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# Current Knowledge on the Antagonists and Inverse Agonists of Cannabinoid Receptors

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**Abstract:** Ten years elapsed since the discovery by Sanofi of SR141716A the first selective $CB_1$ cannabinoid receptor antagonist. Shortly after, Sanofi also reported the synthesis of the first selective $CB_2$ cannabinoid receptor antagonist, SR144528. Since these two milestones in the cannabinoid field, many other compounds, more or less related to the Sanofi compounds, or based on a completely different scaffold appeared. Several of these compounds are currently involved in clinical trials for diseases such as obesity, nicotine and alcohol addictions, or allergies. Further, the cannabinoid receptors knock-out mice production strengthened the hypothesis of the existence of several other "cannabinoid" receptors for which the first antagonists begin to appear. The large amount of patents taken by many different pharmaceutical companies prove, if necessary, the great therapeutic potential expected for the cannabinoid receptors antagonists.

**Keywords:** Cannabinoid, antagonism, inverse agonism, rimonabant, SR144528, SLV319.

## INTRODUCTION

For many years, pharmacological actions of plant derived cannabinoids were ascribed to membrane disruption effects, rather than to specific receptor-mediated interactions [1]. The development of synthetic high-affinity ligands made easier the discovery [2], and the cloning from the rat [3] and human [4] of the first cannabinoid receptor christened $CB_1$, for cannabinoid type 1 receptor. This receptor, highly expressed in the CNS, especially in the allocortex, the substantia nigra, the globus pallidus, and the cerebellum [5], is also present outside the CNS, like in the testis, ileum, urinary bladder, and vas deferens. Two splice variants of this receptor, called $CB_{1A}$ [6] and $CB_{1B}$ [7], have also been cloned from the human. The $CB_{1A}$ cannabinoid receptor exhibits all the properties of the $CB_1$ isoform [8], this is not the case for the 1B isoform, which essentially differs in the endocannabinoid binding [7]. However, their physiological and pharmacological significances remain, to date, unknown. Shortly after the cloning of the $CB_1$ receptor, a second cannabinoid receptor, the $CB_2$ cannabinoid receptor, was found by sequence homology analysis [9]. This receptor, sharing 44% homology with the $CB_1$ receptor, is mainly expressed in the immune system.

The cloning of these two receptors was the main milestone and since, cannabinoids became a widely explored field. These cannabinoid receptors are G-protein coupled receptors (GPCR), acting mainly through $G_{i/0}$-type G-proteins [10]. Even if a $G_s$ coupling was shown to occur with the $CB_1$ cannabinoid receptor by Glass *et al.* [11] and by Calandra *et al.* [12], the $G_{i/0}$ pathway seems to be the preferred one. The major cannabinoid signalling pathways described so far include the adenylyl cyclase inhibition [13], the inwardly rectifying potassium channels [14] and the voltage-dependent calcium channels [15], the Mitogen Activated Protein Kinase cascade [16,17], and the phosphokinase B pathway [18].

Consequently to the discovery of the receptors, were characterized their endogenous ligands, the so-called "endocannabinoids". Important representatives are arachidonylethanolamide (anandamide, AEA) [19] and 2-arachidonoylglycerol (2-AG) [20,21]. Other compounds were described as endocannabinoids – i.e. 2-arachidonyl glyceryl ether [22] (noladin ether) – but their endogenous occurrence is still under debate [23].

Nowadays, there is a growing literature dealing with the physiological role of the endocannabinoid system and some potential therapeutic applications, either for agonists or for antagonists, are already well explored [24]. This is the case for example for the anti-anorectic effect of dronabinol [25] (synthetic $^{9}$-tetrahydrocannabinol, $^{9}$-THC), the analgesic effect of $^{9}$-THC containing cannabis preparations [26], or in contrast, for the anti-obesity effect of rimonabant (Acomplia®). However, several actions remain unclear and are currently under investigation. Since the reports, by Ledent *et al*. [27] and by Zimmer *et al.* [28], $CB_1$ receptor knockout mice strains became a very useful tool to further explore some of the physiological roles of this receptor. Moreover, they strengthened the hypothesis of the existence of additional non-$CB_1$ and non-$CB_2$ cannabinoid receptors in the brain and in the periphery. Wiley and Martin in 2002 reviewed the evidences for the existence of these additional "cannabinoid" receptors [29]. Despite that these putative new receptors are not yet fully characterised, the last section of this paper will describe the known antagonists of these receptors.

The interest in the synthesis of new antagonists is still present as testified by the great number of new compounds reported either by the pharmaceutical companies or the academic research laboratories. Due to the great interest in the field, several reviews dealing with the cannabinoid ligands have already been published [30,31]. However, the

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first review only devoted to cannabinoids antagonists, was published by Barth and Rinaldi-Carmona back in 1999 [32].

The aim of this paper will be to review the cannabinoid antagonists and inverse agonists, with a particular emphasis on the newest compounds. Nevertheless, as a matter of completeness, this review will also cover the earliest development in the field of cannabinoid antagonists. Further, the great interest of the pharmaceutical companies for the field, led to the publication of a great amount of patents. Therefore, this paper will cover, in addition to the scientific papers, the patents covering the cannabinoid antagonists.

One of the most used *in-vivo* assay to characterise the pharmacological properties of the cannabinoids, is the cannabinoid tetrad of effects. An agonist of the $CB_1$ cannabinoid receptor must induce analgesia, catalepsia, hypomotility, and hypothermia upon administration in mice [33]. An antagonist should reverse the effect obtained with the agonist.

Several *ex-vivo* assays are also used to characterise the agonists or antagonists properties of the cannabinoid receptors ligands. The inhibition of the electrically evoked contractions in the guinea pig ileum and in the mouse *vas deferens* are among the most widely used *ex-vivo* assays, the latter being more sensitive to cannabinoids [34].

However, it is quite difficult in these *in*-vivo and *ex-vivo* assays, to distinguish between antagonists and inverse-agonist effects.

Therefore, several *in-vitro* assays are currently used to explore the functionality of known ligands. cAMP quantification is one of the most widely used methods, based

**Table 1. $CB_1$ Cannabinoid Receptor Antagonists: 1. Tetrahydrocannabinol and Cannabidiol Derivatives**

Some $^{8}$-Tetrahydrocannabinol Derivatives Possessing a Rigidified Side Chain. The Affinity for the $CB_1$ Receptor (Radioligand, Cells) and the Function (Assay Used) are Given

OH, O, R

| Cpd. | n° | R= | $CB_1$ (Ki) | Function |
|---|---|---|---|---|
| O-823 | **1** | CN | 0.77 nM ([$^3$H]-CP-55,940; rat brain) [c] | • inactive in Tetrad Test (up to 30 mg/kg, mouse) [e]<br>• partial agonist (mouse *vas deferens*) [c]<br>• antagonist (guinea pig myenteric plexus) [c]<br>• antagonist ([$^{35}$S]-GTP S, rat cerebella) [b] |
| O-1184 | **2** | $N_3$ | 5.24 nM ([$^3$H]-CP-55,940, h$CB_1$-CHO cells) [a] | • partial agonist in Tetrad Test (mouse) [e]<br>• antagonist (guinea pig myenteric plexus) [d]<br>• partial agonist (cAMP, h$CB_1$-CHO cells) [a]<br>• antagonist ([$^{35}$S]-GTP S, rat cerebella) [b] |
| O-584 | **3** | | 4.26 nM ([$^3$H]-CP-55,940, h$CB_1$-CHO cells) [a] | • agonist (cAMP, h$CB_1$-CHO cells) [a]<br>• antagonist ([$^{35}$S]-GTP S, rat cerebella) [b] |
| O-806 | **4** | Br | 1.2 nM ([$^3$H]-CP-55,940; rat brain) [e] | • partial agonist in Tetrad Test (mouse) [e]<br>• antagonist ([$^{35}$S]-GTP S, rat cerebella) [b] |
| O-1176 | **5** | NCS | 11.5 nM ([$^3$H]-CP-55,940; rat brain) [e] | • inactive in Tetrad Test (up to 30 mg/kg, mouse) [e]<br>• antagonist ([$^{35}$S]-GTP S, rat cerebella) [b] |
| O-1238 | **6** | $N_3$ | 3.54 nM ([$^3$H]-CP-55,940, h$CB_1$-CHO cells) [a] | • partial agonist ([$^{35}$S]-GTP S, rat cerebella) [b]<br>• agonist (cAMP, h$CB_1$-CHO cells) [a] |
| / | **7** | H N S O O | 30 nM [f] | • agonist in Tetrad Test (mouse) [f] |
| O-2050 | **8** | H N S O O | 2.5 nM [f] | • no effect *per se* in Tetrad Test (mouse) [f]<br>• antagonist (mouse *vas deferens*) [f] |

[a][39] [b][38] [c][36] [d][37] [e][40] [f][41]

on the negative coupling of cannabinoid receptors to adenylyl cyclase. The binding of an agonist will produce a decrease in cAMP production, which can be measured either directly (EIA) or through a gene-reporter system (firefly luciferase). However, due to the existence of a dual-coupling for the $CB_1$ cannabinoid receptor, the [$^{35}$S]-GTP S assay should be preferred. It is based on the property shared by all the GPCRs to bind to a GTP molecule upon activation by an agonist. Therefore, the binding of an agonist will increase the [$^{35}$S]-GTP S, a radiolabelled non-hydrolysable analogue of GTP, binding [35]. These assays allow to distinguish between full agonists (positive intrinsic activity), partial agonists, neutral antagonists (no intrinsic activity) and inverse agonists (negative intrinsic activity).

## II. $CB_1$ CANNABINOID RECEPTOR ANTAGONISTS AND INVERSE AGONISTS

Compounds having antagonist, or inverse agonist, properties at the $CB_1$ cannabinoid receptor are reviewed in this part of the paper. They are classified depending on their chemical structures.

### 1. Tetrahydrocannabinol and Cannabidiol Derivatives

The first attempts to obtain cannabinoid ligands having antagonist properties were conducted using the tricyclic structure of classical cannabinoids, such as $^9$-tetrahydrocannabinol ( $^9$-THC), as a scaffold. These early researches have been previously reviewed by Barth and Rinaldi-Carmona [32]. The most promising modulations involved the side-chain of $^8$-tetrahydrocannabinol ( $^8$-THC), as it is the case in O-823 (**1**) (Table **1**). This compound (Ki = 0.77 nM) acts as a partial agonist in mouse *vas deferens* preparations, but as an antagonist in guinea-pig myenteric plexus preparations [36]. Ross and co-workers [37] showed that O-1184 (**2**) binds to the $CB_1$ receptor (Ki = 2.85 nM) and, as O-823, possesses antagonist properties in the myenteric plexus-longitudinal muscles. O-823 and O-1184 were shown to act as surmountable antagonists in a [$^{35}$S]-GTP S assay, with $K_B$ values of 4.85 and 2.97 nM, respectively, against CP-55,940 in rat cerebellar membranes [38]. Three other $^8$-tetrahydrocannabinol-3'-ynyl derivatives, O-584 (**3**), O-806 (**4**), and O-1176 (**5**), also behaved as antagonists. However, in a cAMP production assay, conducted in $hCB_1$ transfected CHO cells, O-1184 behaved as an agonist equipotent to CP-55,940 [39]. Taken together, these examples illustrate that the introduction of an acetylenic moiety into the side chain of $^8$-THC, affects the activation of the receptor more than the affinity of these derivatives. Several of these compounds were tested *in-vivo* in the cannabinoid tetrad test by Martin and co-workers [40]. For instance, O-823 which was inactive in the tetrad, acted as an antagonist in the [$^{35}$S]-GTP S assay [38].

More recently, Martin *et al.* described three new compounds possessing an alkyl sulfonamide at the end of the alkyne side chain [41]. The ethyl (**7**) (Ki = 30 nM) and butyl (Ki = 70 nM) derivatives were shown to behave as agonists in the cannabinoid tetrad, while the methyl derivative (**8**) (Ki = 2.5 nM) acted as a silent antagonist. This compound, O-2050, does not induce neither antinociception, nor hypothermia, in mice, and lacks of agonist effects in mouse *vas deferens* preparation. In this assay, it behaved as an antagonist devoid of inverse agonist properties.

Recently, Thomas *et al.* reported the synthesis and characterisation of O-2654 (**9**) (Fig. **1**) obtained by modifying the structure of cannabidiol (or cbd) (**10**), a non psychoactive cannabinoid [42]. This compound, unlike cannabidiol, binds to the $CB_1$ receptor with a Ki value of 114 nM, against [$^3$H]-CP-55,940 on mouse brain membranes. In the mouse *vas deferens* model, O-2654 antagonises the WIN-55,212-2 inhibition of current-induced contractions, causing a rightward shift in the log concentration response curve of the agonist ($K_B$ = 85.7 nM). Further, in the *vas deferens* model, O-2654 behaves as a neutral antagonist. However, this neutral antagonism has to be further confirmed using other models.

O-2654 (**9**)

**Fig. (1).** $CB_1$ cannabinoid receptor antagonists: 1. Tetrahydrocannabinol and cannabidiol derivatives. Chemical structure of (-)-6''-azidohex-2''-yne-cannabidiol (O-2654, **9**).

### 2. Aminoalkylindole Derivatives

This family of compounds was introduced by Sterling's researchers in the early nineties, with a derivative of the anti-inflammatory drug pravadoline called WIN-55,212. Albeit this compound acts as an agonist, some related compounds showed interesting antagonist properties in the mouse *vas deferens* assay by dose-dependently antagonising $^9$-THC and levonantradol effects. This is the case, for instance, for

WIN-56,098 (**11**)

X=Br : WIN-54,461 (**12**)
X=I : AM630 (**13**)

**Fig. (2).** $CB_1$ cannabinoid receptor antagonists: 2. Aminoalkylindole derivatives. Structures of WIN-56,098 (**11**), WIN-54,461 (**12**), and AM630 (**13**) three aminoalkylindole derivatives acting as $CB_1$ cannabinoid receptor antagonists.

WIN-56,098 (**11**) and WIN-54,461 (**12**), the latter being a bromo derivative of pravadoline (Fig. **2**). These two compounds, however, have a low affinity for the cannabinoid receptor ($IC_{50}$ > 500 nM) [43,44].

In 1995, Pertwee *et al*. [45] described the effect of AM630 (**13**), another close analogue of pravadoline. This compound behaved as an antagonist in a [$^{35}$S]-GTP S assay on mouse brain preparations antagonising WIN-55,212-2-induced [$^{35}$S]-GTP S binding [46], and as an inverse agonist on $hCB_1$-CHO cells [47] ($EC_{50}$ = 0.9 µM). However, Ross *et al.* found a weak partial agonist activity for this compound as it decreases cAMP production by $hCB_1$-CHO cells [48]. AM630 was subsequently characterised as a $CB_2$ ligand (see the $CB_2$ section). The pharmacological properties of the aminoalkylindole family were reviewed by John Huffman [49].

### 3. Diarylpyrazole Derivatives

The lead of this class of compounds, SR141716A (**14**), was introduced by Sanofi back in 1994 [50]. This compound was shown by Rinaldi-Carmona and co-workers to be a highly selective $CB_1$ ligand with Ki values of 5.6 nM for the $hCB_1$ and over 1000 nM for the $hCB_2$ receptors expressed in CHO cells ([$^3$H]-CP-55,940). However, more recently, evidences appeared showing that SR141716A binds to other(s) receptor(s) described as anandamide and/or cannabinoid receptors. Thus, it is possible that some of the *in-vivo* effects caused by this compound are, at least, not solely $CB_1$ mediated (see the fourth section of this paper).

In a mouse *vas deferens* preparation, SR141716A causes a rightward shift of the CP-55,940 concentration-response curve, behaving as a competitive antagonist having a $pA_2$ value of 7.98. Furthermore, in the cAMP accumulation model, SR141716A produces no effect by itself, but antagonizes the CP-55,940 inhibition of forskolin-induced cAMP accumulation in $hCB_1$-CHO cells, with an $IC_{50}$ value of 5.6 nM. SR141716A is able, after *i.p.* or *p.o.* administration, to inhibit the [$^3$H]-CP-55,940 binding to mice brain measured *ex-vivo*, with $ED_{50}$ values around 2 mg/kg [51]. In addition to the inhibition of the classical cannabinoid tetrad effects – hypothermia, ring immobility, analgesia, and hypolocomotion – already shown by Rinaldi-Carmona *et al*. in their first report [50], SR141716A was shown to antagonise other *in-vivo* effects of cannabinoid agonists. For instance, the agonist-induced hypotension and bradycardia in mice are abolished by SR141716A [52], as well as the antihyperalgesic effects of the cannabinoid agonists in a neuropathic model of pain in rat [53]. This is also the case for the behavioral effects of agonists treated rats [54], or the cannabinoid tetrad effects induced by $^9$-THC in mice [55].

Despite various papers described SR141716A as an antagonist [56-58], today this compound is considered to act as an inverse agonist based on [$^{35}$S]-GTP S [59-62] and cAMP accumulation assays [62-64] (Table **2**). An interesting review dealing with inverse agonism at the cannabinoid receptors, and mostly with SR141716A effects, was recently published by Roger Pertwee [65].

In 1998, Pan *et al*. [66] demonstrated that the lysine residue K3.28 (192), located in the third transmembrane domain (TMH3) of the $hCB_1$ receptor, is a key residue for the inverse agonist action of SR141716A (Table **3**). They showed that SR141716A enhances calcium current in $hCB_1$ transfected neurons, but not in K(192)A mutant receptor transfected neurons. However, SR141716A still antagonized WIN-55,212-2 inhibition of calcium currents, proving that it is able to bind to the mutated receptor. Later on, Hurst *et al*. [67] demonstrated, using molecular modeling techniques, as well as *in-vitro* experiments, that lysine residue K3.28(192) is a direct interaction site for hydrogen bonding with the $C_3$ substituent of SR141716A in $CB_1$ receptor. Binding results

**Table 2. $CB_1$ Cannabinoid Receptor Antagonists: 3. Diaryl-Pyrazole Derivatives**

*In-Vitro* Functional Characterisation of SR141716A (14). The Assay Used, the Cell Type, and the Obtained Effect are Given

| Cell type | Assay | Effect | Function | References |
|---|---|---|---|---|
| $hCB_1$-CHO cells | cAMP accumulation | no effect by itself | antagonist | [50] |
| $hCB_1$-CHO cells | cAMP accumulation | [cAMP] | inverse agonist | [64] |
| $hCB_1$-CHO cells | [$^{35}$S]-GTP S | [$^{35}$S]-GTP S binding | inverse agonist | [59] |
| $hCB_1$-neurons | $Ca^{2+}$ currents | $Ca^{2+}$ currents | inverse agonist | [66] |
| $rCB_1$ (rat cerebella) | [$^{35}$S]-GTP S | No effect by itself | antagonist | [56] |
| $rCB_1$ (rat cerebella) | [$^{35}$S]-GTP S | No effect by itself | antagonist | [57] |
| $rCB_1$ (rat cerebella) | [$^{35}$S]-GTP S | No effect by itself | antagonist | [58] |
| $rCB_1$ (rat brain) | [$^{35}$S]-GTP S | [$^{35}$S]-GTP S binding | inverse agonist | [62] |
| $rCB_1$ (rat cerebella) | [$^{35}$S]-GTP S | [$^{35}$S]-GTP S binding | inverse agonist | [60] |
| $rCB_1$ (rat cerebella) | [$^{35}$S]-GTP S | [$^{35}$S]-GTP S binding | inverse agonist | [61] |
| $rCB_1$ (rat brain) | cAMP accumulation | [cAMP] | inverse agonist | [63] |
| $mCB_1$ (mouse brain) | cAMP accumulation | [cAMP] | inverse agonist | [62] |

obtained using HEK293 cells transfected with either the K3.28A mutant or the wild type receptor, remarkably confirmed the modeling results (Kd values of 39.6 and 2.3 nM respectively). Interestingly, a vinyl-cyclohexyl SR141716A derivative, VCHSR1 (**15**) (Table **4**), lacking of hydrogen bonding sites in $C_3$ position, is not affected by this $CB_1$ receptor mutation, as its affinity remains unchanged with Ki values of 31 and 35 nM for the wild-type and K3.28A receptors, respectively. Four additional compounds, CHASR1 (**16**), CHMSR1 (**17**), VPSR1 (**18**), and PIMSR (**19**), differing by their potential to form hydrogen bonds were evaluated in affinity and functional assays. The results further confirm the crucial interaction between the C-3 carboxamide oxygen and residue K3.28, to obtain an inverse agonist effect as highlighted in Table **4** [68]. However, it has to be said that this lysine residue is also crucial for agonist binding (CP-55,940) and/or receptor activation (WIN-55,212-2) [69, 70].

