Different Classes of CB2 Ligands Potentially Useful in the Treatment of Pain

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Abstract: The search of new drugs and targets to treat the pain is an intriguing challenge both for several companies and researchers from academia. In this context, since the modulation of the endocannabinoid system with the non selective phytocannabinoid Δ⁹-THC produces analgesia and potentiates opioid analgesia in animal models, CB₂ ligands studies aimed to explore the involvement of endocannabinoid system in management of pain were started. Several selective CB₂ receptor agonists exhibited analgesic activity in preclinical models of acute, inflammatory and neuropathic pain, therefore this class of modulators could be useful as analgesic agents for pain, migraine, inflammation and osteoarthritis. This review is an update of our previously manuscript “A survey of recent patents on CB₂ agonists in the management of pain” and provides an overview of patents and advances in CB₂ agonist studies in the treatment of pain.

Keywords: Cannabinoid, CB₂ receptors, CB₂ receptor ligands, chronic pain, inflammatory pain, neuropathic pain.

1. INTRODUCTION

Pain is an unpleasant sensorial and emotional experience, associated with actual or potential tissue damage. It is a homeostatic and protective mechanism, but is liable to change in disease states and thus requires treatment.

The physiopathology of pain is complex and variable and involves several mechanisms, neurotransmitters and receptors.

In general terms, pain may be classified as being acute or chronic. The aim of acute pain is to warn the body of the presence of dangerous stimuli in the surroundings or in the body itself. Chronic pain is present in degenerative, neurological and oncological illness involving sensorial and emotional aspects.

Three major physiopathogenetic mechanisms are stimulated in chronic pain: the nociceptive or “physiological” pain, a strong, sudden and localized pain which can derive from an internal or external trauma; the neuropathic, due to damage of, or a dysfunction in, peripheral or central nervous system tissue, which is characterized by symptoms such as a burning sensation, constraint, and pins and needles; finally, the idiopathic pain whose cause is unknown.

Chronic pain represents a major health problem throughout the world. It is estimated that, at any given time, 22% of primary care patients between the ages of 18 and 65 suffer from chronic pain. In Europe, as in the United States, approximately half population seeks medical help for pain at some point in their lives.

The mediation of analgesia in the central nervous system (CNS) involves the use of different types of compounds such as opioids, which have been used for centuries to relieve pain and whose analgesic effect is due to the interaction with three types of receptors: µ, δ and κ. Nowadays, opioids represent the only analgesics able to achieve acceptable pain relief in more than 50% of treated patients, thus justifying the challenge faced by different research groups to find new analgesics.

For a long time marijuana and hashish, the common nouns for the dried blossom tips and dried resin, respectively, of Cannabis sativa, have been used as drugs for medicinal and recreational purposes. In the last decades, the chemical and physiological effects of cannabinoids have been investigated. Recently, this research has highlighted the presence of an endogenous cannabinoid system (ECS) in the human body whose biological organization includes two subtypes of G-protein coupled membrane receptors, CB₁ and CB₂, their endogenous ligands anandamide (AN) and 2-arachidonoyl-glycerol (2-Ara-GI), and their multiple metabolic pathways for synthesis, degradation and reuptake.

Animal studies have clearly demonstrated that the non-selective cannabinoid, Δ⁹-tetrahydrocannabinol (Δ⁹-THC), the principal component of Cannabis, and synthetic cannabinoids, which act on the two receptors of the ECS, produce analgesia and potentiate opioid analgesia. Furthermore, recent studies would suggest that cannabinoids could be effective in specific chronic pain states.

To this end, an initial study with cannabinoid ligands was aimed at evaluating the involvement of endogenous cannabinoids in the control of pain [1, 2]. The analgesic properties of non-selective cannabinoid receptor agonists have been known for many years and several behavioral and electrophysiological studies in animal models have shown antinoci-
ceptive and antihyperalgesic effects in different models of pain [3]. However, selective CB1 ligands proved not to be useful as analgesic agents in humans, because their administration was associated with undesirable central nervous system side effects due to the doses of cannabinoids that can be administered [4]: for this reason their clinical development was limited. Recently CB2 agonists have been proposed as a valid alternative, either because of their mainly peripheral distribution, so they do not cause adverse CNS effects, or because of their capability to inhibit signs of acute nociceptive, inflammatory and neuropathic pain in preclinical studies [5-7]. Although there is a large body of evidence supporting the potential utility of selective cannabinoid CB2 receptor agonists for the treatment of pain [8, 9], the mechanism and site of action responsible for CB2-mediated analgesia remain unexplained.

A plethora of reviews on CB2 receptors [10, 11], their distribution, molecular analysis and signalling pathways have been published [12, 13]: the present review focuses on recent patents, from 2009 onwards, concerning CB2 receptor ligands acting as analgesic agents.

The review has been structured taking into account the number of patents, updated to January 2013, from different applicants and the related articles published recently.

Table I [14-129] summarizes the classes of CB2 agonists reviewed in this article, and shows the key compounds for each of the classes of derivatives presented. The table has been organized in the same order in which key compounds appear in the text.

When available from the patents, CB2 selectivity against CB1 receptors is given.

<table>
<thead>
<tr>
<th>Classes</th>
<th>Key Compounds</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiazoles, isothiazoles and thiazoles</td>
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<td></td>
</tr>
<tr>
<td></td>
<td><img src="image" alt="Thiazole" /></td>
<td>hCB2 Ki = 0.64 nM</td>
</tr>
<tr>
<td>Dihydropyrazoles, bicyclic pyrazoles and indazoles</td>
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<td></td>
<td><img src="image" alt="Dihydropyrazole" /></td>
<td>hCB2 Ki = 0.40 nM</td>
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<tr>
<td>Indoles, indole-indanes and arylsulfonylindoles</td>
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<td></td>
<td><img src="image" alt="Indole" /></td>
<td>hCB2 Ki = 0.21 nM</td>
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(Table 1) contd…..

<table>
<thead>
<tr>
<th>Classes</th>
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<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azetidines, pyrrolidines, azepanes, diazepanes and deca-hydroquinolines</td>
<td><img src="image_url" alt="Compound Image" /></td>
<td>[52-57, 66, 68, 97]</td>
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<tr>
<td>Amidosulfones</td>
<td><img src="image_url" alt="Compound Image" /></td>
<td>[58-65]</td>
</tr>
<tr>
<td>Butyramides and ureas</td>
<td><img src="image_url" alt="Compound Image" /></td>
<td>[83, 84, 87]</td>
</tr>
<tr>
<td>(Sulfamoyl)benzamides</td>
<td><img src="image_url" alt="Compound Image" /></td>
<td>[88-91]</td>
</tr>
</tbody>
</table>

- CB$_2$ EC$_{50}$ = 0.015 nM
- CB$_2$ EC$_{50}$ = 0.04 nM
- CB$_2$ $K_i$ = 0.11 nM
- CB$_2$ $K_i$ = 1.7 nM
<table>
<thead>
<tr>
<th>Classes</th>
<th>Key compounds</th>
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<tr>
<td>Pyridines and furopyridines</td>
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<td>[49, 92, 93, 98-101]</td>
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<tr>
<td></td>
<td><strong>76</strong></td>
<td></td>
</tr>
<tr>
<td>CB$<em>2$ IC$</em>{50}$ = 3 nM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetrahydroimidazoles and imidazopyridines</td>
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<td>[75-78, 94-96]</td>
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<td></td>
<td><strong>81</strong></td>
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<tr>
<td>CB$_2$ $K_i$ = 1.92 nM</td>
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<tr>
<td>Triaryl derivatives</td>
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<td></td>
<td><strong>85</strong></td>
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<td>CB$_2$ $K_i$ = 0.27 nM</td>
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<td>Pyrimidines, pyrazines, pyridazinones and purines</td>
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<td>[106-109, 128, 129]</td>
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<td></td>
<td><strong>91</strong></td>
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<tr>
<td>hCB$_2$ $K_i$ = 43 nM</td>
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<td>Thienoderivatives</td>
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<tr>
<td></td>
<td><strong>98</strong></td>
<td></td>
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<tr>
<td>(For these derivatives, patents lack of detailed binding data)</td>
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(Table 1) contd…..

<table>
<thead>
<tr>
<th>Classes</th>
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<td>Dihydrobenzofuranes</td>
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<td>[115]</td>
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<tr>
<td>Imidazoles, (heteroaryl)benzimidazoles</td>
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<td>[117-121]</td>
</tr>
<tr>
<td>Benzimidazolones, biphenylimidazolidine-2,4-diones</td>
<td></td>
<td>[122-124]</td>
</tr>
</tbody>
</table>

2. ABBOTT LABORATORIES

The compounds acting as CB2 agonists which were patented by Abbott Laboratories between 2009 and January 2013 can be classified into three different groups: the largest group is that containing heterocyclic ring systems as thiazole [14-28], and the section on this will also include a discussion on the thiazole sulfonamides published by Taisho Pharmaceuticals Co. [29, 30], the isothiazoles [31-34] and the thiadiazoles [35-38]; the second group covers dihydropyrazole derivatives [35, 39-45]; the last encompasses indole [46-48], (hetero)arylcarboxamides [49] and tricyclic thiophene compounds [50, 51].

