 and K_i = 0.3±0.2 nM at CB_2 [17]. While WIN-55,212-2 is extensively used for receptor binding evaluation of potential cannabinoid ligands, it is not very useful pharmacologically as a selective ligand for either cannabinoid receptor because it has high affinity for both receptors.

At present few detailed ligand docking, mutation and modeling studies have been reported for the CB_2 receptor. Salo *et al*. employed the structure of rhodopsin as a template to construct a model of the CB_2 receptor for use in docking studies [18]. These researchers reported based on docking studies with CB_2 selective ligands, CP-55,940 and WIN-55,212-2, that hydrophobic and aromatic stacking interactions were more important than hydrogen bonding. Numerous aromatic stacking interactions were observed for the binding of WIN-55,212-2 at the CB_2 receptor site. Mutation studies performed by Tao and Abood [19] determined that substitution of a highly conserved aspartate residue by asparginine or glutamate in the second transmembrane domain of the CB_2 receptor did not affect binding. It has also been shown that mutation of a lysine residue in the third transmembrane domain of the CB_2 receptor to alanine did not significantly affect binding of several cannabinoid receptor ligands including CP-55,940 and WIN-55,212-2 [20]. Rhee *et al*. also performed mutation studies in which two tryptophan residues (W158 and W172) on the fourth transmembrane domain of the CB_2 receptor were individually replaced with alanine, lysine, phenylalanine or tyrosine [21]. These studies showed that replacement with phenylalanine did not affect binding of several cannabinoid receptor ligands, inclusive of WIN-55,212-2, however, replacement of W172 with alanine completely disrupted binding to the CB_2 receptor. The location of some of these residues suggests that they are not in direct interaction with the ligands and hence the effect of residue mutation on ligand binding may be due to conformational changes of the receptor.

Subsequent to the synthesis of WIN-55,212-2, several other CB_2 selective ligands have been synthesized, which include cannabimimetic indoles such as 1-(2,3-dichlorobenzoyl)-2-methyl-3-(2-[1-morpholino]ethyl)-5-methoxyindole [L768242, **4**, Fig. (**2**)] [22] and 1-propyl-2-methyl-3-(1-naphthoyl)indole [JWH-015, **5**, Fig. (**2**)] [17]. Indole L768242 has exceptionally high selectivity for the CB_2 receptor (146-fold) with significantly high affinity at CB_2 (K_i = 14 nM).

The Huffman group has synthesized a variety of CB_2 selective 1-deoxy and 1-methoxy-Δ^8–THC analogues, which include 1-deoxy-3-(1',1'-dimethylbutyl)-Δ^8-THC [JWH-133, **6**, Fig. (**2**)], one of the most highly selective ligands for the CB_2 receptor with K_i = 677±132 nM at CB_1 and K_i = 3.4±1.0 nM at CB_2 [23]. 1-Methoxy-3-(1',1'-dimethylhexyl)-Δ^8-THC [JWH-229, **7**, Fig. (**2**)] is another highly selective ligand with over 170 fold selectivity for the CB_2 receptor (K_i = 3134±110 nM at CB_1 and K_i = 18±2 nM at CB_2) [24].

PHARMACOLOGY METHODS

In-vitro evaluation of affinity for the CB_1 receptor is determined by the ability of the ligand to displace tritiated CP-55,940 from its binding site in a membrane preparation as described by Compton *et al*. [25]. The displacement of tritiated WIN-55,212-2 has also been employed to assess CB_1 receptor affinity [26]. Affinity for the CB_2 receptor measures the ability of a ligand to displace tritiated CP-55,940 from its binding site in transfected cell lines [9, 22, 17] or a mouse spleen membrane preparation [27].

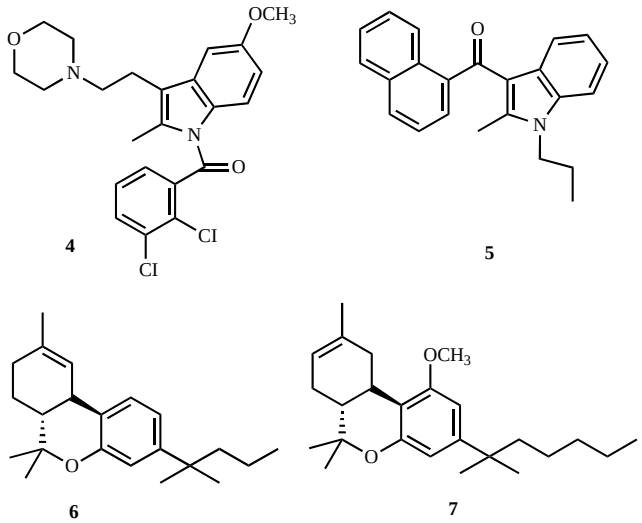


Fig. (2). Structures of L768242, JWH-015, JWH-133 and JWH-229.

Alternatively, *in-vitro* inhibition of electrically evoked contractions of the isolated mouse *vas deferens* (MVD) has been used to assess cannabinoid activity [28]. A common source of disparity in reported affinities by various research groups is the difference in receptor homogeneity between brain or spleen membrane preparations and also membranes obtained from transfected cell lines [7]**.**

Two functional *in-vitro* bioassays have been employed for the determination of the efficacy of ligands at the CB_1 and CB_2 receptors. One measures the agonist induced attenuation of the ability of forskolin to stimulate the production of cyclic adenosine monophosphate (cAMP) and the other the ability of a cannabinoid receptor ligand to stimulate GTPγS binding [28, 29]. The GTPγS binding assay measures G-protein coupled receptor activation using [^{35}S]GTPγS binding.

The mouse model is the most frequently used and most widely accepted protocol for the *in vivo* evaluation of cannabinoid receptor ligands [30]. This procedure involves a series of three or four determinations; measuring spontaneous activity (SA), hypothermia (as a decrease in rectal temperature, RT), catalepsy (as ring immobility, RI) and antinociception (as tail flick, TF).

MODE OF ACTION

Stimulation of the CB_1 receptor inhibits adenylyl cyclase activity and also the voltage-dependent N-type calcium channel activity in neurons [31, 32]. Signaling pathways triggered by the activation of the CB_1 receptor have been found to be sensitive to pertussis toxin (PTX) [33]. Similarly, it has been observed in human lymphocytes and mouse spleen cells that stimulation of the CB_2 receptor inhibits cyclic AMP production. This cAMP inhibition is however blocked by PTX implicating Gi/o proteins as transducers [16, 34, 35].

Cannabinoid receptor agonists are able to stimulate mitogen-activated protein kinase (MAPK) in cultured HL-60 cells expressing CB_2 receptors and Chinese Hamster Ovary (CHO) cells with transfected CB_2 receptors. This subsequently promotes the expression of the growth related gene Krox-24, strongly suggesting that the CB_2 receptor is a functional participant in the MAPK mediated gene induction [33].

A novel signal transduction pathway regulated by CB_2 receptors appears to be directed by the synthesis of ceramide. Remarkably cannabinoids selectively induce apoptosis of cancer cells. This is exemplified in the case of glioma cells (one of the most malignant forms of cancer) in which the CB_2 selective agonist JWH-133 was found to induce apoptosis of glioma cells without affecting healthy non-transformed cells [36, 37]. Apoptotic death is thought to be dependent on sustained ceramide generation (*De Novo* Ceramide Synthesis) [38]. In glioma cells cannabinoid induced ceramide accumulation inhibits serine protein kinase (AKT kinase) and results in apoptosis. In normal glial cells cannabinoids activate AKT, thereby preventing ceramide-induced AKT inhibition and promoting survival [39].

CB_2 receptors are expressed in chronically activated microglial cells, particularly at the leading edges of lamellipodia protrusions involved in cell migration [40]. Microglial cells are thought to play a role in neurodegenerative disorders such as dementia, Alzheimer's, multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) [41]. Recent studies have shown that CB_2 agonists reduce the release of cytotoxins by immune cells and increase their rate of proliferation [41]. This implies that proliferation of these immune cells could be involved in the immune-mediated repair of damaged tissue. If this is the case, CB_2 receptor immune-mediated repair of damaged tissues holds promise as a non-psychotropic therapy for treatment of neuro-inflammation [40, 41].

While recent evidence from experiments performed with palmitoylethanolamide (PEA) strongly suggests the existence of at least one additional cannabinoid receptor expressed in immune cells [42], this review will focus primarily on the impact of the CB_2 receptor as an immunomodulatory target. PEA has been found to reduce the pain associated with inflammatory responses [43, 44]. Researchers observed that SR144528 [**8**, Fig. (**3**)], a well known CB_2 antagonist, was able to block the analgesic effect of

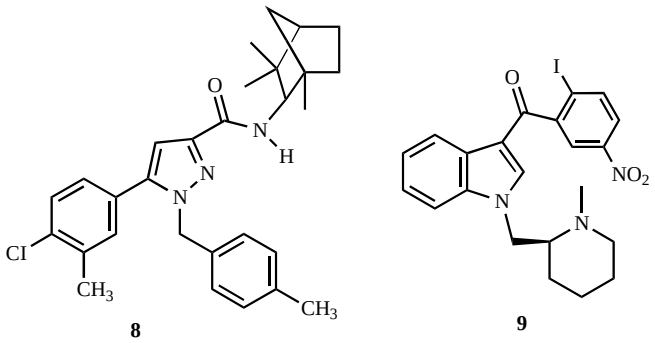


Fig. (3). Structures of SR144528 and AM1241.

PEA, however PEA does not bind to CB_2 receptors [45]. There are two proposals for the explanation of this finding: (i) PEA might interact with a novel cannabinoid receptor that is antagonized by SR144528 (hence this antagonist is not specific for CB_2 receptors) or (ii) PEA might stimulate a novel cannabinoid receptor that couples to phospholipase C and diacylglycerol lipase, increases 2-arachidonylglycerol (2-AG) production and subsequently indirectly activates CB_2 receptors. In any case more research is required to understand the signal transduction pathway involving PEA in order to determine the impact it has on the possible stimulation of CB_2 receptors or any other existing cannabinoid receptor in immune cells [46].

Tissue mast cells are involved in various biological responses and have been associated with the onset of different inflammatory reactions as well as being a contributing factor in the development of autoimmune diseases such as multiple sclerosis and rheumatoid arthritis [47-49]. It has been reported that AM1241 [**9**, Fig. (**3**)], a well known CB_2 receptor selective agonist diminishes oedema produced as a result of mast cell degranulation *in vivo* [50]. Palmitoylethanolamide and JWH-133 have been found to reduce the oedema response triggered by local mast cell degranulation *in vivo*, without affecting the release of the preformed granular enzyme β-hexosaminidase. Jonsson *et al*. [50] determined that data obtained using JWH-133 supported the suggestion that the ability of AM1241 to reduce substance P induced oedema *in vivo* is related to its ability to activate CB_2 receptors rather than *via* another consequence of its chemical structure. As a result of these findings additional questions are raised regarding the exact role of the CB_2 receptor in the regulation of inflammatory processes.

TRADITIONAL CANNABINOID LIGANDS

The chemical structure of traditional cannabinoids is comprised of a partially reduced dibenzopyran moiety and a flexible alkyl side chain at C-3. In the years since the identification of cannabinoid receptors various groups have investigated and developed SAR at both the CB_1 and CB_2 receptors for different classes of ligands such as traditional cannabinoids, indenes, cannabimimetic indoles and pyrroles. However, much more work has been documented based upon ligand interaction with the CB_1 receptor than with the CB_2 receptor. In the years following 1996, research groups described the design and synthesis of several CB_2 selective ligands based upon the traditional cannabinoid template. During 1996-1999, 1-methoxy-$\Delta^{9(11)}$-THC-DMH [L759656, JWH-142, **10**, Fig. (**4**)] and 1-methoxy-Δ^8-THC-DMH [L759633, **11**, Fig. (**4**)] synthesized by Gareau *et al.* [51] were found to exhibit high affinity for the CB_2 receptor combined with low affinity for the CB_1 receptor. 1-Deoxy-Δ^8-THC-DMH [JWH-057, **12**, Fig. (**4**)] synthesized by Huffman *et al*. exhibits high affinity for the CB_2 receptor (K_1 = 2.9 ± 1.6 nM, Table **1**), however it also has rather high affinity for the CB_1 receptor (K_1 = 23 ± 7 nM, Table **1**) [52]. These findings led to further investigations into the development of receptor selective ligands. Subsequently the Huffman group prepared several 1-deoxy-Δ^8-THC analogues. This 1-deoxy series yielded a number of compounds, such as 1-deoxy-Δ^8-THC [JWH-056, **13**, Fig. (**5**)], 1-deoxy-3-(1',1'-dimethylpentyl)-Δ^8-THC [JWH-065, **14**, R = C_4H_9, Fig. (**5**)], 1-deoxy-3-(1',1'-dimethylbutyl)-Δ^8-THC [JWH-133, **6**, Fig. (**2**)] and 1-deoxy-3-(1',1'-dimethylpropyl)-Δ^8-THC [JWH-139, **14**, R = C_2H_5, Fig. (**5**)] with high affinity and significant selectivity for the CB_2 receptor [23]. It had been determined that an 11-hydroxyl substituent enhanced CB_2 receptor affinity [23, 52], hence it was assumed that 1-deoxy-11-hydroxy-1',1'-dimethylalkyl-Δ^8-THC analogues [**15**, R = CH_3 to C_6H_{13}, Fig. (**5**)] and 1-methoxy-11-hydroxy-1',1'-dimethylalkyl-Δ^8-THC [**16**, R = CH_3 to C_6H_{13}, Fig. (**5**)] analogues would exhibit a combination of increased affinity and selectivity for the CB_2 receptor [24] (Table **1**). The series

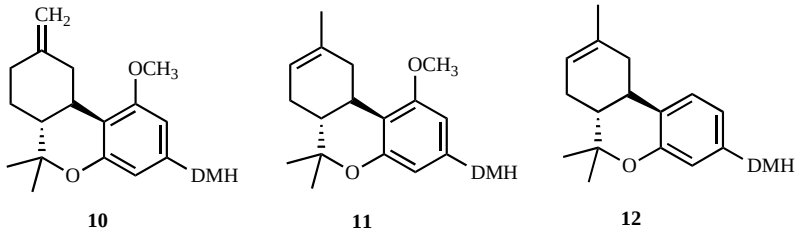


Fig. (4). Structures of 1-methoxy-$\Delta^{9(11)}$-THC-DMH, 1-methoxy-Δ^8-THC-DMH and 1-deoxy-Δ^8-THC-DMH.