Several other studies were undertaken to determine the critical residues for the binding of cannabinoid compounds. Unfortunately, in most of the studies, SR141716A was not used. However, McAllister *et al*. [71], using modeling tools and mutagenesis, explored the importance of aromaticity in position 5.39(275) in the $CB_1$ receptor. The substitution of tyrosine by phenylalanine (Y275F) has no effect on the binding of SR141716A. In contrast, the substitution of tyrosine by isoleucine (Y275I) resulted in the loss of ligand recognition. Calculation studies revealed that, while the Y5.39F mutant is very similar to the wild type receptor, the Y5.39I mutant shows topology changes in the 3-4-5 transmembrane region. This region is considered to be crucial for agonist/antagonist binding at $CB_1$ receptor, since the previous report by Shire *et al.* on $CB_1$/$CB_2$ receptor chimeras [72]. In contrast, the first and third extracellular (EC1 and EC3) loops of the $hCB_1$ receptor are not essential for the binding of SR141716A as illustrated by Murphy *et al.* The authors constructed several receptors mutated in their EC1 or EC3, and none of the tested mutations affected the SR141716A binding [73]. McAllister *et al.*, due the highly aromatic nature of SR141716A, further explored the hypothesis that an aromatic microdomain, comprised in transmembrane helix 3-4-5-6, is the SR141716A binding site. The modelling and mutation studies undertaken suggested to the authors that this aromatic microdomain, comprised of F3.36, W4.64, Y5.39, W5.43, and W6.48, should represent the binding site of SR141716A. Moreover, they identified F3.36 and W5.43 as direct interaction sites for SR141716A [74]. Residue F3.36 was further shown by the same group to be a key residue for both ligand binding (WIN-55,212-2 and SR141716A) and receptor activation. Mutation of phenylalanine 3.36(201) to alanine, resulted in

**Table 3. $CB_1$ Cannabinoid Receptor Antagonists: 3. Diaryl-Pyrazole Derivatives**

**Summary of the Reported $CB_1$ Cannabinoid Receptor Single Point Mutations for which Pharmacological Data Concerning the SR141716A (14) are Available**

| Mutation | Species | Effect | References |
|---|---|---|---|
| S114A [a] | human | no significant effect | [73] |
| S115A [a] | human | no significant effect | [73] |
| D2.50(163)N | human | no significant effect | [263] |
| D2.50(163)E | human | no significant effect | [263] |
| H181A [b] | human | no significant effect | [73] |
| R182A [b] | human | no significant effect | [73] |
| K183A [b] | human | no significant effect | [73] |
| D184A [b] | human | no significant effect | [73] |
| V3.24(188)A | human | no significant effect | [73] |
| F3.25(189)A | human | no significant effect | [73] |
| F3.25(190)A | mouse | no significant effect | [74] |
| K3.28(192)A | human | loss of function | [66] |
| K3.28(192)A | human | reduction of affinity (20 fold) | [67] |
| F3.36(201)A | mouse | reduction of affinity (3 fold) | [74] |
| Y5.39(275)F | human | no significant effect | [71] |
| Y5.39(275)I | human | loss of affinity | [71] |
| W5.43(280)A | mouse | loss of affinity | [74] |
| W6.48(357)A | mouse | reduction of affinity (7fold) | [74] |

a. *N*-terminus. b. First extra-cellular loop

**Table 4. $CB_1$ Cannabinoid Receptor Antagonists: 3. Diaryl-Pyrazole Derivatives. Five SR141716A (14) Derivatives Illustrating the Importance of the C-3 Carboxamide Oxygen in the SR141716A Inverse Agonism**

The structure, affinity ([$^3$H]-SR141716A, $hCB_1$-HEK293 cells), and function ($Ca^{++}$ currents) are given for each compound.

| Cpd. | n° | R= | Affinity | Function | References |
|---|---|---|---|---|---|
| SR141716A | **14** | | Kd=2.3nM | Inverse agonist | [67] |
| VCHSR1 | **15** | | Ki=31.3nM | Neutral antagonist | [67] |
| CHASR1 | **16** | | Ki=1.7nM | Inverse agonist | [68] |
| CHMSR1 | **17** | | Ki=29nM | Inverse agonist | [68] |
| VPSR1 | **18** | | Ki=261nM | Neutral antagonist | [68] |
| PIMSR | **19** | | Ki=6.7nM | Neutral antagonist | [68] |

an increased constitutive activity of the receptor as demonstated by [$^{35}$S]-GTP S binding [75].

A new 3D model of the $CB_1$ cannabinoid receptor, based on the X-ray structure of the bovine rhodopsin, was recently developed by Salo and co-workers [76]. It would be very interesting to see whether or not, the results obtained with previous models are confirmed using this new model. For instance, the lysine K3.28 appeared as a key residue in this model too.

The tritiated analogue of SR141716A, the [$^3$H]-SR141716A, was described by Rinaldi-Carmona *et al.* in 1996. It binds with high affinity to rat brain synaptosomes (Kd=0.61nM). It is competitively displaced by known cannabinoids like CP-55,940 or WIN-55,212-2. Using autoradiography, its rat brain distribution is similar to [$^3$H]-CP-55,940 one [77]. It is now a commercially available, and widely used radioligand for competition studies.

Interestingly, other SR141716A radiolabeled derivatives were synthesised as radioimaging tools, among them, [$^{123}$I]-AM251 (**20**) [78,79], [$^{123}$I]-AM281 (**21**) [80-82], and [$^{18}$F]-SR144385 (**22**) [83,84] for Positon Emission Tomography or Single Photon Emission Computed Tomography. Indeed, cannabinoid antagonists are a much more useful tool for

human radioimaging applications than agonists, as they are devoid of cannabinoid-like effects. For instance, Berding *et al*. very recently reported the use of [$^{123}$I]-AM281 for Single Photon Emission Computed Tomography imaging of the $CB_1$ cannabinoid receptor in six human patients [85]. In order to further optimize the brain uptake of such SR141716A derivatives, Sanofi researchers synthesised two methoxylated SR141716A analogues using $^{11}$C as PET tracer [86]. These two compounds, SR149080 (**24**) and SR149568 (**25**), possess high affinity, with Ki values of 1.5 and 38 nM respectively, and selectivity for the $CB_1$ receptor, as well as an improved penetration in the brain evaluated by measuring the radioactivity present in various brain regions after tail vein injection in CD-1 mice. Some of the representative radiolabelled SR141716A derivatives are summarised in Table **5**.

From a more therapeutic point of view, SR141716A (rimonabant, Acomplia®) is one of the promising agents to treat obesity [87]. It is currently in Phase III clinical trials for the treatment and prevention of obesity. Final results are expected for this year, and FDA application filling for 2005 [88]. Preliminary data based on a one year treatment with rimonabant (RIO-Lipids study, 1036 patients) showed a 5% weight loss in 72% of the treated patients [89,90]. Several reports were published before clinical trials aiming to demonstrate the anti-obesity properties of this compound. The first report by Sanofi Recherche, published in 1997, described the selective inhibition of sucrose intake in rats upon SR141716A treatment (0.3-3 mg/kg) [91]. The anorectic and weight loss effects were firstly published by Colombo *et al*. using Wistar rats [92]. Simiand *et al.* observed that SR141716A selectively reduces sweet food intake in primates [93]. However, several authors [94-96] showed that high palatability of food is not necessary to observe a SR141716A-induced anorectic effect, at doses that do not cause major behavioural alterations or reduced water intake. Interestingly, Gomez *et al.* demonstrated the implication of the peripheral $CB_1$ receptors on the modulation of feeding, and therefore, the possible role of these receptors on the SR141716A influence on food intake [97]. In addition to its effects on food consumption, SR141716A seems to be able to lower the hyperglycemia, the hyperinsulinemia, as well as the insulin resistance in diet-induced obese (DIO) mice. In the same DIO mice, a decrease in adiposity was also observed after treatment [98]. Bensaid *et al.* using another model of obesity, the obesefa/fa rats, observed that SR141716A increases mRNA expression of Acrp30, or adiponectin, a plasmatic protein exclusively secreted by adipose tissue, through a $CB_1$-mediated pathway. Inductions of free fatty acid oxidation, body weight reduction, and hyperinsulinemia decrease are some of the known physiological actions of this protein. Thus, along the authors, an enhanced expression of Acrp30, following SR141716A administration could be responsible for the metabolic effects of the compound leading to body weight reduction [99]. Vickers *et al.* using the same model (i.e. fa/fa rats) showed that SR141716A significantly decreases food consumption and weight gain in both the obese fa/fa rats and the lean Zucker rats [100]. This decrease was greater in the fa/fa group, and reversible upon SR141716A withdrawal.

**Table 5. $CB_1$ Cannabinoid Receptor Antagonists: 3. Diaryl-Pyrazole Derivatives**

Structure and Affinity of SR141716A Radiolabeled Derivatives Developed as Potential Radio-Imaging Tools

| Cpd. | n° | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Affinity $CB_1$ | References |
|---|---|---|---|---|---|---|---|
| [$^{123}$I]-AM251 | **20** | $^{123}$I | piperidinyl | $CH_3$ | Cl | Ki=2.5 nM[a] | [78] |
| [$^{123}$I]-AM281 | **21** | $^{123}$I | morpholinyl | $CH_3$ | Cl | Ki=14 nM[b] | [80] |
| [$^{18}$F]-SR144385 | **22** | Cl | piperidinyl | $CH_2$-$^{18}$F | Cl | $IC_{50}$=2.9 nM[a] | [83] |
| [$^{18}$F]-SR147963 | **23** | Cl | morpholinyl | $CH_2$-$^{18}$F | Cl | $IC_{50}$=120 nM | [84] |
| [$^{11}$C]-SR149080 | **24** | O-$^{11}CH_3$ | piperidinyl | $CH_3$ | Cl | $IC_{50}$=1.5 nM[a] | [86] |
| [$^{11}$C]-SR149568 | **25** | O-$^{11}CH_3$ | morpholinyl | $CH_3$ | Cl | $IC_{50}$=38 nM[a] | [86] |
| [$^{18}$F]-NIDA-42033 | **26** | O-$CH_3$ | piperidinyl | $^{18}$F | H | Ki=18 nM[c] | [155,156] |
| / | **27** | O-$^{11}CH_3$ | piperidinyl | $CH_3$ | H | Ki=8nM[c] | [155,157] |

[a]rat brain homogenates, [$^3$H]-CP-55,940
[b]mouse cerebellum homogenates, [$^3$H]-SR141716
[c]rat brain homogenates, [$^3$H]-AM251

Another recent study by Higgs and co-workers further highlighted the role of endocannabinoids in food taste-perception, and the reduction of orosensory reward of sucrose in SR141716A treated rats [101]. As it was expected, SR141716A induced effects on food consumption are absents in $CB_1^{-/-}$ mice [102]. Very recently, Cota *et al.* obtained results showing that endocannabinoid system modulates homeostasis *via* a dual mechanism: it regulates at a central level food intake, while it blocks at the periphery lipogenetic processes [103]. Moreover, Cani *et al.* showed that ghrelin (an orexigenic peptide) plasma levels are significantly reduced 45 minutes after SR141716A administration (5mg/kg, *i.p.*) to fasted rats, in accordance with the rapid decrease of food intake measured by the authors. It is therefore likely that SR141716 effects on body weight are due to a conjunction of central and peripheral actions [104].

In addition to the anti-obesity potential, the ability of SR141716A to reduce alcohol and tobacco consumption are currently investigated in phase III clinical trials. The effect of SR141716A on alcohol consumption in rats was reported by the GianLuigi Gessa team [105-108] and by Gallate *et al.* [109]. The inhibition of alcohol and nicotine induced dopamine release by SR141716A (1-3 mg/kg, rats) was reported by Cohen and co-workers using a brain microdialysis device [110]. Concerning the smoking cessation, preliminary results of a clinical trial (360 subjects, 40 mg SR141716A) showed an increased abstinence of smoking [111]. More recent results from the STRATUS-US study (787 patients) were reported, 36.2% of patients receiving SR141716A (20 mg/day) quit smoking, against 20.6% in the placebo group [90]. Recently, Le Fol and Goldberg reviewed the development of cannabinoid $CB_1$ antagonists as a new class of therapeutic agents for drugs addictions [112].

Since the late sixties, concerns exist on the association between cannabis use and schizophrenia. Recent studies showed an association between cannabis use and an increased risk of developing schizophrenia [113, 114]. Moreover, a possible role of the endocannabinoid system in schizophrenia has been suggested since its pharmacologicalcharacterisation [115]. Several experimental data obtained on human subjects tend to confirm such hypothesis. On the one hand, Leweke *et al*. found elevated levels of endocannabinoids in cerebrospinal fluid from patients with schizophrenia [116]. Later, De Marchi *et al.* measured higher amounts of anandamide in blood of schizophrenic patients, compared to controls [117]. On the other hand, Dean *et al.* obtained elevated [$^3$H]-CP-55,940 binding in the dorsolateral prefrontal cortex of patients suffering from schizophrenia as compared to controls [118]. Zavitsanou, using [$^3$H]-SR141716A, found elevated binding in the anterior cingulate cortex of subjects with schizophrenia [119]. Recently, Meltzer and collaborators published the results of a trial conducted to evaluate the potential of four new compounds in treating schizophrenia and schizoaffective disorders [120]. SR141716A (20 mg/day) was one of the compounds evaluated during a six weeks study. The authors found no effect of the $CB_1$ cannabinoid receptor antagonist in improving patient's schizophrenia (72 subjects).

It is known since the early nineties that the administration of cannabinoid agonists impairs memory in rodents (for a review, see Castellano *et al.* [121]). Thus, administration of a cannabinoid antagonist was expected to somehow improve memory [122,123]. However, depending on the authors, SR141716A when administered alone, was reported to impair (5-10 mg/kg, i.m.) [124], to have no effect (1-32 mg/kg, i.p.) [125] or to improve memory (3 mg/kg, i.p.) [126]. In a more recent paper, Wolff and Leander, showed that SR141716A (1mg/kg, i.p.) improves memory in rats by apparently enhancing the consolidation processes of memory [127]. Further, they found that, at higher doses (3 mg/kg), this effect was lost. The discrepancy in the results reported in the literature could be ascribed either to the differences in the tests used, or in the doses administered. Further experiments are needed to assess whether or not a cannabinoid antagonist could be helpful in memory diseases.

To conclude, several patents were taken by Sanofi concerning the therapeutic applications of their lead compound, among them being anti-obesity, smoking cessation, neuroinflammatory diseases and anti-diarrhoea. One of the last patents, to our knowledge, concerns the treatment of sexual dysfunctions with a cannabinoid antagonist such as SR141716A [128]. However, da Silva *et al.* showed that, even if this compound (2 mg/kg, *ip*) enhances the effects of apomorphine (20-80 µg/kg), it has no effect alone on penile erection [129]. Meanwhile, Melis *et al.*

**Fig. (3).** $CB_1$ cannabinoid receptor antagonists: 3. Diarylpyrazole derivatives. The chemical structures of 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-ethylpyrazole-3-piperidine-carboxamide (SR147778, **28**) and 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-ethylpyrazole-3-(4-hydroxypiperidine)-carboxamide (**29**) described by Rinaldi-Carmona *et al*.

reported an increased rate of penile erection after direct injection of SR141716A in the paraventricular nucleus of the hypothalamus [130]. This effect was dose dependent, and a significant effect was obtained with a dose of 1µg/kg.

From the clinical trials involving the SR141716A, the most frequently reported side-effects are nausea, dizziness and diarrhoea. Depression and anxiety were not higher than in the placebo groups. Regarding one of the known side effects of SR141716A administration, the enhancement of intestinal motility, Carai *et al*. reported recently that chronic administration of the inverse agonists to mice induced a tolerance to this prokinetic effect [131]. This is not the first report of tolerance onset after administration of SR141716A [132], however, few is known on the mechanisms responsible for that phenomenon.

One close analogue of SR141716A, 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-ethylpyrazole-3-piperidinecarboxamide (SR147778, **28**), was described very recently by Rinaldi-Carmona *et al.* [133, 134] (Fig. **3**). It possesses high affinity and selectivity for the $hCB_1$ cannabinoid receptor with Ki values for the $hCB_1$ and $hCB_2$ receptors of 3.5 and 400 nM, respectively ([$^3$H]-CP-55,940, $hCB_{1\&2}$-CHO cells). Further, SR147778 antagonised CP-55,940 effects on mouse *vas deferens* contractions ($pA_2$=8.1) and on forskolin-stimulated adenylyl cyclase activity in U373MG cells ($pA_2$=8.2), but had no effects alone. *In-vivo*, SR147778 after oral administration, reversed WIN-55,212-2 induced hypothermia and analgesia. As SR141716A, SR147778 dose-dependently reduced ethanol and sucrose solution intake with significant effects starting at 0.3 mg/kg (s.c.) and 3 mg/kg (p.o.), respectively. This compound is currently investigated in Phase I clinical trials for the treatment of obesity, as well as nicotine and alcohol addictions.

Another derivative, the 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-ethylpyrazole-3-(4-hydroxypiperidine)-carboxamide (**29**), was described in a very recent patent from Sanofi [135] (Fig. **3**). This antagonist possesses an $IC_{50}$ value for the $hCB_1$ cannabinoid receptor of 32 nM ([$^3$H]-CP-55,940, $hCB_1$-CHO cells).

The first structure-affinity relationships for the SR141716A derivatives were reviewed by Barth and Rinaldi-Carmona in 1999 [32]. Since then, many papers describing new derivatives were published. In 1999, Lan *et al.* described around thirty 1,5-diarylpyrazoles derivatives [136]. Among them was AM251, previously reported as a radioimaging ligand for the $CB_1$ receptor [78], and that appeared to be more potent (Ki = 7.49 nM) and selective (selectivity ratio of 306) than SR141716A (Ki = 11.5 nM, selectivity ratio of 143). They reported a Kd value of 0.5 nM in the mouse *vas deferens* model, using WIN-55,212 as agonist. Interestingly, New *et al.* described for the same compound an inverse agonist effect on the $hCB_2$ receptor, using a forskolin-induced cAMP accumulation assay ($EC_{50}$ = 650 nM) [137]. Recently, the anti-obesity effects of AM251 were reported and are, not surprisingly, similar to those of SR141716A [138, 139, 140]. Chen *et al.* demonstrated that there are synergistic effects on food intake suppression between AM251 and nalmefene, an opioid antagonist [141]. This synergistic effect was also present between SR141716A and naloxone, as shown by Kirkham [142]. Shearman *et al.* found antidepressant-like effects of AM251 in mice at a same dose range (10-30 mg/kg, *ip*) than the anorectic effects [143]. These effects are reversed by administration of CP-55,940, and are absent in $CB_1^{-/-}$ mice, proving the implication of the $CB_1$ receptor in the antidepressant-like effect of AM251. In 2004, Liao *et al.* reported that AM251 was able to displace the binding of [$^3$H]-batrachotoxin A from its binding site on sodium channels in mice brain synaptic preparations [144]. They obtained an $IC_{50}$ value of 11.2 µM, and a competitive mechanism of action. The authors suggested that AM251 is capable of reducing neuronal excitability through blockade of voltage-sensitive sodium channels in brain.

Wiley and collaborators reported in 2001, a study describing new structure-activity relationships using the pyrazole nucleus as a central scaffold [145]. Starting from SR141716A structure, they alternatively substituted one of the four substituents, while retaining the others, by substituents known to impart agonist activity in classical cannabinoids. One of the authors expectations was to determine which positions are responsible respectively for the antagonism and for the affinity of SR141716A. The affinity of thirty compounds was assessed previous to *in-vivo* evaluation of their function in mice using the spontaneous activity, the tail-flick, and the rectal temperature assays. The authors showed that phenyl group in position 5 is critical for affinity, as compound O-1559 (**30**), lacking this phenyl has a decreased affinity (Table **6**). This was already shown by Lan and co-workers [136], along with the need of a substituent in *para* position on the phenyl. Thomas *et al.* previously showed that this substituent could be a bromine or an iodine atom [146]. However, an alkyl chain is also tolerated as it is the case in compounds O-1302 (**31**), O-1691 (**32**) or O-1704 (**33**). All these compounds antagonise the anti-nociceptive and hypothermic effects of $^9$-THC. The authors suggested that the 5-substituent of pyrazoles is involved in receptor recognition and antagonism. The modulations of the substitution pattern of the phenyl in position 1 demonstrate that the 2,4-di-chloro substitution is the preferred one for the affinity, as well as for the activity. Several compounds support this assertion. Thomas *et al.* showed that additional halogens result in a decreased affinity as in compounds 6-I-SR141716A (**34**) or 4',6-di-I-SR141716A (**35**) (Ki values of 166 and 126 nM, respectively) [146]. Suppression of the two chlorines (O-1300, **39**) or replacement of these two chlorines by an alkyl chain like in O-1254 (**40**) and O-1255 (**41**), led to less active compounds with Ki values ranging from 150 to 430 nM. However, O-1254 and O-1255 behaved as antagonists in a [$^{35}$S]-GTP S assay [61]. Lan *et al.* [136] and Wiley *et al.* [145] also modified the substitution in position 3. Replacement of the amido piperidinyl substituent by alkyl amides as in O-1269 (**42**) or O-1270 (**43**), ethers, like in O-848 (**44**) and O-853 (**45**), ketones (O-1272, **46**), alcohols (O-1876, **47**) or alkanes (O-1877, **48**), resulted mostly in decreased affinity (Table **6**), but also with a change of functionality in some compounds as revealed by *in-vivo* assays. For instance, replacement of the piperidinyl by a pentyl (O-1269) or by an heptyl chain (O-1270) gave agonist compounds having Ki values of 32 and 48 nM, respectively. Thus, the authors suggested that the 3-substituent region is involved in receptor recognition and agonist activity. Wiley *et al*. on the basis of their results concluded that, while the 3-position seems to be involved in agonism, the 1-, 4-, and

5-positions appear to be involved in antagonism. The O-derivatives reported were also described in a patent, along with their synthetic pathways which were not given in the paper [147].