2.1. 1,3-Thiazoles, Isothiazoles and 1,3,4-Thiadiazoles

Over the last three years Abbot has published several patents and articles which propose different 1,3-thiazol-2(3H)-ylidene derivatives as cannabinoid receptor ligands [14-17]. In patents WO071783 (2010) [14] and WO28338 (2010) [15], the authors claim that these compounds are novel CB2 ligands. Amongst them, both (Z)-N-(5-tert-butyl-3-isobutylthiazol-2(3H)-ylidene)-2-(2-hydroxyethoxy)-5-(trifluoromethyl)benzamide (1) and (Z)-N-(5-tert-butyl-3-[2R]-tetrahydrofuran-2-ylmethyl)-1,3-thiazol-2(3H)-ylidene)-2-[(isopropylamino)oxy]-5-(trifluoromethyl)benzamide (2) showed a high affinity for both human and rat CB2 receptors, with Ki values of 0.74 and 0.15 nM for the former, and 0.30 and 0.50 nM for the latter, respectively, Fig. (1).

The modulation of the 1,3-thiazol-2(3H)-ylidene-benzamide, by condensation of 6-membered hetero(aryl) rings to the thiazolic core [18, 19], furnished the N’-(2Z)-3-
butyl[1,3]thiazolo[4,5-b]pyrazin-2(3H)-ylidene]-2-[[2S]-2-hydroxypropoxy][oxy]-5-(trifluoromethyl)benzamide (3) and N-[(2Z)-3-butyl-6-methyl-7-oxo-6,7-dihydro[1,3]thiazolo[4,5-d]pyrazin-2(3H)-ylidene]-2-[[2S]-2-hydroxypropoxy][oxy]-5-(trifluoromethyl)benzamide (4) which showed interesting $K_{CB2}$ values, Fig. (2). The condensation of the pyrazine ring to the thiazole system gave the new CB2 receptor ligand 3 with $K_i$ values for human CB2 and rat CB2 receptors of 2.0 and 4.1 nM, respectively, whereas the substitution of the pyrazine ring with pyridazine (4) showed an inversion of affinity for human and rat CB2 receptors, with $K_i$ values of 6.2 and 1.5 nM, respectively.

**Derivative 7** demonstrated a CB2 receptor-mediated broad spectrum of efficacy in *in vivo* animal models of nociceptive pain, using complete Freund’s adjuvant (CFA) assay, and of neuropathic pain, measuring allodynia in L5 and L6 spinal nerve ligation (SNL) and in a rat human constriction injury (CCI) model, following systemic administration [28]. Therefore, compound 7 provides a useful tool for studying the CB2 receptor pharmacology *in vitro* and for assessing the biological consequences of CB2 receptor activation in animals.

**Fig. (2).** N-[(2Z)-3-Butyl[1,3]thiazolo[4,5-b]pyrazin-2(3H)-ylidene]-2-[[2S]-2-hydroxypropoxy][oxy]-5-(trifluoromethyl)benzamide (3) and N-[(2Z)-3-butyl-6-methyl-7-oxo-6,7-dihydro[1,3]thiazolo[4,5-d]pyrazin-2(3H)-ylidene]-2-[[2S]-2-hydroxypropoxy][oxy]-5-(trifluoromethyl)benzamide (4). The introduction of a second aliphatic ring condensed with various heterocycles [20-22] and the synthesis of carbboximidamide derivatives [23-26], gave new CB2 receptor ligands with general structures 5 and 6, some of which were shown to possess an analgesic effect in animal models of neuropathic and nociceptive pain, although no *in vitro* and *in vivo* data were given in the patents for these derivatives Fig. (3).

**Fig. (3).** Modulation of the 1,3-thiazol-2(3H)-ylidene-benzamide.

In contrast, compound 7, (Z)-N-[3-(2-methoxyethyl)-4,5-dimethylthiazol-2(3H)-ylidene]-2,2,3,3-tetramethylcyclopropanecarboxamide (A-836339), has been extensively characterized using *in vitro* binding and functional assays, *in vivo* pain models and CNS behavior tests, and phMRI studies Fig. (4). It was found to exhibit high affinities and agonist potencies at both human and rat CB2 receptors, with $K_i$ values of 0.64 and 0.76 nM, respectively, and an excellent selectivity against the CB1 receptors in human ($K_{IC50}$ = 270 nM) and rat ($K_{IC50}$ = 143 nM), with a selectivity ratio, $\frac{K_{IC50,CB1}}{K_{IC50,CB2}}$, of 422- and 189-fold, respectively [27]. In addition, 7 did not exhibit significant species selectivity between human and rat for either CB1 or CB2 receptors.

**Fig. (4).** (Z)-N-[3-(2-Methoxyethyl)-4,5-dimethylthiazol-2(3H)-ylidene]-2,2,3,3-tetramethylcyclopropanecarboxamide.

A series of compounds with the same 1,3-thiazole core as the structures just reviewed, were identified by Taisho Pharmaceuticals Co. through a random screening program of their libraries. In particular, (Z)-N-[5-tert-butyl-3,4-dimethyl-1,3-thiazol-2(3H)-ylidene]phenyl-1-sulfonamide (8), acting as a full CB2 receptor agonist in the [35S]GTPyS binding assay (EC50 = 7.2 nM, Emax = 100%), showed a moderate CB2 receptor affinity (IC50 = 340 nM) [29]. Structure-Activity Relationship (SAR) studies, aimed at identifying related compounds with a better CB2 affinity, allowed to obtain the (Z)-N-[5-tert-butyl-3-(cyclopropylmethyl)-4-methyl-1,3-thiazol-2(3H)-ylidene]napththalene-1-sulfonamide (9) which exhibited high CB2 affinity (IC50 = 16 nM) and selectivity (IC50/CB1 IC50/CB2 = 106) in comparison to 8 Fig. (5) [29].

**Fig. (5).** (Z)-N-[5-tert-Butyl-3,4-dimethyl-1,3-thiazol-2(3H)-ylidene]phenyl-1-sulfonamide (8) and (Z)-N-[5-tert-butyl-3-(cyclopropylmethyl)-4-methyl-1,3-thiazol-2(3H)-ylidene]napththalene-1-sulfonamide (9).

Modulation of the sulfonamide group, responsible for the poor metabolic stability of these compounds in human liver microsomes, led to the synthesis of N-alkylidenearyl-carboxamide analogues shown in Fig. (6). In particular,
(Z)-N-(5-tert-butyl-3-(cyclopropylmethyl)-4-methylthiazol-2(3H)-ylidene)-3-(trifluoromethyl)benzamide (10) exhibited a high affinity for the CB2 receptor and a good selectivity (IC$_{50}$CB$_2$ = 13 nM, IC$_{50}$CB$_1$ = 3500 nM, selectivity CB$_1$/CB$_2$ = 269). Moreover, an evaluation of plasma levels after oral administration of compound 10 in rats gave significant results with a good oral bioavailability [30].

Further optimization of this class of compounds led to the discovery of N-[5-tert-butyl-2-(cyclopropylmethyl)-1-methyl-1,2-dihydro-3H-pyrazol-3-ylidene]-2-fluoro-3-(trifluoromethyl)benzamide (11), which exhibited good affinity for CB$_2$ receptors (IC$_{50}$CB$_2$ = 2.9 nM) and good metabolic stability in human and rat liver microsomes. The analgesic effect of compound 11 was also tested by its oral administration that significantly reversed the mechanical hyperalgesia in the Randall-Selitto model of inflammatory pain in rats.

This class of compounds will be also discussed under Abbot Laboratories in section 2.2.

Four different applications [31-34] relate to benzothiazolylidene derivatives. The representative compound is (Z)-N-(1-tert-butyl-5-(trifluoromethyl)-4,5,6,7-tetrahydrobenzo[2,1-c]isothiazol-3(1H)-ylidene)-5-chloro-2-methoxybenzamide (12), with a $K_i$ value of 0.24 nM for hCB2 receptors Fig. (7). Patents WO054024 (2010) [34] also describes compounds related to (Z)-methyl 3-([(2-tert-butyl-4-butylisothiazol-5(2H)-ylidene)amino]carbonyl)-2,2,3-trimethylcyclopentanecarboxylate (13) shown in Fig. (7). This compound showed high affinities and agonist potencies for both human and rat CB2 receptors with $K_i$ values of 0.20 and 0.25 nM, respectively. The separation of two enantiomers showed that the (1S,3R) isomer, 14, still retained high $K_i$ values (hCB2 = 0.16 nM and rCB$_2$ = 0.10 nM), whereas its (1R,3S) enantiomer was less potent ($K_i$ hCB2 = 1.6 nM and $K_i$ rCB$_2$ = 1.4 nM).