Table 1. Receptor Affinities for Δ^9-THC (1a), Δ^8-THC (1b), 1-Methoxycannabinoids (7, 11, 17), 1-Deoxycannabinoids (6, 12, 13, 14) and 11-Hydroxycannabinoids (15, 16)

Compound	CB_1 / K_i (nM)	CB_2 / K_i (nM)
Δ^9-THC (**1a**)	41 ± 2[a]	36 ± 10[a]
Δ^8-THC (**1b**)	44 ± 12[b]	44 ± 17[b]
1-Methoxy-3-(1',1'-dimethylheptyl)-$\Delta^{9(11)}$-THC (JWH-142, **10**)	529 ± 49[c]	35 ± 14[c]
1-Deoxy-Δ^8-THC-DMH (JWH-057, **12**, R= C_6H_{13})	23 ± 7[c]	2.9 ± 1.6[c]
1-Methoxy-3-(1',1'-dimethylheptyl)-Δ^8-THC (JWH-143, **11**)	924 ± 104[c]	65 ± 8[c]
1-Deoxy-Δ^8-THC (JWH-056, **13**)	>10,000[c]	32 ± 9[c]
1-Deoxy-3-(1',1'-dimethylpentyl)-Δ^8-THC (JWH-065, **14**, R= C_4H_9)	399 ± 76[c]	10 ± 2[c]
1-Deoxy-3-(1',1'-dimethylbutyl)-Δ^8-THC (JWH-133, **6**)	677 ± 132[c]	3.4 ± 1.0[c]
1-Deoxy-3-(1',1'-dimethylpropyl)-Δ^8-THC (JWH-139, **14**, R= C_2H_5)	2290 ± 505[c]	14 ± 10[c]
11-Hydroxy-1-deoxy-Δ^8-THC-DMH (JWH-051, **15**, R= C_6H_{13})	1.2 ± 0.1[d]	0.032 ± 0.019[d]
11-Hydroxy-3-(1',1'-dimethylpropyl)-Δ^8-THC (JWH-186, **15**, R = C_2H_5)	187 ± 23[e]	5.6 ± 1.7[e]
11-Hydroxy-3-(1',1'-dimethylbutyl)-Δ^8-THC (JWH-187, **15**, R = C_3H_7)	84 ± 16[e]	3.4 ± 0.5[e]
11-Hydroxy-3-(1',1'-dimethylethyl)-Δ^8-THC (JWH-188, **15**, R = CH_3)	270 ± 58[e]	18 ± 2[e]
11-Hydroxy-3-(1',1'-dimethylpentyl)-Δ^8-THC (JWH-190, **15**, R = C_4H_9)	8.8 ± 1.4[e]	1.6 ± 0.03[e]
11-Hydroxy-3-(1',1'-dimethylhexyl)-Δ^8-THC (JWH-191, **15**, R = C_5H_{11})	1.8 ± 0.3[e]	0.52 ± 0.03[e]
11-Hydroxy-3-(1',1'-dimethylethyl)-1-methoxy-Δ^8-THC (JWH-216, **16**, R = CH_3)	1856 ± 148[e]	333 ± 104[e]
11-Hydroxy-3-(1',1'-dimethylpropyl)-1-methoxy-Δ^8-THC (JWH-215, **16**, R = C_2H_5)	1008 ± 117[e]	85 ± 21[e]
11-Hydroxy-3-(1',1'-dimethylbutyl)-1-methoxy-Δ^8-THC (JWH-224, **16**, R = C_3H_7)	347 ± 34[e]	28 ± 1[e]
11-Hydroxy-3-(1',1'-dimethylpentyl)-1-methoxy-Δ^8-THC (JWH-227, **16**, R = C_4H_9)	40 ± 6[e]	4.4 ± 0.3[e]
11-Hydroxy-3-(1',1'-dimethylhexyl)-1-methoxy-Δ^8-THC (JWH-230, **16**, R = C_5H_{11})	15 ± 3[e]	1.4 ± 0.12[e]
11-Hydroxy-3-(1',1'-dimethylheptyl)-1-methoxy-Δ^8-THC (JWH-233, **16**, R = C_6H_{13})	14 ± 3[e]	1.0 ± 0.3[e]
1-Methoxy-3-(1',1'-dimethylethyl)-Δ^8-THC (**17**, R = CH_3)	>10,000[e]	1867 ± 867[e]
1-Methoxy-3-(1',1'-dimethylpropyl)-Δ^8-THC (JWH-217, **17**, R = C_2H_5)	>10,000[e]	1404 ± 66[e]
1-Methoxy-3-(1',1'-dimethylbutyl)-Δ^8-THC (JWH-225, **17**, R = C_3H_7)	>10,000[e]	325 ± 70[e]
1-Methoxy-3-(1',1'-dimethylpentyl)-Δ^8-THC (JWH-226, **17**,R = C_4H_9)	4001 ± 282[e]	43 ± 3[e]
1-Methoxy 3-(1',1'-dimethylhexyl)-Δ^8-THC (JWH-229, **7**)	3134 ± 110[e]	18 ± 2[e]

[a]Ref. 17; [b]Ref. 52; [c]Ref. 23; [d]Ref. 51; [e]Ref. 24.

of 1-deoxy-11-hydroxy-1',1'-dimethylalkyl-Δ^8-THC analogues ranges in affinity for the CB_2 receptor from exceptionally high affinity for the 1',1'-dimethylheptyl analogue [JWH-051, **15**, R = C_6H_{13}, Fig. (**5**)] to significant affinity as the length of the alkyl side chain decreases. Cannabinoid JWH-051 with K_i = 0.03±0.02 nM at CB_2 has extraordinarily high affinity for the CB_2 receptor, however with K_i = 1.2±0.1 nM at CB_1, it also has a very high affinity for the CB_1 receptor and has been found to be a very potent cannabinoid *in vivo* [52].

In the 1-deoxy-11-hydroxy-1',1'-dimethylalkyl-Δ^8-THC series affinity for both receptors increases as the length of the C-3 side chain increases, as indicated by increased affinity moving up the homologous series, JWH-188 (**15**, R = CH_3, Table **1**), JWH-186 (**15**, R = C_2H_5, Table **1**), JWH-187 (**15**, R = C_3H_7, Table **1**), JWH-190 (**15**, R = C_4H_9, Table **1**), JWH-191 (**15**, R = C_5H_{11}, Table **1**) and JWH-051 (**15**, R = C_6H_{13}, Table **1**) . These compounds exhibit modest selectivity for the CB_2 receptor with JWH-186 and JWH-051 being the most selective having 33 and 37-fold selectivity for the CB_2 receptor respectively. The other two cannabinoids in the series, 1-deoxy-11-hydroxy-1',1'-dimethylpentyl-Δ^8-THC [JWH-190, **15**, R = C_4H_9, Fig. (**5**)] and 1-deoxy-11-hydroxy-1',1'-dimethylhexyl-Δ^8-THC [JWH-191, **15**, R = C_5H_{11}, Fig. (**5**)] show exceptionally high affinity for both

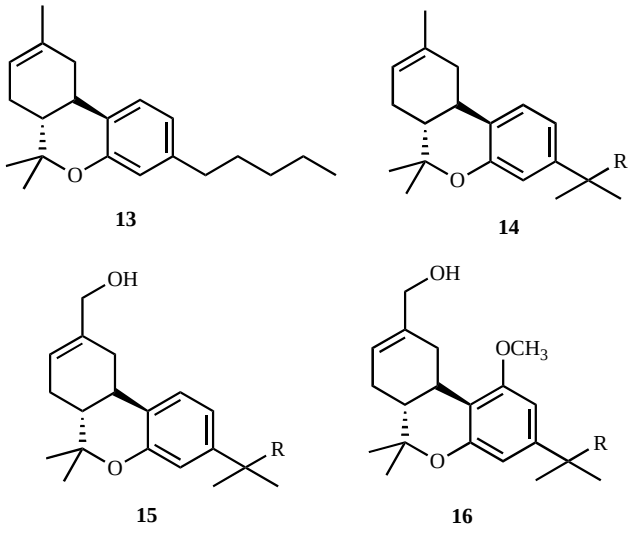


Fig. (5). Structures of JWH-056, 1-deoxy-Δ^8-THC, 1-deoxy-11-hydroxy-Δ^8-THC and 1-methoxy-11-hydroxy-Δ^8-THC analogues.

receptors, with no significant selectivity for the CB_2 receptor and if these compounds prove to be agonists, they would be expected to elicit the unwanted psychotropic effects associated with CB_1 receptor activation.

A trend similar to that for the 1-deoxy-11-hydroxy-1',1'-dimethylalkyl-Δ^8-THC series is observed for the 1-methoxy-11-hydroxy-1',1'-dimethylalkyl-Δ^8-THC series [**16**, Fig. (**5**)]. In this series the affinity for both cannabinoid receptors increases as the length of the alkyl side chain is increased. The CB_2 selectivity for these 1-methoxy analogues ranges between 14-5 fold from the highest to the lowest member of the homologous series (Table **1**), which is low in comparison to the corresponding 1-deoxy analogues. For both the 1-deoxy-11-hydroxy-1',1'-dimethylalkyl-Δ^8-THC and the 1-methoxy-11-hydroxy-1',1'-dimethylalkyl-Δ^8-THC series, it can be concluded that an 11-hydroxy substituent signify-cantly enhances affinity at both the CB_1 and CB_2 receptors with resultant negligible selectivity for the CB_2 receptor. In the 1-deoxy series JWH-051, JWH-186 and JWH-187 exhibit the highest selectivity for the CB_2 receptor with 37, 33 and 25-fold selectivity, respectively.

In the 1-methoxy-3-(1',1'-dimethylalkyl)-Δ^8-THC series, two compounds JWH-226 [**17**, R = C_4H_9, Fig. (**6**)] and JWH-229 [**7**, Fig. (**2**)] show high selectivity for the CB_2 receptor with 93 and 174-fold selectivity, respectively [24] (Table **1**). JWH-226 has modest affinity at the CB_2 receptor (K_i = 43±3 nM) and poor affinity at CB_1 (K_i = 4001±282 nM). JWH-229 has significant affinity at the CB_2 receptor (K_i = 18±2 nM) combined with poor affinity at CB_1 (K_i = 3134±110 nM) and is one of the most selective CB_2 receptor partial agonists to be reported [EC_{50} = 4.6±2.0 nM and E_{max} = 75.7±8.3% relative to CP-55,940 [53]. The other members of the series show very poor affinity for both receptors, especially the lower members of the homologous series with K_i >10,000 nM at CB_1 and K_i = 325-1867 nM at CB_2.

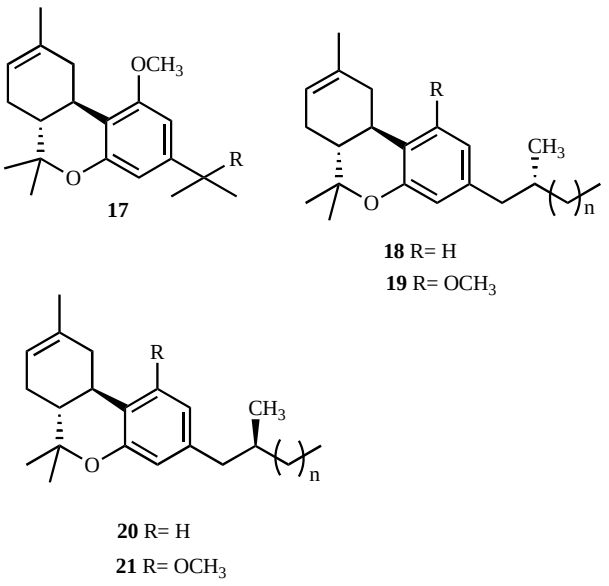


Fig. (6). Structures of 1-methoxy-3-(1',1'-dimethylalkyl)-Δ^8-THC analogues and (2'*R*) – and (2'*S*)-1-methoxy and 1-deoxy-Δ^8-THC analogues.

Some tentative SAR based on the data collected from the 1-deoxy [**14**, Fig. (**5**)], 1-methoxy-Δ^8-THC [**16**, Fig. (**5**)] and the corresponding 11-hydroxy-Δ^8-THC analogs [**15**, Fig. (**5**)] have been outlined [23, 24, 52]. The length of the alkyl chain at C-3 is a significant factor in determining the affinity for both receptors, however, its impact is considered to be less critical in determining the affinity for the CB_2 receptor than for the CB_1 receptor. In the 1-deoxy-Δ^8-THC series there is a dramatic increase in affinity for the CB_1 receptor as the length of the alkyl side chain at C-3 is increased. This is observed with JWH-139 (R= C_2H_5, K_i= 2290±505 nM at CB_1) and JWH-057 (R= C_6H_{13}, K_i= 23±7 nM at CB_1). All members of the 1-deoxy-Δ^8-THC series have good affinity for the CB_2 receptor ranging from K_i = 14 nM to 2.9 nM, with affinity increasing as the length of the alkyl side chain at C-3 increases. Overall, an 11-hydroxyl substituent enhances affinity for both the CB_1 and CB_2 receptors.