Another investigation of the SR141716A aminopiperidine region was conducted by Francisco *et al.* [148]. They synthesised 21 analogues possessing either an alkyl amide or an alkyl hydrazide substituent of various

**Table 6. CB$_1$ Cannabinoid Receptor Antagonists: 3. Diaryl-Pyrazole Derivatives**

Structure of some of the characterised 1-, 3-, 4-, and 5-pyrazole derivatives. Binding affinities (Ki values, nM) were obtained on rat brain homogenates using [$^3$H]-CP-55,940 [136, 145, 146, 148, 150] or [$^{125}$I]-AM-251 [155].

| Cpd. | n° | R$_1$ | R$_3$ | R$_4$ | R$_5$ | [136] | [145] | [146] | [148] | [150] | [155] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SR141716 | **14** | 2,4-di-Cl-Ph | CO-NH-piperidinyl | Me | 4-Cl-Ph | 11.5 | 6.2 | 6.2 | 6.2 | 1.3 | 1.8 |
| O-1559 | **30** | 2,4-di-Cl-Ph | CO-NH-piperidinyl | Me | 1-Methylpentyl | / | 233 | / | / | / | / |
| O-1302 | **31** | 2,4-di-Cl-Ph | CO-NH-piperidinyl | Me | 4-((CH$_2$)$_5$)-Ph | / | 2.1 | / | / | 1 | / |
| O-1691 | **32** | 2,4-di-Cl-Ph | CO-NH-piperidinyl | Br | 4-((CH$_2$)$_5$)-Ph | / | 1.5 | / | / | / | / |
| O-1704 | **33** | 2,4-di-Cl-Ph | CO-NH-piperidinyl | I | 4-((CH$_2$)$_5$)-Ph | / | 2.2 | / | / | / | / |
| 6-I-SR141716 | **34** | 2,4-di-Cl-6-I-Ph | CO-NH-piperidinyl | Me | 4-Cl-Ph | / | / | 166 | / | / | / |
| 4',6-di-I-SR141716 | **35** | 2,4-di-Cl-6-I-Ph | CO-NH-piperidinyl | Me | 4-I-Ph | / | / | 126 | / | / | / |
| 4'-I-SR141716 | **36** | 2,4-di-Cl-Ph | CO-NH-piperidinyl | Me | 4-I-Ph | 7.5 | / | 2.5 | / | 6 | / |
| 4'-Br-SR141716 | **37** | 2,4-di-Cl-Ph | CO-NH-piperidinyl | Me | 4-Br-Ph | 16.8 | / | 3 | / | / | / |
| Cpd. 25 in ref. [136] | **38** | 2,4-di-Cl-Ph | CO-NH-morpholinyl | Me | 4-Br-Ph | 54 | / | / | / | / | / |
| O-1300 | **39** | Ph | CO-NH-piperidinyl | Me | 4-Cl-Ph | / | 150 | / | / | / | / |
| O-1254 | **40** | 4-(CH$_2$)$_4$-Ph | CO-NH-piperidinyl | Me | 4-Cl-Ph | / | 226 | / | / | 256 | / |
| O-1255 | **41** | 4-(CH$_2$)$_5$-Ph | CO-NH-piperidinyl | Me | 4-Cl-Ph | / | 433 | / | / | / | / |
| O-1269 | **42** | 2,4-di-Cl-Ph | CO-NH-pentyl | Me | 4-Cl-Ph | / | 32 | / | 11.4 | 3 | / |
| O-1270 | **43** | 2,4-di-Cl-Ph | CO-NH-heptyl | Me | 4-Cl-Ph | / | 48 | / | 46.2 | 3 | / |
| O-848 | **44** | 2,4-di-Cl-Ph | CH$_2$-O-(CH$_2$)$_2$-piperidinyl | Me | 4-Cl-Ph | / | 2450 | / | / | 232 | / |
| O-853 | **45** | 2,4-di-Cl-Ph | CH$_2$-O-CH$_2$-cyclohexyl | Me | 4-Cl-Ph | / | 388 | / | / | 100 | / |
| O-1272 | **46** | 2,4-di-Cl-Ph | CO-heptyl | Me | 4-Cl-Ph | / | 221 | / | / | / | / |
| O-1876 | **47** | 2,4-di-Cl-Ph | 1'-OH-heptyl | Me | 4-Cl-Ph | / | 657 | / | / | / | / |
| O-1877 | **48** | 2,4-di-Cl-Ph | Heptyl | Me | 4-Cl-Ph | / | 422 | / | / | / | / |
| MF9725-64-17 | **49** | 2,4-di-Cl-Ph | CO-NH-butyl | Me | 4-Cl-Ph | / | / | / | 13.4 | / | / |
| MF9725-179-32 | **50** | 2,4-di-Cl-Ph | CO-NH-NH-butyl | Me | 4-Cl-Ph | / | / | / | 51 | / | / |
| MF9725-66-11 | **51** | 2,4-di-Cl-Ph | CO-NH-cyclohexyl | Me | 4-Cl-Ph | / | / | / | 2.5 | / | / |
| MF9725-95-31 | **52** | 2,4-di-Cl-Ph | CO-NH-(4-hydroxybutyl) | Me | 4-Cl-Ph | / | / | / | 154 | / | / |
| Cpd. 15 [150] | **53** | n-pentyl | CO-NH-piperidinyl | Me | Ph | / | / | / | / | 23 | / |
| Cpd. 16 [150] | **54** | n-pentyl | CO-NH-piperidinyl | Me | 4-Br-Ph | / | / | / | / | 63 | / |
| Cpd. 17 [150] | **55** | n-hexyl | CO-NH-piperidinyl | Me | Ph | / | / | / | / | 21 | / |
| Cpd. 18 [150] | **56** | n-heptyl | CO-NH-piperidinyl | Me | Ph | / | / | / | / | 47 | / |
| NIDA-41109 | **57** | 2,4-di-Cl-Ph | CO-NH-piperidinyl | Br | 4-Cl-Ph | / | / | / | / | / | 1.4 |
| NIDA-41119 | **58** | 2,4-di-Cl-Ph | CO-NH-piperidinyl | H | 4-Cl-Ph | / | / | / | / | / | 9 |
| NIDA-41057 | **59** | 2,4-di-Cl-Ph | CO-NH-piperidinyl | Me | 4-OH-Ph | / | / | / | / | / | 104 |
| NIDA-41020 | **60** | 2,4-di-Cl-Ph | CO-NH-piperidinyl | Me | 4-OMe-Ph | / | / | / | / | / | 4.1 |
| NIDA-41087 | **61** | 2-Cl-Ph | CO-NH-piperidinyl | Me | 4-OMe-Ph | / | / | / | / | / | 8 |
| NIDA-42055 | **62** | 2,4-di-Cl-Ph | CO-NH-piperidinyl | Br | 4-OMe-Ph | / | / | / | / | / | 6.2 |

lengths in position 3 of the pyrazole moiety. They observed that, until five carbons, the affinity increases along with the carbon chain length. This is observed with the alkyl hydrazide, the alkyl amide, and the hydroxyalkyl amide series. Moreover, the hydrazide analogues exhibit a lower affinity for the $rCB_1$ than the amide analogues, as illustrated by compounds MF9725-64-17 (**49**) and MF9725-179-32 (**50**) having Ki values of 13.4 and 51 nM, respectively (Table **6**). From their structure-activity relationships (SAR) studies, the authors concluded that the pharmacophoric requirement of the amidopiridine region is a chain not longer than 3 Å, and that a substituent having a positive charge density would probably result in increased affinity and potency. The same team described in a patent several other compounds [149]. Binding affinities (Ki) against [$^3$H]-CP-55,940, [$^3$H]-SR141716A, or [$^3$H]-WIN55,212-2 on whole rat brain or on $hCB_1$-transfected cells were given. Moreover, activity data were obtained using the [$^{35}$S]-GTP S assay demonstrating that these alkyl amide and hydrazide analogs act as antagonists or inverse agonists. Despite the great number of derivatives claimed, none of them posses a significantly greater affinity for the $CB_1$ receptor than the SR141716A. Nevertheless, they showed a slight enhancement of the selectivity for the $CB_1$ receptor over the $CB_2$ cannabinoid receptor.

The affinity for the rat $CB_1$ receptor of seven derivatives possessing an alkyl chain in position 1, instead of the 2,4-dichlorophenyl substituent, was reported by Shim *et al.* in a paper describing a molecular mechanism for the antagonist and inverse agonist activity of SR141716A [150]. The affinity of these derivatives increases with the length of the alkyl chain, with an optimal length of 5-6 carbons. The best compound of this series, *N*-(piperidin-1-yl)-5-phenyl-1-hexyl-4-methyl-1*H*-pyrazole-3-carboxamide (**55**), possesses a Ki value of 21 nM, determined using [$^3$H]-CP-55,940 on rat brain membranes, which is higher than the 1.3 nM determined for SR141716A in the same conditions (Table **6**). In the same paper, the authors, starting from the hypothesis that antagonism by SR141716A is caused by binding to the same region of the receptor as do the agonists (CP-55,940 and WIN-55-212,2), but preventing the agonist promoted conformational change, conducted extensive conformational analysis, as well as superimposition models and 3D-QSAR to propose a molecular mechanism supporting the action of SR141716A. Along with the authors, the C-5 aryl substituent of SR141716A, occupying a unique region, could contribute in conferring the antagonist properties. Moreover, the C-3 substituent could be responsible for the antagonist or inverse agonist properties depending on the interaction with the receptor.

About this topic, a very interesting review dealing with the cannabinoid receptors pharmacophores, as well as with the activation/inactivation of these receptors was recently published by Reggio [151].

More recently, Dyck *et al.* described seven other derivatives varying at the amide position [152]. The only compound possessing a higher affinity for the $CB_1$ cannabinoid receptor than SR141716A was the 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(hexahydrocyclopenta-

**Fig. (4).** $CB_1$ cannabinoid receptor antagonists: 3. Diarylpyrazole derivatives. Structures of 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-*N*-(hexahydrocyclopenta[c]pyrrol-2(1*H*)-yl)-4-methyl-pyrazole-3-carboxamide (**63**), 2-[1-(2-chlorophenyl)-5-(4-chlorophenyl)-4-methyl-pyrazol-3-yl]-4-[(6-methyl-2-pyridinyl)methyl]-morpholine (**64**), *N*-(piperidin-1-yl)-5-(3'-chloro-biphenyl-4-yl)-1-(2,4-dichloro-phenyl)-4-methyl-pyrazole-3-carboxamide (**65**), and *N*-phenyl-1-(2-chlorophenyl)-4-cyano-5-(4-methoxyphenyl)-pyrazole-3-carboxamide (CP-272-871, **66**).

[c]pyrrol2(1*H*)-yl)-4-methyl-pyrazole-3-carboxamide (**63**) (Fig. **4**). The Ki values were 5 and 12 nM, respectively ([$^3$H]-CP-55,940, hCB$_1$-HEK cells).

Over 200 other pyrazole derivatives acting at the CB$_1$ cannabinoid receptor were claimed in a patent from Pfizer [153]. The affinity for the hCB$_1$ receptor of 2-[1-(2-chlorophenyl)-5-(4-chlorophenyl)-4-methylpyrazol-3-yl]-4-[(6-methyl-2-pyridinyl)methyl]–morpholine (**64**) (Fig. **4**) was 79 nM ([$^3$H]-SR141716A, hCB$_1$-HEK cells). The others compounds affinity was between 0.1 and 100 nM.

Seventy five pyrazole derivatives were described in a very recent patent from Makriyannis *et al.* [154]. The affinity of the compounds for the cannabinoid receptors is given. The Ki values for the best compounds are lower than 6 nM ([$^3$H]-CP-55,940, rat brain). For instance, *N*-(piperidin-1-yl)-5-(3'-chloro-biphenyl-4-yl)-1-(2,4-dichloro-phenyl)-4-methyl-pyrazole-3-carboxamide(**65**) (Fig. **4**) showed a Ki value of 1.5 nM. However, no data were provided concerning the functionality of such compounds.

In an effort to develop new SR141716A analogues possessing lower lipophilicity to be used as PET tracers, Katoch-Rouse and colleagues further explored the substitution pattern of the two phenyls [155]. Binding assays showed that a decreased lipophilicity lead to a decreased affinity for the CB$_1$ receptor, as determined by displacement of [$^3$H]-AM251. For instance, compound NIDA-41057 (**59**) has a lipophilicity of 4.2 ( expressed as ElogD$_{oct}$) and a Ki value of 104 nM, whereas SR141716A has a ElogD$_{oct}$ value of 5.4 and a Ki value of 1.8 nM. In

**Table 7. CB$_1$ Cannabinoid Receptor Antagonists: 3. Diaryl-Pyrazole Derivatives**

Structures, and affinities for the cannabinoid receptors of some representative tricyclic pyrazole derivatives. Values were obtained using [$^3$H]-CP-55,940 as radioligand and mouse [160, 228], rat [159, 227] or human [158] cannabinoid receptors. Compounds **69**-**78** are CB$_1$ cannabinoid receptor ligands, Compounds **79**-**86** are CB$_2$ cannabinoid receptor ligands.

| n° | X | R$_1$ | R$_2$ | n | R$_3$ | Ki CB$_1$ (nM) | Ki CB$_2$ (nM) | Selectivity | References |
|---|---|---|---|---|---|---|---|---|---|
| **69** | -(CH$_2$)$_3$- | Cl | 2,4-di-Cl | 0 | piperidine | 126 | / | / | [158] |
| **70** | -(CH$_2$)$_3$- | H | 2,4-di-Cl | 0 | azepane | 100 | / | / | [158] |
| **71** | -(CH$_2$)$_3$- | H | 2,4-di-Cl | 0 | piperidine | 398 | / | / | [158] |
| **72** | -(CH$_2$)$_3$- | Cl | 2,4-di-Cl | 0 | azepane | 125 | / | / | [158] |
| **73** | -(CH$_2$)$_3$- | NO$_2$ | 2,4-di-Cl | 0 | azepane | 63 | / | / | [158] |
| **74** | -(CH$_2$)$_2$-O- | H | 2,4-di-Cl | 0 | piperidine | 501 | / | / | [158] |
| **75** | -CH$_2$-S- | Br | 2,4-di-Cl | 0 | piperidine | < 500 | / | >10 | [159] |
| **76** | -CH$_2$-SO$_2$- | Cl | 2,4-di-Cl | 0 | piperidine | < 500 | / | >10 | [159] |
| **77** | -(CH$_2$)$_2$- | Cl | 2,4-di-Cl | 0 | piperidine | < 500 | / | >10 | [159] |
| **78** | -(CH$_2$)$_3$- | Cl | 2,4-di-Cl | 0 | piperidine | 0.00035 | 21 | 60000 | [160] |
| **79** | -CH$_2$- | Cl | 2,4-di-Cl | 0 | piperidine | 2050 | 0.34 | 0.0002 | [228] |
| **80** | -CH$_2$- | Br | 2,4-di-Cl | 0 | piperidine | 1570 | 0.27 | 0.0002 | [228] |
| **81** | -CH$_2$- | CH$_3$ | 2,4-di-Cl | 0 | piperidine | 363 | 0.037 | 0.0001 | [228] |
| **82** | -CH$_2$- | Cl | 4-Cl | 0 | piperidine | 1787 | 0.9 | 0.0005 | [228] |
| **83** | -CH$_2$- | H | 2,4-di-Cl | 0 | piperidine | 1152 | 0.385 | 0.0003 | [228] |
| **84** | -CH$_2$- | Cl | 3,4-di-Cl | 1 | 1,3,3-trimethylbicyclo [2.2.1]heptyl | / | <500 | >10 | [227] |
| **85** | -CH$_2$- | Br | 2,4-di-Cl | 1 | bicyclo[3.2.1]oct-3-yl | / | <500 | >10 | [227] |
| **86** | -CH$_2$- | Br | 4-Me | 1 | 7,7-dimethylbicyclo[4.1.0] hept-3-yl | / | <500 | >10 | [227] |

another paper, Katoch-Rouse described the synthesis of [$^{18}$F]-NIDA-42033 (**26**), a ligand useful as PET radiotracer, starting from its bromo derivative [156]. Recently, Kumar *et al*. [157] proposed the synthesis of a $^{11}$C derivative of NIDA-41087 (**61**), a compound previously described by Katoch-Rouse (Table **5**). Post-mortem binding of this compound to human prefrontal cortex was also investigated. Interestingly, CP-272,871 (**66**), described by Meschler as a $CB_1$ inverse agonist (Ki=57 nM, [$^3$H]-CP-55,940, rat brain homogenates), possesses a lipophilicity similar to the NIDA-41087 one [62]. However, despite the presence of a methoxy function, no radiolabelled derivative has been described to date. One explanation could be the compound low selectivity (2 fold) for the $CB_1$ cannabinoid receptor.

Several new attempts to increase the affinity of the diarylpyrazole derivatives were recently made by rigidifying the SR141716A structure (Table **7**). Six fused ring analogues of SR141716A, obtained by fusion of the 5-(4-chlorophenyl) substituent with the central pyrazole to form an indazole ring, were published by Bass *et al.* in 2002 [61]. The compound possessing the highest affinity, O-1248 (**67**), has a Ki value of 475 nM, as determined by displacement of [$^3$H]-CP-55,940 from rat brain membranes.

Stoit and colleagues from Solvay Pharmaceuticals, published a paper describing the synthesis and pharmacological characterisation of new benzocyclohepta-pyrazole derivatives as $CB_1$ antagonists (**69**-**74**) [158]. The affinity of these compounds was more or less one order of magnitude lower than the SR141716A one, with pKi values ranging from 6.4 to 7.2, compared to 7.6 for the SR compound (Table **7**). Compounds were shown to be antagonists, with $pA_2$ values from 7.0 to 8.9, as they prevent CP-55,940-induced cAMP accumulation. However, the authors investigating the bioavailability of their compounds, either *po* or after *ip* injection, found negligible blood plasma levels.

However, just before the publication of Stoit results, Sanofi-Synthelabo took a patent describing tricyclic pyrazolecarboxylic acid amide derivatives having antagonist properties on the $CB_1$ cannabinoid receptor and submicromolar affinities (**75**-**77**) [159]. Thirty examples were given, along with their synthetic pathway, but no individual pharmacological data were shown.

More recently, Ruiu and colleagues published a paper describing the synthesis and complete pharmacological characterisation of a very potent $CB_1$ receptor ligand christened NESS 0327 (**78**) [160]. This compound has the same structure as compound **69** published by Stoit *et al.* (compound 20 in the reference [158]). Nevertheless, Ruiu has obtained an affinity for the $CB_1$ cannabinoid receptor 5000 times bigger than the SR141716A, one with Ki values of 0.35 pM and 1.8 nM, respectively, using mouse forebrain homogenates and [$^3$H]-CP-55,940 as radioligand. Furthermore, the selectivity ratio obtained for this compound by Ruiu is 60,000, with a Ki value for the $CB_2$ receptor of 21 nM against the [$^3$H]-CP-55,940. The discrepancy between the data obtained independently by Stoit *et al.* and Ruiu *et al.* could hardly be explained by the differences in the origin of the receptor used, respectively $hCB_1$-CHO and mouse forebrain.

To further explore the pharmacology of this compound, a functional assay using the mouse *vas deferens* model was performed by Ruiu and colleagues, enlightening the competitive antagonist properties of NESS ($pA_2$= 12.46, against WIN-55,212-2) which had no effect by itself up to 1µM. Moreover, NESS 0327 was unable to affect basal binding of the [$^{35}$S]-GTP S, demonstrating its lack of negative intrinsic activity. *In-vivo* studies were conducted using the hot plate and tail flick tests, NESS dose-dependently abolished the antinociceptive effect of WIN-55,212-2, but had no effect by itself. However, the authors suggested, based on the affinity and *in-vivo* activity of their compound, that NESS 0327 possesses a poor central bioavailability.

Another example of ring-constrained biarylpyrazole was described by Herbert Seltzman team [161]. They investigated a photocyclisation reaction, starting from the SR141716A, leading to a pyrazolo[1,5-*f*]phenanthridine structure (Fig. **5**). The new compound (**68**) was tested on the $CB_1$ receptor (whole rat brain), against [$^3$H]-CP-55,940, [$^3$H]-SR141716A and [$^3$H]-WIN-55,212-2, and exhibited Ki values of 48, 35 and 50 nM, respectively. The $CB_2$ affinity was assessed using [$^3$H]-CP-55,940 and was found to be 100 times lower (Ki = 3340 nM). No effect on the [$^{35}$S]-GTP S assay (rat brain) was observed by the authors.

O-1248, **67** **68**

**Fig. (5).** $CB_1$ cannabinoid receptor antagonists: 3. Diarylpyrazole derivatives. Two ring-constrained diarypyrazole derivatives, O-1248 (**67**) and a pyrazolo[1,5-*f*]phenanthridine derivative (**68**).

It appears from these attempts to increase SR141716A affinity by rigidifying its structure that only a limited increase in the $CB_1$ cannabinoid receptor affinity can be expected, with the apparent exception of NESS 0327. Moreover, rigidifying the structure often results in a more lipophilic compound in a family of compounds, being yet lipophilic.

## 4. Phenyl Benzofuranone Derivatives

In a 1997 patent from Eli Lilly, was described the synthesis of aryl-benzothiophene and aryl-benzofurane derivatives (**87**-**89**, Fig. **6**) having $CB_1$ receptor affinity and antagonist properties [162]. From ten compounds having a Ki value lower than 25 µM ([$^3$H]-CP-55,940, $hCB_1$-CHO cells), one was selected and claimed to have a Ki of 170 nM. Compound **88** was shown in the patent to antagonise the effect of anandamide on cAMP accumulation with an $IC_{50}$ of

**87** LY320135, **88** **89**

**Fig. (6).** $CB_1$ cannabinoid receptor antagonists: 4. Phenyl benzofuranone derivatives. Chemical structures of two aryl-benzofurane derivatives (**87**-**88**) and one aryl-benzothiophene (**89**) derivative from Eli Lilly.

500 nM, and the effect of WIN-55,212-2 on calcium channels at 1μM. Moreover, the intra-peritoneal injection (20 mg/kg) of this compound antagonised the *in-vivo* effect of anandamide in the Open Field Assay, a mouse behaviour model. Later on, Felder and colleagues gave a further description of this compound, called LY320135, in a paper describing its effect on cAMP accumulation and on ionic currents [163]. The selectivity ratio of LY320135 was 106, with Ki values of 141 and 14900 nM, for $hCB_1$ and $hCB_2$ receptors ([$^3$H]-CP-55,940) respectively. LY320135 antagonised anandamide inhibition of forskolin-induced cAMP accumulation with an $IC_{50}$ value of 734 nM. Christopoulos *et al.*, in 2001, reported, for LY320135, a $pK_b$ value of 5.27 in the inhibition of WIN-55,212-2 mediated response in the rat electrically-stimulated *vas deferens*, while the pKb value for SR141716A was 7.5 [164].

## 5. Azetidine Derivatives

The family of azetidine compounds, illustrated by compounds **90** and **91** (Fig. **7**), was developed at Aventis by Daniel Achard and colleagues and was described in a series of patents [165-168]. They claimed the $IC_{50}$ values of these compounds for the $CB_1$ receptor to be less than or equal to 100 nM. These values were obtained following the procedure described by Kuster *et al.* [169]. The antagonist property was shown using an *in-vivo* model, the reversal of hypothermia induced by CP-55,940 in mice, and the $ED_{50}$ obtained were lower than 50 mg/kg.

Other patents were taken by Aventis in which some potential applications for their compounds were described. For instance, an association between an azetidine derivative having $CB_1$ antagonists properties and sibutramine, a serotonin and norepinephrine reuptake inhibitor, was claimed [170] as a treatment against obesity. Oral administration of compound **90** (3 mg/kg) and sibutramine (0.6 mg/kg) to fa/fa Zucker rats, which are genetically obese rats, has resulted in a decreased food intake, compared to control lean Zucker rats. In another patent, the association of an azetidine derivative (1-10 mg/kg, *p.o.*) and a $D_2/D_3$ agonist like quinpirole (0.1 mg/kg, *ip*) was claimed to have a therapeutic potential in the treatment of Parkinson's disease [171]. The effect has been evidenced using an akinesia model in the rat. Evidences supporting the use of $CB_1$ antagonists as adjuvant in the treatment of Parkinson's disease begin to appear. It seems that such compounds would be helpful in the treatment of Parkinsonism [172] and levodopa-induced dyskinesia [173]. Brotchie published an interesting paper reviewing the $CB_1$ cannabinoid receptor signaling in Parkinson's disease [174]. Nevertheless, further clinical studies should be conducted to further confirm the usefulness of $CB_1$ antagonists in the treatment of Parkinson's disease.

## 6. Aryl-Imidazolidine-2,4-Diones Derivatives

In 1999, the Didier Lambert team published a paper describing the affinity of 24 new 3-alkyl-5,5'-diphenyl-imidazolidine-2,4-dione derivatives for the $hCB_1$ receptor [175]. The preliminary structure-activity relationships

**90** **91**

**Fig. (7).** $CB_1$ cannabinoid receptor antagonists: 5. Azetidine derivatives. Two examples (**90**-**91**) of the azetidine derivatives developed by Aventis.