The bioisosteric replacement of the carbon atom in the 4 position of the thiazoline ring with a nitrogen atom enabled a series of 1,3,4-thiadiazolidene derivatives to be obtained. Within this class, compounds 15-17 Fig. (8) proved to be potent hCB2 receptor ligands, with $K_i$ values ranging from 0.32 to 0.60 nM [35-38], the best compound in this series (Z)-2-tert-Butyl-){[(2Z)-3-butyl-5-tert-butyl-1,3,4-thiadiazol-2(3H)-ylidene]carbamoyl}-4-(trifluoromethyl)phenylcarbamate (15) and other 1,3,4-thiadiazolylidene carboxamides.
being \((Z)-2\text{-}2\text{-}\text{tert-}\text{butyl}[([2Z]\text{-}3\text{-}\text{butyl-5\text{-}tert-}\text{butyl-1,3,4-}\text{thiadiazol-2(3H)-ylidene}][\text{carbamoyl}]-4\text{-}(\text{ trifluoromethyl})\) phenylcarbamate \((15)\) \((K_i\ hCB_2 = 0.32 \text{ nM})\).

2.2. 1,2-Dihydro-3H-pyrazoles

The substitution of the sulfur atom of previous compounds with a carbon led to the 1,2-dihydro-3H-pyrazolylidine derivatives \([31, 39-45]\). Among them, compounds \(18\) and \(19\) Fig. (9), namely \(N\text{-}[(3E)\text{-}2\text{-}\text{butyl-5\text{-}tert-}\text{butyl-1-methyl-1,2-dihydro-3H-pyrazol-3-ylidene}]-2\text{-}[(2,2-\text{dimethylpropanoyl})\text{hydrazine}]-5\text{-}(\text{ trifluoromethyl})\) benzamide and \((E)\text{-}N\text{-}[(5\text{-}tert-}\text{butyl-1-methyl-2-}\text{[(tetrahydrofuran-2-yl)methyl]}\text{-}1H\text{-}\text{pyrazol-3(2H)-ylidene}]-2\text{-}\text{fluoro-3\text{-}(}{(\text{trifluoromethyl)})\} benzamide, respectively, showed the best hCB2 affinity with \(K_i\) values of 0.4 nM for \(18\) and 0.7 nM for \(19\).

![Fig. (9).](image)

Different 1,2-dihydro-3H-pyrazolidines from those just reviewed are described in patent US8188135 \((2012)\) \([45]\), whose representative compound is the \(N\text{-}[(3E)\text{-}5\text{-}\text{tert-}\text{butyl-1-methyl-1,2-dihydro-3H-pyrazol-3-ylidene}]-2\text{-}[(2,2-\text{dimethylpropanoyl})\text{hydrazine}]-5\text{-}(\text{ trifluoromethyl})\) benzamide \((18)\) and \((E)\text{-}N\text{-}[(5\text{-}tert-}\text{butyl-1-methyl-2-}\text{[(tetrahydrofuran-2-yl)methyl]}\text{-}1H\text{-}\text{pyrazol-3(2H)-ylidene}]-2\text{-}\text{fluoro-3\text{-}(}{(\text{trifluoromethyl)})\} benzamide \((19)\).

Fig. (9). \(N\text{-}[(3E)\text{-}2\text{-}\text{Butyl-5\text{-}tert-}\text{butyl-1-methyl-1,2-dihydro-3H-pyrazol-3-ylidene}]-2\text{-}[(2,2-\text{dimethylpropanoyl})\text{hydrazine}]-5\text{-}(\text{ trifluoromethyl})\) benzamide \((18)\) and \((E)\text{-}N\text{-}[(5\text{-}tert-}\text{butyl-1-methyl-2-}\text{[(tetrahydrofuran-2-yl)methyl]}\text{-}1H\text{-}\text{pyrazol-3(2H)-ylidene}]-2\text{-}\text{fluoro-3\text{-}(}{(\text{trifluoromethyl)})\} benzamide \((19)\).

![Fig. (9).](image)

![Fig. (10).](image)

Fig. (10). \(N\text{-}[(3E)\text{-}5\text{-}\text{tert-Butyl-1-methyl-2-}\text{[(2R)\text{-tetrahydrofuran-2-ylmethyl]}\text{-}1,2-dihydro-3H-pyrazol-3-ylidine}]-2\text{-}[(2S)\text{-tetrahydrofuran-2-ylmethyl}][\text{amino}]-5\text{-}\text{(trifluoromethyl)}\) benzamide.

2.3. Other Structures

In other patents \([46-48]\), Abbott Laboratories claim other classes of CB2 receptor ligands, not related to those previously described, whose structures are shown in Fig. (11). In particular, the authors assert that indole derivatives bearing a 3-keto-tetramethylcyclopropyl group, such as \([1\text{-}\text{[(2-morpholinoethyl)\text{-}1H-indol-3-yl]}\text{-}[2,2,3,3\text{-}\text{tetramethylcyclopropyl}][\text{methanone} \(21\) and \([1\text{-}\text{[2\text{-}\text{tetrahydro-2H-pyran-4-yl}]}\text{-}\text{1H-indol-3-yl]}\text{-}[2,2,3,3\text{-}\text{tetramethylcyclopropyl}][\text{methanone} \(22\), have been shown to possess high hCB2 receptor binding affinities with \(K_i\) values of 4.6 and 0.21 nM, respectively. The activity of these compounds was also assessed in human and rat cyclase assays, compound \(21\) being found to be highly selective for the hCB2 receptors (\(CB_2/CB_1 = 1385\)). These derivatives display an analgesic effect in two types of animal pain model relating to neuropathic and nociceptive pain, that is, the complete Freund’s adjuvant and the spinal nerve ligation model, respectively.

![Fig. (11).](image)

Fig. (11). \([1\text{-}(\text{2-Morpholinoethyl})\text{-}1H-indol-3-yl]\text{-}[2,2,3,3\text{-}\text{tetramethylcyclopropyl}][\text{methanone} \(21\) and its tetrahydro-2H-pyran-4-yl-ethanol analog \(22\))

Compounds having the general structure \(23\) and \(24\) Fig. \((12)\) were claimed in EP2176219 \((2010)\) \([49]\), whereas \(25\)-\(27\) were claimed in US7868038 \((2011)\) \([50, 51]\). The authors declare that some of these derivatives, which bound to CB2 receptors with a \(K_i\) between 1 and 400 nM, showed a statisti-
cally significant change in paw withdrawal latency versus a saline vehicle at less than about 300 micromol/Kg in the spinal nerve ligation model of neuropathic pain, and showed efficacy at less than about 50 micromol/Kg in the same model. No data were provided concerning specific structures and activities.

3. BOEHRINGER INGELHEIM

Recent Boehringer Ingelheim patents [52-57] on CB$_2$ receptor modulators have claimed aliphatic heterocyclic compounds bearing a carboxamide moiety at the C2 position. In particular, these have covered azetidine and azepane-type structures, including pyrrolidine and piperidine homologues.

All of the CB$_2$ agonist derivatives claimed in the patents exhibited CB$_2$-receptor-mediated modulation of cAMP synthesis, expressed as EC$_{50}$ (nM). However, though the inventors identified a range of binding affinities for many compounds, they did not report the binding data values of individual compounds on both the CB$_1$ and CB$_2$ receptors. As a consequence it is difficult to assess whether any of the compounds are selective for one receptor over another and to infer whether they are particularly well suited for the treatment of pain.

Seven patents [58-64] covered different sulfonyl derivatives which were strictly related to amidosulfones reported in 2009 by Agmen Inc. [65] and, therefore, all of these derivatives will be presented in the second section on Boehringer Ingelheim.

Finally, the last section deals with a series of 1,4-diazepane carboxamides [66-68] and covers recently granted patents concerning amine [69], ether [70], triazole and tetrazoles derivatives [71-74] which modulate CB$_2$ receptors.