In 2006 two new series of CB_2 receptor selective ligands were reported by the Huffman group [54]. These are the (2'*R*)- and (2'*S*)-1-methoxy- and 1-deoxy-3-(2'-methylalkyl)-Δ^8-tetrahydrocannabinols with alkyl side chains of three to seven carbon atoms. Most of these compounds exhibit greater affinity for the CB_2 receptor than the CB_1 receptor. Exceptionally high CB_2 receptor selectivity was observed for (2'*R*)-1-methoxy-3-(2'-methylbutyl)-Δ^8-THC [JWH-359, **19**, n = 1, Fig. (**6**), 224-fold], (2'*S*)-1-deoxy-3-(2'-methylbutyl)-Δ^8-THC [JWH-352, **20**, n = 1, Fig. (**6**), 212-fold], (2'*S*)-1-deoxy-3-(2'-methylpentyl)-Δ^8-THC [JW H-255, **20**, n = 2, Fig. (**6**), 179-fold] and all have good affinity for the CB_2 receptor (K_i = 13-47 nM) combined with very little affinity for the CB_1 receptor (K_i = 1493 to >10,000 nM) (Table **2**). The (2'S)-1-deoxy compounds have overall greater affinity for the CB_2 receptor than the corresponding (2'*R*) isomers. These (2'*S*)-1-deoxy compounds also exhibit maximum CB_2 receptor affinity with little CB_1 receptor affinity when the length of the alkyl side chain is in the range of 4 to 6 carbon atoms.

Table 2. Receptor Affinities of 1-Methoxy-2'-Methylcannabinoids (19, 21), 1-Deoxy-2'-Methylcannabinoids (18, 20), 1-Methoxy-11-Nor-9-Hydroxyhexahydrocannabinols (22, 24) and 1-Deoxy-11-Nor-9-Hydroxyhexahydrocannabinols (23, 25)

Compound	CB_1/ K_i (nM)	CB_2/ K_i (nM)
1-Methoxy-3-(2'-methylpropyl)-Δ^8-THC (JWH-339, **19**, n = 0)	>10,000[a]	2317 ± 93[a]
(2'*S*)-1-Methoxy-3-(2'-methylbutyl)-Δ^8-THC (JWH-351, **21**, n = 1)	>10,000[a]	295 ± 3[a]
(2'*R*)-1-Methoxy-3-(2'-methylbutyl)-Δ^8-THC (JWH-359, **19**, n = 1)	2918 ± 450[a]	13 ± 0.2[a]
(2'*S*)-1-Methoxy-3-(2'-methylpentyl)-Δ^8-THC (JWH-254, **21**, n = 2)	4724 ± 509[a]	319 ± 16[a]
(2'*R*)-1-Methoxy-3-(2'-methylpentyl)-Δ^8-THC (JWH-354, **19**, n = 2)	1961 ± 21[a]	241 ± 14[a]
(2'*S*)-1-Methoxy-3-(2'-methylhexyl)-Δ^8-THC (JWH-256, **21**, n = 3)	4300 ± 888[a]	97 ± 18[a]
(2'*R*)-1-Methoxy-3-(2'-methylhexyl)-Δ^8-THC (JWH-356, **19**, n = 3)	5837 ± 701[a]	108 ± 17[a]
(2'*S*)-1-Methoxy-3-(2'-methylheptyl)-Δ^8-THC (JWH-247, **21**, n = 4)	427 ± 31[a]	99 ± 4[a]
(2'*R*)-1-Methoxy-3-(2'-methylheptyl)-Δ^8-THC (JWH-358, **19**, n = 4)	1243 ± 266[a]	52 ± 3[a]
1-Deoxy-3-(2'-methylpropyl)-Δ^8-THC (JWH-338, **18**, n = 0)	>10,000[a]	111 ± 16[a]
(2'*S*)-1-Deoxy-3-(2'-methylbutyl)-Δ^8-THC (JWH-352, **20**, n = 1)	>10,000[a]	47 ± 2[a]
(2'R)-1-Deoxy-3-(2'-methylbutyl)-Δ^8-THC (JWH-360, **18**, n = 1)	2449 ± 606[a]	160 ± 8[a]
(2'*S*)-1-Deoxy-3-(2'-methylpentyl)-Δ^8-THC (JWH-255, **20**, n = 2)	4307 ± 649[a]	24 ± 9[a]
(2'*R*)-1-Deoxy-3-(2'-methylpentyl)-Δ^8-THC (JWH-353, **18**, n = 2)	1493 ± 10[a]	31 ± 1[a]
(2'*S*)-1-Deoxy-3-(2'-methylhexyl)-Δ^8-THC (JWH-257, **20**, n = 3)	25 ± 2[a]	1.0 ± 0.3[a]
(2'*R*)-1-Deoxy-3-(2'-methylhexyl)-Δ^8-THC (JWH-355, **18**, n = 3)	2162 ± 220[a]	108 ± 17[a]
(2'*S*)-1-Deoxy-3-(2'-methylheptyl)-Δ^8-THC (JWH-264, **20**, n = 4)	332 ± 43[a]	146 ± 28[a]
(2'*R*)-1-Deoxy-3-(2'-methylheptyl)-Δ^8-THC (JWH-357, **18**, n = 4)	647 ± 78[a]	185 ± 4[a]
1-Deoxy-11-nor-9β-hydroxy-3-(1',1'dimethylheptyl)-HHC (JWH-102, **23**, n = 4)	7.9 ± 0.9[b]	5.2 ± 2.0[b]
1-Deoxy-11-nor-9α-hydroxy-3-(1',1'dimethylheptyl)-HHC (JWH-103, **25**, n = 4)	28± 3[b]	23 ± 7[b]
1-Methoxy-11-nor-9β-hydroxy-3-(1',1'dimethylbutyl)-HHC (JWH-298, **22**, n = 1)	812 ± 67[c]	198 ± 23[c]
1-Methoxy-11-nor-9α-hydroxy-3-(1',1'dimethylbutyl)-HHC (JWH-277, **24**, n = 1)	3905 ± 91[c]	589 ± 65[c]
1-Methoxy-11-nor-9β-hydroxy-3-(1',1'dimethylpentyl)-HHC (JWH-299, **22**, n = 2)	415 ± 50[c]	30 ± 2[c]
1-Methoxy-11-nor-9α-hydroxy-3-(1',1'dimethylpentyl)-HHC (JWH-278, **24**, n = 2)	906 ± 80[c]	69 ± 6[c]
1-Methoxy-11-nor-9β-hydroxy-3-(1',1'dimethylhexyl)-HHC (JWH-350, **22**, n = 3)	395 ± 50[c]	12 ± 1[c]
1-Methoxy-11-nor-9α-hydroxy-3-(1',1'dimethylhexyl)-HHC (JWH-349, **24**, n = 3)	376 ± 1[c]	38 ± 4[c]
1-Methoxy-11-nor-9β-hydroxy-3-(1',1'dimethylheptyl)-HHC (JWH-341, **22**, n = 4)	100 ± 8[c]	10 ± 0.1[c]
1-Methoxy-11-nor-9α-hydroxy-3-(1',1'dimethylheptyl)-HHC (JWH-340, **24**, n = 4)	135 ± 6[c]	30 ± 1[c]
1-Deoxy-11-nor-9β-hydroxy-3-(1',1'dimethylbutyl)-HHC (JWH-310, **23**, n = 1)	1059 ± 51[c]	36 ± 3[c]
1-Deoxy-11-nor-9α-hydroxy-3-(1',1'dimethylbutyl)-HHC (JWH-336, **25**, n = 1)	4589± 367[c]	153 ± 15[c]
1-Deoxy-11-nor-9β-hydroxy-3-(1',1'dimethylpentyl)-HHC (JWH-300, **23**, n = 2)	118 ± 16[c]	5.3 ± 0.1[c]
1-Deoxy-11-nor-9α-hydroxy-3-(1',1'dimethylpentyl)-HHC (JWH-301, **25**, n = 2)	295± 64[c]	48 ± 4[c]
1-Deoxy-11-nor-9β-hydroxy-3-(1',1'dimethylhexyl)-HHC (JWH-361, **23**, n = 3)	63 ± 3[c]	2.7 ± 0.1[c]
1-Deoxy-11-nor-9α-hydroxy-3-(1',1'dimethylhexyl)-HHC (JWH-362, **25**, n = 3)	127± 8[c]	34 ± 5[c]

[a]Ref. 54; [b]Ref. 23; [c]Ref. 55.

In the (2'*R*)- and (2'*S*)-1-methoxy-3-(2'-methylalkyl)-Δ^8-THC series [**19**, **21**, Fig. (**6**)], there is no significant affinity for the CB_1 receptor with K_i = 427 to >10,000 nM (Table **2**) which is reminiscent of the results obtained for the 1-methoxy-3-(1',1'-dimethylalkyl)-Δ^8-THC series in which the CB_1 receptor affinities ranged from 924 to >10,000 nM. (2'R)-1-Methoxy-3-(2'-methylbutyl)-Δ^8-THC [JWH-359, **19**, n = 1, Fig. (**6**)] has high CB_2 affinity (K_i = 13±0.2 nM) and is an extremely selective ligand for the CB_2 receptor with 224-fold selectivity. In contrast, the epimer, (2'S)-1-methoxy-3-(2'-methylbutyl)-Δ^8-THC [JWH-351, **21**, n = 1, Fig. (**6**)] has little affinity for the CB_2 receptor (K_i = 295±3 nM) and is significantly less selective.

All the 1-methoxy-3-(2'-methylalkyl)-Δ^8-THC analogues are selective for the CB_2 receptor, however, only JWH-359 and JWH-358 exhibit the combination of significant affinity for the CB_2 receptor with little affinity for the CB_1 receptor. In general the (2'R)-1-methoxy-3-(2'-methylalkyl)-Δ^8-tetrahydrocannabinols have greater affinity for the CB_2 receptor than the corresponding (2'S) isomers. With the exception of (2'*S*)-1-deoxy-3-(2'-methylhexyl)-Δ^8-THC [JWH-257, **20**, n = 3, Fig. (**6**)], none of these new (2'*R*)- and (2'*S*)-1-methoxy- and 1-deoxy-3-(2'-methylalkyl)-Δ^8-tetrahydrocannabinols have significant affinity for the CB_1 receptor.

Very recently the Huffman group investigated the CB_2 receptor selectivity of several new 11-*nor*-1-methoxy- and 11-*nor*-1-deoxy-9-hydroxyhexahydrocannabinols [**22**, **23**, **24**, **25**, Fig. (**7**)], with 1',1'-dimethylalkyl side chains of four to seven carbon atoms at C-3 of the cannabinoid skeleton [55]. These cannabinols exhibit greater affinity for the CB_2 receptor than for the CB_1 receptor. 1-Deoxy-9β-hydroxy-dimethylhexylhexahydrocannabinol [JWH-361, **23**, n = 3, Fig. (**7**)] and 1-deoxy-9β-hydroxy-dimethylpentylhexahydrocannabinol [JWH-300, **23**, n=2, Fig. (**7**)] have exceptionally high affinity for the CB_2 receptor with K_i = 2.7 nM and 5.3 nM respectively (Table **2**).

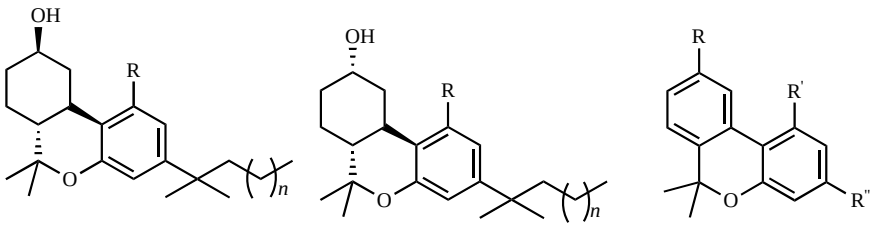
Moderate, 22- to 33-fold selectivity for the CB_2 receptor with the desirable combination of good affinity for the CB_2 receptor and little affinity for the CB_1 receptor was observed for three of these 1-methoxy and 1-deoxy-11-nor-9-hydroxy-3-(1',1'-dimethylalkyl)-hexahydrocannabinols. Two of these selective ligands are 1-deoxy analogues, JWH-310 [**23**, n = 1, Fig. (**7**)] and JWH-300 [**23**, n = 2, Fig. (**7**)] and one is a 1-methoxy-hexahydrocannabinol JWH-350 [**22**, n = 3, Fig. (**7**)]. These compounds however are not nearly as selective as JWH-133 (**6**), JWH-139 (**14**) or several aforementioned 2'-methyl-Δ^8-THC analogs. With the hexahydrocannabinols as with other cannabinoids, affinity for the CB_1 receptor increases as the length of the C-3 alkyl side chain increased from 4 to 7 carbons. In general the 9β-hydroxy analogues have greater affinity for both the CB_1 and CB_2 receptors than their corresponding α-epimers. This steric effect was observed to have a greater impact on CB_2 receptor affinity than on CB_1 receptor affinity.

Several years ago, Reggio *et al*. performed an insightful computational study pertaining to the CB_1 receptor that indicated a correlation between the stereochemical orientation of substituents at C-9 of the traditional cannabinoid moiety and the cannabinoid activity of the compound [56]. This study indicated that traditional cannabinoid ligands with a substituent at C-9 in the α-orientation had little or no cannabinoid activity. These results were based upon drug discrimination studies in rhesus monkeys and were performed prior to the recognition of the existence of cannabinoid receptor subtypes. Although the original observations regarding stereochemical orientation of substituents at C-9 by Reggio *et al*. pertained to the CB_1 receptor, the same trends were observed at the CB_2 receptor in the 11-nor-9-hydroxy-hexahydrocannabinol series.