**Table 8. $CB_1$ Cannabinoid Receptor Antagonists: 6. Aryl-Imidazolidine-2,4-Diones Derivatives**

Structures, affinities ([$^3$H]-SR141716A, $hCB_1$-CHO cells) and pKb values ([$^{35}$S]-GTP S, HU210, rat brain) of three aryl-imidazolidine-2,4-dione derivatives (**92**-**94**).

| Cpd. | n° | R | Ki (nM) [a] | pKb [b] |
|---|---|---|---|---|
| DML20 | **92** | ethylmorpholine | 70 | 6.11 |
| DML21 | **93** | 1-hydroxypropyl | 103 | 6.25 |
| DML23 | **94** | heptyl | 98 | 5.74 |

[a] [175]
[b] [177]

showed that the substitution at the nitrogen in position 3, as well as a bromine atom in *para* of the phenyl rings, are mandatory for the compounds affinity. Later on, three compounds termed DML20 (**92**), DML21 (**93**), and DML23 (**94**) were characterised as neutral antagonists using the [$^{35}$S]-GTP S assay on rat cerebellum membranes [176] (Table **8**). In the same model, they competitively inhibited HU-210-induced [$^{35}$S]-GTP S binding with $pK_B$ values of 6.11, 6.25, and 5.74, respectively. Moreover, these compounds were proven to be quite selective for the $CB_1$ receptor.

Very recently, the functionality of these DML compounds was explored using the [$^{35}$S]-GTP S assay, both on rat and human $CB_1$ cannabinoid receptors [177]. The data obtained confirm that the 3-alkyl-5,5'-diphenyl-imidazolidinedione derivatives behave as neutral antagonists on rat $CB_1$ cannabinoid receptor (rat brain). However, DML 20, DML21 and DML23 acted as inverse agonists on the human receptor ($hCB_1$-CHO cells). Furthermore, the authors showed that this different functionality is not due to the $hCB_1$ receptor level of expression in the recombinant cell line, as whatever the level of expression (Bmax of 44 pmol/mg or 3.2 pmol/mg), DML derivatives behaved as inverse agonists of the $hCB_1$ cannabinoid receptor.

## 7. Diarylimidazoles Derivatives

Finke and colleagues, at Merck, developed a new class of $CB_1$ receptor antagonists based on an imidazole nucleus. Over eighty 4,5-diaryl-imidazoles derivatives having $CB_1$ antagonist properties were claimed in a patent published in 2003 [178]. Some representative compounds of the patent are illustrated in Table **9** (**95-100**). The affinity of the new compounds was determined using [$^3$H]-CP-55,940 and $hCB_1$-CHO cells. Although no detailed pharmacological data were given in the patent, *in-vitro* and *in-vivo* properties of one compound, termed Cpd A (**100**), have been described elsewhere [179]. Its affinity for the $CB_1$ cannabinoid receptor was shown to be nanomolar ($IC_{50}$ = 4 nM) and 75 times higher than the one for the $CB_2$ cannabinoid receptor ($IC_{50}$ = 300 nM). The inverse agonism of the compound was extrapolated by inhibiting CP-55,940-induced hypothermia in mice ($EC_{50}$ = 18 nM). Moreover, one oral dose (10 mg/kg) reduces the food intake in diet-induced obese rats. Interestingly, food intake of $CB_1^{-/-}$ mice was not altered. Body weight loss was maintained all over the study during a chronic administration (14 days) of 10 mg/kg of Cpd A.

Later on, Merck published a patent [180] in which some of the 4,5-diaryl-pyrazoles derivatives previously described were claimed to be useful in the treatment, or the prevention of obesity in association with compounds inhibiting the 11 -hydroxysteroid dehydrogenase type 1 (1 -HSD1) enzyme. The 1 -HSD1 enzyme is responsible for the synthesis of cortisol, increased levels of which, according to the inventors, are associated with obesity. However, neither results on appetite suppression, nor on weight loss, were given even if some indications on the affinity of the compounds for the cannabinoid receptors were given.

Another imidazole ring pattern of substitution was proposed by Hagmann and collaborators [181]. The compounds described, 43 examples are given, possess two phenyls, the first one in position 1, linked to the nitrogen, and the second one in position 2. The carboxamide substituent is in position 4, instead of in position 2 for the diarylimidazoles described by Finke. These 1,2-diaryl-imidazoles are claimed to be $CB_1$ cannabinoid receptor antagonists or inverse agonists, but the absence of precise pharmacological values do not allow any comparison between the two substitutions patterns. However, a same pattern of substitution was used by other researchers. Among them, Dyck *et al*. described in 2004, ten 1-(4-chlorophenyl)-2-(2,4-dichlorophenyl)-imidazole derivatives varying by their carboxamide substituent in position 4 (**101-103**, Table **9**) [152]. For instance, with a 3-azabicyclo[3.3.0]octan-3-yl substituent (**101**), they obtained a Ki value of 14 nM ([$^3$H]-CP-55,940, $hCB_1$-HEK-EDNA cells). In a [$^{35}$S]-GTP S assay, **101** exhibited an $IC_{50}$ value of 19 nM.

**Table 9. $CB_1$ Cannabinoid Receptor Antagonists: 7. Diaryl-Imidazole Derivatives**

Structure, and affinity range of some 4,5-diaryl-imidazole derivatives for the human cannabinoid receptors determined using [$^3$H]-CP-55,940 as radioligand and $hCB_{1\&2}$-CHO cells.

| n° | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $hCB_1$ ($IC_{50}$ or Ki) | $hCB_2$ ($IC_{50}$ or Ki) | References |
|---|---|---|---|---|---|---|---|
| **95** | $CH_3$ | CO-NH-piperidinyl | 2,4-di-Cl-Ph | 4-Cl-Ph | 10 nM [a] | 100 –1000 nM [a] | [178] |
| **96** | $CH_3$ | CO-NH-piperidinyl | 4-Me-Ph | 4-Me-Ph | 100 –1000 nM [a] | > 1000 nM [a] | [178] |
| **97** | $CH_3$ | CO-NH-cyclohexyl | 4-Me-Ph | 4-Me-Ph | 100 –1000 nM [a] | > 1000 nM [a] | [178] |
| **98** | $CH_3$ | CO-NH-phenyl | 2,4-di-Cl-Ph | 4-Cl-Ph | 10 – 100 nM [a] | 100 –1000 nM [a] | [178] |
| **99** | $CH_3$ | CO-NH-cyclohexyl | 2,4-di-Cl-Ph | 4-Cl-Ph | 10 nM [a] | 100 –1000 nM [a] | [178] |
| **100** | $CH_3$ | CO-NH-cyclohexyl | 2,4-di-Cl-Ph | 4-Cl-Ph | 4 nM [a] | 300 nM [a] | [179] |
| **101** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-(3-azabicyclo[3.3.0] octan-3-yl) | $CH_3$ | 14 nM [b] | N.D. | [152] |
| **102** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-(3-azabicyclo[3.3.0] octan-3-yl) | H | 66 nM [b] | N.D. | [152] |
| **103** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-piperidinyl | H | 85 nM [b] | N.D. | [152] |
| **104** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-piperidinyl | H | 23 nM [b] | 542 nM [b] | [182] |
| **105** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-piperidinyl | $CH_3$ | 30 nM [b] | 608 nM [b] | [182] |
| **106** | 4-Br-Ph | 2,4-di-Cl-Ph | CO-NH-piperidinyl | $CH_3$ | 60 nM [b] | 489 nM [b] | [182] |
| **107** | 4-$CF_3$-Ph | 2,4-di-Cl-Ph | CO-NH-piperidinyl | $CH_3$ | 29 nM [b] | 634 nM [b] | [182] |
| **108** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-morpholinyl | $CH_3$ | 197 nM [b] | 3297 nM [b] | [182] |
| **109** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-piperidin-4-ol | $CH_3$ | 172 nM [b] | 3959 nM [b] | [182] |
| **110** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-piperidinyl | CN | 30 nM [b] | 1590 nM [b] | [182] |
| **111** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH- piperidinyl | $CH_2F$ | 36 nM [b] | 906 nM [b] | [182] |
| **112** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-piperidinyl | $CH_2$-$CH_3$ | 14 nM [b] | 430 nM [b] | [182] |
| **113** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-piperidinyl | Cl | 27 nM [b] | 823 nM [b] | [182] |
| **114** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-piperidinyl | Br | 23 nM [b] | 746 nM [b] | [182] |
| **115** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-tetrahydroisoquinoline | $CH_3$ | 34 nM [b] | 696 nM [b] | [182] |
| **116** | 4-Cl-Ph | 2,4-di-Cl-Ph | CO-NH-cycloheptyl | $CH_3$ | 35 nM [b] | 349 nM [b] | [182] |
| **117** | 2,4-di-Cl-Ph | 4-Cl-Ph | CO-NH-piperidinyl | $CH_3$ | 403 nM [b] | 208 nM [b] | [182] |

[a] $IC_{50}$ value ; [b] Ki value

Lange and co-workers from Solvay described twenty-eight imidazole derivatives [182]. The 2,4-dichlorophenyl substituent at position 2 was kept constant, while the substitutions at the other positions were explored (Table **9**). Position 5 can accommodate a large range of little substituents like hydrogen (**104**), methyl (**105**), ethyl (**112**), chlorine (**113**), bromine (**114**), fluoromethyl (**111**) , or cyano (**110**), without major changes in the affinity. At position 4, more bulky substituents like 1,2,3,4-tetraisoquinoline (**115**) or cycloheptyl (**116**) were well tolerated, whereas the presence of a more hydrophilic moiety such as morpholine (**108**) or piperidin-4-ol (**109**) was detrimental to the affinity. Interestingly, position exchange between the 2,4-diphenyl and the 4-chlorophenyl substituents led to a 13 fold lower affinity with Ki values of 30 and 403 nM for **105** and **117**, respectively ([$^3$H]-CP-55,940, $hCB_1$-CHO cells). In the functional assay, which was inhibition of WIN-55,212-2-induced [$^3$H]-arachidonic acid release by $hCB_1$-CHO cells, all tested compounds behaved as antagonists. Compound **105** showed a $pA_2$ value of 8.6, which is the same the authors obtained for SR141716A. It would be interesting to assess if these compounds are true antagonists or if they act more as inverse agonists. Compound **105** when administered to rats was able to inhibit CP-55,940-induced hypotension

with an $ED_{50}$ value of 2.4 mg/kg, which is close to the 3 mg/kg obtained for SR141716A. The compound was also active in another *in-vivo* model, the WIN-55,212-2-induced hypothermia in mice. Compound **104**, which has no substituent in position 5 of the imidazole ring, was less active in the hypotension model and inactive in the hypothermia model. Thus, a methyl group is the preferred substituent in position 5 of the imidazole moiety. Other properties, either experimental (P-glycoprotein affinity, $logP_{HPLC}$), or computational (molecular volume, polar surface area), obtained for compounds **105** were similar to those obtained for SR141716A. Finally, molecular modelling studies revealed a close structural overlap between the two compounds.

## 8. 3,4-Diaryl-Pyrazoline Derivatives

Lange and collaborators also discovered 3,4-diaryl-pyrazoline derivatives, after a screening of compounds resembling to SR141716A [183,184]. Compound **118,** that was initially identified during the screening, possesses a Ki value of 197 nM, is selective for $hCB_1$ and is as potent as SR141716A. The structural modulations of **118** led to some interesting compounds (Table **10**). Six compounds possess a Ki value for the $hCB_1$ cannabinoid receptor equal to or lower than 25 nM, antagonist properties in the inhibition of [$^3$H]-arachidonic acid release by WIN-55,212-2-stimulated $hCB_1$-CHO cells, and Ki values for the $hCB_2$ receptor over 1μM. The diaryl-pyrazoline derivatives contained a chiral center at position 4. The authors resolved the racemic mixture of two compounds (**121** and **130**). The levorotatory enantiomers appeared to be the eutomers. For compound SLV319, levorotatory enantiomer of **121**, the Ki and $pA_2$ values were 7.8 nM and 9.9, respectively. The Ki and $pA_2$ values of SLV326, levorotatory enantiomer of **130**, were 35.9 nM and 9, respectively.

Two *in-vivo* models, CP-55,940-induced hypotension in rat, and WIN-55,212-2-induced hypothermia in mouse, were used to assess the potential of these compounds. The results are of the same order of magnitude than those obtained for SR141716A. For instance, in the hypotension model, SLV319 ((**-**)**121**) and SLV326 ((**-**)**130**) showed $ED_{50}$ values

**Table 10. $CB_1$ Cannabinoid Receptor Antagonists: 8. 3,4-Diaryl-Pyrazolines Derivatives**

Structure, affinity for the $hCB_1$ and $hCB_2$ cannabinoid receptors (Ki, nM) expressed in CHO transfected cells ([$^3$H]-CP-55,940) and potency ($pA_2$) of some 3,4-dirylpyrazolines derivatives. Table adapted from [184].

| n° | $R_1$ | $R_2$ | $R_3$ | $R_4$ | Ki $hCB_1$ (nM) | Ki $hCB_2$ (nM) | $pA_2$ ($CB_1$) |
|---|---|---|---|---|---|---|---|
| **118** | H | H | H | 4-$CH_3$ | 197 | > 1000 | 8.4 |
| **119** | H | H | H | 2,4,6- $CH_3$ | 24 | > 1000 | 9.4 |
| **120** | H | H | H | 4-Cl | 16 | > 1000 | 9.5 |
| **121** | H | $CH_3$ | H | 4-Cl | 25 | > 1000 | 8.7 |
| **122** | H | $CH_3$ | $CH_3$ | 4-Cl | 280 | > 1000 | 8.5 |
| **123** | H | H | H | 4-F | 53 | > 1000 | 9 |
| **124** | H | $CH_3$ | H | 4-F | 338 | > 1000 | 8.5 |
| **125** | H | $CH_3$ | $CH_3$ | 4-F | > 1000 | > 1000 | < 7.5 |
| **126** | H | $CH_3$ | H | 3-Cl | 14 | > 1000 | 8.6 |
| **127** | 4-Cl | $CH_3$ | H | 4-Cl | 255 | N.D. | N.D. |
| **128** | 4-F | $CH_3$ | H | 4-Cl | 584 | N.D. | N.D. |
| **129** | H | $CH_3$ | H | H | 170 | N.D. | 7.5 |
| **130** | H | $CH_3$ | H | 4- $CF_3$ | 221 | > 1000 | 9.3 |
| SR141716A | / | / | / | / | 25 | 1580 | 8.6 |

**Fig. (8).** $CB_1$ cannabinoid receptor antagonists: 9. Diaryl-pyrazine, diphenyl-pyridine, diphenyl-phenyl, and diaryl-pyrimidines derivatives. Chemical structures of two 5,6-diaryl-pyrazine-2-amide derivatives (**131**-**132**) developed as $CB_1$ cannabinoid receptor antagonists by AstraZeneca.

of 5.5 and 2 mg/kg (p.o.), respectively, proving their *in-vivo* efficacy after oral administration. The authors also demonstrated that SLV319 is devoid of affinity for the P-glycoprotein pump, and that it possessed a good CNS/plasma ratio (1.7). These two compounds (SLV319 and SLV326) were chosen as development candidates by Solvay, and entered clinical Phase I trials in 2003 [185].

## 9. Diaryl-Pyrazine, Diphenyl-Pyridine, Diphenyl-Phenyl, and Diaryl-Pyrimidines Derivatives

In 2003, appeared several patents describing new families of compounds that bind to the $CB_1$ cannabinoid receptor. These compounds have in common, a central, six atoms aromatic ring, which could be a pyrazine, a pyridine, a phenyl, or a pyrimidine, and that is substituted by at least two phenyl rings.

The first patents were from Berggren and collaborators, from AstraZeneca, who published two patents describing the synthesis of 5,6-diaryl-pyrazine-2-amide derivatives (**131**-**132**, Fig. **8**), useful as $CB_1$ cannabinoid receptor antagonists [186, 187]. The activity of twenty derivatives was determined using a $[^{35}S]$-GTP S assay and $hCB_1$-CHO transfected cells. The concentration required to give half maximal inhibition of CP-55,940-induced $[^{35}S]$-GTP S binding ($IC_{50}$) is lower than 200 nM for the preferred compounds. No affinity data were given for these compounds.

The same year, in a patent from Merck, Finke and collaborators described several 5,6-di-phenyl-pyridine derivatives as $hCB_1$ antagonists or inverse agonists [188]. The compounds have a substituent in position 3 of the pyridine core (Fig. **9**). This substituent could be a cyano (**133**-**134**) or a nitro group, a halogen, an ester or an amide (**135**-**136**). Over 150 compounds were synthesised to illustrate the invention, but no pharmacological data was disclosed in the patent.

A few months later, another patent was taken by Sanofi, claiming the antagonist properties of 5,6-di-phenyl-2-pyridine carboxamide derivatives [189]. These compounds,

**Fig. (9).** $CB_1$ cannabinoid receptor antagonists: 9. Diaryl-pyrazine, diphenyl-pyridine, diphenyl-phenyl, and diaryl-pyrimidines derivatives. 5,6-Di-phenyl-pyridine derivatives patented by Merck (**133**-**136**) and by Sanofi (**137**) as $CB_1$ cannabinoid receptors antagonists.

**Fig. (10).** $CB_1$ cannabinoid receptor antagonists: 9. Diaryl-pyrazine, diphenyl-pyridine, diphenyl-phenyl, and diaryl-pyrimidines derivatives. Representative structures of the diphenyl-phenyl (**138**-**139**) and 4,5-diphenyl-pyrimidine (**140**) derivatives developed as cannabinoid antagonists at Sanofi and Merck, respectively.

28 examples are given, structurally related to the diphenyl-phenyl derivatives, have their pyridine core substituted by two phenyl rings and by an amide moiety (**137**, Fig. **9**). The $IC_{50}$ values were lower than 100 nM, but no data were given showing the antagonist properties, although the compounds were assayed in the adenylate cyclase inhibition assay.

Along this line, Sanofi Synthelabo developed new diphenyl-phenyl derivatives claimed to have $CB_1$ cannabinoid receptor antagonist properties [190]. They are based on a central phenyl ring, substituted by two phenyls and by an amide moiety (**138**-**139**, Fig. **10**). Thirteen compounds were claimed along with their synthesis. Their $IC_{50}$ values were lower than 100 nM ([$^3$H]-CP,55-940, $hCB_1$-CHO cells), but no other specific data (selectivity, $pA_2$...) was given.

Finally, Kopka *et al.*, from Merck, described more than one hundred 4,5-diaryl-pyrimidine derivatives as $CB_1$ cannabinoid receptor antagonists (**140**, Fig.**10**) [191]. However, no precise data were provided concerning the affinity and functionality of the compounds.

## 10. Other Derivatives

Several amide derivatives structures recently appeared among the $CB_1$ cannabinoid receptor antagonists. In a series of patents taken by Merck in 2003, Hagmann and colleagues described a large amount of compounds obtained by parallel synthesis which are claimed to be $CB_1$ cannabinoid receptor antagonists [192-195]. They were synthesised by reacting a library of substituted amines with a library of carboxylic acids (**141**-**144**, Fig. **11**). However, as no value was given for the affinity, or activity of these amides, we will not further discuss this class of compounds.

Several 1,5-diaryl-pyrrole-3-carboxamide derivatives were synthesised and claimed to be $CB_1$ cannabinoid receptor antagonists by Berggren *et al*. [196]. The affinity values were not disclosed in the patent (**145**-**146**, Fig. **12**). Other pyrrole derivatives were synthesised by Guba *et al.* at Hofmann-La Roche [197]. More specifically, 2-(thiazol-4-yl)pyrrole derivatives were claimed as $CB_1$ cannabinoid receptor antagonist ($IC_{50}<2\mu M$) (**147**-**148**, Fig. **12**).

**Fig. (11).** $CB_1$ cannabinoid receptor antagonists. 10. Other derivatives. Retrosynthetic scheme of a $CB_1$ cannabinoid antagonist library of amide derivatives developed at Merck. The structures of four representative compounds are illustrated (**141**-**144**).

**Fig. (12).** $CB_1$ cannabinoid receptor antagonists. 10. Other derivatives. Two examples of 1,5-diaryl-pyrrole derivatives described by Berggren *et al.* (**145**-**146**). While the 2-(thiazol-4yl)pyrroles derivatives **147** and **148** were described by Guba *et al*. from Hofmann-La Roche. All these pyrroles derivatives were claimed to be $CB_1$ cannabinoid receptor antagonists.

Another new structure is represented by the 1,2,4-triazole derivatives developed by Jagerovic and collaborators. In a recent paper, were described the synthesis and pharmacological properties of five new 1,5-diphenyl-3-alkyl-triazole derivatives [198]. Among these compounds, only one (**149**) behaved as a $CB_1$ antagonist, inhibiting the WIN-55,212-2-induced contractions in a mouse *vas deferens* preparation. However, despite its effects in isolated tissue

**Table 11. $CB_1$ Cannabinoid Receptor Antagonists: 10. Other Derivatives**

Structure and affinity of diaryl-triazole derivatives.

| Cpd. | $R_1$ | $R_2$ | $R_3$ | Ki $CB_1$ (nM) | References |
|---|---|---|---|---|---|
| **149** | 2,4-diCl | 4-Cl | hexyl | 855 [a] | [198] |
| **150** | 2,4-diCl | 4-Cl,2-OMe | CO-NH-(3-azabicyclo[3.3.0]octan-3-yl) | 270 [b] | [152] |
| **151** | 2,4-diCl | 4-Cl | CO-NH-(3-azabicyclo[3.3.0]octan-3-yl) | 164 [b] | [152] |
| **152** | 4-Cl | 2,4-diCl | CO-NH-(3-azabicyclo[3.3.0]octan-3-yl) | 137 [b] | [152] |
| **153** | 4-Cl | 2,4-diCl | CO-NH-benzylpyrrolidin-3-yl | 29 [b] | [152] |
| **154** | 4-Cl | 2,4-diCl | CO-NH-1-(4-chlorophenyl)ethyl | 66 [b] | [152] |
| **155** | 4-Cl | 2,4-diCl | CO-NH-piperidinyl | 356 [c] | [182] |
| **156** | 2,4-diCl | 4-Cl | CO-NH-piperidinyl | 382 [c] | [182] |

[a] $[^3H]$-SR141716A, rat cerebellar membranes
[b] $[^3H]$-CP-55,940, $hCB_1$-HEK EDNA cells
[c] $[^3H]$-CP-55,940, $hCB_1$-CHO cells

**156** **157** **158** **159**

**Fig. (13).** $CB_1$ cannabinoid receptor antagonists: 10. Other derivatives. Structures of 4,5-diaryl-thiazole derivatives patented as $hCB_1$ cannabinoid receptor antagonists by Solvay Pharmaceuticals (**156, 157, 158**) or by AstraZeneca (**157, 158, 159**).

assays (mouse *vas deferens* and guinea pig ileum), the compound possessed only a reduced $rCB_1$ affinity with Ki values of 855 and 748 nM using [$^3$H]-SR141716A and [$^3$H]-WIN-55-212-2, respectively (Table **11**). Further, four series of compounds, differing by the nature of the substituent around the triazole core, were also described in a patent [199]. Usually the compounds claimed possess two aromatic rings possibly substituted and one linear alkyl chain. The affinity for the cannabinoid receptors was not given. Nevertheless, **149** was evaluated in two isolated tissue models, the inhibition of the electrically evoked contractions in the guinea pig ileum and in the mouse *vas deferens*. In the two models, compound **149** inhibited the effect of WIN-55,212-2, but had no effect by itself. In the *vas deferens* model, the authors obtained a $pA_2$ value of 7.48, to be compared with 7.63 obtained for AM251.