3.1. Azacycloalkane Carboxamides

3.1.1. Azetidine Carboxamides

The azetidine-2-carboxylic acid core [52, 53] was modulated both at position N1 and on the C2 carboxylic function, with the introduction of a number of aromatic and/or aliphatic substituents on the azetidinic N1, and the simultaneous evaluation of different amine groups in the carboxylic portion. The N-1-[3-chloro-5-(trifluoromethyl)pyridin-2-yl] group was the preferred substituent on the N1 position of the azetidine, this group being present in the two most potent derivatives claimed in the patent, namely (S)-N-(5-tert-butyl-isoazol-3-yl)-1-[3-chloro-5-(trifluoromethyl)pyridin-2-yl]azetidine-2-carboxamide (28) and the (S)-N-(5-tert-butyl-isoazol-3-yl) isomer 29. Fig. (13).
From this series further indications could be derived regarding the carboxamidic function since the two derivatives 28 and 29, in addition to possessing the best CB2 receptor affinity amongst related compounds, showed little difference in cAMP EC50 values, 0.020 and 0.050 nM, respectively, indicating that the bond position of the isoxazole ring relative to the carboxylic function of the azetidine core plays a significant role.

3.1.2. Pyrrolidine Carboxamides

The same trend, regarding its azetidinic homologs, is also reported in the pyrrolidine-carboxamides series claimed in patents WO147791 (2010) [54] and US0142677 (2012) [55]. Again, (S)-N-(5-tert-butyl-isoxazol-3-yl)-2-methyl-1-[5-(trifluoromethyl)pyridin-2-yl]pyrrolidine-2-carboxamide (30) was found to be the agonist with the highest CB2 receptor affinity (EC50 = 0.015 nM), while its (S)-N-(3-tert-butyl-isoxazol-5-yl) analogs, 31 and 32, showed a slight CB2 receptor affinity decrease (cAMP, EC50 = 0.020 and 0.023 nM, respectively) Fig. (14).

3.1.3. Piperidine and Azepane Carboxamides

(S)-N-(5-tert-Butyl-isoxazol-3-yl)-1-[5-(trifluoromethyl) pyridin-2-yl]piperidine-2-carboxamide (33) and (S)-N-(5-tert-butyl-isoxazol-3-yl)-1-(tetrahydro-pyran-4-ylmethyl) azepane-2-carboxamide (34) Fig. (15) are the most representative derivatives of piperidine and azepane-carboxamides related to previously discussed compounds [56, 57]. The homologation of the aza-ring to piperidine, as in compound 33, resulted in a decrease in CB2 receptor affinity (cAMP, EC50 = 0.093 nM), as compared with the pyrrolidine series, while for the azepane derivative, 34, the N1-piperidine group substitution with a tetrahydro-pyran-4-ylmethyl retained a CB2 receptor affinity (cAMP, EC50 = 0.030 nM) with respect to pyrrolidine.

3.2. Sulfonyl-Isoxazolyl-Amides and Benzensulfonyl-Carboxamides

SAR studies and cannabinergic profiles of 2-(azetidin-1-ylsulfonyl)-N-(5-tert-butylisoxazol-3-yl)-2-methylpropnamide (35) and related compounds were evaluated by Boehringer Ingelheim [58]. Fig. (16). Compound 35 showed agonistic activity for the CB2 receptor with an EC50 value of 3.6 nM. Ring expansion of the azetidine nucleus to pyrrolidine, and its homologation to piperidine, furnished compounds 36 and 37 which still retained a good CB2 affinity: more precisely, the potency of pyrrolidin-1-ylsulfonyl-propanamide, compound 36, was 3-fold higher than that of compound 35 (EC50/CB2 = 1.3 nM), whilst the potency of the...
piperidine analogue, 37, was 2-fold less than that of compound 35 (EC\textsubscript{CB2} = 6.5 nM).

In two different patents [59, 60] derivatives N-(3-tert-butylisoxazol-5-yl)-2-(isobutylsulfonyl)-2-methylpropanamide (38) and 2-methyl-N-[3-[2-(methylthiazol-5-yl)propan-2-yl]isoxazol-5-yl]-2-(tetrahydro-2\textsubscript{H}-pyran-4-ylsulfonyl)propanamide (39) Fig. (17) are reported as selective CB\textsubscript{2} agonists. The in vitro cannabinoid activity of all compounds was evaluated by the modulation of cAMP synthesis. Compound 38 resulted the derivatives with the highest CB\textsubscript{2} affinity (EC\textsubscript{50CB2} = 0.04 nM), whereas the EC\textsubscript{50CB1} value was only reported for compound 39 (EC\textsubscript{50CB2} = 3.4 nM, EC\textsubscript{50CB1} > 50000 nM).

In 2009 Agmen Inc. described the synthesis, SARs and pharmacological properties of a series of \(\alpha\)-amidosulfones strictly related to structure 42 [65]. In general, these studies showed that the substituent resulting in a high CB\textsubscript{2} profile was a \(\text{bis-aryl}\) system on the carboxamide nitrogen, as in the case of N-[4-chloro-3-(furan-2-yl)phenyl]-2-(4-chlorophenylsulfonyl)-2-methylpropanamide (44) and 2-(4-chlorophenylsulfonyl)-2-methyl-N-[5-(5-methylthiophen-2-yl)pyridin-3-yl]propanamide (45) (GTP binding; EC\textsubscript{50} hCB\textsubscript{2} = 0.92 nM).
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= 4 nM and 9 nM, respectively), while the 3-tert-butyl-1-methyl-1H-pyrazol-5-yl-carboxamide, compound 46, which maintained a good CB2 affinity (GTP binding; EC50hCB2 = 26 nM), was the more CB2 selective full agonist of the series (CB1/CB2 = 86) Fig. (21). Thus, compound 46 was selected for in vitro and in vivo pharmacokinetic evaluations. The in vitro assays for 46 demonstrated a low intrinsic clearance, both in human (14 μL/min/mg) and rat (20 μL/min/mg) liver microsomes, and a lower plasma protein binding across species (roughly 94%). The i.v. administration to Sprague-Dawley rats resulted in a moderate clearance (CL = 1.2 L/h/Kg), a short half-life (t1/2 = 1.9 h) and a small distribution volume (Vss = 2.0 L/Kg). Upon oral administration in rats, compound 46 was well absorbed with an oral bioavailability of 43%. Given that it displays all of these good pharmacokinetic properties, compound 46 should be a valuable candidate for use in in vivo pain models.

3.3. 1,4-Diazepanecarboxamides

Patents US0261708 (2010) [66] and US8173638 (2012) [67] claim diazepane compounds able to modulate the CB2 receptors function. The CB2 agonists [4-(5-tert-butylbenzooxazol-2-yl)-[1,4]-diazepan-1-yl]-(4,4-difluoro-cyclohexyl)-methanone (47) and [4-(5-tert-butyl-benzooxazol-2-yl)-[1,4]-diazepan-1-yl]-(4-fluoro-phenyl)-methanone (48), shown in Fig. (22), have the best CB2 receptor affinities. The former showed an EC50 value of 0.017 nM, while the latter an EC50CB2 = 0.14 nM, indicating that the planarity of the phenyl ring is responsible for an 8-fold decrease in affinity to CB2 receptors.

From SAR studies on a hit compound derived from high-throughput screening, N-(5-tert-butylisoxazol-3-yl)-4-(tetrahydro-2H-pyran-4-carbonyl)-1,4-diazepane-1-carboxamide (49) emerged as an interesting new lead compound for further optimization on selective CB2 agonists because of its different binding mode to CB2 receptors, as compared with classical and non-classical cannabinoids, and its good stability in liver microsome assays Fig. (23) [68].

The different binding mode was inferred because compound 49, which had an EC50CB2 value of 67 nM in cAMP assays, did not demonstrate competitive binding for the CB2 receptor agonist [3H]-CP-55940, suggesting that its binding site was not identical to that of CP-55940. Furthermore, when incubated with human liver microsomes, it showed a significant higher half-life of two hours. Finally, i.v. administration to Wistar rats showed a low volume of distribution (Vss = 0.87 L/Kg), a medium clearance (CL = 34 mL/min/Kg) and short half-life (t1/2 = 1.4 h).

3.4. Other Structures

Between June 2011 and March 2012 Boerhinger Ingelheim filed six extensions of previously claimed patents [69-74]. Two of these, US0136869 (2011) [69] and US0190256 (2011) [70] relate to the CB2 ligands whose structures are reported in Fig. (24). Inventors assert that isoxazole amides
bearing an aminoalkyl or ether group, such as \(N-[3-(1-hydroxy-2-methylpropan-2-yl)isoxazol-5-yl]-2-methyl-2-\{methyl[[3(S)-5-(methylsulfonyl)pyrrolidin-3-yl]methyl]amino\}propanamide \(50\) and \(N-[3-(\text{tert-butyl})isoxazol-5-yl]-2-\{(5-(trifluoromethyl)pyridin-2-yl)oxy\} propanamide \(51\), have been shown to act as CB\(_2\) receptor agonists with EC\(_{50}\) values of 1.7 and 0.10 nM, respectively, compound \(50\) being found to be highly selective for the CB\(_2\) receptors (EC\(_{50}\)CB\(_2\) > 20,000 nM). Although these derivatives are claimed as suitable weapons for treatment of several types of pain as well as of inflammation, nowhere is indicated any animal pain model employed to test the neuropathic and/or nociceptive pain.