Razdan *et al.* synthesized and studied the SAR of three series of cannabinols [**26**, R = CH_3, CH_2OH or CO_2CH_3, $CO_2CH_2C_6H_5$, Fig. (**7**)], in which R' = OH, OCH_3, or H and R'' = C_3H_7 or C_6H_{13}. Only compounds with a hydroxyl substituent at C-11 and/or C-9 exhibited significant affinity for either receptor [57]. Negligible selectivity for the CB_2 receptor was observed for this class of cannabinols as those compounds that showed good affinity for the CB_2 receptor, also showed good affinity for the CB_1 receptor. The 3-(1',1'-dimethylbutyl) analogue of cannabinol [**26**, R = CH_3, R' = OH and R'' = C_6H_{13}, Fig. (**7**)] has the greatest selectivity for the CB_2 receptor (7-fold), with K_i = 6±2 nM at CB_2 and K_i = 42±2 nM at CB_1.

INDOLES

In the years since Sterling Winthrop reported that pravadoline [**27**, Fig. (**8**)] binds to the cannabinoid receptor, considerable progress has been made in the synthesis and pharmacology of cannabimimetic indoles [58]. Pravadoline and structurally related aminoalkylindole (AAI) ligands are inhibitors of electrically stimulated MVD contractions, adenylate cyclase and are also antinociceptive *in vivo* [58]. Aminoalkylindoles interact with a G-protein coupled receptor in the brain and some of these compounds have



22 R = OCH_3
23 R = H

24 R= OCH_3
25 R= H

26

Fig. (7). Structures of 11-*nor*-1-methoxy-9-hydroxyhexahydrocannabinol, 11-*nor*-1-deoxy-9-hydroxyhexahydrocannabinol and cannabinol analogues.

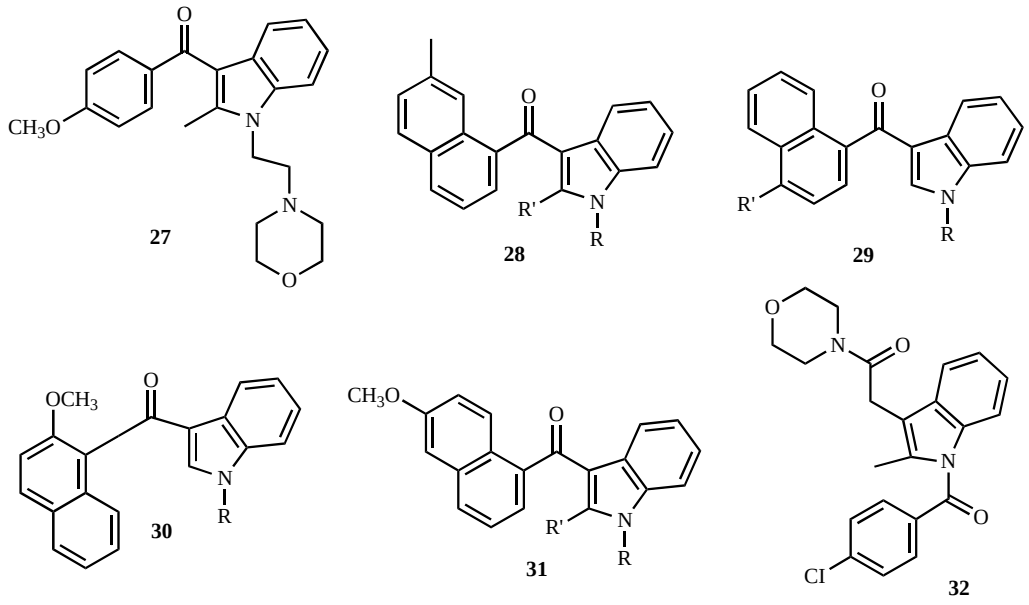


Fig. (8). Structures of pravadoline, cannabimimetic indole analogues (**28**, **29**, **30**, **31**) and BML-190.

significantly high affinity for the cannabinoid brain receptor [59]. WIN-55,212-2 [**3**, Fig. (**1**)] a structurally rigid AAI exhibits significant CB_2 selectivity (K_i = 1.9 nM at CB_1 and K_i = 0.3 nM at CB_2) [17].

In 1996 Showalter *et al*. reported the structure-activity relationship studies of several cannabinoid receptor ligands at the CB_2 receptor site. These ligands included several aminoalkylindoles and in particular it was found that JWH-015, 1-propyl-2-methyl-3-(1-naphthoyl)indole [**5**, Fig. (**2**)] has high affinity for the CB_2 receptor and is 27-fold selective for the CB_2 receptor (K_i = 13.8 nM at CB_2 and K_i = 383 nM at CB_1) [17].

The Huffman group carried out further investigations into the development of CB_2 selective cannabimimetic indoles using JWH-015 as a lead compound [60-62]. This resulted in the synthesis of one other CB_2 selective ligand, 1-propyl-2-methyl-3-(7-methyl-1-naphthoyl) indole [JWH-046, **28**, R = propyl, R' = methyl, Fig. (**8**)] which like JWH-015 has a propyl substituent on the nitrogen. JWH-046 has 21-fold selectivity for the CB_2 receptor with significant affinity for the CB_2 receptor (K_i = 16±5 nM at CB_2 and K_i = 343±38 nM at CB_1) [62]. SAR developed based on the data collected from these AAIs determined that the aminoalkyl group is not necessary for cannabimimetic activity, whereas an *N*-alkyl substituent of four to six carbons is necessary for CB_1 affinity [60-62].

Very recently forty-seven new indole derivatives were reported by the Huffman group, in which substituents on the naphthalene moiety are varied and include either a pentyl or propyl substituent on the indole nitrogen [53, 63]. Four of these cannabimimetic indoles possess the desired characteristics of high CB_2 receptor affinity and low CB_1 receptor affinity and may be classified as selective CB_2 receptor ligands. The compounds are 1-propyl-3-(4-methyl-1-naphthoyl)indole [JWH-120, R = *n*-propyl, R' = methyl, **29**, Fig. (**8**)], 1-pentyl-3-(2-methoxy-1-naphthoyl)indole [JWH-267, **30**, Fig. (**8**)], 1-propyl-2-methyl-3-(6-methoxy-1-naphthoyl)indole [JWH-151, R = *n*-propyl, R' = methyl, **31**, Fig. (**8**)] and 1-pentyl-2-methyl-3-(6-methoxy-1-naphthoyl)indole [JWH-153, R = *n*-pentyl, R' = methyl, **31**, Fig. (**8**)] with K_i = 6.1, 7.2, 30 and 11 nM at CB_2 and 170-, 54-, >300- and 23-fold CB_2 selectivity, respectively. The receptor affinities for these compounds are summarized in Table **3**. The efficacy of compounds JWH-015, JWH-120, JWH-267 and JWH-151 was evaluated based on their ability to stimulate GTPγS binding. The stimulation is normalized to that produced by 3 μM CP-55,940 [**2**, Fig. (**1**)], a maximally effective concentration of this standard cannabinoid agonist. All compounds evaluated were found to be potent in the [^{35}S]GTPγS assay with EC_{50} values ranging from 5.1±1.0 nM for JWH-120 to 17.7±1.0 nM for JWH-015. 1-Propyl-2-methyl-3-(6-methoxy-1-naphthoyl)indole (JWH-151) was found to be highly efficacious with an E_{max} of 108.5±13.0% relative to CP-55,940. The other three indoles JWH-015, JWH-120 and JWH-267 are partial agonists relative to CP-55,940 with E_{max} values ranging from 65.7±6.4% (JWH-015) to 78.1±10.7% (JWH-120).

In contrast to CB_2 agonists, WIN-55,212-2 [**3**, Fig. (**1**)] and JWH-015 [**5**, Fig. (**2**)], which decrease the levels of cAMP, it has been reported that indomethacin morpholinoamide [BML-190, **32**, Fig. (**8**)] dose dependently increases the forskolin stimulated levels of cAMP in HEK-293 cells transfected with the human CB_2 receptor [64]. BML-190 is a CB_2 selective inverse agonist, however, it has only modest affinity for the CB_2 receptor (K_i = 435±43 nM) [22]. CB_2 receptor selective inverse agonists have been extremely useful in the investigation of the roles played by CB_2 receptors. Another indole derived CB_2 selective inverse agonist is AM630 [**33**, Fig. (**9**)] which has significantly greater affinity for the CB_2 receptor (K_i = 37.5±15.4 nM) than BML-190 [65]. AM630 potently reverses the actions of CP-55,940, which is known to induce inhibition of forskolin-stimulated cAMP production by human CB_2 transfected

Table 3. Receptor Affinities of WIN-5512-2 (3) and Related Cannabimimetic Indole Analogues (5, 28, 29, 30, 31)

Compound	CB_1/ K_i (nM)	CB_2/ K_i (nM)
WIN-55212-2 (**3**)	1.9 ± 0.1[a]	0.3 ± 0.2[a]
28, R = *n*-Propyl, R' = Methyl (JWH-046)	343 ± 38[b]	16 ± 5[b]
5, (JWH-015)	383 ± 72[a]	14 ± 5[a]
29, R = *n*-Propyl, R' = Methyl (JWH-120)	1054 ± 31[a]	6.1 ± 0.7[a]
30, R = *n*-Pentyl (JWH-267)	381 ± 16[c]	7.2 ± 0.14[c]
31, R = *n*-Propyl, R' = Methyl (JWH-151)	>10,000[c]	30 ± 1.1[c]
31, R = *n*-Pentyl, R' = Methyl (JWH-153)	250 ± 24[c]	11 ± 0.5[c]

[a]Ref. 17; [b]Aung, M. M.; Griffin, G.; Huffman, J. W.; Wu, M.-J.; Keel, C.; Yang, B.; Showalter, V. M.; Abood, M. E.; Martin, B. R. *Drug Alcohol Depend.* **2000**, *60*, 133. [c] Ref. 53.

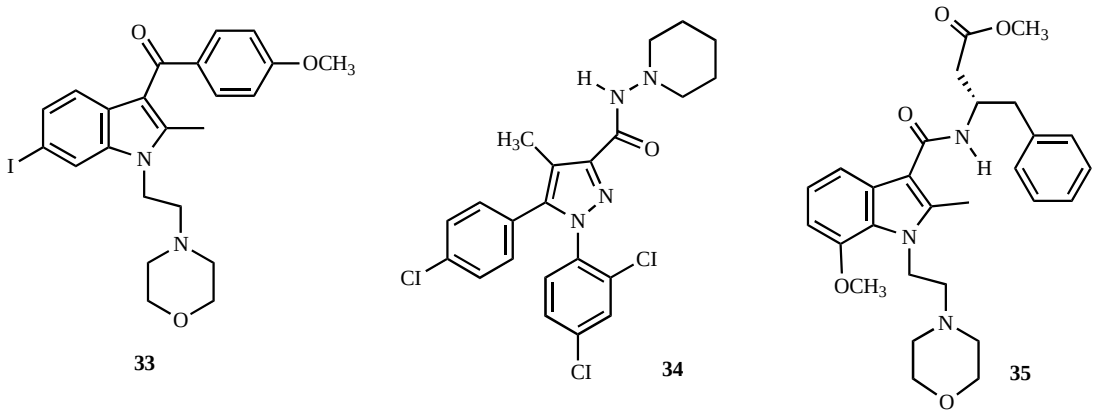


Fig. (9). Structures of AM630, SR141716A (Rimonabant®) and phenylalanine derived amide.

CHO cell preparations. Several investigations have also suggested that AM630 has mixed agonist/antagonist properties and is a low-affinity partial CB_1 agonist [65-67]. At least one research group has reported AM630 as a low-potency CB_1 inverse agonist [68]. This investigation studied the effect of AM630 at the cloned human CB_1 receptor stably expressed in CHO cells, in which AM630 inhibited basal [^{35}S]GTPγS binding by 20.9% with an EC_{50} value of 0.90 μM.

Cannabimimetic indole (*R*)-3-(2-Iodo-5-nitrobenzoyl)-1-(1-methyl-2-piperidinylmethyl)-1H-indole [AM1241, **9**, Fig. (**3**)] has been reported to produce antinociception to thermal stimuli [69]. This is an effect which is blocked by the CB_2 receptor antagonist AM630 [**33**, Fig. (**9**)] [70]. AM1241 is a highly selective CB_2 receptor ligand with 82-fold selectivity and combines high CB_2 receptor affinity (K_i = 3.4±0.5 nM) with low CB_1 receptor affinity (K_i = 280±41 nM) [69]. Antihyperalgesic and antiallodynic effects of AM1241 are reportedly blocked by the CB_2 antagonist SR144528 [**8**, Fig. (**3**)] but not by the CB_1 antagonist SR141716A [Rimonabant®, **34**, Fig. (**9**)], suggesting that these effects are mediated through the CB_2 receptor [71].

Bristol-Myers Squibb recently described a series of amides derived from a substituted indole 3-carboxylic acid, which are highly selective for the CB_2 receptor [72]. A phenylalanine derived amide [**35**, Fig. (**9**)] was found to be the most selective ligand in this series with K_i = 8 nM at CB_2 and K_i = 4000 nM at CB_1. This amide (**35**) has exceptionally high (500-fold) CB_2 receptor selectivity, coupled with high affinity for the receptor.