Others 1,2,4-triazole derivatives were described by Dyck *et al*., compounds **150-154**, unlike the Jagerovic ones, have an amide moiety in position 3 [152]. The highest affinity was obtained with a 1-benzyl-pyrrolidin-3-yl substituent. The Ki value was 29 nM ([$^3$H]-CP-55,940, $hCB_1$-HEK-EDNA cells), to be compared to 12 nM obtained for the SR141716A.

Lange *et al.* described a triazole derivative (**155**) which can be superimposed with SR141716A. Compound **155** showed a Ki value of 356 nM ([$^3$H]-CP-55,940, hCB1-CHO cells), a $pA_2$ value of 8.3 in the inhibition of WIN-55,212-induced release of [$^3$H]-arachidonic acid by $hCB_1$-CHO cells, and was active *in vivo* in the CP-55,940-induced hypotension in rat ($ED_{50}$ = 23.6 mg/kg) [182].

Yet, another five membered ring, a thiazole, has been used as central moiety of a new class of cannabinoid ligands. The thiazole ring is substituted by two aryls, in position 4 and 5, and by an amide (position 2) as in compounds **156-159** (Fig. **13**). Forty derivatives of this type were synthesised by Lange and colleagues from Solvay Pharmaceuticals, and claimed in a patent as agonists or antagonists of the $CB_1$ cannabinoid receptor [200]. No pharmacological data were provided, however, their activity was assessed by measuring the cAMP in transfected $hCB_1$-CHO cells. In a recent paper from Lange *et al.*, two of these thiazole derivatives were further described [182]. Compound 13 (**157**), the most closely related to SR141716A, has a Ki value of 227 nM, while for compound 14 (**158**), Ki value is over 1000 nM. Thus, as in the imidazole series (see Table **9**), the aromatic substitution pattern is of great importance for the $CB_1$ cannabinoid receptor affinity. Compound 13, however, was devoid of *in vivo* activity.

Seventeen 4,5-diaryl-thiazole derivatives were also synthesised by Berggren *et al.* and patented by AstraZeneca (**157-159**, Fig. **13**) [201].

In a patent from Merck, Toupence *et al*. described over 190 substituted furo[2,3]pyridines claimed to be $CB_1$ cannabinoid receptor antagonists [202]. The affinity ($IC_{50}$ < 1μM) was measured using recombinant CHO cells and [$^3$H]-CP-55,940, and the activity using cAMP dosage (**160**, Fig. **14**).

Very recently, Alanine, from Hoffmann-LaRoche, found that diarylbenzo[1,3]dioxole derivatives act as $CB_1$

**160** **161** **162**

**Fig. (14).** $CB_1$ cannabinoid receptor antagonists: 10. Other derivatives. One example of a furo[2,3]pyridine derivative (**160**) and two examples of diarylbenzo[1,3]dioxole derivatives (**161**-**162**) described as $hCB_1$ cannabinoid receptor antagonists by Toupence *et al*. and Alanine *et al*. respectively.

cannabinoid receptor antagonists (**161-162**, Fig. **14**) [203]. Approximate $IC_{50}$ values for twelve compounds ([$^3$H]-CP-55,940, $hCB_1$-HEK cells) , among the three hundred and eighty compounds described, were given ($IC_{50}$<2μM).

Griffith, from Pfizer, described three new series of $CB_1$ cannabinoid receptor antagonists, based on a bicyclic central aromatic ring. Purine, pyrazoltriazines, and pyrazolo[1,5a]pyrimidines derivatives were synthesised and investigated for their ability to interact with the $CB_1$ cannabinoid receptor. Albeit a [$^{35}$S]-GTP S assay was described, no data were given concerning the antagonist or inverse agonist properties of such compounds. Two hundred and eighty purine derivatives were synthesised [204]. The affinities of **163** and **164** were given in the patent (2.8 and 1.2 nM, [$^3$H]-SR141716A). In a following patent, the synthesis of sixty pyrazoltriazine derivatives was described (**165**, Fig. **15**) [205]. The affinity of each compound was not given, but was said to be ranging between 0.1 and 590 nM. And finally, more than forty pyrazolo[1,5a]pyrimidines were synthesised (**166**, Fig. **15**) [206]. Their affinity for the $CB_1$ cannabinoid receptor was lower than 155 nM. Despite the fact that a great number of derivatives share the same substitution pattern around the central bicyclic aromatic ring, the comparison of these three new series of compounds is made difficult by the absence of precise affinity data.

## III. $CB_2$ LIGANDS RECEPTOR ANTAGONISTS AND INVERSE AGONISTS

The compounds acting as antagonist, or inverse agonists, at the $CB_2$ cannabinoid receptor are reviewed in this third part of the paper. As for the CB1 cannabinoid receptor ligands, they are classified depending on their chemical structures.

### 1. Classical Cannabinoid Derivatives

The first report of an antagonism mediated by $^9$-THC at the peripheral cannabinoid receptor was made by Bayewitch *et al*. in 1995 [207]. In their hands, $^9$-THC was not able to inhibit the forskolin-stimulated adenylyl cyclase activity in an assay performed on $hCB_2$-CHO cells. Subsequently, they showed that $^9$-THC was able to antagonise HU210-induced inhibition of adenylyl cyclase activity, thus acting as an antagonist of the $hCB_2$ cannabinoid receptor expressed in CHO cells [208]. Furthermore, very recently, Govaerts *et al.* [209] reported an inverse agonist effect of $^9$-THC and $^8$-THC on $hCB_2$-CHO cells using the [$^{35}$S]-GTP S assay. The $pEC_{50}$ values were 7.63 and 8.88, respectively, while the $E_{max}$ values were –27% and –16%, as compared to basal.

Some other classical cannabinoids were shown to possess an antagonist activity at the $CB_2$ receptor. This is the case, for instance, for the non-selective O-1184 (**2**) (Ki = 7.4 nM), which enhances the forskolin-induced cAMP production in $CB_2$-CHO cells ($E_{Max}$ = 161%, $EC_{50}$ = 6.3 nM), and also for O-584 (**3**) ($E_{Max}$ = 246%, $EC_{50}$ = 138 nM) [39].

### 2. Indole Derivatives

Since the disclosure, in 1993, of a second cannabinoid receptor, many attempts have been made to synthesise $CB_2$ cannabinoid receptor selective ligands. The first compound reported to have a somehow greater affinity for the peripheral cannabinoid receptor was WIN-55,212-2. The compound was introduced by D'Ambra and colleagues [210], back in 1992, as a new cannabinoid ligand having a nanomolar affinity and acting as an agonist. In 1996, Showalter *et al.* used a transfected cell line expressing the $CB_2$ cannabinoid receptor, in order to identify selective ligands for this receptor [211]. They showed that the $CB_2$ affinity of WIN-55,212-2 was 7 times greater than the $CB_1$, with Ki values of 1.89 and 0.28 nM for the $CB_1$ and $CB_2$ cannabinoid receptors, respectively. However, WIN-55,212-2 is an agonist of the cannabinoid receptors. Interestingly, WIN-55,212-3 (**167**, Fig. **16**), the so-called inactive enantiomer of WIN-55,212, was recently shown to behave as a low affinity (Ki=36.3 μM) inverse agonist of the $hCB_2$ cannabinoid receptor, as it decreased the [$^{35}$S]-GTP S binding [209].

**Fig. (15).** $CB_1$ cannabinoid receptor antagonists: 10. Other derivatives. Examples of purine (**163-164**), pyrazoltriazines (**165**), and pyrazolo[1,5-a]pyrimidines (**166**) derivatives described as $CB_1$ cannabinoid receptor antagonists by Griffith.

The $pEC_{50}$ value was 5.54 and the $E_{max}$ value –26% as compared to basal.

(S)-WIN-55,212-3, **167** BML-190, **168**

**Fig. (16).** $CB_2$ cannabinoid receptor antagonists: 2. Indole derivatives. Chemical structures of WIN-55,212-3 (**167**) and BML-190 (**168**), two indoles derivatives acting as inverse agonists at the $hCB_2$ cannabinoid receptor.

Further, on the basis of WIN-54,461 (**12**), a $CB_1$ antagonist, AM630 (**13**) was synthesised by replacement of the bromine atom by an iodine one. In the initial report, Pertwee *et al*. showed the antagonist effects of AM630, but also suggested that the $CB_1$ cannabinoid receptor may not be the preferential receptor of AM630 [45]. Later on, Ross *et al.* reported, using $hCB_{1\&2}$-CHO cells and [$^3$H]-CP-55,940, that AM630 binds to the $CB_2$ cannabinoid receptor (Ki = 31.2 nM) with a selectivity ratio over 160 [48]. Furthermore, using a [$^{35}$S]-GTP S assay, they showed its inverse agonist properties ($EC_{50}$ = 76 nM). However, AM630 also acts as an inverse agonist at the $hCB_1$ cannabinoid receptor ($EC_{50}$ = 900 nM) as Landsman *et al.* demonstrated [47]. Recently, Zhang and colleagues reported the characterisation of the microsomal metabolism of AM630 [212].

In 1996, Gallant and colleagues identified, by submitting a large number of compounds to a binding assay, another indole analog as $CB_2$ cannabinoid receptor ligand [213]. On the basis of this compound, christened BML-190 (**168**, Fig. **16**) or called indomethacin morpholinylamide, they synthesised several derivatives that possessed a selectivity ratio for the $CB_2$ cannabinoid receptor of up to 140. These were the first compounds specifically designed to be $CB_2$ cannabinoid receptor ligands. The first report on the pharmacological properties of BML-190 appeared only recently. New and colleagues highlighted in the paper the inverse agonist properties of the compound. BML-190 dose-dependently increases (103%, $EC_{50}$= 980±70 nM) the forskolin-stimulated levels of cAMP in $hCB_2$-HEK cells, while WIN-55,212-2 decreases (44%, 4.5±4.2nM) this accumulation [137].

Surprisingly, in 2002, Melck and collaborators used BML-190 as a $CB_2$ receptor agonist in a cell proliferation assay [214]. BML-190 was also tested as inhibitor of the cyclooxygenase-2 enzyme by Kalgutkar *et al.* [215]. They obtained $IC_{50}$ values higher than 33 and 66 µM for the COX-I and COX-II enzymes, respectively. These values have to be compared with the submicromolar activity of indomethacin, the parent compound. Very recently, Klegeris and colleagues [216] looking for an antineurotoxic action of cannabinoids showed that BML-190 increases TNF- secretion by stimulated THP-1 monocytic cells, but is not effective on the IL-1 secretion.

**Table 12. $CB_2$ Cannabinoid Receptor Antagonists: 3. Diaryl-Pyrazole Derivatives**

Reported binding affinities (Ki) and selectivity ratio ($CB_1/CB_2$) of SR144528 (**169**) for rat and human cannabinoid receptors determined using [$^3$H]-CP 55,940 as radioligand.

SR144528, **169**

| $rCB_2$ (spleen) | $rCB_1$ (whole brain) | Selectivity | Ki $hCB_2$ (transfected CHO cells) | Ki $hCB_1$ (transfected CHO cells) | Selectivity | References |
|---|---|---|---|---|---|---|
| 0.38 nM | 305 nM | 800 | 0.6 nM | 437 nM | 730 | [217] |
| / | / | / | 0.67 nM | 54.8 nM | 80 | [219] |
| / | / | / | 5.6 nM | >10µM | >1780 | [48] |
| / | / | / | 2.5 nM [a] | > 1µM [a] | >400 | [224] |
| 0.24 nM | 27.6 nM | 115 | 1.99 nM | 50.3 nM | 25 | [232] |

a. COS cells

### 3. Diarylpyrazole Derivatives

The same Sanofi team which synthesised the $CB_1$ antagonist SR141716A, reported in 1998, the synthesis and pharmacological characterisation of a $CB_2$ cannabinoid receptor selective antagonist, the SR144528 (**169**) [217, 218]. The structure of the new compound is derived from SR141716A one. SR144528 is the first selective $CB_2$ receptor antagonist reported, as the inverse agonist properties of BML-190 were shown only recently by New *et al.* [137]. The authors reported for **169**, using [$^3$H]-CP-55,940 as radioligand, Ki values of 0.4 nM and 0.6 nM for the rat and the human $CB_2$ receptors, respectively, and found a $CB_1$-$CB_2$ selectivity ratio around 700. Table **12** sums up the binding affinities of SR144528 for the cannabinoid receptors obtained by different authors. Interestingly, while the $CB_2$ affinity is more or less constant, the affinity obtained for the $CB_1$ receptor varies by one order of magnitude [219]. The antagonist effect of SR144528 was highlighted by evaluating its effect on cAMP production by h$CB_2$-CHO cells exposed to 3 nM CP-55,940 ($EC_{50}$=10nM). The antagonism was also observed on the activation of the MAP kinase pathway by CP-55,940 ($IC_{50}$=39nM).

Early after the report of the synthesis and characterisation of SR144528, the same authors further explored the function of this compound and showed its inverse agonist properties using $CB_2$-CHO cells co-transfected with the luciferase reporter gene, with either the CRE or the murine krox24 regulatory sequence [220]. In these models, SR144528 not only inhibits CP-55,940 effects, but also reproduces the effects of a pertussin toxin treatment, proving its inverse agonist properties. SR144528 functionality was also explored by [$^{35}$S]-GTP S binding in h$CB_2$-CHO cells ($IC_{50}$=3 nM) [221]. The inverse agonist function of SR144528 was further illustrated by Rhee and Kim using COS cells co-transfected with h$CB_2$ receptor and adenylyl cyclase [222].

Bouaboula and co-workers investigated SR144528 effects on $CB_2$ cannabinoid receptor phosphorylation status [223]. Firstly, they showed that the $CB_2$ receptor transfected in CHO cells is constitutively active and phosphorylated on Ser 352. Upon treatment by SR144528, this phosphorylation of the receptor was inhibited. Moreover, the SR compound was able to induce dephosphorylation of agonist-induced $CB_2$ phosphorylation. The question whether SR144528 induces a $CB_2$ receptor conformational change making it a better phosphatase-specific substrate, or if SR144528 binding activates specific phosphatases, remains open. However, this remains a nice example of inverse agonist-induced receptor resensitisation.

The Sanofi research team working on cannabinoids conducted investigations in order to determine the key residues for SR144528 interaction with the receptor (Table **13**), as well as the binding mode of the compound. Firstly, they identified the TM4-EL2-TM5 region as a region containing crucial residues for the affinity of several ligands, among them, SR144528 [224]. Among these residues, the mutation of two cysteine of the second extracellular loop (C174 and C179) abolishes the affinity for the $CB_2$ receptor. Later on, the implication of S4.53(161) and S4.57(165), in the binding of SR144528 was evidenced by mutational studies. In the model proposed for the docking of SR144528 into the $CB_2$ receptor, the crucial contacts are comprised into the TM4, this is the case for S4.53(161) and S4.57(165), which are proposed to form hydrogen bonds with the ligand, and W4.64(172) proposed to have aromatic interactions with the 4-methylbenzyl of the SR144528 [225]. In 2003, Feng and Song showed that point mutations D3.49(130)A and A6.34(241)E abolished ligand binding. However, mutation of the arginine R3.50(131), member of the highly conserved DRY motif to alanine has no influence on SR144528 binding [226].

As for the $CB_1$ cannabinoid receptor ligands, Sanofi developed tricyclic derivatives based on the 1-benzylpyrazole-3-carboxylic scaffold. More than twenty compounds were disclosed in a patent [227] in 2001 (**84-86**, Table **7**). The benzyl in position 4, characteristic of the $CB_2$ diarylpyrazoles from Sanofi, is conserved as well as the bicyclic residue in position 3. The length of the second link between the phenyl and the pyrazole can be made of one or

**Table 13. $CB_2$ Cannabinoid Receptor Antagonists: 3. Di-Aryl-Pyrazole Derivatives**

Some of the single point mutations reported for the h$CB_2$ cannabinoid receptor for which pharmacological data concerning the SR144528 are available.

| Mutant | Effect | References |
|---|---|---|
| R3.50(131)A | no effect | [226] |
| S4.53(161)A | loss of affinity | [225] |
| V4.56(164)I | no effect | [225] |
| S4.57(165)A | loss of affinity | [225] |
| C174S [a] | loss of affinity | [224, 225] |
| C175S [a] | loss of affinity | [225] |
| C177S [a] | no effect | [225] |
| C179S [a] | loss of affinity | [224, 225] |
| S5.44(193)G | no effect | [225] |

a. Second extra-cellular loop

two methylene, but according to the patent, one methylene bridge is preferred. The Ki values of these compounds for the $CB_2$ receptor were said to be lower than 500 nM, and their antagonist properties have been assessed using the adenylate cyclase inhibition assay.

In a recent paper, Mussinu and colleagues reported the synthesis of other tricyclic derivatives starting from SR141716A structure [228]. *N*-piperidin-1-yl-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide derivatives are $CB_2$ receptor ligands, and some of them possess high affinity and selectivity for the $CB_2$ receptor as shown in Table **7** (**79-83**). These tricyclic derivatives differ from the Sanofi tricyclic derivatives, as they do have a phenyl ring instead of a benzyl in position 1. Another difference is the nature of the substituent in position 3, which is not a bicycloalkyl, but a cyclic amine. One of the compounds synthesised by Mussinu *et al.*, **81**, possesses the highest affinity for the $CB_2$ receptor ever reported with a Ki value of 0.037 nM for the murine $CB_2$ receptor ([$^3$H]-CP-55,940, mice spleen homogenate). Interestingly, **79**, the closest SR141716A analog lost almost all its affinity for the $CB_1$ receptor, and increased by four orders of magnitude its affinity for the $CB_2$ receptor. Even if the structure, quite similar to the SR compounds, could suggest that these derivatives behave as antagonists, no pharmacological data were given in the paper to support this hypothesis.

Finally, it is interesting to strengthen that the five-membered ring derivatives (**79-86**) of the diarylpyrazole family are reported to possess a high $CB_2$ receptor affinity, while the six- and seven-membered ring derivatives (**69-78**) are described as $CB_1$ receptor ligands.

## 4. Other Pyrazole Derivatives

Other $CB_2$ ligands based on a pyrazole nucleus were introduced by SmithKline Beecham, in a patent describing the synthesis of about sixty new compounds [229]. Their affinity (Ki) for the $hCB_2$ receptor transfected in HEK293 cells was reported to be ranging between 25nM and 10µM. Their structures (**170-171**, Fig. **17**) differ from the Sanofi compounds by the presence of an alkyl substituent in position 5 instead of a phenyl, and of course by position 3, which remains unsubstituted. However, except a lecture at the 1998 ACS meeting [230], where compound **171** was reported to have a Ki value of 835 nM and a selectivity ratio over 100, no further report on these compounds was published untill now.

**Fig. (17).** $CB_2$ cannabinoid receptor antagonists: 4. Other pyrazole derivatives. Structure of two compounds (**170-171**) among those claimed by SmithKline Beecham to be $hCB_2$ antagonists.

## 5. 2-Oxoquinoline Derivatives

Inaba and colleagues, from Japan Tobacco, introduced in 2001, a completely new structure in the cannabinoid field, based on a 2-oxoquinoline scaffold. Over eighty derivatives were disclosed in a patent [231], and one compound, JTE-907 (**173**), was subsequently characterised *in-vitro* as well as *in-vivo* [232]. In Table **14**, are listed some of the compounds (**172-178**) disclosed in the patent, along with their affinity for the cannabinoid receptors. These values were obtained on human receptors against [$^3$H]-WIN-55,212-2. For twelve compounds, the anti-inflammatory activity *per os* was assessed in a carrageenin-induced paw edema model. The highest effect reported was obtained with JTE-907 with an $ED_{50}$ value lower than 0.1 mg/kg. Therefore, these compounds were claimed to be useful as anti-inflammatory and anti-allergic agents.

To further characterize JTE-907, the authors determined its affinity for cannabinoid receptors of different species using [$^3$H]-CP-55,940 as radioligand. In their hands, JTE-907 behaved as a very selective $hCB_2$ ligand with Ki values of 36 and 2370 nM for the $CB_2$ and $CB_1$ cannabinoid receptors, respectively, even more selective than the SR144528 (Ki values of 1.99 and 50 nM, respectively). However, it has to be mentioned that the affinities reported in the paper, for JTE-907 are quite different from those reported in the patent ($hCB_1$ Ki values of 35nM and 0.09 nM, respectively). It also appears that the SR144528 affinity for the $CB_1$ receptor determined by Iwamura *et al.* is higher than the affinity reported by Rinaldi-Carmona *et al.* with values of 50 and 437nM, respectively. The authors ascribed the difference to the variability of SR144528 binding affinities to the assay conditions. The function of JTE-907 was evaluated by measuring the increase in cAMP production by forskolin-stimulated $hCB_2$-CHO cells. Maximum stimulation was attained at a concentration of 1µM of JTE-907. In the same assay, WIN-55,212-2 decreased the cAMP production in a dose-dependent manner.

In conclusion, JTE-907 was shown to be a $CB_2$ selective inverse-agonist with anti-inflammatory and anti-allergic properties. This compound is currently undergoing Phase I clinical trials as anti-allergic drug [233].

# IV. NON-$CB_1$ NON-$CB_2$ CANNABINOID RECEPTORS LIGANDS

In the last years, the possible presence of additional non-$CB_1$ non-$CB_2$ cannabinoid receptors was raised from pharmacological evidences.

One of the putative receptors possesses an endothelial localisation, while other ones are localized in the CNS or at peripheral nerves. For instance, an additional target for $^9$-

**Table 14. $CB_2$ Cannabinoid Receptor Antagonists: 5. 2-Oxoquinoline Derivatives**

Structure of six compounds among the oxoquinoline derivatives patented by Japan Tobaco. Affinity (Ki, nM) and selectivity ($CB_1/CB_2$) for the cannabinoid receptors are given. The affinity was measured by binding against [$^3$H]-WIN-55,212-2 on either $hCB_2$-CHO or $hCB_1$-CHO cells. Adapted from [231].

| Cpd. | n° | Structure | $hCB_2$ | $hCB_1$ | Selectivity |
|---|---|---|---|---|---|
| 1-2 | **172** | | 0.014 | 3671 | 262215 |
| JTE-907 | **173** | | 0.087 | 3436 | 39495 |
| 2-2 | **174** | | 0.77 | 3247 | 4215 |
| 3-27 | **175** | | 0.021 | 336 | 16000 |
| 3-32 | **176** | | 0.036 | 2398 | 66610 |
| 3-38 | **177** | | 0.085 | 935 | 11000 |
| 3-43 | **178** | | 0.043 | 864 | 20100 |

tetrahydrocannabinol was described in capsaicin-sensitive sensory nerves by Zygmunt *et al*. [234].