The \(2-\{2\text{-fluoro}-4\text{-}(methylsulfonyl)phenyl\}sulfonyl\}-N-[5-(4-methoxyphenyl)-4\text{-H}-1,2,4-triazol-3-yl]-2-methylpropanamide \(52\) and \(N\)-\([2\text{-}(\text{tert-butyl})-2\text{-H-tetrazol-5-yl}]\)-2-\{[(1\text{R},4\text{R})-4-hydroxycyclohexyl]sulfonyl\}-2-methylpropanamide \(53\) Fig. (25), were claimed in US0312932 \(2011\) \([71]\) and US8048899 \(2012\) \([72]\), and in US0071529 \(2012\) \([73]\) and WO109324 \(2011\) \([74]\), respectively. The triazole \(52\) and the tetrazole \(53\) showed good CB\(_2\) receptors affinity and selectivity over CB\(_1\), demonstrating a low activation of the CB\(_1\) receptors (EC\(_{50}\) > 20,000 and 50,000 nM, respectively), as congeners cited in patents. Authors state that these compounds have good oral bioavailability, low clearance \textit{in vitro} human liver microsomes and are useful for treatment of several diseases as acute, visceral, neuropathic, inflammatory/nociceptive and cancer pain, but no data were provided concerning specific structures and activities.

### 4. CARA THERAPEUTICS

Twelve patents covering tetrahydroimidazoazepine derivatives \([75-78]\), heterocyclic-pyrazole-fused compounds \([79-82]\), arylureido-butyramides \([83, 84]\), benzofused heterocycles \([85]\) and pyridoxazines \([86]\) were issued by Cara therapeutics. For these last derivatives detailed biological data were not reported in the patents.

#### 4.1. Tetrahydroimidazoazepine Derivatives

In 2010 Cara Therapeutics claimed \([75] 6,7,8,9\)-tetrahydroimidazo[1,5-\(a\)]azepine derivatives typified by compounds \(54-57\) Fig. (26).

The compounds were tested \textit{in vitro} to assess their affinity to cannabinoid receptors. All four compounds \(54-57\) were found to act as CB\(_2\) receptor agonists, with EC\(_{50}\)CB\(_2\) values between 0.1 and less than 10 nM and EC\(_{50}\)CB\(_1\) values between more than 100 nM and 100 \(\mu\)M values, thus possessing good CB\(_2\) selectivity (data for individual compounds were not reported in the patent).

The \textit{in vivo} evaluation of their utility in the treatment of pain was examined by: the anti-hyperalgesic effects of the compounds in a CFA model of inflammatory pain, measuring the mechanical hyperalgesia using the Randall-Selitto paw pressure device model in rats; the inhibition of acetic acid-induced writhing in mice for the determination of analgesic activity against visceral pain or pain associated with activation of low pH-sensitive nociceptors; and the anti-hyperalgesic effects of the compounds in a CFA model of pain.
acute inflammation, measuring the thermal hyperalgesia determining the paw withdrawal latencies (PWLs) in response to a noxious thermal stimulus.

4.2. Tetrahydroimidazo-Oxazepine and –Diazepine Derivatives

Compound (S)-3-(3,4-difluorophenyl)-N-(3,3-dimethyl-1-(methylamino)-1-oxobutan-2-yl)-5,6,7,9-tetrahydroimidazo[5,1-c][1,4]oxazepine-1-carboxamide (58) Fig. (27), with EC_{50}CB2 and EC_{50}CB1 values between less than 10 nM and 100 nM, respectively, produced a significant reversal of CFA-induced thermal hyperalgesia following oral administration (3 mg/Kg) and a significant increase in PWLs which persisted for at least four hours post-dosing [76].

Compound (S)-N-(3,3-dimethyl-1-(methylamino)-1-oxo-butan-2-yl)-8-methyl-3-phenyl-6,7,8,9-tetrahydro-5H-imidazo[1,5-a][1,4]diazepine-1-carboxamide (59) and its 3-(4-chloro-2-fluorophenyl) analogue (60) Fig. (28) were also shown to be CB2 receptor agonists, with EC_{50}CB2 values in the nanomolar range [77, 78].

The in vivo tests on these compounds demonstrated a better profile for derivative 60. In the chronic neuropathic pain rat model (SNL), compound 60 resulted in a 50% attenuation of mechanical hypersensitivity after an oral dose of 3 mg/Kg, whilst the same attenuation was recorded for analogue 59 with a dose of 10 mg/Kg. A similar trend was observed in the analgesic activity assay against visceral pain, the inhibition of acetic acid-induced writhing in mice, and in the carrageenan model of acute inflammation in rats, in which a 10 mg/Kg oral dose of 60 resulted in a response similar to that of a 30 mg/Kg dose of 59.

4.3. Bicyclic Pyrazolo-Heterocycles

The 4,5,6,7-tetrahydro-1H-pyrazolo[4,3-b]pyridines 61 and 62, 4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine 63, 1,4,6,7-tetrahydropyrano[4,3-c]pyrazole 64 and 1,4,5,7-tetrahydropyrano[4,3-c]pyrazole 65, have been claimed in recent patents as useful in modulating cannabinoid receptors and in the treatment of pain and inflammation [79-82].

Patents US7741350 (2010) [79] and US0144121 (2011) [80] report the syntheses and biological data of (S)-N-[3,3-dimethyl-1-(methylamino)-1-oxobutan-2-yl]-5-methyl-3-(2,4,5-trifluorophenyl)-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-c]pyridine-1-carboxamide (62) and (S)-3-(3,4-difluorophenyl)-N-[1-(2-hydroxyethylamino)-3,3-dimethyl-1-oxobutan-2-yl]-4-methyl-4,5,6,7-tetrahydro-1H-pyrazolo[4,3-b]pyridine-1-carboxamide (63), which were claimed as CB2 receptors agonists having EC_{50}hCB2 values less than 1 μM Fig. (29).

Both derivatives were evaluated in anti-hyperalgesia assay, in anti-inflammatory pain model and in the test of inhibition of acetic acid-induced writhing test in mice. Compound 62, administered at a dose of 10 mg/Kg, gave 100% reversal of the mechanical hyperalgesia induced by the acetic acid treatment, whereas carboxamide 63 gave a
47% of maximum permissible effect (MPE) in paw withdrawal latency after complete Freund’s adjuvant model of inflammation pain.

The pyrano-homologs (S)-3-(5-chloro-2-fluorophenyl)-N-[1-(2-hydroxyethylamino)-3,3-dimethyl-1-oxobutan-2-yl]-4,5-dihydropyrano[3,4-c]pyrazole-1(7H)-carboxamide \((64)\) and (S)-1-(3,4-difluorophenyl)-N-[1-(2-hydroxyethylamino)-3,3-dimethyl-1-oxobutan-2-yl]-1,4,5,7-tetrahydroprano[3,4-c]pyrazole-3-carboxamide \((65)\) were reported in patents WO088050 \((2010)\) [81] and US0275609 [82] Fig. (30).

As was the case for the aforementioned bicyclic pyrazolo-pyridines, the inventors did not report detailed binding data for these compounds, but merely stated that had values of EC\(_{50}\) hCB\(_2\) less than 1 \(\mu\)M and a comparable percentage of maximum possible effect in the anti-hyperalgesia test \((64\) MPE = 52%, \(65\) MPE = 60\%). From these data it can be argued that the aryl groups and the diamidic chain are important structural features for the activity of such compounds, also their reversal position on pyrazole ring is well tolerated.

### 4.4. Arylureido-Butyramides

4-[1-(3-Cyclohexylpropyl)-3-phenylureido]butanamide \((66)\) Fig. (31) represents the best CB\(_2\) agonist ligand \((K_{CB2} = 0.11 \, \text{nM})\) claimed by Cara in the class of arylureido-butyramides [83, 84]. Compound \(66\) resulted in a 90\% anti-allodynic effect upon administration into the paw. Furthermore, \(66\) resulted in a 100\% anti-hyperalgesic response to the thermal testing in L5/L6 spinal nerve ligation after the same administration.