Researchers at Abbott Laboratories very recently reported the synthesis of a series of 1-alkyl-3-keto indoles [Fig. (**10**), Table **4**] [73]. These new indoles possess a variety of pendant nitrogen side chains with saturated cyclic ketones as the C-3 aryl substituent. Cell lines expressing recombinant human CB_2 or CB_1 receptors were used in radioligand binding assays. Calcium flux (FLIPR) assay was used to determine the functional efficacy of these ligands at the human CB_2 receptor. The most selective ligand from this new series has a tetramethylcyclopropyl substituent at C-3 [A-796260, **36**, Fig. (**10**)]. A-796260 has extremely high affinity at the CB_2 receptor, K_i = 0.77 nM combined with 2700-fold selectivity for the CB_2 receptor. A-796260 is a novel high affinity ligand for the human and rat CB_2 receptor with very high selectivity for the CB_2 receptor. A-796260 exhibits full or near full agonist efficacy in *in-vitro* functional assays and is fully effective in a range of pre-clinical models of pathological pain including models of chronic inflammatory pain, chronic neuropathic pain and moderate to severe post-operative pain. The analgesic effects of A-796260 are selectively blocked by CB_2 antagonists and

Table 4. Receptor Affinities of 1-Alkyl-3-Keto Indoles

Compound	CB_1/ K_i (nM)	CB_2/ K_i (nM)	CB_1/CB_2
36	2100	0.77	2700
37	1100	1.9	555
38	1100	2.4	469
39	950	2.7	351
40	340	0.49	694
41	730	2.5	292
42	230	0.18	1278
43	>1000	2.1	>490
44	210	0.23	913
45	1050	3.0	350
46	600	2.7	222
47	1270	3.0	423
48	3160	5.3	596

[a]Ref. 73.

not CB_1 antagonists supporting a CB_2 mediated mechanism of action. Unlike AM1241, the analgesic effects of A-796260 are not blocked by the μ-opioid antagonist naloxone.

Variations in the nitrogen side chains resulted in the production of a number of highly selective ligands for the CB_2 receptor including aminoalkylindole agonists **37** and **38** with 555 and 469-fold selectivity respectively. Very high affinities at the CB_2 receptor were observed for the tetrahydrofuran analogues (**39**, K_i = 2.7 nM, Table **4**) and (**40**, K_i = 0.49 nM, Table **4**) with 351 and 694-fold selectivity, respectively. Oxazolidinone analogue **42** has combined extremely high affinity and selectivity at the CB_2 receptor with K_i = 0.18 nM and 1278-fold selectivity. Hydroxyalkyls (**43**, K_i = 2.1 nM, Table **4**) and (**44**, K_i = 0.23 nM, Table **4**) also exhibit high affinity and >490- and 913-fold selectivity for the CB_2 receptor, respectively.

Small substituents on the indole ring maintain potent CB_2 affinity and have a significant impact on selectivity as is exhibited by indoles **45**, **46**, **47** and **48** [Fig. (**10**), Table **4**] which have 350, 222, 423 and 596-fold selectivity for the CB_2 receptor, respectively.

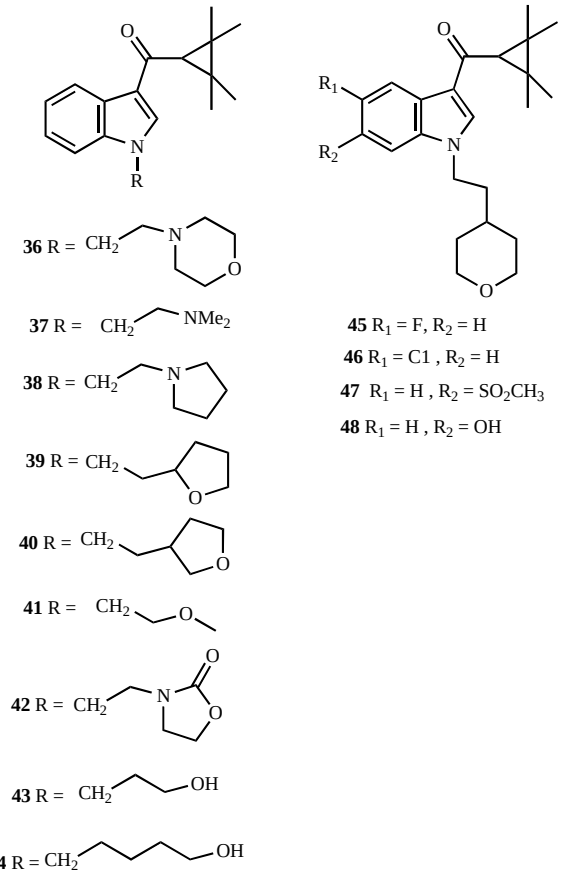


Fig. (10). Structures of 1-alkyl-3-keto indoles.

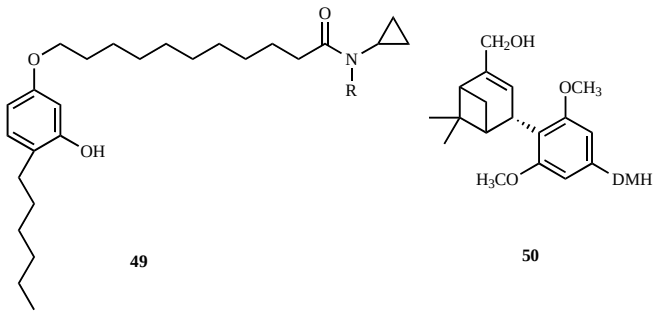


Fig. (11). Structures of resorcinol derivatives.

OTHER STRUCTURAL CLASSES

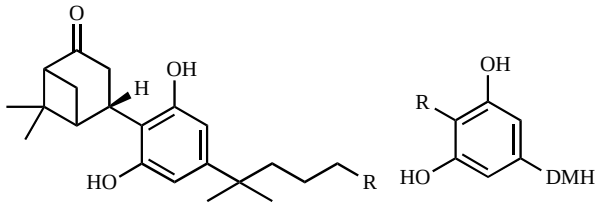
Brizzi *et al.* synthesized a variety of potent cannabinoid ligands that are resorcinol derivatives [74]. The investigation was primarily aimed at the synthesis of a series of compounds that retained the rigid traditional cannabinoid structure combined with a flexible portion similar to that of anandamide. Some of these compounds have high affinity for both cannabinoid receptors, however, only 11-(3-hydroxy-4-hexylphenoxy)undecanoic acid cyclopropylamide [**49**, Fig. (**11**)] showed significant selectivity for the CB_2 receptor over the CB_1 receptor. Compound **49** has >25-fold selectivity for the CB_2 receptor with K_i = 0.35 μM at CB_2 and K_i >10 μM at CB_1. Brizzi's hypothesis is that active cannabinoid receptor ligands can be obtained by linking a stable and rigid structure typical of aromatic compounds such as THC, olivetol and its analogues to a flexible chain carrying an amide "head" as in anandamide [74]. This hypothesis is apparently valid and led to the synthesis of a new structural class of potent cannabinoid receptor ligands.

Bicyclic CB_2 selective ligands based on the structure of HU308 (**50**, Fig. (**11**), Table **5**), a highly selective CB_2 ligand, have been reported by Makriyannis *et al.* and Pharmos [75-77]. Compounds such as **51**, **52**, **53** and AM1703 (**54**) shown in Fig. (**12**) exhibit very high affinity for the CB_2 receptor (1.8 to 0.006 nM) as well as very high

Table 5. Receptor Affinities of Resorcinol Derivatives

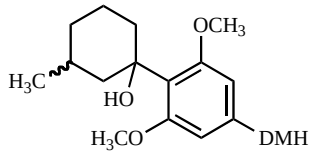
Compound	CB_2/ K_i (nM)	CB_1/CB_2
51	1.8[a]	234
52	1.55[a]	146
53	0.36[a]	139
54, AM-1703	0.006[a]	500
56, O-1966A	23[b]	220
50, HU-308	22.7[a]	440

[a]Ref. 75, 76, 77; [b]Ref. 78.



55

51 R = CH_3
52 R = CH_2CN
53 R = CH_2Br
54 R = $CH_2C{\equiv}CH$



56

Fig. (12). Structures of resorcinol derivatives.

selectivity for the receptor ranging from 139-500 fold selectivity. Receptor affinity data for these compounds are summarized in Table **5**.

A number of resorcinol derivatives have also been described by Wiley *et al.* [78]. Some of these compounds have selectivity for the CB_2 receptor ranging from 14-50 fold, however, as was the case with Brizzi's compounds, the most highly selective ligands for the CB_2 receptor also had significantly high affinity for the CB_1 receptor. Two series of compounds were prepared (**55**, Fig. (**12**), R = various cycloalkyl and heterocyclic groups) in which the phenolic hydroxyl groups were not derivatized and all but two of the compounds have a 1,1-dimethylheptyl group at C-5 of the resorcinol. The cyclopentyl analogue [**55**, R = cyclopentyl, Fig. (**12**)] with 14-fold CB_2 selectivity (K_i = 95±6 nM at CB_1 and K_i = 7±0.4 nM at CB_2) and the isomeric mixture of *cis*- and *trans*-4-phenylcyclohexyl derivatives [**55**, R = 4-phenylcyclohexyl, Fig. (**12**)] with 16-fold CB_2 selectivity (K_i = 144±22 nM at CB_1 and K_i = 9±2 nM at CB_2) have a combination of modest affinity for the CB_1 receptor and weak activity *in vivo*.

Two series of resorcinol dimethyl ethers were also reported by Wiley *et al.* One of these compounds, O-1966A [**56**, Fig. (**12**)] has very high, 220-fold, selectivity for the CB_2 receptor combined with very low affinity for the CB_1 receptor (K_i = 5,055±984 nM at CB_1 and K_i = 23±2.1 nM at CB_2).

In 2003 Mussinu *et al.* described several highly CB_2 selective tricyclic pyrazoles based upon CB_1 antagonists SR141716A [**34**, Rimonabant®, Fig. (**9**)] [79] and CB_2 inverse agonist SR144528 [**8**, Fig. (**3**)] [80-82]. These pyrazoles are among the most highly selective ligands for the CB_2 receptor to be reported to date, with selectivity ranging from 32-fold [**57**, R = OCH_3, Fig. (**13**)] to 9810-fold [**57**, R = CH_3, Fig. (**13**)]. Selectivity decreases drastically with the introduction of an iodide or methoxy substituent at C-6 of the aryl group and also with the lack of a substituent at this position (Table **6**).

Table 6. Receptor Affinities of Tricyclic Pyrazole Analogues

Compound 57	CB_1/ K_i (nM)	CB_2/ K_i (nM)	CB_1/CB_2
R = Cl	2050 ± 90	0.34 ± 0.06	6029
R = F	1268 ± 0.02	0.225 ± 0.02	5635
R = Br	1570 ± 15	0.27 ± 0.02	5814
R = I	333 ± 0.5	5.5 ± 0.5	60
R = H	1152 ± 65	0.385 ± 0.04	2992
R = CH_3	363 ± 30	0.037 ± 0.003	9810
R = OCH_3	399 ± 24	12.3 ± 1	32

[a]Ref. 82.

Iwamura *et al.* have synthesized several 2-oxoquinoline analogues with exceptionally high selectivity for the CB_2 receptor [83]. JTE-907 [**58**, Fig. (**13**)] in particular, is described as an inverse agonist *in vitro* for the CB_2 receptor and possesses anti-inflammatory properties *in vivo*. JTE-907 has modest affinity (K_i = 35.9 nM) for the CB_2 receptor, with an exceptionally high selectivity of 2,760 fold at this receptor. Structural variations in these 2-oxoquinolines include an *N*-methyl substituent at the quinoline ring, variable alkoxy substituents in the 6, 7 and 8 positions of the quinoline moiety and substituents at the 3-position that vary from carboxylic acid groups to various amide and ester groups. Compound **59** [Fig. (**13**)] in particular has very high CB_2 receptor affinity (K_i = 0.014 nM) and extremely high selectivity of 262,202-fold for this receptor.

Recently Raitio *et al.* synthesized a new series of CB_2 inverse agonists with responses comparable to SR144528 [**8**, Fig. (**3**)], based on the structure of 2-oxoquinoline JTE-907 [**58**, Fig. (**13**)] in which the piperonylamide end is replaced with a variety of aromatic amide structures [**60**-**67**, Fig. (**13**)] [84]. These researchers determined the CB_2 receptor activities of the ligands using a functional assay monitoring G protein activation, assessed by [^{35}S]GTPγS binding to

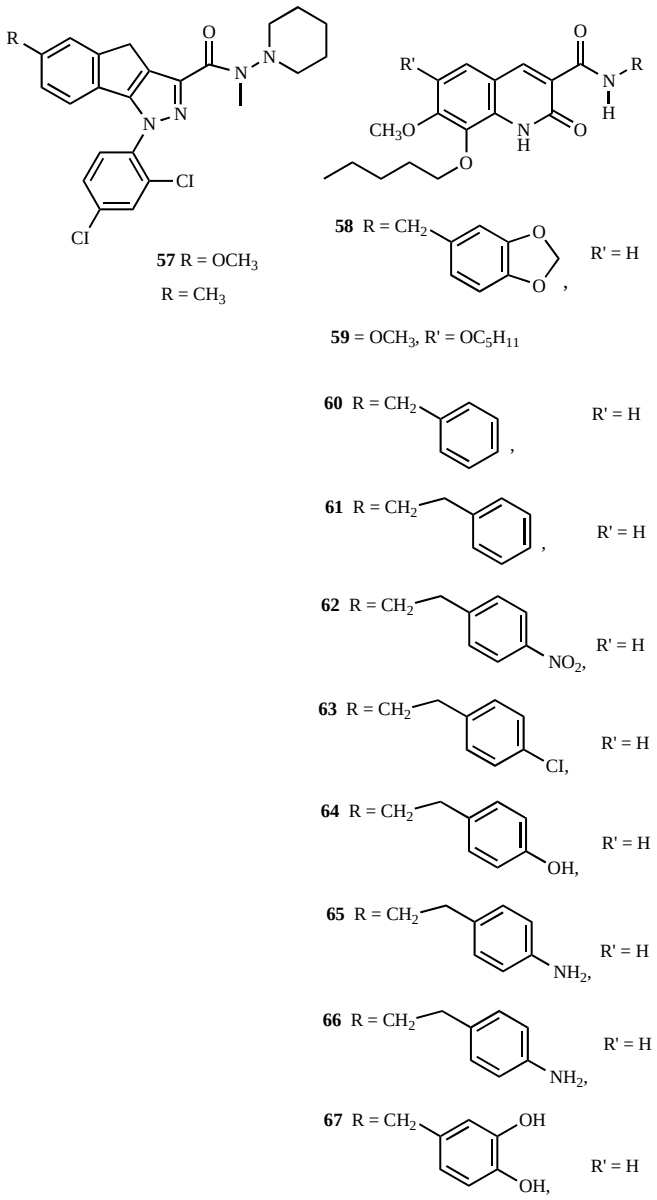


Fig. (13). Structures of tricyclic pyrazole and 2-oxoquinoline derivatives.