Pharmacological evidences for the existence of additional cannabinoid receptors were reviewed by Wiley and Martin [29] and by Di Marzo *et al.* [235]. Thus, in the present review, we will focus on the description of the so far identified ligands of the non-$CB_1$ non-$CB_2$ receptors, with an emphasis on the compounds having antagonist properties

(Table **15**). In particular, the knowledge about three not yet cloned targets, has grown. The growing molecular pharmacology of the three putative new cannabinoid receptors is reviewed in the three following sections. The fourth section summarises the data on the other proposed cannabinoid targets.

### 1. Endothelial Anandamide Receptor – Abnormal-Cannabidiol Receptor

In 1999, Wagner *et al.* showed that anandamide could induce mesenteric vasodilatation through an endothelially located "anandamide receptor", pharmacologically distinct from $CB_1$ [236]. Other cannabinoids such as $^{9}$-THC, HU-210, or WIN-55,212-2 were devoid of mesenteric vasodilatator activity. In addition, the anandamide effect is partially sensitive to SR141716A inhibition. The authors proposed the existence of two distinct receptors responsible for the vasodilatatory effect of anandamide, one located in the endothelium is SR141716A-sensitive, while the other one, located on the vascular smooth muscle, is SR141716A-insensitive. Thus, SR141716A is an antagonist of an endothelial anandamide receptor. Several studies were conducted in order to understand the molecular pharmacology of the endothelial anandamide receptor. It is pertussis toxin-sensitive [237]. Cannabidiol acts as antagonist, and abnormal-cannabidiol (Abn-cbd, **179**) and its analogue O-1602 (**180**) act as agonists [238]. The synthesis of O-1602 and of its analogues are disclosed in a patent describing the vasodilatatory activity of cannabinoid

**Table 15. Non-$CB_1$ Non-$CB_2$ Cannabinoid Receptors Antagonists**

An overview of the putative new non-$CB_1$ non-$CB_2$ anandamide-receptors ligands. The antagonists are highlighted in bold.

cannabidiol (**10**)

Abnormal-cannabidiol, **179**

O-1602, **180**

O-1918, **181**

capsaicin, **182**

capsazepine, **183**

| Compound | Endothelial AEA receptor | Brain AEA receptor | Cannabinoid-vanilloid brain receptor |
|---|---|---|---|
| AEA | agonist [a] | agonist [d,e] | / |
| WIN-55,212-2 | no effect [a] | agonist [e] | agonist [g, h] |
| CP-55,940 | / | no effect [e] | agonist [i] |
| SR141716A | antagonist [a, b, j] | no effect ([$^{35}$S]-GTP S binding) [d, e, f] | antagonist [g, h] |
| AM251 | no effect [k] | / | no effect [h, i] |
| Cannabidiol | antagonist [b] | no effect [e] | / |
| O-1918 | antagonist [c, j,m] | / | / |
| Abd-cbd | agonist [b, l] | / | / |
| O-1602 | agonist [b] | / | / |
| Capsaicin | no effect[j] | / | agonist [i] |
| Capsazepine | no effect [b] | / | antagonist [h] |

[a] [236]; [b] [238]; [c] [240]; [d] [249]; [e] [250]; [f] [251]; [g] [254]; [h] [258]; [i] [255]; [k] [244]; [j] [243]; [l] [242]; [m] [241]

analogues [239]. More recently, Offertaler *et al.* showed that O-1918 (**181**), a cannabidiol analog, acts as an antagonist of the endothelial anandamide receptor [240]. In addition, O-1918 does not bind to the $CB_1$ and $CB_2$ cannabinoid receptors. Furthermore, O-1918, unlike cannabidiol, does not cause vasorelaxation in the mesenteric artery preparation model used and, thus, acts as a true neutral antagonist. Using O-1918 as a pharmacological tool, Begg *et al.* proposed a coupling of this new G protein-coupled endothelial receptor through a $G_i/G_o$ mechanism to ion channels in primary cultured human vascular endothelial cells [241]. However, in their hands, SR141716A was ineffective in antagonising Abd-cbd. Ho and Hiley further demonstrated that Abn-cbd relaxes rat small mesenteric arteries through an activation of $K^+$ channels *via* a SR141716A-sensitive pathway [242]. Further, using the same model, they characterized the vasorelaxant activity of virodhamine, a novel endocannabinoid, apparently acting through the same receptor [243]. The virodhamine-induced vasorelaxation is sensitive to SR141716A and O-1918, but insensitive to AM251, SR144528, and AM630. Moreover, vanilloid receptor desensitisation by capsaicin (**182**) had no effect.

More recently, Mo *et al*. obtained results showing that the Abnormal-cannabidiol receptor is also responsible for an increased endothelial cell migration [244]. Abn-cbd enhanced human umbilical vein endothelial cell migration, while O-1918 antagonised Abn-cbd effect. The $CB_1$ cannabinoid receptor inverse agonist SR141716A, partially inhibited Abd-cbd action, whereas AM251 and SR144528 had no effect.

In another paper from George Kunos team, Batkai *et al.* reported that SR141716A is able to inhibit the endotoxic hypotension by a cardiac mechanism involving neither the $CB_1$, nor the $CB_2$ cannabinoid receptors [245]. The involvement of a non-$CB_1$ non-$CB_2$ site of action was shown using $CB_1^{-/-}$ and $CB_1^{-/-}/CB_2^{-/-}$ knock-out mice. Further studies are needed to identify the SR141716A sensitive myocardial site of action.

In a recent patent, Kunos described O-1918 and its analogues, as vasoconstrictor agents, useful to reverse pathological vasodilatation of blood vessels [246]. The effects of O-1918 on mouse blood pressure, on LPS-induced hypotension in mouse, as well as the reversal of hypotension by O-1918 injection are described. Furthermore, the synthesis of O-1918 based antagonists is described, starting from *p*-metha-2,8-dien-1-ol and the appropriate resorcinols.

Interestingly, the Abd-cbd receptor, which was first detected in the vascular system, also seems to be present in the CNS [247]. Indeed, Walter *et al.* found that microglial cells (BV-2) migration induced by 1 µM 2-AG is inhibited by cannabidiol (300 nM) and O-1918 (1 µM). SR141716A was found ineffective in this assay. Thus, 2-AG is an agonist of the Abd-cbd receptor. Moreover, Abd-cbd is able to elicit a dose-dependent cell migration in the same model ($EC_{50}$ = 600nM) [248].

From a more structural point of view, SR141716A behaves as an antagonist, while on the opposite, AM251 (**20**), a close analogue which differs from the former only by the nature of one halogen, has no effect on this receptor [242, 244]. Thus, position 4 on the 5-phenyl could be further explored to obtain selective ligands.

To come to an end, despite the fact that the endothelial anandamide receptor is neither identified nor cloned, at least three antagonists are identified : SR141716A, cannabidiol, and O-1918. It is likely that a growing number of ligands and therapeutic applications will appear in the very near future.

## 2. Brain Anandamide Receptor

Following an in-depth evaluation of anandamide effects in $CB_1^{-/-}$ mice, Di Marzo and co-workers suggested that a non-$CB_1$ non-$CB_2$ G protein-coupled receptor might mediate some of the actions of AEA in mice [249]. Actually, effects of AEA, but not those of $^9$-THC, were not decreased in $CB_1$ cannabinoid receptor knock-out mice. For instance, 30 mg/kg of AEA were still effective in hot-plate test, whereas 10 mg/kg of $^9$-THC had no effect on anti-nociception. Moreover, in a [$^{35}$S]-GTP S assay, AEA stimulated with an $EC_{50}$ value of 2.23 µM, the [$^{35}$S]-GTP S binding to $CB_1^{-/-}$ mice brain membranes ($E_{max}$= 28%). $^9$-THC showed no effect in this assay. The addition of SR141716A did not affect the [$^{35}$S]-GTP S binding to $CB_1^{-/-}$ mice brain membranes. Shortly after, other evidences for the existence of a new G protein-coupled cannabinoid receptor with a distinct distribution in the central nervous system were published [250]. From a set of 24 commonly used cannabinoids (AEA, WIN-55,212-2, JWH-030, THC…), only AEA and WIN-55,212-2 produced an increased binding of [$^{35}$S]-GTP S on $CB_1^{-/-}$ mice brain membranes. Interestingly, in this study SR141716A alone produced a significant inhibition of [$^{35}$S]-GTP S binding, similar to the inhibition obtained using $CB_1^{+/+}$ mice brain membranes ($IC_{50}$ values of 5.7 µM and 4.7 µM, respectively). However, SR141716A did not affect the anandamide-induced [$^{35}$S]-GTP S binding in $CB_1^{-/-}$ mice brain membranes. Moreover, [$^3$H]-SR141716A exhibited significant binding in some brain regions of $CB_1^{-/-}$ mice, which are different from those exhibiting stimulation of [$^{35}$S]-GTP S binding by WIN-55,212-2 and AEA. Thus, the authors proposed that the SR141716A effects on $CB_1^{-/-}$ mice are mediated, neither by the $CB_1$ cannabinoid receptor, nor by the putative new anandamide brain receptor. Monory and co-workers, using $CB_1^{-/-}$ mice, described a novel, non adenylyl cyclase-coupled, cannabinoid binding site in mice cerebellum [251]. Two agonists, AEA and WIN-55,212-2, but not $^9$-THC or HU210, were found to bind to this receptor as they enhanced [$^{35}$S]-GTP S binding. Neither SR141716A, nor SR144528, were able to affect the WIN-55,212-2 induced [$^{35}$S]-GTP S binding in $CB_1^{-/-}$ mice, as found by Breivogel *et al.* [250]. However, the distribution pattern of the receptor described by Monory *et al.* differs from the distribution found by Breivogel *et al.* It has to be mentioned that the knock-out strains used were different. The mice used by Monory were from a CD-1 strain, while the one used by Breivogel were from a C57BL/61 strain. Interestingly, Muthane *et al.* detected differences in nigral neurons number, and sensitivity to MPTP in the two mouse strains used [252].

To conclude, to our knowledge, no compounds having antagonist or inverse agonist properties at this receptor have been identified so far.

### 3. Cannabinoid-Vanilloid Brain Receptor

Evidences for the presence of an additional "cannabinoid" receptor, differing from the $CB_1$ receptor and from the brain anandamide receptor, appeared from studies focused on the hippocampus (for a review see Hajos *et al.* [253]). Hajos *et al.*, studied the cannabinoid actions on GABAergic and glutamatergic transmission, using the whole-cell patch-clamp technique [254]. WIN-55,212-2 induced a dose-dependent reduction in glutamatergic transmission reversed by SR141716A (1µM). Thus, the authors concluded that the cannabinoid actions on the glutamatergic transmission are mediated by a non-$CB_1$, but SR141716A-sensitive receptor. In another paper, Hajos and Freund further characterised this putative receptor [255]. They showed that, in addition to WIN-55212-2, CP-55,940 and capsaicin (**182**), a TRPV1 receptor agonist, also act as agonists. Furthermore, in their hands, SR141716A, as well as capsazepine (**183**), a TRPV1 receptor antagonist, were able to antagonise this effect on the glutamatergic transmission, while AM251 was not. SR141716A effects on $CB_1$ knock-out mice were further studied using a test of anxiety [256]. Haller *et al.* showed that on the one hand, the distribution of the $CB_1$ receptor induces anxiety, and SR141716A has an anxiolytic effect, and on the other hand, SR141716A is still active in $CB_1^{-/-}$ mice. In a following paper, they reported that AM-251 induces anxiogenic effects in wild-type and in knock-out mice [257]. Thus, SR141716A, in addition to $CB_1$, binds to a not yet identified receptor. Another evidence for this additional receptor for SR141716A was given by Bass and co-workers [61]. They conducted a structure-activity relationship study on the stimulation of locomotor activity induced by SR141716A and twenty analogues. No correlation was found between the affinity for the $CB_1$ cannabinoid receptor and the stimulation of locomotor activity. Albeit SR141716A and five of its analogues, O-1803, O-1710, O-1253, O-1254 (**40**), O-1255 (**41**), behaved as inverse agonists in [$^{35}$S]-GTP S assay, none of these analogues stimulated the locomotor activity. In conclusion, the SR141716A-induced stimulation of locomotor activity is neither due to inverse agonism nor to inhibition of an endogenous tone, but to a brain receptor distinct from the $CB_1$ receptor. Di Marzo *et al*. previously provided evidences that the cannabinoid-vanilloid brain receptor could mediate some of the cannabinoid effects on locomotion [249].

Very recently, Pistis *et al.* published a study on the neurophysiological effects of cannabinoids on the basolateral amygdala neurons *in-vivo* [258]. One of their findings is that HU-210 and WIN-55,212-2 did not elicit similar effects on basal amygdala projection neurons firing rate. While the former had no effect, the latter decreased the firing rate. Moreover, SR141716A reversed the WIN-55,212-2 effects, while AM251 had no effect. The vanilloid receptor antagonist capsazepine, also antagonised the WIN-55,212-2 effect on firing rate of these neurons. Therefore, the authors suggested the presence of a novel cannabinoid-vanilloid receptor.

From these lines, it appears that in addition to the known $CB_1$ cannabinoid receptor, it is now likely that two other cannabinoid receptors do exist in the brain. The first one should be an anandamide brain receptor, for which SR141716A has no affinity, while the second one should be a cannabinoid-vanilloid receptor, at which SR141716A and capsazepine behave as antagonists.

### 4. Other Non-$CB_1$ Non-$CB_2$ Cannabinoid Receptors

Evidences for the existence of additional non-$CB_1$ non-$CB_2$ cannabinoid receptors localised in the rat isolated mesenteric arterial bed, appeared from the works of Ralevic and collaborators. Several cannabinoids were evaluated in their model of sensory neurogenic vasorelaxation, evoked by electrical field stimulation.

HU210 was found to attenuate vasorelaxation in a $CB_1$ and $CB_2$ independent way, as SR141716A, LY320135, and SR144528 were ineffective in inhibiting HU210 effect [259]. Similar results were obtained with $^{9}$-THC [260]. Similarly, noladin ether, inhibited sensory neurogenic relaxation in a concentration-dependent manner, which was also unaffected by SR141716A, LY320135, and SR144528. Moreover, this effect, as the $^{9}$-THC one, was pertussis toxin sensitive, suggesting the involvement of a $G_{i/o}$ protein-coupled receptor [261].

However, the inhibitory effects of CP-55,940 and WIN-55,212-2 were affected by the presence of SR141716A (1µM) or LY320135 (1µM), but not by the addition of SR144528 (1µM) [262].

It appears that, at least, two receptors should be involved in cannabinoid-mediated attenuation of sensory nerve-mediated vasorelaxation. The first one is SR141716A and LY320135 sensitive, while the other one is resistant to SR141716A.

Some evidences for the existence of a "$CB_2$-like" receptor appeared from the studies on palmitoylethanolamide (PEA), an endogenous fatty acid amide, devoid of $CB_1$ and $CB_2$ affinity [264], antinociceptive potential. Calignano and co-workers showed that PEA was able to alleviate pain in several animal models [265, 266]. For instance, PEA dose-dependently inhibits kaolin-evoked writhing in mice. The $CB_2$ cannabinoid receptor inverse agonist SR144528 (0.2 mg/kg), inhibits PEA anti-nociceptive effect [266]. Thus, the authors suggest that PEA acts as agonist on a SR144528-sensitive, non-$CB_2$ cannabinoid receptor, ("$CB_2$-like" receptor) at which SR144528 acts as an antagonist.

Further studies are needed in order to better characterise these additional "cannabinoid" receptors. However, it is likely that several therapeutic applications will soon spring for these receptors.

## V. CONCLUSION

Since their development, cannabinoid antagonists proved to be essential tools in the understanding of cannabinoid pharmacology and biochemistry. Moreover, the interest shown by the pharmaceutical industry in the development of new cannabinoid antagonists prove, if necessary, the real therapeutic potential of this class of compounds. The treatment of eating and movement disorders, memory deficits, psychosis, and dependencies from various addictive drugs are some of the most cited applications for the $CB_1$ cannabinoid receptor antagonists.

Much less wide is the knowledge on the $CB_2$ cannabinoid receptor antagonists. However, potential applications could be disorders involving the immune system such as inflammation or allergies.

Finally, the growing literature on the non $CB_1$ non $CB_2$ cannabinoid receptors should allow the development of new drugs. First examples are given by the cannabidiol and abnormal-cannabidiol derivatives described by Kunos *et al.* for which potential therapeutic applications on the vascular system are already patented.

It clearly appears from this paper that the antagonists of the cannabinoid receptors are still an active research field from which should emerge new promising therapeutic tools as well as innovative drugs in the near future.

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## REFERENCES

[1] Hillard, C.J.; Harris, R.E.; Bloom, A.S. *J. Pharmacol. Exp. Ther.*, **1985**, *232*, 579.
[2] Devane, W.A.; Dysarz, F.A.; Johnson, M.R.; Melvin, L.S.; Howlett, A.C. *Mol. Pharmacol.*, **1988**, *34*, 605.
[3] Matsuda, L.A.; Lolait, S.J.; Brownstein, M.J.; Young, A.C.; Bonner, T.I. *Nature*, **1990**, *346*, 561.
[4] Gerard, C.M.; Molerau, C. Vassart, G.; Parmentier, M. *Biochem. J.*, **1991**, *279*, 129.
[5] Glass, M.; Dragunow, M.; Faull, R.L.M. *Neuroscience*, **1997**, *2*, 299.
[6] Shire, D.; Carillon, C.; Kaghad, M.; Calandra, B.; Rinaldi-Carmona, M.; LeFur, G.; Caput, D.; Ferrara, P. *J. Biol. Chem.*, **1995**, 270, 3726.
[7] Ryberg, E.; Khang Vu, H.; Groblewski, T.; Larsson, N.; Elebring, T.; Hjorth, S.; Sjogren, S.; Greasley, P.J. **2004** *Symposium on the Cannabinoids*, Burlington Vermont, International Cannabinoid Research Society, page 119.
[8] Rinaldi-Carmona, M.; Calandra, B.; Shire, D.; Bouaboula, M.; Oustric, D.; Barth, F.; Casellas, P.; Ferrara, P.; LeFur, G. *J. Pharmacol. Exp. Ther.*, **1998**, *278*, 871.
[9] Munro, S.; Thomas, K.L.; Abu-Shaar, M. *Nature*, **1993**, *365*, 61.
[10] Howlett, A.C.; Qualy, J.M.; Khachatrian, L.L. *Mol. Pharmacol.*, **1986**, *29*, 307.
[11] Glass, M.; Felder, C.C. *J. Neurosci.*, **1997**, *17*, 5327.
[12] Calandra, B.; Portier, M.; Kernéis, A.; Delpech, M.; Carillon, C .; LeFur, G.; Ferrara, P.; Shire, D. *Eur. J. Pharmacol.*, **1999**, *374,* 445.
[13] Howlett, A.C. *Life Sci.*, **1984**, *35*, 1803.
[14] Deadwyler, S.; Hampson, R.E.; Mu, J.; Whyte, A.; Childers, S.R. *J. Pharmacol. Exp. Ther.*, **1995**, *273*, 734.
[15] Mackie, K.; Lai, Y.; Westenbroek, R.; Mitchell, R. *J. Neurosci.*, **1995**, *15*, 6552.
[16] Bouaboula, M.; Poinot, C.C.; Bourrie, B.; Canat, X.; Calandra, B.; Rinaldi-Carmona, M.; LeFur, G.; Casellas, P. *Biochem. J.*, **1995**, *312*, 637.
[17] Bouaboula, M.; Poinot, C.C.; Marchand, J.; Canat, X.; Bourrie, B.; Rinaldi-Carmona, M.; Calandra, B.; LeFur, G.; Casellas, P. *Eur. J. Biochem.*, **1996**, *237*, 704.
[18] delPulgar, T.; Velasco, G.; Guzman, M. *Biochem. J.*, **2000**, *347*, 369.
[19] Devane, W.A.; Hanus, L.; Breuer, A.; Pertwee, R.G.; Stevenson, L.A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. *Science*, **1992**, *258*, 1946.
[20] Mechoulam, R.; Ben-Shabat, S.; Hanus, L.; Ligumsky, M.; Kaminski, N.E.; Schatz, A.R; Gopher, A.; Almong, S.; Martin, B.R.; Compton, D.R. *Biochem. Pharmacol.*, **1995**, *50*, 83.
[21] Sugiura, T.; Kondo, S.; Sukagawa, A.; Nakane, S.; Shinoda, A.; Itoh, K.; Yamashita, A.; Waku, K. *Biochem. Biophys. Res. Co.*, **1995**, *215*, 89.
[22] Hanus, L.; Abu-Lafi, S.; Fride, E.; Breuer, A.; Vogel, Z.; Shalev, D.E.; Kustanovich, I.; Mechoulam, R. *Proc. Natl. Acad. Sci. U.S.A.*, **2001**, *98*, 3662.
[23] Oka, S.; Tsuchie, A.; Tokumura, A.; Muramatsu, M.; Suhara, Y.; Takayama, H.; Waku, K.; Sugiura, T. *J. Neurochem.*, **2003**, *85*, 1374.
[24] Goutopoulos, A. and Makriyannis, A. *Pharmacol. Therapeut.*, **2002**, *95*, 103.
[25] Beal, J.E.; Olson, R.; Lefkowitz, L.; Laubenstein, L.; Bellman, P.; Yangco, B.; Morales, J.O.; Murphy, R.; Powderly, W.; Plasse, T.F.; Mosdell, K.W.; Shepard, K.V. *J. Pain Symptom Manage.*, **1997**, *14*, 7.
[26] Notcutt, W.; Price, M.; Miller, R.; Newport, S.; Phillips, C.; Simmons, S.; Sansom, C. *Anaesthesia*, **2004**, *59*, 440.
[27] Ledent, C.; Valverde, O.; Cossu, G.; Petitet, F.; Aubert, J.F.; Beslot, F.; Bohme, G.A.; Imperato, A.; Pedrazzini, T.; Roques, B.P.; Vassart, G.; Fratta, W.; Parmentier, M. *Science*, **1999**, *283*, 401.
[28] Zimmer, A.; Zimmer, A.M.; Hohmann, A.G.; Herkenham, M.; Bonner, T.I. *Proc. Natl. Acad. Sci. U. S. A.*, **1999**, *96*, 5780.
[29] Wiley, J.L. and Martin, B.R. *Chem. Phys. Lipids*, **2002**, *121*, 57.
[30] Adam, J. and Cowley, P. *Expert. Opin. Ther. Patents*, **2002**, *12*, 1475.
[31] Razdan, R.K. and Mahadevan, A. *Chem. Phys. Lipids*, **2002**, *121*, 21.
[32] Barth, F. and Rinaldi-Carmona, M. *Curr. Med. Chem.*, **1999**, *6*, 745.
[33] Little, P.J.; Compton, D.R.; Johnson, M.R.; Melvin, L.S.; Martin, B.R. *J. Pharmacol. Exp. Ther.*, **1988**, *247*, 1046.
[34] Pertwee, R.G.; Stevenson, L.A.; Elrick, D.B.; Mechoulam, R.; Corbett, A.D. *Brit. J. Pharmacol.*, **1992**, *105*, 980.
[35] Harrison, C.; Traynor, J.R. *Life Sci.*, **2003**, *74*, 489-508.
[36] Pertwee, R.G.; Fernando, S.R.; Griffin, G.; Ryan, W.; Razdan, R.J.; Compton, D.R.; Martin, B.R. *Eur. J. Pharmacol.*, **1996**, *315*, 195.
[37] Ross, R.A.; Brockie, H.C.; Fernando, S.R.; Saha, B.; Razdan, R.K.; Pertwee, R.G. *Brit. J. Pharmacol.*, **1998**, *125*, 1345.
[38] Griffin, G.; Wray, E.J.; Rorrer, W.K.; Crocker, P.J.; Ryan, W.J.; Saha, B.; Razdan, R.K.; Martin, B.R.; Abood, M.E. *Brit. J. Pharmacol.*, **1999**, *126*, 1575.
[39] Ross, R.A.; Gibson, T.M.; Stevenson, L.A.; Saha, B.; Crocker, P.; Razdan, R.K.; Pertwee, R.G. *Brit. J. Pharmacol.*, **1999**, *128*, 735.
[40] Martin, B.R.; Winckler, J.R.; Wiley, J.L.; Huffman, J.W.; Crocker, P.J.; Saha, B.; Razdan, R.K. *J. Pharmacol. Exp. Ther.*, **1999**, *290*, 1065.
[41] Martin, B.R.; Stevenson, L.A.; Pertwee, R.G.; Breivogel, C.S.; Williams, A.; Mahadevan, A.; Razdan, R.K. **2002** *Symposium on the Cannabinoids*, Burlington Vermont, International Cannabinoid Research Society, page 2.
[42] Thomas, A.; Ross, R.A.; Saha, B.; Mahadevan, A.; Razdan, R.K.; Pertwee, R.G. *Eur. J. Pharmacol.*, **2004**, *487*, 213.
[43] Compton, D.R.; Gold, L.H.; Ward, S.J.; Balster, R.L.; Martin, B.R. *J. Pharmacol. Exp. Ther.*, **1992**, *263*, 1118.
[44] Eissenstat, M.A.; Bell, M.R.; D'Ambra, T.E.; Alexander, E.J.; Daum, S.J.; Ackerman, J.H.; Gruett, M.D.; Kumar, V.; Estep, K.G.; Olefirowicz, E.M.; Wetzel, J.R.; Alexander, M.D.; Weaver, J.D.; Haycock, D.A.; Luttinger, D.A.; Casiano, F.M.; Chippari, S.M.; Kuster, J.E.; Stevenson, J.I.; Ward, S.J. *J. Med. Chem.*, **1995**, *38*, 3094.
[45] Pertwee, R.; Griffin, G.; Fernando, S.; Li, X.; Hill, A.; Makriyannis, A. *Life Sci.*, **1995**, *56*, 1949.
[46] Hosohata, Y.; Quock, R.M.; Hosohata, K.; Makriyannis, A.; Consroe, P.; Roeske, W.R.; Yamamura, H.I. *Eur.J.Pharmacol.*, **1997**, *321*, R1.
[47] Landsman, R.S.; Makriyannis, A.; Deng, H.; Consroe, P.; Roeske, W.R.; Yamamura, H.I. *Life Sci.*, **1998**, 62, 109.