### 4.5. Other Structures

On June 2011 two patents by Cara Therapeutics, US7960376 \((2011)\) [85] and US7960377 \((2011)\) [86], have been granted. The former covers benzofused heterocycles as \(N\)-cyclopentyl-4-[(4-fluorophenyl)sulfonyl]-3,4-dihyro-2H-benzo[\(b\)]1,4-thiazine-6-carboxamide \((67)\), whereas the latter deals with substituted pyridoxazines typified by \(N\)-(tert-butyl)-1-(4-fluorobenzyl)-2-oxo-2,3-dihydro-1H-pyrido[2,3-b][1,4]oxazine-7-carboxamide \((68)\) Fig. (32).
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Although in these patents not detailed binding values have been reported, these compounds are claimed as CB2 receptors agonists. Moreover, only for derivative 67, have been described the results of the complete Freund’s adjuvant model of inflammatory pain: from the data reported in the patent, the local administration (1 mg/paw) of compound 67 didn’t significantly alter the withdrawal thresholds of the contralateral paw [85].

5. ADOLOR CORPORATION

Urea derivatives [87], sulfamoyl-benzamides [88, 89] and morpholinomethyl-aryl amides [90, 91] have been published by Adolor Corporation and an outline of these classes of compound is given below.

5.1. Biphenylmethyl Ethyl Ureas

The compounds, claimed by Adolor under the generic term of phenyl derivatives in US0168108 (2010) [87], displayed agonistic activity to both CB1 and CB2 receptors, and various degrees of selectivity. The limited information concerning in vitro data suggests that only 1-[(4'-1-butoxy-2-methylpropan-2-yl)-2'-hydroxybiphenyl-3-yl]methyl)-3-ethylurea (69) and 1-[(4'-1-butoxy-2-methylpropan-2-yl)-2'-hydroxybiphenyl-2-yl]methyl)-3-ethylurea (70) possess a modest selectivity for CB2 receptors, the former having a $K_i = 0.40$ nM and a 4-fold selectivity for CB2 ($K_i=1.6$ nM), the latter having a $K_i=0.56$ nM and a 2.85-fold selectivity for CB2 ($K_i=1.56$ nM) Fig. (33).

The behavioral assays to evaluate the activity of these compounds against neuropathic pain were performed above all on CB1 agonists.

5.2. Sulfamoyl-Benzamides

Adolor identified compounds with the sulfamoyl-benzamide structure which had a good affinity and selectivity for CB2 receptors [88, 89]. $(JS,2S,4R)-1,3,3$-trimethyl-$N$-(4-methyl-3-(morpholinosulfonyl)phenyl)bicyclo[2.2.1]heptane-2-carboxamide (71) showed a good CB2 receptor affinity ($K_i=1.7$ nM) and a good selectivity (CB2/CB1 = 310) with a value of $EC_{50}=9.6$ nM from the GTP assay Fig. (34).

SAR studies based around the scaffold of 71 led to its tetramethylecyclopropane analogue 72 (EC50/CB2 = 11 nM) Fig. (34) which exhibited a robust antiallodynic activity in the hind-paw incision model, though only when administered i.p. and not following oral administration. This was due to its rapid metabolism as confirmed by studies on human and rat liver microsomes (RLM = 2%; HLM = 1%).

To improve the metabolic stability of 72, whilst retaining affinity and selectivity for CB2 receptors, at first a second methyl group was introduced in the phenyl ring, with the aim of blocking the postulated metabolic pathway, and then a dimethyl butanoyl group was introduced into the amide portion. These modifications led to N-[3,4-dimethyl-5-(morpholinosulfonyl)phenyl]-2,2-dimethylbutanamide (73) Fig. (34), which displayed a much improved metabolic stability profile and interesting pharmacokinetic parameters both after i.v. and per os administration. Compound 73 exhibited a high systemic clearance (i.v.: 4.2 L/h/Kg) and a half-life of 0.7 hour. Moreover, the in vivo efficacy of compound 73 was demonstrated in a rat model of postsurgical pain.

5.3. Morpholinomethyl-Aryl-Amides

SAR studies on 73, directed at the replacement of the sulfonamide moiety with a methylene amino group, led to N-(3,4-dimethyl-5-(morpholinomethyl)phenyl)-3,3,3-trifluoro-2-methyl-2-(trifluoromethyl)propanamide (74) Fig. (35), which still retained a high affinity for CB2 receptors ($K_i=2.7$ nM), a good selectivity towards CB1...
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Fig. (34). (1S,2S,4R)-1,3,3-Trimethyl-N-(4-methyl-3-(morpholinosulfonyl)phenyl)bicyclo[2.2.1]heptane-2-carboxamide (71) and sulfamoylbenzamidesimplified analogs.

Fig. (35). N-(3,4-Dimethyl-5-(morpholinomethyl)phenyl)-3,3,3-trifluoro-2-methyl-2-(trifluoromethyl)propanamide (74) and 2,2-dimethyl-N-(5-methyl-4-(morpholinomethyl)pyridin-2-yl)butanamide (75).

Moreover, compound 74 showed a good metabolic stability, both in human and rat liver microsomes. Therefore, given its good profile, compound 74 was evaluated in vivo in the hind paw incision model of postoperative pain. It was found that, following i.p. administration, a dose of 10 mg/Kg produced an antiallodynic effect comparable to that produced by morphine (3mg/Kg i.p.) with an enhanced duration of action.

Further optimization of 73 led to 2,2-dimethyl-N-(5-methyl-4-(morpholinomethyl)pyridin-2-yl)butanamide (75) a potent ($K_{iCB2} = 4$ nM) and selective CB2 agonist ($CB1/CB2 = 158$), which exhibited in vivo efficacy after oral administration in the L5 spinal nerve ligation rat model [91].

6. MERCK

Over the period reviewed in this article, Merck’s CB2 agonist patents concerned three different classes of compounds: firstly, the pyridine-containing derivatives [92, 93], secondly, a class encompassing imidazopyridine compounds [94-96], and, thirdly, a class based on decahydroquinoline structures [97].

6.1. Pyridine Derivatives

The pyridine derivatives, described in two patents [92, 93], were claimed to be selective CB2 agonists. The compounds 5-(2-butoxypyridin-3-yl)-3-(2,3-dichlorophenyl)-1,2,4-oxadiazole (76) and its 2,6-dichlorophenyl-isomer 77, shown in Fig. (36), had high CB2 affinity ($IC_{50CB2} = 3$ nM and 4 nM, respectively) and good CB2 selectivity ($CB1/CB2 = 103$ and $28.5$, respectively), whilst 3-[3-(3-fluorophenyl)-1H-pyrazol-5-yl]-2-[4-(methylsulfonyl)piperidin-1-yl]pyridine (78) showed a higher CB2 selectivity ($CB1/CB2 = 128.7$) as measured by the HTRF assay for the determination of intracellular cAMP level.

The efficacy of these compounds against inflammatory pain was determined by intradermal injection (100 μL, 1
mg/mL) of complete Freund’s adjuvant and testing mechanical hind paw withdrawal thresholds after dosing the compounds at 20 mg/Kg per os. These compounds were also evaluated for their efficacy against chronic osteoarthritic pain in a rat iodoacetate model, however, the data provided on these assays were very limited.

6.2. Imidazopyridine Derivatives

Another class of compounds claimed by Merck [94, 95] consists of the imidazopyridine derivatives, such as 1-[(4,4-difluoropiperidin-1-yl)methyl]-3-[3-(trifluoromethyl)phenyl] imidazo[1,5-α]pyridine (79) and its pyrazole-analog 80. These compounds were also evaluated in vitro (IC₅₀:CB₂ = 17 nM and 22 nM, respectively) and in vivo, but, as in the case for derivatives 76-78, the data provided were very limited.

Fig. (37). 1-[(4,4-Difluoropiperidin-1-yl)methyl]-3-[3-(trifluoromethyl)phenyl]imidazo[1,5-α]pyridine (79) and its pyrazole-analog 80.

6.3. Decahydroquinoline Derivatives

A class of decahydroquinolines is claimed in patent US99673 (2010) [97]. A selected compound is (cis)-1-[4-(2-hydroxypropan-2-yl)pyridin-2-yl]-4-phenyldecahydroquinolin-4-ol (82). This compound had a CB₂ value of 1.3 nM and a CB₁/CB₂ selectivity of 8077-fold (HTRF assay).

Fig. (39). (cis)-1-[4-(2-Hydroxypropan-2-yl)pyridin-2-yl]-4-phenyldecahydroquinolin-4-ol.

7. UNIVERSITY OF TENNESSEE

Bicyclic furanes [98-101] and tri-aryl/heteroaromatic systems [102, 103], showing activity as selective agonists on CB₂ receptors, have been claimed in five patents by a research group from the University of Tennessee.

7.1. Furo-Pyrimidines and –Pyridines

In 2011 Moore et al. applied for four patents [98-101] on furo-pyrimidines and –pyridines, of which the most interesting compounds, in terms of their cannabinoidergic profile, were 2-(2-methyloctan-2-yl)-5-metatolylfuro[2,3-d]pyrimidin-4-ol (83) and 3-(3,5-dichlorophenyl)-6-(2-methyloctan-2-yl)furo[2,3-b]pyridin-4-ol (84), shown in Fig. (40).