CHO cell membranes stably expressing the human CB_2 receptor (hCB_2). All compounds maintained efficacy relative to JTE-907, however there were differences in potency. Compound **61**, the unsubstituted phenylethylamide exhibited significantly higher potency (IC_{50} = 9.2±0.4) than the benzylamide derivative **60** (IC_{50} = 7.2±0.4). The effect of a phenyl ring *para*-substituent on CB_2 activity was investigated, which determined that only a nitro-substituent (**62**, IC_{50} = 7.0±0.7), but not a chloro (**63**, IC_{50} = 8.3±0.2), hydroxy (**64**, IC_{50} = 8.0±0.4) or amino-substituent (**65**, IC_{50} = 8.5±0.3) decreased potency compared to compound **61.** Catecholamides (**66** and **67**) both exhibit lower potencies (IC_{50} = 6.4±0.5 and 5.4±0.3, respectively) compared to SR144528.

Innovet Italia described the synthesis of a number of covalent amide derivatives of dicarboxylic and monocarboxylic acids [**68**, **69**, **70, 71,** Fig. (**14**)], which are CB_2 selective agonists and have the added benefit of possessing the desired lipid/water partition coefficient and solubility properties suitable for interactions with biological membranes [85]. These covalent amide derivatives are stable, highly lipophilic in nature and are rapidly absorbed both at the gastrointestinal and topical levels. The lipophilic characteristics of these ligands were evaluated by the determination of the partition parameter RM between the lipid and the aqueous phase, extrapolated from thin-film partition chromatography. Selectivity of these compounds for the CB_2 receptor ranges from >1600 to >15000 fold. In particular compound **69** has extremely high selectivity of >15,000 fold for the CB_2 receptor (K_i = 1 nM). Compounds **68, 70** and **71** have high affinity for the CB_2 receptor with K_i = 2, 6 and 9 nM combined with extremely good selectivity at this receptor, >7500, >2500 and >1600 fold, respectively.

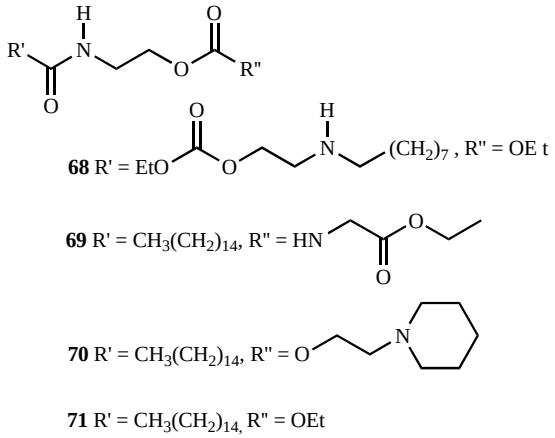


Fig. (14). Structures of monocarboxylic and dicarboxylic acid derivatives.

Scientists at Schering-Plough recently reported the synthesis of a new class of CB_2 receptor selective triaryl bissulfones as inverse agonists at the hCB_2 receptor [86]. These sulfones are promoted as potential immunomodulatory agents for the treatment of a broad range of acute and chronic inflammatory disorders. In particular, Sch.336 [**72**, Fig. (**15**)] is a CB_2 receptor selective inverse agonist which inhibits leukocyte trafficking in numerous rodent *in vivo* models, when administered orally. Sch.336 exhibits excep-

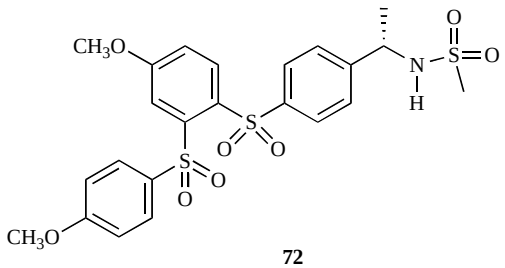


Fig. (15). Structure of Sch. 336.

tionally high selectivity for the CB_2 receptor with 4633-fold selectivity (K_i = 0.3 nM at CB_2 and K_i = 1390 nM at CB_1).

Kai *et al.* and Hanasaki *et al.* [87, 88] have reported the synthesis of 1,3-thiazine derivatives [**73**, **74** and **75** Fig. (**16**)] with either antagonistic or agonist properties, some of which have significant CB_2 receptor selectivity. In this series the 1,3-thiazine-3-carbothioic acid esters are particularly interesting. These compounds exhibit high affinity for the CB_2 receptor and exceptional CB_2 selectivity ranging from 2273-16,667 fold [87]. Compound **73** exhibits exceptionally high (16,667-fold) selectivity for the CB_2 receptor as well as very high affinity K_i = 0.3 nM.

Huffman *et al.* [89] reported the synthesis of several new 1-alkyl-2-aryl-4-(1-naphthoyl)pyrroles [**76,** Fig. (**16**)] with high affinity for both CB_1 and CB_2 receptors. *N*-alkyl substituents vary from *n*-propyl to *n*-heptyl with a 2-phenyl substituent on the pyrrole ring. JWH-372 [**76**, R = C_5H_{11}, Ar = *o*-trifluoromethylphenyl, Fig. (**16**)] has the highest selectivity for the CB_2 receptor, 9-fold (CB_1, K_i = 77±2 nM and CB_2, K_i = 8.2±0.2 nM) in this class. Negligible variation in CB_2 receptor affinities is observed for these compounds with K_i values ranging from 3.4 to 71 nM. An *ortho*, *meta* or *para* substituent on the phenyl ring has a significant impact on CB_1 receptor affinity. Small *ortho* electron-releasing substituents slightly enhance CB_1 receptor affinity. An inductively electron-withdrawing, but electron releasing by resonance, fluoro or chloro substituent also enhances CB_1 receptor affinity. Larger or strongly electron-withdrawing groups attenuate affinity. With the exception of fluorine, a *meta* or *para* substituent diminishes CB_1 receptor affinity, however, a *p*-ethyl or *p*-butyl phenyl group has only a slight effect. This variation in CB_1 receptor affinities appears to be due to a subtle combination of steric and electronic effects.

New 1,8-naphthyridin-4(1*H*)-on-3-carboxamide [**77**, Fig. (**17**)] and quinolin-4(1*H*)-on-3-carboxamide [**78** and **79**, Fig. (**17**)] derivatives have been synthesized by Manera *et al.* [90] based on published docking studies by Tuccinardi *et al.* [91]. In particular, 1,8-naphthyridine-4-one derivative **77** consisting of *p*-fluorobenzyl and carboxycycloheptylamide substituents exhibited very high affinity for the CB_2 receptor with K_i = 1±0.1 nM. Compound **77** however, also has high affinity for the CB_1 receptor with K_i = 4.3±0.6 nM and so is not significantly CB_2 selective. Quinoline-4-one derivative **79** has combined high affinity and good selectivity with K_i = 3.3±0.4 nM and >303-fold selectivity at the CB_2 receptor. Compound **78** with an *N*-benzyl substituent on the quinoline-4-one moiety also exhibited the desired combination of high affinity and selectivity with K_i value of 4.8±0.4 nM and >210-fold selectivity for the CB_2 receptor. Based on docking studies [91], it has been suggested that interaction of the chlorine atom in position-7 of compound **79** with the non-conserved residue S6.58(268) in the CB_2 receptor may be one of the reasons for the high CB_2 selectivity observed. [^{35}S]GTPγS binding assay and functional studies on human basophils indicate that the 1,8-naphthyridin-4(1*H*)-on-3-carboxamide derivatives behave as CB_1 and CB_2 agonists.

CONCLUSION

The almost exclusive distribution of the CB_2 receptor in tissues of the immune system makes this receptor an ideal target for therapeutic immune intervention and has promoted widespread interest in the generation of CB_2 selective ligands. The discovery of the CB_2 receptor has generated considerable interest in the scientific community with emphasis on determining the physiological role of this receptor.

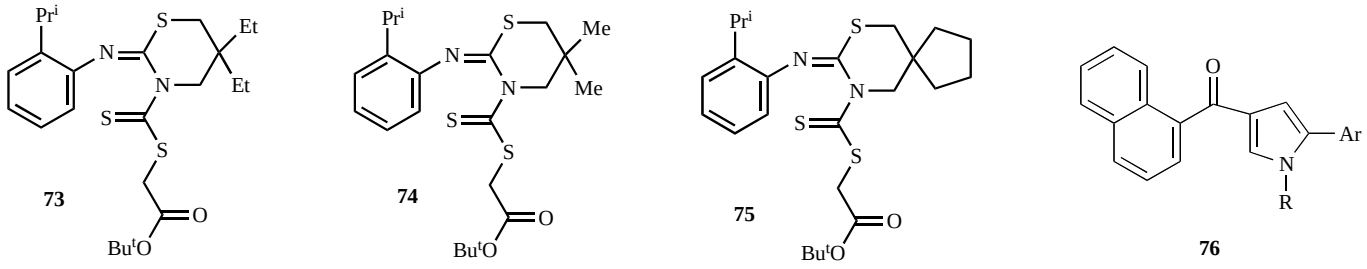


Fig. (16). Structures of 1,3-thiazine derivatives and 1-alkyl-2-aryl-4-(1-naphthoyl)pyrroles.

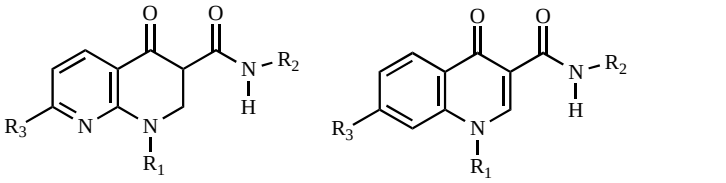


Fig. (17). Structures of 1,8-naphthyridine-4-one and quinoline-4-one derivatives.

This review has made mention of numerous CB_2 selective ligands from a variety of structural classes, inclusive of traditional cannabinoids, indoles, resorcinol derivatives, covalent amides of carboxylic acids, 1,3-thiazine derivatives and triaryl bis-sulfones with selectivity for the CB_2 receptor ranging from modest to extremely selective. Several highly selective traditional cannabinoids have been synthesized including some very selective 1-deoxy-Δ^8-THC analogues, JWH-133 [**6**, Fig. (**2**)], JWH-065 [**14**, R= C_4H_9, Fig. (**5**)] and JWH-056 [**13**, Fig. (**5**)]. The (2'*R*)- and (2'*S*)-1-methoxy-3-(2'-methylalkyl)-Δ^8-THC series recently synthesized by Huffman *et al.* has produced a highly selective traditional cannabinoid JWH-359 [**19**, Fig. (**6**)].

Researchers at Abbott Laboratories recently presented the synthesis of a series of 1-alkyl-3-keto indoles [Fig. (**10**), Table **4**], with the most selective ligand from this new series possessing a tetramethylcyclopropyl substituent at C-3 [A-796260, **36**, Fig. (**10**)]. A-796260 is a novel high affinity ligand for the human and rat CB_2 receptor with very high selectivity (2700-fold) for the CB_2 receptor and exhibits full or near full agonist efficacy in *in-vitro* functional assays.

Mussina *et al.* have described the synthesis of highly selective tricyclic pyrazoles in particular **57** [R = CH_3, Fig. (**13**)] with 9810-fold selectivity for the CB_2 receptor. Numerous CB_2 selective covalent amide derivatives of dicarboxylic and monocarboxylic acids [**68-71**, Fig. (**14**)] were synthesized by Iwamura *et al.* and have extremely high selectivity for the CB_2 receptor ranging from >1600 to >15000 fold. Raitio *et al.* synthesized a new series of CB_2 inverse agonists based on the structure of the 2-oxoquinoline JTE-907 [**58**, Fig. (**13**)] in which the piperonylamide end was replaced by a variety of aromatic amide structures [**60-67**, Fig. (**13**)]. A highly selective triaryl bis-sulfone, Sch.336 [**72**, Fig. (**15**)] was recently synthesized by Schering-Plough and suggested as a potential immunomodulatory agent for the treatment of a broad range of acute and chronic inflammatory disorders. The 1,3-thiazine-3-carbothioic acid ester [**73**, Fig. (**16**)] synthesized by Kai *et al.* is one of the most selective ligands to be reported with a selectivity of 16,667 fold for the CB_2 receptor. The newly synthesized 1,8-naphthyridin-4(1*H*)-on-3-carboxamide [**77**, Fig. (**17**)] and quinolin-4(1*H*)-on-3-carboxamide [**78** and **79**, Fig. (**17**)] derivatives reported by Manera *et al.* have added to the currently available SAR for cannabinoid agonist ligands, expanding research efforts towards the development of CB_2 receptor selective ligands. Although quite a number of CB_2 receptor selective ligands have been documented it is still not possible to determine SAR for structural classes such as indoles, pyrazoles and resorcinols.

The diverse structural features of CB_2 selective ligands suggest that there are different binding sites at the CB_2 receptor enabling ligand-receptor interactions. Salo *et al.* performed docking studies using four CB_2 receptor ligands including CP-55,940 [**2**, Fig. (**1**)] and WIN-55,212-2 [**3**, Fig. (**1**)] and concluded from their model of the CB_2 receptor that hydrophobic and aromatic stacking interactions were more important than hydrogen bonding.