[48] Ross, R.A.; Brockie, H.C.; Stevenson, L.A.; Murphy, V.L.; Templeton, F.; Makriyannis, A.; Pertwee, R.G. *Brit. J. Pharmacol.*, **1999**, *126*, 665.
[49] Huffmann, J.W. *Curr. Med. Chem.*, **1999**, *6*, 705.
[50] Rinaldi-Carmona, M.; Barth, F.; Heaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Neliat, G.; Caput, D.; Ferrara, P.; Soubrié, P.; Brelière, J.C.; Le Fur, G. *FEBS Lett.*, **1994**, *350*, 240.
[51] Rinaldi-Carmona, M.; Barth, F.; Heaulme, M.; Alonso, R.; Shire, D.; Congy, C.; Soubrie, P.; Breliere, J.C.; Le Fur, G. *Life Sci.*, **1995**, *56*, 1941.
[52] Jarai, Z.; Wagner, J.A.; Goparaju, S.K.; Wang, L.; Razdan, R.K.; Sugiura, T.; Zimmer, A.M.; Bonner, T.I.; Zimmer, A.; Kunos, G. *Hypertension*, **2000**, *35*, 679.
[53] Fox, A.; Kesingland, A.; Gentry, C.; McNair, K.; Patel, S.; Urban, L.; James, I. *Pain*, **2001**, *92*, 91.
[54] Costa, B.; Vailati, S.; Colleoni, M. *Behav. Pharmacol.*, **1999**, *10*, 327.
[55] Compton, D.R.; Aceto, M.D.; Lowe, J.; Martin, B.R. *J. Pharmacol. Exp. Ther.*, **1996**, *277*, 586.
[56] Petitet, F.; Jeantaud, B.; Capet, M.; Doble, A. *Biochem. Pharmacol.*, **1997**, *54*, 1267.
[57] Griffin, G.; Atkinson, P.J.; Showalter, V.M.; Martin, B.R.; Abood, M.E. *J. Pharmacol. Exp. Ther.*, **1998**, *285*, 553.
[58] Kearn, C.S.; Greenberg, M.J.; DiCamelli, R.; Kurzawa, K.; Hillard, C.J. *J. Neurochem*., **1999**, *72,* 2379.
[59] MacLennan,S.J.; Reynen, P.H.; Kwan, J.; Bonhaus, D.W. *Brit. J. Pharmacol.*, **1998**, *124*, 619.
[60] Sim-Selley, L.J.; Brunk, L.K.; Selley, D.E. *Eur. J. Pharmacol.*, **2001**, *414*, 135.
[61] Bass, C.E.; Griffin, G.; Grier, M.; Mahadevan, A.; Razdan, R.K.; Martin, B.R. *Pharmacol. Biochem. Behav*., **2002**, *74*, 31.
[62] Meschler, J.P.; Kraichely, D.M.; Wilken, G.H.; Howlett, A.C. *Biochem. Pharmacol.*, **2000**, *60*, 1315.
[63] Mato, S.; Pazos, A.; Valdizan, E.M. *Eur. J. Pharmacol.*, **2002**, *443*, 43.
[64] Bouaboula, M.; Perrachon, S.; Milligan, L.; Canat, X.; Rinaldi-Carmona, M.; Portier, M.; Barth, F.; Calandra, B.; Pecceu, F.; Lupker, J.; Maffrand, J.P.; Le Fur, G.; Casellas, P. *J. Biol. Chem.*, **1997**, *272*, 22330.
[65] Pertwee, R. *International Congress Series*, **2003**, *1249*, 75.
[66] Pan, X.; Ikeda, S.R.; Lewis, D.L. *Mol. Pharmacol.*, **1998**, *54*, 1064.
[67] Hurst, D.P.; Lynch, D.L.; Barnett-Norris, J.; Hyatt, S.M.; Seltzman, H.H.; Zhong, M.; Song, Z.H.; Nie, J.; Lewis, D.; Reggio, P.H. *Mol. Pharmacol.*, **2002**, *62*, 1274.
[68] Reggio, P.; Umijiego, U.; Hurst, D.; Seltzman, H.; Hyatt, S.; Roche, M.; McAllister, S.; Fleisher, D. **2004** *Symposium on the Cannabinoids,* Burlington Vermont, International Cannabinoid Research Society, page 2.
[69] Song, Z.H. and Bonner, T.I. *Mol. Pharmacol.*, **1996**, *49*, 891.
[70] Chin, C.; Lucas-Lenard, J.; Abadjii, V.; Kendall, D.A. *J. Neurochem.*, **1998**, *170, 366.*
[71] McAllister, S.D.; Tao, Q.; Barnett-Norris, J.; Buehner, K.; Hurst, D.P.; Guarnieri, F.; Reggio, P.H.; Nowell Harmon, K.W.; Cabral, G.A.; Abood, M.E. *Biochem. Pharmacol.*, **2002**, *63*, 2121.
[72] Shire, D.; Calandra, B.; Delpech, M.; Dumont, X .; Kaghad, M.; LeFur, G.; Caput, D.; Ferrara, P. *J. Biol. Chem.*, **1996**, *271*, 6941.
[73] Murphy, J.W. and Kendall, D.A. *Biochem. Pharmacol.*, **2003**, *65*, 1623.
[74] McAllister, S.D.; Rizvi, G.; Anavi-Goffer, S.; Hurst, D.P.; Barnett-Norris, J.; Lynch, D.L.; Reggio, P.H.; Abood, M.E. *J. Med. Chem.*, **2003**, *46*, 5139.
[75] McAllister, S.D.; Hurst, D.P.; Barnett-Norris, J.; Lynch, D.; Reggio, P.H.; Abood, M.E. *J. Biol. Chem.*, **2004**, *279*, 48024.
[76] Salo, O.M.; Lahtela-Kakkonen, M.; Gynther, J.; Jarvinen, T.; Poso, A. *J. Med. Chem*., **2004**, *47*, 3048.
[77] Rinaldi-Carmona, M.; Pialot, F.; Congy, C.; Redon, E.; Barth, F.; Bachy, A.; Breliere, J.C.; Soubrie, P.; Le Fur, G. *Life Sci.*, **1996**, *58*, 1239.
[78] Gatley, S.J.; Gifford, A.N.; Volkow, N. D.; Lan, R.; Makriyannis, A. *Eur. J. Pharmacol.*, **1996**, *307*, 331.
[79] Gatley, S.J.; Lan, R.; Volkow, N.D.; Pappas, N.; King, P.; Wong, C.T.; Gifford, A.N.; Pyatt, B.; Dewey, S.L.; Makriyannis, A. *J. Neurochem.*, **1998**, *70*, 417.
[80] Gifford, A.N.; Tang, Y.; Gatley, S.J.; Volkow, N.D.; Lan, R.; Makriyannis, A. *Neurosci. Lett.*, **1997**, *238*, 84.
[81] Gatley, S.J.; Lan, R.; Volkow, N.D.; Pappas, N.; King, P.; Wong, C.T.; Gifford, A.N.; Pyatt, B.; Dewey, S.L.; Makriyannis, A. *J. Neurosci.*, **1998**, *70*, 417.
[82] Lan, R.; Lu, Q.; Fan, P.; Gatley, J.; Volkow, N.D.; Fernando, S.R.; Pertwee, R.; Makriyannis, A. *AAPS Pharmsci.*, **1999**, *1*, article 4.
[83] Mathews, W.B.; Ravert, H.T.; Musachio, J.L.; Franck, R.A.; Rinaldi-Carmona, M.; Barth, F.; Dannals, R.F. *J. Labelled. Compd. Rad.*, **1999**, *42*, 589.
[84] Mathews, W.B.; Scheffel, U.; Finley, P.; Ravert, H.T.; Frank, R.A.; Rinaldi-Carmona, M.; Barth, F.; Dannals, R.F. *Nucl. Med. Biol.*, **2000**, *27*, 757.
[85] Berding, G.; Müller-Vahl, K.; Schneider, U.; Gielow, P.; Fitschen, J.; Stuhrmann, M.; Harke, H.; Buchert, R.; Donnerstag, F.; Hofmann, M.; Knoop, B.O.; Brooks, D.J.; Emrich, H.M.; Knapp, W.H. *Biol. Psychiat.*, **2004**, *55*, 904.
[86] Mathews, W.B.; Scheffel, U.; Rauseo, P.A.; Ravert, H.T.; Frank, R.A.; Ellames, G.J.; Herbert, J.M.; Barth, F.; Rinaldi-Carmona, M.; Dannals, R.F. *Nucl. Med. Biol.*, **2002**, *29*, 671.
[87] Bays, H.E. *Obes. Res.*, **2004,** *12*, 1197.
[88] Comment in *Nat. Rev. Drug Discov*., **2004**, 3, 288.
[89] LeFur, G. **2004** *Symposium on the Cannabinoids*, Burlington Vermont, International Cannabinoid Research Society, page 67.
[90] Cleland, J.G.F.; Ghosh, J.; Freemantle, N.; Kaye, G.C.; Nasir, M.; Clark, A.L.; Coletta, A.P. *Eur. J. Heart Fail.*, **2004**, *6*, 501.
[91] Arnone, M.; Maruani, J.; Chaperon, F.; Thiebot, M.H.; Poncelet, M.; Soubrie, P.; Le Fur, G. *Psychopharmacology*, **1997**, *132*, 104.
[92] Colombo, G.; Agabio, R.; Diaz, G.; Lobina, C.; Reali, R.; Gessa, G.L. *Life Sci*., **1998**, *63*, PL113.
[93] Simiand, J.; Keane, M.; Keane, P.E.; Soubrie, P. *Behav. Pharmacol.*, **1998**, *9*, 179.
[94] Freedland, C.S.; Poston, J.S.; Porrino, L.J. *Pharmacol. Biochem. Behav*., **2000**, *67*, 265.
[95] Rowland, N.E.; Mukherjee, M.; Robertson, K. *Psychopharmacology*, **2001**, *159*, 111.
[96] Verty, A.N.; McGregor, I.S.; Mallet, P.E. *Neurosci. Lett.*, **2004**, *354*, 217.
[97] Gomez, R.; Navarro, M.; Ferrer, B.; Trigo, J.M.; Bilbao, A.; Del Arco, I.; Cippitelli, A.; Nava, F.; Piomelli, D.; Rodriguez de Fonseca, F. *J. Neurosci.*, **2002**, *22*, 9612.
[98] Ravinet Trillou, C.; Arnone, M.; Delgorge, C.; Gonalons, N.; Keane, P.; Maffrand, J.P.; Soubrie, P. *Am. J. Physiol.- Reg. I.*, **2003**, *284*, R345.
[99] Bensaid, M.; Gary-Bobo, M.; Esclangon, A.; Maffrand, J.P.; Le Fur, G.; Oury-Donat, F.; Soubrie, P. *Mol. Pharmacol.*, **2003**, *63*, 908.
[100] Vickers, S.P.; Webster, L.J.; Wyatt, A.; Dourish, C.T.; Kennett, G.A. *Psychopharmacology*, **2003**, *167*, 103.
[101] Higgs, S.; Williams, C.M.; Kirkham, T.C. *Psychopharmacology*, **2003**, *165*, 370.
[102] Ravinet Trillou, C.; Delgorge, C.; Menet, M.; Arnone, M.; Soubrie, P. *Int. J. Obesity*, **2004**, *28*, 640.
[103] Cota, D.; Marsicano, G.; Tschöp, M.; Grübler, Y.; Flachskamm, C.; Schubert, M.; Auer, D.; Yassouridis, A.; Thöne-Reineke, C.; Ortmann, S.; Tomassoni, F.; Cervino, C.; Nisoli, E.; Linthorst, A.C.E.; Pasquali, R.; Lutz, B.; Stalla, G.K.; Pagotto, U. *J. Clin. Invest.*, **2003**, *112*, 423.
[104] Cani, P.D.; Lasa Montoya, M.; Neyrinck, A.M.; Delzenne, N.M.; Lambert, D.M. *Br. J. Nutr*., **2004**, *92*, 757.
[105] Colombo, G.; Agabio, R.; Fa, M.; Guano, L.; Lobina, C.; Loche, A.; Reali, R.; Gessa, G.L. *Alcohol Alcohol*., **1998**, *33*, 126.
[106] Serra, S.; Carai, M.A.; Brunetti, G.; Gomez, R.; Melis, S.; Vacca, G.; Colombo, G.; Gessa, G.L. *Eur. J. Pharmacol.*, **2001**, *430*, 369.
[107] Serra, S.; Brunetti, G.; Pani, M.; Vacca, G.; Carai, M.A.M.; Gessa, G.L.; Colombo, G. *Eur. J. Pharmacol.*, **2002**, *443*, 95.
[108] Colombo, G.; Vacca, G.; Serra, S.; Carai, M.A.M.; Gessa, G.L. *Eur. J. Pharmacol.*, **2004**, *498*, 119.
[109] Gallate, J.E. and McGregor, I.S. *Psychopharmacology*, **1999**, *142*, 302.
[110] Cohen, C.; Perrault, G.; Voltz, C.; Steinberg, R.; Soubrie, P. *Behav. Pharmacol.*, **2002**, *13*, 451.
[111] Le Fur, G.; Cohen, C.; Steinberg, R.; Soubrie, P. **2003** *Symposium on the Cannabinoids*, Burlington Vermont, International Cannabinoid Research Society, page 55.
[112] Le Fol, B.; Goldberg, S.R. *J. Pharmacol. Exp. Ther.*, **2005**, *12*, 875.
[113] Zammit, S.; Allebeck, P.; Andreasson, S.; Lundberg, I.; Lewis, G. *Br. Med. J.*, **2004**, *325*, 1199.