Fig. (40). 2-(2-Methyloctan-2-yl)-5-metatolylfuro[2,3-d]pyrimidin-4-ol (83) and 3-(3,5-dichlorophenyl)-6-(2-methyloctan-2-yl)furo[2,3-b]pyridin-4-ol (84).
For compound 83 cannabinoid receptors binding data are not available, but the two patents on furopyrimidine derivatives [98, 99] reported the affinity and selectivity of its 5-phenyl and 5-thienyl derivatives (EC50CB1 = 1.71 μM, EC50CB2 = 679 nM, selectivity CB1/CB2 = 2.52, and EC50CB1 = 2.36 μM, EC50CB2 = 808 nM, selectivity CB1/CB2 = 2.92, respectively). In contrast, binding data for derivative 84 are known. This compound exhibited micromolar affinity for the CB2 receptor and good selectivity (IC50CB2 = 1.15 μM, IC50CB1 = 31.9 μM, selectivity CB1/CB2 = 33) [100, 101]. These compounds showed anti-inflammatory activity that was determined by cytokine or chemokine release profiles in various stimulated cells.

7.2. Try-Aryl/Heteroaromatic Derivatives

Compound (3',5'-dichloro-2,6-dimethoxybiphenyl-4-yl)(thiophen-2-yl)methanol (85), shown in Fig. (41), was claimed as a selective CB2 agonist having a KiCB2 = 0.27 nM and a CB1/CB2 ratio of 3700 (KiCB1 > 1000 nM) [102, 103].

The anti-inflammatory activity of 85 was assessed both in vitro and in vivo. The in vitro anti-inflammatory activity was evaluated in the A549 cell line by determining the reduction in the inflammatory effect of TNF-α. Following treatment with TNF-α and compound 85 (4 μM), the production of inflammatory mediators IL-6 and CXCL-8, by cells previously transfected with plasmid pNF-kB, was found to be reduced by 90 and 80%, respectively.

The in vivo anti-inflammatory activity was assessed using a murine acute lung injury model, but no data were made available.

8. BRISTOL-MYERS SQUIBB

In 2009 Bristol-Meyers Squibb patented a class of compounds with an indole-indane carboxamide skeleton whose representative compounds are (R)-N-(6-cyano-2,2-dimethyl-2,3-dihydro-1H-inden-1-yl)-5-fluoro-1H-indole-2-carboxamide (86) and its 6-chloroindole analogue (87).

Among these compounds, derivative 86 displayed a 3-fold greater activity for CB2 receptors than its analogue 87, having a KCB2 value of 0.24 nM, as compared with a value of 0.71 nM for the latter. Although claimed to be an useful compound in treating pain, inflammation and other diseases, no in vivo data were reported.

9. ASTELLAS PHARMA

Compound 4-[[2-(1R,2S,4S)-bicyclo[2.2.1]heptan-2-ylamino)-4-(trifluoromethyl)pyrimidin-5-yl]carbonyl]thiomorpholine 1,1-dioxide (88) Fig. (43) was the most potent CB2 receptor agonist in the series of compounds claimed by Astellas Pharma Inc. [106], showing a 99% inhibitory rate of intracellular cAMP increase.

Furthermore, the in vivo activity of 88 was assayed measuring the inhibitory effect on hind-paw weight distribution in adjuvant-induced arthritis in rats. Calculations showed that compound 88 inhibited weight distribution by 40% after an oral dose of 0.1 mg/kg.

10. GLENMARK PHARMACEUTICALS

The hCB2-CHO cell line assay was employed for testing the in vitro CB2 receptor binding affinity of N-(3)-(tert-butyl)-1-(2,4-difluorophenyl)-4,5,6,7-tetrahydro-1H-4,7-methano-indazole-3-carboxamide (89) Fig. (44) [107, 108].
Compound 89 showed hCB2 agonist activity, with an IC_{50} value of 0.61 nM (vs IC_{50} hCB1 = 857 nM) and a 17.6\% (10\mu M) hCB2 antagonism (hCB1 antagonism = 0\% at 10 mM).

In these patents no in vivo data for compound 89 were reported.

11. HOFFMANN-LA ROCHE

The cannabinoid CB2 receptor agonistic activities of compounds (S)-6-(cyclopropylmethoxy)-N-(1-hydroxy-4-methylpentan-2-yl)-5-(pyrrolidin-1-yl)pyrazine-2-carboxamide (90) and its 5-cyclopropylmethoxy-6-piperidin-1-yl-analogue 91 Fig. (45) were determined both by measuring the decrease in intracellular cAMP level and by a GTP\gamma S binding assay. These compounds showed good affinities for the hCB2 receptor, with compound 90 having a K_{i} hCB2 = 44 nM and its piperidinyl isomers 91 a value of K_{i} hCB2 = 43 nM, and exhibited a 10-fold selectivity against CB1 receptors [109].

The in vivo activity of compounds was assessed by their ability to influence inflammation parameters in a model of acute peritonitis in mice. Some compounds claimed in the patent have been shown to act as CB2 selective agonists due to inhibition of the migration of macrophages, produced by inflammatory cytokines, and increased production of anti-inflammatory IL-10.

12. IPPSEN PHARMA

Novel 2-alkyl-4-aryl-thiazoles and oxazoles were claimed in patent US0059970 (2011) [110]. From the limited data available it is possible to identify compounds 92-97 as those derivatives having the best CB2 affinity with K_{i} CB2 \leq 100 nM Fig. (46), however, no in vitro data on selectivity against CB1 receptors, or in vivo data on their activity, were reported.

Fig. (44). N-(3)-(tert-Butyl)-1-(2,4-difluorophenyl)-4,5,6,7-tetrahydro-1H-4,7-methano-indazole-3-carboxamide.

Fig. (45). Pyrazine-2-carboxamide derivatives.

Fig. (46). 2-Alkyl-4-aryl-thiazoles and -oxazole derivatives.
13. IRONWOOD PHARMACEUTICALS

From partial data on hCB binding and functional assays, compound N-{3-[3-(4-methoxybenzoyl)-5-methyl-6H-thieno[2,3-b]pyrrol-6-yl]propyl}methanesulfonamide (98) Fig. (47) displayed a good hCB₂ receptor affinity [111].

![Fig. (47). N-{3-[3-(4-methoxybenzoyl)-5-methyl-6H-thieno[2,3-b]pyrrol-6-yl]propyl}methanesulfonamide.](image)

Compound 98 was also tested in vivo, both to determine its analgesic activity, by the phenylbenzoquinone (PBQ) induced writhing model, and to measure its anti-hyperalgesic activity (CFA). From these studies derivative 98 was found to have a good analgesic activity following an oral dose of 30 mg, and the best anti-hyperalgesic activity of the thieno-pyrrole derivatives following an oral dose of 10 mg/kg.

(Hetero)aryl thieno-pyrrole ketones have been also claimed in two PCT filed on 2011 by Ironwood [112, 113]. In WO100324 [112] are described compounds related to (2,3-dichlorophenyl)[5-methyl-6-(2-morpholinoethyl)-6H-thieno[2,3-b]pyrrol-4-yl]methanone (99), which displayed a good hCB₂ receptor affinity with an IC₅₀ less than 0.1 μM (IC₅₀ hCB₂ = 1-10 μM) Fig. (48). In GTPᵦS functional assays, derivative 99 showed hCB₂ receptor agonist activity with an EC₅₀ less than 0.1 μM, whereas the EC₅₀/CB₁ value less than 30% was not significant when compared to the positive control. Moreover, compound 99 have been tested also in vivo by phenylbenzoquinone (PBQ) induced writhing model, predictive for analgesic activity: an i.p. administered dosage of 30 mg/Kg produced a number of writhes of 0.1 ± 0.1 (vs 15.6 ± 2.6 control), therefore compound 99 act as analgesic.

![Fig. (48). (2,3-Dichlorophenyl)[5-methyl-6-(2-morpholinoethyl)-6H-thieno[2,3-b]pyrrol-4-yl]methanone.](image)

The 3-{2-[3-(6-ethyl-6H-thieno[2,3-b]pyrrole-5-carbonyl)-2-methyl-1H-indol-1-yl]ethyl}oxazolidin-2-one (100) and 6-ethyl-6H-thieno[2,3-b]pyrrol-5-yl-(1-propyl-1H-indol-3-yl)methanone (101) are claimed as hCB₂ receptor agonists, whose binding and GTPᵦS functional assay data are reported in Fig. (49) [113].

Patent WO100359 (2011) has also reported the analgesic activity for an oral dose of 30 mg/Kg of derivative 101, which was assayed by PBQ writhes (1.9 ± 0.8 vs 15.6 ± 2.6 control) [113].