As mounting evidence suggests the CB_2 receptor has an important physiological role and potential application in immune therapy, the next few years should see increased studies concerning this receptor. There remains much work ahead to determine the complex manner in which CB_2 receptors impact the immune system, tumor cells and inflammatory responses. Investigations employing PEA have suggested the possible existence of another cannabinoid receptor that is expressed in immune cells. The signal transduction pathway involving PEA is not clearly understood and more research will be necessary to determine the role of PEA in the activation of CB_2 receptors or any other cannabinoid receptors located in immune cells.

Synthesis of additional CB_2 selective ligands is extremely important to achieving an understanding of the signal transduction pathway and ultimately an understanding of the requirements for various structural classes in the development of potential immunotherapeutic agents.

ABBREVIATIONS

AAI	=	Aminoalkylindole
cAMP	=	Cyclic Adenosine Monophosphate
CHO cells	=	Chinese Hamster Ovary Cells
GTPγS	=	Guanosine-5'-(γ-thio)-triphosphate
MAPK	=	Mitogen-Activated Protein Kinase
mRNA	=	Messenger Ribonucleic Acid
MVD	=	Mouse Vas Deferens
PEA	=	Palmitoylethanolamide
PTX	=	Pertussis Toxin
THC	=	Tetrahydrocannabinol

REFERENCES

[1] Huffman, J. W. CB_2 Receptor ligands. *Mini Rev. Med. Chem.* **2005**, *5*, 641-649.

[2] Gaoni, Y.; Mechoulam, R. Hashish. III. Isolation, structure, and partial synthesis of an active constituent of hashish. *J. Am. Chem. Soc.* **1964**, *86*, 1646-1647.

[3] Razdan, R. K. Structure-activity relationships in cannabinoids. *Pharmacol. Rev.* **1986**, *38*, 75-149.

[4] Devane, W. A.; Dysarz, F. A.; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.* **1988**, *34*, 605-613.

[5] Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. H. Structures of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **1990**, *346*, 561-564.

[6] Gérard, C. M.; Mollereau, C.; Vassart. G.; Parmentier, M. Molecular cloning of a human cannabinoid receptor which is also expressed in testis. *Biochem. J.* **1991**, *279*, 129-134.

[7] Howlett, A. C.; Barth, F.; Bonner, T. I.; Cabral, G.; Casellas, P.; Devane, W. A.; Felder, C. C.; Herkenham, M.; Mackie, K.; Martin, B. R.; Mechoulam, R.; Pertwee, R. G. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol. Rev.* **2002**, *54*, 161-202.

[8] Munro, S.; Thomas, K. L.; Abu-Shar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **1993**, *365*, 61-65.

[9] Bouaboula, M.; Rinaldi, M.; Carayon, P.; Carillon, C.; Delpech, B.; Shire, D.; Le Fur, G.; Casellas, P. Cannabinoid receptor expression in human leukocytes. *Eur. J. Biochem.* **1993**, *214*, 173-180.

[10] Facci, L.; Dal Taso, R.; Romanello, S.; Buriani, A.; Skaper, S. D.; Leon, A. Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 3376-3380.

[11] Galiègue, S.; Mary, S.; Marchand, J.; Dussossoy, D.; Carrière, D.; Carayon, P.; Bouaboula, M.; Shire, D.; Le Fur, G.; Casellas, P.

Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte sub-populations. *Eur. J. Biochem.* **1995**, *232*, 54-61.

[12] Condie, R.; Herring, A.; Koh, W. S.; Lee, M.; Kaminski, N. E. Cannabinoid inhibition of adenylate cyclase-mediated signal transduction and interleukin 23 (IL-2) expression in the murine T-cell line, EL4.IL-2. *J. Biol. Chem.* **1996**, *271*, 13175-13183.

[13] Pettit, D. A.; Anders, D. L.; Harrison, M. P.; Cabral, G. A. Cannabinoid receptor expression in immune cells. *Adv. Exp. Med. Biol.* **1996**, *402*, 119-129.

[14] Schatz, A. R.; Lee, M.; Condie, R. B.; Pulaski, J. T.; Kaminski, N. E. Cannabinoid receptor CB_1 and CB_2: a characterization of expression and adenylate cyclase modulation within the immune system. *Toxicol. Appl. Pharmacol.* **1997**, *142*, 278-287.

[15] Huffman, J. W. The search for selective ligands for the CB2 receptor. *Curr. Pharm. Design* **2000**, *6*, 1323-1337.

[16] Felder, C. C.; Joyce, K. E.; Briley, E. M.; Mansouri, J.; Mackie, K.; Blond, O.; Lai, Y.; Ma, A. L.; Mitchell, R. L. Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. *Mol. Pharmacol.* **1995**, *48*, 443-450.

[17] Showalter, V. M.; Compton, D. R.; Martin, B. R.; Abood, M. E. Evaluation of binding in a transfected cell line expressing a peripheral cannabinoid receptor (CB2): identification of cannabinoid receptor subtype selective ligands. *J. Pharmacol. Exp. Ther.* **1996**, *278*, 989-999.

[18] Salo, O. M. H.; Raitio, K. H.; Savinainen, J. R.; Nevalainen, T.; Lahtela-Kakkonen, M.; Laitinen, J. T.; Jarvinen, T.; Poso, A. Virtual screening of novel CB2 ligands using a comparative model of the human cannabinoid CB2 receptor. *J. Med. Chem.* **2005**, *48*, 7166-7171.

[19] Tao, Q.; Abood, M. E. Mutation of a highly conserved aspartate residue in the second transmembrane domain of the cannabinoid receptors, CB1 and CB2, disrupts G-protein coupling. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 651-658.

[20] Tao, Q.; McAllister, S. D.; Andreassi, J.; Nowell, K. W.; Cabral, G. A.; Hurst, D. P.; Bachtel, K.; Ekman, M. C.; Reggio, P. H.; Abood, M. E. Role of a conserved lysine residue in the peripheral cannabinoid receptor (CB_2): Evidence for subtype specificity. *Mol. Pharmacol.* **1999**, *55*, 605-613.

[21] Rhee, M-H.; Nevo, I.; Bayewitch, M. L.; Zagoory, O.; Vogel, Z. Functional role of tryptophan residues in the fourth transmembrane domain of the CB_2 cannabinoid receptor. *J. Neurochem.* **2000**, *75*, 2485-2491.

[22] Gallant, M.; Dufresne, C.; Gareau, Y.; Guay, D.; Leblanc, Y.; Prasit, P.; Rochette, C.; Sawyer, N.; Slipetz, D. M.; Tremblay, N.; Metters, K. M.; Labelle, M. New class of potent ligands for the human peripheral cannabinoid receptor. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2263-2268.

[23] Huffman, J. W.; Liddle, J.; Yu, S.; Aung, M. M.; Abood, M. E.; Wiley, J. L.; Martin, B. R. 3-(1',1'-Dimethylbutyl)-1-deoxy-Δ^8-THC and related compounds: synthesis of selective ligands for the CB2 receptor. *Bioorg. Med. Chem.* **1999**, *7*, 2905-2914.

[24] Huffman, J. W.; Bushell, S. M.; Miller, J. R. A.; Wiley, J. L.; Martin, B. R. 1-Methoxy-, 1-deoxy-11-hydroxy- and 11-Hydroxy-1-methoxy-Δ^8-tetrahydrocannabinols: new selective ligands for the CB2 receptor. *Bioorg. Med. Chem.* **2002**, *10*, 4119-4129.

[25] Compton, D. R.; Rice, K. C.; De Costa, B. R.; Razdan, R. K.; Melvin, L. S.; Johnson, M. R.; Martin, B. R. Cannabinoid structure-activity relationships: Correlation of receptor binding and *in vivo* activities. *J. Pharmacol. Exp. Ther.* **1993**, *265*, 218-226.

[26] D'Ambra, T. E.; Estep, K. G.; Bell, M. R.; Eissenstat, M. A.; Josef, K. A.; Ward, J.; Haycock, D. A.; Baizman, E. R.; Casiano, F. M.; Beglin, N. C.; Chippari, S. M.; Grego, J. D.; Kulling, R. K.; Daley, G. T. Conformationally restrained analogs of pravadoline: nanomolar potent, enantioselective, (aminoalkyl)indole agonists of the cannabinoid receptor. *J. Med. Chem.* **1992**, *35*, 124-135.

[27] Busch-Peterson, J.; Hill, W. A.; Fan, P.; Khanolkar, A.; Xie, X.-Q.; Tius, M. A.; Makriyannis, A. Unsaturated Side Chain β-11-Hydroxyhexahydrocannabinol Analogs. *J. Med. Chem.* **1996**, *39*, 3790-3796.

[28] Pertwee, R. G. The evidence for the existence of cannabinoid receptors. *Gen. Pharmacol.* **1993**, *24*, 811-824.

[29] Selley, D. E.; Stark, S.; Sim, L. J.; Childers, S. R. Cannabinoid receptor stimulation of guanosine-5'-O-(3-[35S]thio)triphosphate binding in rat brain membranes. *Life Sci.* **1996**, *59*, 659-668.

[30] Little, P. J.; Compton, D. R.; Johnson, M. R.; Melvin, L. S.; Martin, B. R. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J. Pharmacol. Exp. Ther.* **1988**, *247*, 1046-1051.

[31] Howlett, A. C.; Fleming, R. M. Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. *Mol. Pharmacol.* **1984**, *26*, 532-538.

[32] Mackie, K.; Hille, B. Cannabinoids inhibit N-type calcium chammels in neuroblastoma-glioma cells. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 3825-3829.

[33] Bouaboula, M.; Poinot-Chazel, C.; Marchand, J.; Canat, X.; Bourrié, B.; Rinaldi-Carmona, M.; Calandra, B.; Le Fur, G.; Casellas, P. Signaling pathway associated with stimulation of CB_2 peripheral cannabinoid receptor. Involvement of both mitogen-activated protein kinase and induction of Krox-24 expression. *Eur. J. Biochem.* **1996**, *237*, 704-711.

[34] Gonsiorek, W.; Lunn, C.; Fan, X.; Narula, S.; Lundell, D.; Hipkin, R. W. Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. *Mol. Pharmacol.* **2000**, *57*, 1045-1050.

[35] Slipetz, D. M.; O'Neil, G. P.; Favreau, L.; Dufresne, C.; Gallant, M.; Gareau, Y.; Guay, D.; Labelle, M.; Metters, K. M. Activation of the human peripheral cannabinoid receptor results in inhibition of adenylyl cyclase. *Mol. Pharmacol.* **1995**, *48*, 352-361.

[36] Sanchez, C., Galve-Roperh, I., Canova, C., Brachet, P.; Guzman, M. Δ^9-Tetrahydrocannabinol induces apoptosis in C6 glioma cells. *FEBS Lett.* **1998**,*436*, 6-10.

[37] Sanchez, C.; de Ceballos, M. L.; Gomez del Pulgar, T.; Rueda, D.; Corbacho, C.; Velasco, G.; Galve-Roperh, I.; Huffman, J. W.; Ramon y Cajal, S.; Guzman, M. Inhibition of glioma growth *in vivo* by selective activation of the CB2 cannabinoid receptor. *Cancer Res.* **2001**, *61*, 5784-5789.

[38] Galve-Roperh, I.; Rueda, D.; Gomez Del Pulgar, T.; Velasco, G.; Guzman, M. Mechanism of extracellular signal-regulated kinase activation by the CB1 cannabinoid receptor. *Mol. Pharmacol.* **2002**, *62*, 1385-1392.

[39] Galve-Roperh, I.; Sanchez, C.; Cortes, M. L.; Del Pulgar, T. G.; Izquierdo, M.; Guzman, M. Anti-tumoral action of cannabinoids: involvement of sustained ceramide accumulation and extracellular signal-regulated kinase activation. *Nature Med.* **2000**, *6*, 313-319.

[40] Walter, L.; Franklin, A.; Witting, A.; Wade, C.; Xie, Y.; Kunos, G.; Mackie, K.; Stella, N. Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *J. Neurosci.* **2003**, *23*, 1398-1405.

[41] Klegeris, A.; Bissonette, C. J.; McGreer, P. L. Reduction of human monocytic cell neurotoxicity and cytokine secretion by ligands of the cannabinoid-type CB_2 receptor. *Br. J. Pharmacol.* **2003**, *139*, 775-786.

[42] Lambert, D. M.; Vandervoorde, S.; Jonsson, K-O.; Fowler, C, J. The palmitoylethanolamide family: A new class of anti-inflammatory agents? *Curr. Med. Chem.* **2002**, *9*, 663-674.

[43] Calignano, A.; La Rana, G.; Giuffrida, A.; Piomelli, D. Control of pain initiation by endogenous cannabinoids. *Nature* **1998**, *394*, 277-281.

[44] Jaggar, S. I.; Hasnie, F. S.; Sellaturay, S.; Rice, A. S. The anti-hyperalgesic actions of the cannabinoid anandamide and the putative CB_2 receptor agonist palmitoylethanolamide in visceral and somatic inflammatory pain. *Pain* **1998**, *76*, 189-199.

[45] Griffin, G.; Tao, Q.; Abood, M. E. Cloning and pharmacological characterization of the rat CB_2 cannabinoid receptor. *J. Pharmacol. Exp. Ther.* **2000**, *292*, 886-894.

[46] Mackie, K.; Stella, N. Cannabinoid receptors and endocannabinoids: evidence for new players. *AAPS Journal* **2006**, *8*, 298-306.

[47] Church, M. K.; Levi-Schaffer, F. The human mast cell. *J. Allergy Clin. Immunol.* **1997**, *99*, 155-160.