[114] Arseneault, L.; Cannon, M.; Witton, J.; Murray, R.M. *Br. J. Psychiatry*, **2004**, *184*, 110.
[115] Emrich, H.M.; Leweke, F.M.; Schneider, U. *Pharmacol. Biochem. Behav.*, **1997**, *56*, 803.
[116] Leweke, F.M.; Giuffrida, A.; Wurster, U.; Emrich, H.M.; Piomelli, D. *NeuroReport*, **1999**, *10*, 1665.
[117] De Marchi, N.; De Petrocellis, L.; Orlando, P.; Daniele, F.; Fezza, F.; Di Marzo, V. *Lipids Health Dis.*, **2003**, *2*, 5.
[118] Dean, B.; Sundram, S.; Bradbury, R.; Scarr, E.; Copolov, D. *Neuroscience*, **2001**, *103*, 9.
[119] Zavitsanou, K.; Garrick, T.; Huang, X.F. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, **2004**, *28*, 355.
[120] Meltzer, H.Y.; Arvanitis, L.; Bauer, D.; Rein, W. *Am. J. Psychiatry*, **2004**, *161*, 975.
[121] Castellano, C.; Rossi-Arnaud, C.; Cestari, V.; Constanzi, M. *Curr. Drug Targets*, **2003**, *2*, 61.
[122] Terranova, J.P.; Storme, J.J.; Lafon, N.; Perio, A.; Rinaldi-Carmona, M.; Le Fur, G.; Soubrie, P. *Psychopharmacology*, **1996**, *126*, 165.
[123] Mallet, P.E.; Beninger, R.J. *Psychopharmacology*, **1998**, *140*, 11.
[124] Nakamura-Palacios, E.M.; Winsaure, P.J.; Moerschbaecher, J.M. *Behav. Pharmacol.*, **2000**, *11*, 377.
[125] Brodkin, J.; Moerschbaecher, J.M. *J. Pharmacol. Exp. Ther.*, **1997**, *282*, 1526.
[126] Lichtman, A.H. *Eur. J. Pharmacol.*, **2000**, *404*, 175.
[127] Wolff, M.C.; Leander, J.D. *Eur. J. Pharmacol.*, **2003**, *477*, 213.
[128] Arnone, M. *World Patent Application*, **2003**, WO03082256.
[129] Da Silva, G.E.; Fernandes, M.S.; Takahashi, R.N. *Neurosci. Lett.*, **2003**, *349*, 49.
[130] Melis, R.M.; Succu, S.; Mascia, M.S.; Argiolas, A. *Neurosci. Lett.*, **2004**, *359*, 17.
[131] Carai, M.A.M.; Colombo, G.; Gessa, G.L. *Eur. J. Pharmacol.*, **2004**, *494*, 221.
[132] Rubino, T.; Vigano, D.; Zagato, E.; Mariaelvina, S.; Parolaro, D. *Synapse,* **2000**, *35*, 8.
[133] Rinaldi-Carmona, M.; Barth, F.; Congy, C.; Martinez, S.; Oustric, D.; Perio, A.; Poncelet, M.; Maruani, J.; Arnone, M.; Finance, O.; Soubrie, P.; LeFur, G. *J. Pharmacol. Exp. Ther.*, **2004**, *310*, 905.
[134] Camus, P.; Martinez, S.; Rinaldi, M. *World Patent Application*, **2000**, WO0046209.
[135] Miscoria, G.; Rinaldi, M.; Schofield, J. *World Patent Application*, **2004**, WO04058744.
[136] Lan, R.; Liu, Q.; Fan, P.; Lin, S.; Fernando, S.R.; McCallion, D.; Pertwee, R.; Makriyannis, A. *J. Med. Chem.*, **1999**, *42*, 769.
[137] New, D.C. and Wong, Y.H. *FEBS Lett.,* **2003**, *536*, 157.
[138] Hildebrandt, A.L.; Kelly-Sullivan, D.M.; Black, S.C. *Eur. J. Pharmacol.*, **2003**, *462*, 125.
[139] McLaughlin, P.J.; Winston, K.; Swezey, L.; Wisniecki, A.; Aberman, J.; Tardif, D.J.; Betz, A.J.; Ishiwari, K.; Makriyannis, A.; Salamone, J.D. *Behav. Pharmacol.*, **2003**, *14*, 583.
[140] Chambers, A.P.; Sharkey, K.A.; Koopmans, H.S. *Physiol. Behav.*, **2004**, *82*, 863.
[141] Chen, R.Z.; Huang, R.-R.C.; Shen, C.-P.; MacNeil, D.J.; Fong, T.M. *Brain Res.*, **2004**, *999*, 227.
[142] Kirkham, T.C. and Williams, C.M. *Psychopharmacology*, **2001**, *153*, 267.
[143] Shearman, L.P.; Rosko, K.M.; Fleischer, R.; Wang, J.; Xu, S.; Tong, X.S.; Rocha, B.A. *Behav. Pharmacol.*, **2003**, *14*, 573.
[144] Liao, C.; Zheng, J.; David, L.S.; Nicholson, R.A. *Basic Clin. Pharmacol. Toxicol.*, **2004**, *94*, 73.
[145] Wiley, J.L.; Jefferson, R.G.; Grier, M.C.; Mahadevan, A.; Razdan, R.J.; Martin, B.R. *J. Pharmacol. Exp. Ther.*, **2001**, *296*, 1013.
[146] Thomas, B.F.; Gillian, A.F.; Burch, D.F.; Roche, M.J.; Seltzman, H.H. *J. Pharmacol. Exp. Ther.*, **1998**, *285*, 285.
[147] Martin, B.R.; Razdan, A.K.; Mahadevan, A. *United States Patent*, **2003**, US6,509,367.
[148] Francisco, M.E.; Seltzman, H.H.; Gilliam, A.F.; Mitchell, R.A.; Rider, S.L.; Pertwee, R.G.; Stevenson, L.A.; Thomas, B.F. *J. Med. Chem.*, **2002**, *45*, 2708.
[149] Thomas, B.; Seltzman, H.H.; Francisco, M.E.E. *World Patent Application*, **2003**, WO03088968.
[150] Shim, J.Y.; Welsh, W.J.; Cartier, E.; Edwards, J.L.; Howlett, A.C. *J. Med. Chem.*, **2002**, *45*, 1447.
[151] Reggio, P.H. *Curr. Pharm. Design*, **2003**, *9*, 1607.
[152] Dyck, B.; Goodfellow, V.S.; Phillips, T.; Grey, J.; Haddach, M.; Rowbottom, M.; Naeve, G.S.; Brown, B.; Saunders, J. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 1151.
[153] Dow, R.L.; Hammond, M. *World Patent Application*, **2004**, WO04052864.
[154] Makriyannis, A.; Liu, Q.; Thotapally, R. *United States Patent Application*, **2004**, US2004/0192667.
[155] Katoch-Rouse, R.; Pavlova, O.A.; Caulder, T.; Hoffman, A.F.; Mukhin, A.G.; Horti, A.G. *J. Med. Chem.*, **2003**, *46*, 642.
[156] Katoch-Rouse, R. and Horti, A.G. *J. Labelelled Compd. Rad.*, **2003**, *46*, 93.
[157] Kumar, J.S.D.; Prabhakaran, J.; Arango, V.; Parsey, R.V ; Underwood, M.D.; Simpson, N.R.; Kassir, S.A.; Majo, V.J.; Van Heertum, R.L.; Mann, J.J. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 2393.
[158] Stoit, A.R.; Lange, J.H.M.; den Hartog, A.P.; Ronken, E.; Tipker, K.; van Stuivenberg, H.H.; Dijksman, J.A.R.; Wals, H.C.; Kruse, C.G. *Chem. Pharm. Bull.*, **2002**, *50*, 1109.
[159] Barth, F.; Congy, C.; Martinez, S.; Rinaldi, M. *World Patent Application*, **2001**, WO0132663.
[160] Ruiu, S.; Pinna, G.A.; Marchese, G.; Mussinu, J.-M.; Saba, P.; Tambaro, S.; Casti, P.; Vargiu, R.; Pani, L. *J. Pharmacol. Exp. Ther.*, **2003**, *306,* 363.
[161] Francisco, M.E.Y.; Burgess, J.P.; George, C.; Bailey, G.S.; Gilliam, A.F.; Seltzman, H.H.; Thomas, B.F. *Magn. Reson. Chem.*, **2003**, *41*, 265.
[162] Cullinan, G.J.; Fahey, K.J.; Koppel, G.A. *United States Patent*, **1997**, US5,596,106.
[163] Felder, C.C.; Joyce, K.E.; Briley, E.M.; Glass, M.; Mackie, K.P.; Fahey, K.J.; Cullinan, G.J.; Hunden, D.C.; Johnson, D.W.; Chaney, M.O.; Koppel, G.A.; Brownstein, M. *J. Pharmacol. Exp. Ther.*, **1998**, *284*, 291.
[164] Christopoulos, A.; Coles, P.; Lay, L.; Lew, M.J.; Angus, J.A. *Brit. J. Pharmacol.*, **2001**, *132*, 1281.
[165] Mailleron, J.-L.; Achard, D.; Grisoni, S.; Mignani, S.; Bouchard, H.; Bouquerel, J.; Capet, M.; Hittinger, A. *World Patent Application*, **2000**, WO0015609.
[166] Achard, D.; Filoche, B.; Grisoni, S.; Myers, M.; Bouchard, H.; Bouquerel, J.; Hittinger, A. *World Patent Application*, **2001**, WO0164632.
[167] Achard, D.; Filoche, B.; Grisoni, S.; Myers, M.; Bouchard, H.; Bouquerel, J.; Hittinger, A. *World Patent Application*, **2001**, WO0164633.
[168] Achard, D.; Filoche, B.; Grisoni, S.; Myers, M.; Bouchard, H.; Bouquerel, J.; Hittinger, A. *World Patent Application*, **2001**, WO0164634.
[169] Kuster, J.E.; Stevenson, J.I.; Ward, S.J.; D'Ambra, T.E.; Haycock, D.A. *J. Pharmacol. Exp. Ther.*, **1993**, *264*, 1352.
[170] Piot-Grosjean, O.; Picaut, P.; Petitet, F. *World Patent Application,* **2002**, WO0228346.
[171] Benavides, J.; Boccio, D.; Henin, Y.; Piot-Grosjean, O. *World Patent Application*, **2003**, WO03020314.
[172] Di Marzo, V.; Hill, M.P.; Bisogno, T.; Crossman, A.R.; Brotchie, J.M. *FASEB J.*, **2000**, *14*, 1432.
[173] Segovia, G.; Mora, F.; Crossman, A.R.; Brotchie, J.M. *Movement Disord.*, **2003**, *18,* 138.
[174] Brotchie, J.M. *Curr. Opin. Pharmacol.*, **2003**, *3*, 54.
[175] Kanyonyo, M.; Govaerts, S.J.; Hermans, E.; Poupaert, J.H.; Lambert, D.M. *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 2233.
[176] Ooms, F.; Wouters, J.; Oscari, O.; Happaerts, T.; Bouchard, G.; Carrupt, P.A.; Testa, B.; Lambert, D.M. *J. Med. Chem.*, **2002**, *45*, 1748.
[177] Govaerts, S.J.; Muccioli, G.G.; Hermans, E.; Lambert, D.M. *Eur. J. Pharmacol.*, **2004**, *495*, 43.
[178] Finke, P.E.; Mills, S.G.; Plummer, C.W.; Shah, S.K.; Truong, Q.T. *World Patent Application*, **2003**, WO03007887.
[179] Shearman, L.P.; Stribling, D.S.; Camacho, R.E.; Rosko, K.M.; Feng, Y.; Marsh, D.J.; MacNeil, D.J.; Fong, T.M.; Goulet, M.; Hagmann, W.; Plummer, C.; Finke, P.; Mills, S.; Shah, S.; Truong, Q.; MacIntyre, D.E.; Strack, A.M. **2003** *Symposium on the Cannabinoids*, Burlington Vermont, International Cannabinoid Research Society, page 150.
[180] Fong, T.M.; Van Der Ploeg, L.H. *World Patent Application*, **2003**, WO03075660.
[181] Hagmann, W.K.; Qi, H.; Shah, S.K. *World Patent Application*, **2003**, WO03063781.

[182] Lange, J.H.M.; van Stuivenberg, H.H.; Coolen, H.K.A.C.; Adolfs, T. J.P.; McCreary, A.C.; Keizer, H.G.; Walls, H.C.; Veerman, W.; Borst, A.J.M.; de Loff, W.; Verveer, P.C.; Kruse, C.G. *J. Med. Chem.*, **2005**, *48*, 1823.

[183] Lange, J.H.M.; Kruse, C.G.; Tipker, J.; Hoogendorn, J. *World Patent Application*, **2002**, WO02076949.

[184] Lange, J.H.M.; Coolen, H. K. A. C.; van Stuivenberg, H.H.; Dijksman, J.A.R.; Herremans, A.H.J.; Ronken, E.; Keizer, H.G.; Tipker, K.; McCreary, A.C.; Veerman, W.; Wals, H.C.; Stork, B.; Verveer, P.C.; den Hartog, A.P.; de Jong, N.M.J.; Adolfs, T.J.P.; Hoogendoorn, J.; Kruse, C.J. *J. Med. Chem.*, **2004**, *47*, 628.

[185] http://www.solvaypharmaceuticals.com/html/science/pipelitext.-html (accessed march 2004).

[186] Wilstermann, J.M. and Berggren, A.I.K. *World Patent Application*, **2003**, WO03051850.

[187] Berggren, A.I.K.; Bostrom, S.J.; Elebring, S.T.; Greasley, P.; Terricabra, E.; Wilstermann, J.M. *World Patent Application*, **2003**, WO03051851.

[188] Finke, P.E.; Meurer, L.C.; Debenham, J.S.; Toupence, R.B.; Walsh, T.E. *World Patent Application*, **2003**, WO03082191.

[189] Barth, F.; Martinez, S.; Rinaldi-Carmona, M. *World Patent Application*, **2003**, WO03084930.

[190] Barth, F.; Martinez, S.; Rinaldi-Carmona, M. *World Patent Application*, **2003**, WO03084943.

[191] Kopka, I. E.; Li, B.; Hagmann, W.K. *World Patent Application*, **2004**, WO04029204.

[192] Hagmann, W.K.; Lin, L.S.; Shah, S.K.; Guthikonda, R.N.; Qi, H.; Chang, L.L.; Liu, P.; Armstrong, H.M. *World Patent Application*, **2003**, WO03077847.

[193] Hagmann, W.K.; Lin, L.S.; Shah, S.K.; Goulet, M.T.; Jewell, J.P. *World Patent Application*, **2003**, WO03082190.

[194] Castonguay, L.A.; Hagmann, W.K.; Lin, L.S.; Shah, S.K. *World Patent Application*, **2003**, WO03086288.

[195] Hagmann, W.K.; Lin, L.S.; Shah, S.K. *World Patent Application*, **2003**, WO03087037.

[196] Berggren, A.I.K.; Bostrom, S.J.; Cheng, L.; Elebring, S.T.; Greasley, P.; Nagard, M.; Wilsterlmann, J.M.; Terricabras, E. *World Patent Application*, **2004**, WO04058249.

[197] Guba, W.; Haap, W.; Marty, H.P.; Narquizian, R. *United States Patent Application*, **2004**, US 2004/0147572.

[198] Jagerovic, N.; Hernandez Folgado, L.; Alkorta, I.; Goya, P.; Navarro, M.; Serrano, A.; de Fonseca, F.R.; Dannert, M.T.; Dannert, M.T.; Alsasua, A.; Suardiaz, M.; Pascual, D.; Martin, M.I. *J. Med. Chem.*, **2004**, *47*, 2939.

[199] Jagerovic, N.; Goya Laza, M.P.; Hernandez Folgado, L.; Alcorta Osoro, I.J.; Martin Fontelles, M.I.; Suardiaz Garcia, M.L.; Dannert, M.T. *World Patent Application*, **2003**, WO03082833.

[200] Lange, J.H.M.; Kruse, C.G.; Herremans, A.H.J.; VanStuivenberg, H.H.; Dijksman, J.A.R.; McCreary, A.C. *World Patent Application*, **2003**, WO03078413.

[201] Berggren, A.I.K.; Bostrom, S.J.; Elebring, S.T.; Fallefors, L.; Wilstermann, J.M.; Greasley, P. *World Patent Application*, **2004**, WO04058255.

[202] Toupence, R.B.; Debenham, J.S.; Goulet, M.T.; Madsen-Duggan, C.B.; Walsh, T.E.; Shah, S.K. *World Patent Application*, **2004**, WO04012671.

[203] Alanine, A.; Bleicher, K.; Guba, W.; Haap, W.; Kube, D.; Luebbers, T.; Plancher, J.-M.; Roche, O.; Rogers-Evans, M.; Schneider, G.; Zuegge, J. *World Patent Application*, **2004**, WO04013120.

[204] Griffith, D.A. *United States Patent Application*, **2004**, US 2004/0092520.

[205] Griffith, D.A. *United States Patent Application*, **2004**, US 2004/0157839.

[206] Griffith, D.A. *United States Patent Application*, **2004**, US 2004/0157838.

[207] Bayewitch, M.; Avidor-Reiss, T.; Levy, R.; Barg, J.; Mechoulam, R.; Vogel, Zvi, *FEBS letters*, **1995**, *375*, 143.

[208] Bayewitch, M.; Rhee, M.-H.; Avidor-Reiss, T.; Breuer, A.; Mechoulam, R.; Vogel, Z. *J. Biol. Chem.*, **1996**, *271*, 9903.

[209] Govaerts, S.J.; Hermans, E.; Lambert, D.M. *Eur. J. Pharm. Sci.*, **2004**, *23*, 233.

[210] D'Ambra, T.E.; Estep, K.G.; Bell, M.R.; Eissenstat, M.A.; Josef, K.A.; Ward, S.J.; Haycock, D.A.; Baizman, E.R.; Casiano, F.M.; Beglin, N.C. *J. Med. Chem.*, **1992**, *35*, 124.

[211] Showalter, V.M.; Compton, D.R.; Martin, B.R.; Abood, M.E. *J. Pharmacol. Exp. Ther.*, **1996**, *278*, 989.

[212] Zhang, Q.; Ma, P.; Wang, W.; Cole, R.B.; Wang, G. *J. Mass Spectrom.*, **2004**, *39*, 672.

[213] Gallant, M.; Dufresne, C.; Gareau, Y.; Guay, D.; Leblanc, Y.; Prasit, P.; Rochette, C.; Sawyer, N.; Slipetz, D. M.; Tremblay, N. *Bioorg. Med. Chem. Lett.*, **1996**, *6*, 2263.

[214] Melck, D.; De Petrocellis, L.; Orlando P.; Bisogno, T.; Laezza, C.; Bifulco, M.; Di Marzo, V. *Endocrinology*, **2000**, *141*, 118.

[215] Kalgutkar, A.S.; Marnett, A.B.; Crews, B.C.; Remmel, R.P.; Marnett, L.J. *J. Med. Chem.*, **2000**, *43*, 2860.

[216] Klegeris, A.; Bissonnette, C.J.; McGeer, P.L. *Brit. J. Pharmacol.*, **2003**, *139*, 775.

[217] 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. *J. Pharmacol. Exp. Ther.*, **1998**, *284*, 644.

[218] Barth, F.; Millan, J.; Casellas, P.; Oustric, D.; Sarran, M.; Rinaldi, M. *World Patent Application*, **1997**, WO9721682.

[219] Griffin, G.; Wray, E.J.; Tao, Q.; McAllister, S.D.; Rorrer, W.K.; Aung, M.M.; Martin, B.R.; Abood, M.E. *Eur. J. Pharmacol.*, **1999**, *377*, 117.

[220] Portier, M.; Rinaldi-Carmona, M.; Pecceu, F.; Combes, T.; Poinot-Chazel, C.; Calandra, B.; Barth, F.; Le Fur, G.; Casellas, P. *J. Pharmacol. Exp. Ther.*, **1999**, *288*, 582.

[221] Bouaboula, M.; Desnoyer, N.; Carayon, P.; Combes, T.; Casellas, P. *Mol. Pharmacol.*, **1999**, *55*, 473.

[222] Rhee, M.-H. and Kim, S.-K. *J. Vet. Sci.*, **2002**, *3*, 179.

[223] Bouaboula, M.; Dussossoy, D.; Casellas, P. *J. Biol . Chem.*, **1999**, *274*, 20397.

[224] Shire, D.; Calandra, B.; Bouaboula, M.; Barth, F.; Rinaldi-Carmona, M.; Casellas, P.; Ferrara, P. *Life Sci.*, **1999**, *65*, 627.

[225] Gouldson, P.; Calandra, B.; Legoux, P.; Kerneis, A.; Rinaldi-Carmona, M.; Barth, F.; Le Fur, G.; Ferrara, P.; Shire, D. *Eur. J. Pharmacol.*, **2000**, *401*, 17.

[226] Feng, W. and Song, Z.H. *Biochem. Pharmacol.*, **2003**, *63*, 1077.

[227] Barth, F.; Millan, J.; Oustric, D.; Rinaldi, M.; Vernhet, M. *World Patent Application*, **2001**, WO0132629.

[228] Mussinu, J.M.; Ruiu, S.; Mule, A.C.; Pau, A.; Carai, M.A.; Loriga, G.; Murineddu, G.; Pinna, G.A. *Bioorg. Med. Chem.*, **2003**, *11*, 251.

[229] Xiang, J.N.; Elliott, J.D.; Atkinson, S.T.; Christensen, S.B. *World Patent Application,* **1998**, WO9831227.

[230] Xiang, J.N.; Atkinson, S.T.; Wang, D.Y.; Lee, J.; Lee, J.C. Siegfried, B. *216th ACS meeting*, Boston (MA), USA, 23-27 Aug **1998**.

[231] Inaba, T.; Kaya, T.; Iwamura, H. *United States Patent*, **2003**, US 6,509,352 B1.

[232] Iwamura, H.; Suzuki, H.; Ueda, Y.; Kaya, T.; Inaba, T. *J. Pharmacol. Exp. Ther.*, **2001**, *296*, 420.

[233] http://www.jti.co.jp/JTI_E/IR/02/P.L.20030212_E.pdf (accessed 02.16.2004).

[234] Zygmunt, P.M.; Andersson, D.A.; Högestätt, E.D. *J. Neurosci.*, **2002**, *22*, 4720.

[235] Di Marzo, V.; De Petrocellis, L.; Fezza, F.; Ligresti, A.; Bisogno, T. *Prostaglandins Leukot. Essent. Fatty Acids*, **2002**, *66*, 377.

[236] Wagner, J.A.; Varga, K.; Jarai, Z.; Kunos, G. *Hypertension*, **1999**, *33*, 429.

[237] Mukhopadhyay, S.; Chapnick, B.M.; Howlett, A.C. *Am. J. Physiol.-Heart Circul. Physiol.*, **2002**, *282*, H2046.

[238] Jarai, Z.; Wagner, J.A .; Varga, K.; Lake, K.D.; Compton, D.R.; Martin, B.R.; Zimmer, A.M.; Bonner, T.I.; Buckley, N.E.; Mezey, E.; Razdan, R.K.; Zimmer, A.; Kunos, G. *Proc. Natl. Acad. Sci. U. S. A.*, **1999**, *96*, 14136.

[239] Kunos, G.; Martin, B.; Razdan, R. *World Patent Application,* **2001**, WO0103690.

[240] Offertaler, L.; Mo, F.-M.; Batkai, S.; Liu, J.; Begg, M.; Razdan, R.K.; Martin, B.R.; Bukoski, R.D.; Kunos, G. *Mol. Pharmacol.*, **2003**, *63*, 699.

[241] Begg, M.; Mo, F.-M.; Offertaler, L.; Batkai, S.; Pacher, P.; Razdan, R .K.; Lovinger, D.M.; Kunos, G. *J. Biol. Chem.*, **2003**, *278*, 46188.

[242] Ho, W.S.V.; Hiley, C.R. *Brit. J. Pharmacol.*, **2003**, *138*, 1320.

[243] Ho, W.S.V.; Hiley, C.R. *J. Pharm. Pharmacol.*, **2004**, *56*, 869.

[244] Mo, F.M.; Offertaler, L.; Kunos, G. *Eur. J. Pharmacol.*, **2004**, *489*, 21.

[245] Batkai, S.; Pacher, P.; Jarai, J.; Wagner, J.A.; Kunos, G. *Am. J. Physiol.-Heart Circul. Physiol.*, **2004**, *287*, H595.

[246] Kunos, G. *World Patent Application,* **2003**, WO03015700.
[247] Franklin, A. and Stella, N. *Eur. J. Pharmacol.*, **2003**, *474*, 195.
[248] Walter, L.; Franklin, A.; Witting, A.; Wade, C.; Xie, Y.; Kunos, G.; Mackie, K.; Stella, N. *J. Neurosci.*, **2003**, *23*, 1398.
[249] Di Marzo, V.; Breivogel, C. S.; Tao, Q.; Bridgen, D.T.; Razdan, R.K.; Zimmer, A.M.; Zimmer, A.; Martin, B.R. *J. Neurochem.*, **2000**, *75*, 2434.
[250] Breivogel, C.S.; Griffin, G.; Di Marzo, V.; Martin, B.R. *Mol. Pharmacol*., **2001**, *60*, 155.
[251] Monory, K.; Tzavara, E.T.; Lexime, J.; Ledent, C.; Parmentier, M.; Borsodi, A.; Hanoune, J. *Biochem. Bioph. Res. Commun.*, **2002**, *292*, 231.
[252] Muthane, U.; Ramsay, K.A.; Jiang, H.; Jackson-Lewis, V.; Donaldson, D.; Fernando, S.; Ferreira, M.; Przedborki, S. *Exp. Neurol.*, **1994**, *126*, 195.
[253] Hajos, N.; Freund, T.F. *Chem. Phys. Lipids*, **2002**, *121*, 73.
[254] Hajos, N.; Ledent, C.; Freund, T.F. *Neuroscience*, **2001**, *106*, 1.
[255] Hajos, N.; Freund, T.F. *Neuropharmacology*, **2002**, *43*, 503.
[256] Haller, J.; Bakos, N.; Szirmay, M.; Ledent, C.; Freund, T.F. *Eur. J. Neurosci.*, **2002**, *16*, 1395.
[257] Haller, J.; Varga, B.; Ledent, C.; Freund, T.F. *Behav. Pharmacol.*, **2004**, *15*, 299.
[258] Pistis, M.; Perra, S.; Pillolla, G.; Melis, M.; Gessa, G.L.; Muntoni, A.L. *Neuropharmacology*, **2004**, *46*, 115.
[259] Ralevic, V. and Kendall, D.A. *Eur. J. Pharmacol.*, **2001**, *418*, 117.
[260] Duncan, M.; Kendall, D.A.; Ralevic, V. *Brit. J. Pharmacol.*, **2003**, *138*, 214P.
[261] Duncan, M.; Millns, P.; Smart, D.; Wright, J.E.; Kendall, D.A.; Ralevic, V. *Brit. J. Pharmacol.*, **2004**, *142*, 509.
[262] Duncan, M.; Kendall, D.A.; Ralevic, V. *J. Pharmacol. Exp. Ther.*, **2004**, *311*, 411.
[263] Tao, Q. and Abood, M.E. *J. Pharmacol. Exp. Ther.*, **1998**, *285*, 651.
[264] Lambert, D.M.; DiPaolo, F.G.; Sonveaux, P.; Kanyonyo, M.; Govaerts, S.J.; Hermans, E.; Bueb, J.-L.; Delzenne, N.M.; Tschirhart, E.J. *Biochim. Biophys. Acta*, **1999**, *1440*, 266.
[265] Calignano, A.; La Rana, G.; Giuffrida, A.; Piomelli, D. *Nature*, **1998**, *394*, 277.
[266] Calignano, A.; La Rana, G.; Piomelli, D. *Eur. J. Pharmacol.*, **2001**, *419*, 191.