[48] Benoist, C.; Mathis, D. Mast cells in autoimmune disease. *Nature* **2002**, *420*, 875-878.

[49] Puxeddu, I.; Piliponsky, A. M.; Bachelet, I.; Levi-Schaffer, F. Mast cells in allergy and beyond. *Int. J. Biochem. Cell Biol.* **2003**, *35*, 1601-1607.

[50] Jonsson, K-O.; Persson, E.; Fowler, C. J. The cannabinoid CB_2 receptor selective agonist JWH133 reduces mast cell edema in response to compound 48/80 *in vivo* but not the release of β-hexosaminidase from skin slices *in vitro*. *Life Sci.* **2006**, *78*, 598-606.

[51] Gareau, Y.; Dufresne, C.; Gallant, M.; Rochette, C.; Sawyer, N.; Slipetz, D. M.; Tremblay, N.; Weech, P. K.; Metters, K. M.; Labelle, M. Structure activity relationships of tetrahydrocanabinol analogs on human cannabinoid receptors. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 189-194.

[52] Huffman, J. W.; Bramblett, R. D.; Yu, S.; Showalter, V.; Abood, M. E.; Wiley, J. L.; Compton, D. R.;Martin, B. R.;Reggio, P. H. Synthesis and pharmacology of a very potent cannabinoid lacking a phenolic hydroxyl with high affinity for the CB2 receptor. *J. Med. Chem.* **1996**, *39*, 3875-3877.

[53] Huffman, J. W.; Zengin, G.; Wu, M.-J.; Lu, J.; Hynd, G.; Bushell, K.; Thompson, A. L. S.; Bushell, S.; Tartal, C.; Hurst, D. P.; Reggio, P. H.; Selley, D. E.; Cassidy, M. P.; Wiley, J. L.; Martin, B. R. Structure-activity relationships for 1-alkyl-3-(1-naphthoyl) indoles at the cannabinoid CB(1) and CB(2) receptors: steric and electronic effects of naphthoyl substituents. New highly selective CB(2) receptor agonists. *Bioorg. Med. Chem.* **2005**, *13*, 89-112.

[54] Huffman, J. W.; Bushell, S. M.; Joshi, S. N.; Wiley, J. L.; Martin, B. R. Enantioselective Synthesis of 1-Methoxy- and 1-Deoxy-2'-methyl-Δ^8-tetrahydrocannabinols: New Selective Ligands for the CB_2 receptor. *Bioorg. Med. Chem.* **2006**, *14*, 247-262.

[55] Marriott, K. C.; Huffman, J. W.; Wiley, J. L.; Martin, B. R. Synthesis and pharmacology of 11-nor-1-methoxy-9-hydroxy-hexahydrocannabinols and 11-nor-1-deoxy-9-hydroxyhexhydro-cannabinols: New selective ligands for the cannabinoid CB_2 receptor. *Bioorg. Med. Chem.* **2006**, *14*, 2386-2397.

[56] Reggio, P. H.; Greer, K. V.; Cox, S. M. The importance of the orientation of the C9 substituent to cannabinoid activity. *J. Med. Chem.* **1989**, *32*, 1630-1635.

[57] Mahadevan, A.; Siegel, C.; Martin, B. R.; Abood, M. E.; Beletskaya, I.; Razdan, R. K. Novel Cannabinol Probes for CB1 and CB2 Cannabinoid Receptors. *J. Med. Chem.* **2000**, *43*, 3778-3785.

[58] Bell, M. R.; D'Ambra, T. E.; Kumar, V.; Eissanstat, M. A.; Herrmann, J. L.; Wetzel, J. R.; Rosi, D.; Philion, R. E.; Daum, S. J.; Hlasta, D. J.; Kullnig, R. K.; Ackerman, J. H.; Haubrich, D. R.; Luttinger, D. A.; Baizman, E. R.; Miller, M. S.; Ward, S. J. Antinociceptive (aminoalkyl)indoles. *J. Med. Chem.* **1991**, *34*, 1099-1110.

[59] D'Ambra, T. E.; Estep, K. G.; Bell, M. R.; Eissanstat, M. A.; Josef, K. A.; Ward, J.; Haycock, D. A. Baizman, E. R.; Casiano, F. M.; Beglin, N. C.; Chippari, S. M.; Grego, J. D.; Kullnig, R. K.; Daley, G. T. Conformationally restrained analogs of pravadoline: nanomolar potent, enantioselective, (aminoalkyl)indole agonists of the cannabinoid receptor. *J. Med. Chem.* **1992**, *35*, 124-135.

[60] Huffman, J. W.; Dai, D.; Martin, B. R.; Compton, D. R. Design, synthesis and pharmacology of cannabimimetic indoles. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 563-566.

[61] Wiley, J. L.; Compton, D. R.; Dai, D.; Lainton, J. A. H.; Phillips, M.; Huffman, J. W.; Martin, B. R. Structure-activity relationships of indole- and pyrrole-derived cannabinoids. *J. Pharmacol. Exp. Ther.* **1998**, *285*, 995-1004.

[62] Huffman, J. W.; Wu, M.-J.; Lainton, J. A. H.; Dai, D.; Phillips, M.; Keel, C.; Wiley, J. L.; Compton, D. R.; Showalter, V.; Abood, M. E.; Martin, B. R. *1997 Symposium on the Cannabinoids*, International Cannabinoid Research Society; Burlington, VT, **1997**, p. 8.

[63] Huffman, J. W.; Padgett, L. W. Recent developments in the medicinal chemistry of cannabimimetic indoles, pyrroles and indenes. *Curr. Med. Chem.* **2005**, *12*, 1395-1411.

[64] New, D. C.; Wong, Y. H. BML-190 and AM251 act as inverse agonists at the human cannabinoid CB2 receptor: signalling *via* cAMP and inositol phosphates. *FEBS Lett.* **2003**, *536*, 157-160.

[65] Ross, R. A.; Brockie, H. C.; Stevenson, L. A.; Murphy, V. L.; Templeton, F.; Makriyannis, A.; Pertwee, R. G. Agonist-inverse agonist characterization at CB1 and CB2 cannabinoid receptors of L759633, L759656 and AM630. *Br. J. Pharmacol.* **1999**, *126*, 665-672.

[66] Pertwee, R. G. Pharmacology of cannabinoid receptor ligands. *Curr. Med. Chem.* **1999**, *6*, 635.

[67] Hosohata, K.; Quock, R. M.; Hosohata, Y.; Burkey, T. H.; Makriyannis, A.; Consroe, P.; Roeske, W. R.; Yamamura, H. I. AM630 is a competitive cannabinoid receptor antagonist in the guinea pig brain. *Life Sci.* **1997**, *61*, 115-118.

[68] Landsman, R. S.; Makriyannis, A.; Deng, H.; Consroe, P.; Roeske, W. R.; Yamamura, H. I. AM630 is an inverse agonist at the human cannabinoid CB1 receptor. *Life Sci.* **1998**, *62*, 109.

[69] Ibrahim, M.; Deng, H.; Zvonok, A.; Cockayne, D. A.; Kwan, J.; Mata, H.; Vanderah, T. W.; Lai, J.; Porreca, F.; Makriyannis, A.; Malan, T. P. Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 10529-10533.

[70] Malan, T. P.; Ibrahim, M.; Deng, H.; Liu, Q.; Mata, H. P.; Vanderah, T.; Porreca, F.; Makriyannis, A. CB2 cannabinoid receptor-mediated peripheral antinociception. *Pain* **2001**, *93*, 239-245.

[71] Nackley, A. G.; Makriyannis, A; Hohmann, A. G. Selective activation of cannabinoid CB2 receptors suppresses spinal fos protein expression and pain behavior in a rat model of inflammation. *Neurosci.* **2003**, *119*, 747-757.

[72] Hynes, J.; Leftheris, K.; Wu, H.; Pandit, C.; Chen, P.; Norris, D. J.; Chen, B.-C.; Zhao, R.; Kiener, P. A.; Chen, X.; Turk, L. A.; Patil-Koota, V.; Gillooly, K. M.; Shuster, D. J.; McIntyre, K. W. Amido-Indole cannabinoid receptor modulators. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2399-2402.

[73] Dart, M.; Frost, J.; Tietje, K.; Daza, A.; Grayson, G.; Fan, Y.; Mukherjee, S.; Garrison, T. R.; Yao, B.; Meyer, M. 1-Alkyl-3-keto-indoles: Identification and *in vitro* characterization of a series of potent cannabinoid ligands. *2006 Symposium on the Cannabinoids*, International Cannabinoid Research Society; Burlington, VT, **2006**, p.125.

[74] Brizzi, A.; Brizzi, V.; Cascio, M. G.; Bisogno, T.; Sirianni, R.; Di Marzo, V. Design, Synthesis, and Binding Studies of New Potent Ligands of Cannabinoid Receptors. *J. Med. Chem.* **2005**, *48*, 7343-7350.

[75] Garzon, A.; Fink, G.; Dar, D. E.; Menashe, N.; Nudelman, A.; Greenberg, O. In *W.O. Patent 03/064359*, **2003**.

[76] Garzon, A.; Fink, G.; Dar, D.E.; Menashe, N.; Nudelman, A.; Greenberg, O. In *W.O. Patent 03/063758*, **2003**.

[77] Makriyannis, A.; Khanolkar, A. In *W.O. Patent 01/028497*, **2001**.

[78] Wiley, J. L.; Beletskaya, I. D.; Ng, E. W.; Dai, Z.; Crocker, P. J.; Mahadevan, A.; Razdan, R. K.; Martin, B. R. Resorcinol derivatives: a novel template for the development of cannabinoid CB1/CB2 and CB2-selective agonists. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 679-689.

[79] Rinaldi-Carmona, M.; Barth, F.; Heaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Neliat, G.; Caput, D.; Ferrara, P.; Soubrie, P.; Breliere, J.-C.; Le Fur, G. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* **1994**, *350*, 240-244.

[80] Rinaldi-Carmona, M.; Barth, F.; Millan, J.; Derocq, J. M.; Casellas, P.; Congy, C.; Oustric, D.; Sarran, M.; Bouaboula, M.; Calandra, B.; Portier, M.; Shire, D.; Breliere, J. C.; Le Fur, G. L. SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J. Pharmacol. Exp. Ther*. **1998**, *284*, 644-650.

[81] Shire, D.; Calandra, B.; Bouaboula, M.; Barth, F.; Rinaldi-Carmona, M.; Casellas, P.; Ferrara, P. Cannabinoid receptor interactions with the antagonists SR 141716A and SR 144528. *Life Sci.* **1999**, *65*, 627-635.

[82] Mussinu, J. M.; Ruiu, S.; Mule, A. C.; Pau, A.; Carai, M. A.; Loriga, G.; Murineddu, G.; Pinna, G. A. Tricyclic Pyrazoles. Part 1: Synthesis and Biological Evaluation of Novel 1,4-Dihydroindeno[1,2-c]pyrazol-based Ligands for CB1and CB2 Cannabinoid Receptors. *Bioorg. Med. Chem*. **2003**, *11*, 251-263.

[83] Iwamura, H.; Suzuki, H.; Ueda, Y.; Kaya, T.; Inaba, T. *In vitro* and *in vivo* pharmacological characterization of JTE-907, a novel selective ligand for cannabinoid CB2 receptor. *J. Pharmacol. Exp. Ther*. **2001**, *296*, 420-425.

[84] Raitio, K. H.; Savinainen, J. R.; Vepsalainen, J.; Laitinen, J. T.; Poso, A.; Jarvinen, T.; Nevalainen, T. Synthesis and SAR studies of 2-oxoquinoline derivatives as CB2 receptor inverse agonists. *J. Med. Chem.* **2006**, *49*, 2022-2027.

[85] Comelli, C.; Della Valle, F.; Della Valle, M.; Marcolongo, G. In *W.O. Patent 01/04083*, **2001**.

[86] Lunn, C. A.; Fine, J. S.; Rojas-Triana, A.; Jackson, J. V.; Fan, X.; Kung, T. T.; Gonsiorek, W.; Schwarz, M. A.; Lavey, B.; Kozlowski, J. A.; Narula, S. K.; Lundell, D. J.; Hipkin, R. W.; Bober, L. A. A novel cannabinoid CB_2 receptor-selective inverse

agonist blocks leukocyte recruitment *in vivo*. *J. Pharmacol. Exp. Ther*. **2006**, *316*, 780-788.
[87] Kai, H.; Murashi, T.; Tomida, M. In *W.O. Patent 02/072562*, **2002**.
[88] Hanasaki, K.; Murashi, T.; Kai, H. In *W.O. Patent 01/19807*, **2001**.
[89] Huffman, J. W.; Padgett, L. W.; Isherwood, M. L.; Wiley, J. L.; Martin, B. R. 1-Alkyl-2-aryl-4-(1-naphthoyl)pyrroles: New high affinity ligands for the cannabinoid CB_1 and CB_2 receptors. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5432-5435.
[90] Manera, C.; Benetti, V.; Castelli, P.; Cavallini, T.; Lazzarotti, S.; Pibiri, F.; Saccomanni, G.; Tuccinardi, T.; Vannacci, A.; Martinelli, A.; Ferrarini, P. L. Design, synthesis, and biological evaluation of new 1,8-naphthyridin-4(1*H*)-on-3-carboxamide and quinolin-4(1*H*)-on-3-carboxamide derivatives as CB_2 selective agonists. *J. Med. Chem.* **2006**, *49*, 5947-5957.
[91] Tuccinardi, T.; Ferrarini, P. L.; Manera, C.; Ortore, G.; Saccomanni, G.; Martinelli, A. Cannabinoid CB_2/CB_1 selectivity. Receptor modeling and automated docking analysis. *J. Med. Chem.* **2006**, *49*, 984-994.

Received: April 23, 2007 Accepted: July 6, 2007