14. SCHERING-PLOUGH

Two compounds, N-(6-(1-(2-fluorophenylsulfonyl)-1H-indol-2-yl)-6-azaspiro[2.5]octan-1-yl)methanesulfonamide (102) and 1,1,1-trifluoro-N-(6-(1-(pyridin-2-ylsulfonyl)-1H-indol-2-yl)-6-azaspiro[2.5]octan-1-yl)methanesulfonamide (103) Fig. (50), were found to exhibit potent affinities for the CB₂ receptors, with Kᵢ values of 0.24 and 0.40 nM, respectively [114].

In addition to their high CB₂ affinity, such compounds are also highly selective in modulating the CB₂ receptors, having a 15000-fold CB₁/CB₂ ratio for 102, and 3500-fold for 103. Compounds with similar structures to 102 and 103 were claimed to exhibit anti-inflammatory activity, but no data were provided.

15. THE UNIVERSITY OF TEXAS SYSTEM

Compound 104, (3-benzyl-3-methyl-2,3-dihydrobenzofuran-6-yl)(piperidin-1-yl)methanone Fig. (51), was claimed in patent WO012221 (2009) [115] by the University of Texas System.

The in vitro characterization of 104 was based on radioligand competition binding assays, GTPᵦS functional and cAMP activation assays. In the binding assays, compound 104 displayed a value of Kᵢ,CB₂ of 422 nM and did not demonstrate affinity for CB₁ receptors. Also in GTPᵦS functional assays there was an EC₅₀ = 128 nM at hCB₂, but no activity at hCB₁, whilst for rCB₂, the EC₅₀ value was 21.7 nM, but again no activity was shown at rCB₁ receptors.
In vivo studies were also conducted for compound 104. Neuropathic pain was induced by segmental nerve ligation and the mechanical allodynia in rats was assessed measuring the paw withdrawal threshold (PWTs). An i.p. administered dosage of 30 mg/Kg of 104 produced an increase in PWTs, the high threshold being maintained for the whole period of observation (2 hours). A similar trend was observed after intrathecal administration at a dosage of 100 μg with an increase in PWTs lasting for 1.5 hours. Data regarding the reversal of allodynia showed that the tested compound 104 exhibited a trend comparable to that of morphine. Therefore, this long-acting compound 104 appears to be an interesting analgesic in the neuropathic pain animal model when i.p. administered.

16. KYOWA HAKKO KIRIN AND ACADIA PHARMACEUTICALS

Two patents by Kyowa Hakko Kirin [116, 117] claim imidazole derivative as 5-{2-(tert-butoxy)-1-[(tetrahydro-2H-pyranyl-4-yl)methyl]-1H-imidazol-4-yl)-2-chloro-N,N-diethylbenzamide (105) and 6-{2-(tert-butoxy)-1-[(tetrahydro-2H-pyranyl-4-yl)methyl]-1H-imidazol-4-yl)-N,N-dipropylpyrazine-2-carboxamide (106), whereas a third patent [118] describes the synthesis and biology of (heteroaryl)benzimidazoles whose main derivative is 2-(tert-butyl)-N,N-diethyl-3-[(tetrahydro-2H-pyranyl-4-yl)methyl] imidazo[1,2-c]pyrimidine-7-carboxamide (107). All these compound showed a Ki,CB2 value less than 1 μM in a binding assay using the radiolabelled [3H]-CP55,940. Also in the GTPγS functional assay compounds 105-107 showed a human ECsoCB2 value less than 1 μM and a percentage of the maximum effect (Emax) more than 30%. These derivatives claimed as CB2 receptor agonists useful as therapeutic agents for pains, migraine, inflammation and osteoarthritis, significantly increase the pain threshold at a dose of 50 mg/Kg or less, in rats with chronic constriction nerve injury.

In the patent application US0206607 (2011) [119] by Acadia Pharmaceuticals were disclosed deaza-analogs of 107, as the N-(benzo[a][1,3]dioxol-5-yl)-2-[4-(fluoromethoxy)phenyl]-5-methoxyimidazo[1,2-a]pyridin-3-amine (108) Fig. (53), with a Ki,CB2 value of 0.36 nM, for the...
treatment of different pains, but not examples or specific data have been reported for these compounds.

17. RAQUALIA PHARMA AND JANSSEN PHARMACEUTICA

Benzimidazole derivatives are described in two different patents by Raqualia Pharma [120] and Janssen Pharmaceutica researchers [121] and are claimed also for treatment of pain and osteoarthritis. Compounds of general structure 109 Fig. (54) are claimed by Raqualia Pharma as CB2 receptor agonist, but the patent lacks both of CB2 receptors binding values, and detailed EC50 values, simply stating that all compounds claimed showed CB2 receptor agonist activity with EC50 less than 0.03 μM and with excellent selectivity against CB1 (EC50 > 25 μM).

Janssen Pharmaceutica [121] claimed the class of compounds typified by 1-{4-[(2-tert-butyl)-5-[(4,4,4-trifluorobutyl)sulfonyl]-1H-benzo[d]imidazol-1-yl]methyl] piperidin-1-yl}-2-methoxyethanone (110) Fig. (54) which possesses a human EC50CB2 of 1.5 μM observed in the cAMP level inhibition assay. As for derivative 109, also for compound 110 not specific data about its analgesic activity were reported in the patent.

18. PFIZER

Benzimidazolones as 111 Fig. (55) were reported in US8138177 (2012) [122] by Pfizer: authors state that all compounds of examples reported in the patent showed CB2 receptor affinity. Although these compounds are claimed for treatment of several different kinds of pain, no specific data are reported.

19. ORGANON

Bi(hetero)aryl imidazolidin-2,4-diones such as 3-(cyclopropylmethyl)-1-[[2'(morpholinomethyl)-[1,1'-biphenyl]-4-yl]methyl]imidazolidin-2,4-dione (112) and 3-isobutyl-1-[4-[6-(piperidin-1-ylmethyl)pyridin-2-yl]benzyl]imidazolidin-2,4-dione (113) Fig. (56) have been patented by Organon as CB2 agonists, potentially useful for the treatment of different kind of pain [123, 124]. These derivatives tested in the agonist-induced cAMP assay in human CB2 and CB1 receptor transfected CHO cells, showed an EC50CB2 less than 0.4 μM and an EC50CB1 less than 1 μM. Moreover, in the rat model of neuropathic pain, induced by tight ligation of the left L5 spinal nerve, an oral dose of compounds 112 (4.5 μmol/Kg) and 113 (4.4 μmol/Kg) attenuated mechanical allodynia in a dose-dependent fashion at 120 min after drug administration. Same compounds reversed mechanical hyperalgesia in the complete Freund’s adjuvant-induced pain model for inflammatory pain with a minimum effective dose of 4.5 μmol/Kg (112) and 4.4 μmol/Kg (113) p.o.

20. ARENA

Arena patents concern CB2 modulators possessing a 4,4a,5,5a-tetrahydro-1H-cyclopenta[4,5]cyclopenta[1,2-c]pyrazole-3-carboxamide scaffold [125-127]. Derivatives (4aS,5aS)-1-(pyrazin-3-yl-1-oxide)-N-[(R)-1-hydroxy-3,3-dimethylbutan-2-yl]-4,4a,5,5a-tetrahydro-1H-cyclopropa[4,5]cyclopenta[1,2-c]pyrazole-3-carboxamide (114), its N-[(1-(trifluoromethyl)cyclobutyl) analogous (115) Fig. (57) were claimed as compounds for the treatment of pain and exhibiting therapeutic efficacy in the monoiodoacetate (MIA) model of osteoarthritis. Each compound of the invention has been assayed in the homogeneous time-resolved fluorescence (HTRF) assay for direct cAMP measurement, in the PathHunter β-arrestin assay and in the commonly used radioligand binding assay, and the functional activity data at the CB2 receptors of compounds 114 (EC50 hCB2 = 5.4 nM) and 115 (EC50 hCB2 =
1.1 nM) are reported in patents WO116276 (2012) [125] and WO116279 (2012) [126].

Patent application US0214766 (2012) [127] relates to (4aS,5aS)-1-(2,4-difluorophenyl)-N-(1-hydroxy-2-methylpropan-2-yl)-4,4a,5,5a-tetrahydro-1H-cyclopenta[4,5]cyclopenta[1,2-c]pyrazole-3-carboxamide (116) Fig. (57), claimed as CB2 agonist with human EC50CB2 ranging from 0.72 nM and about 3 μM, which exhibited therapeutic efficacy in the monoiodoacetate (MIA), in the skin incision and in the complete Freund's adjuvant models. An oral dose of 30 mg/Kg of 116 showed efficacy in the MIA-induced osteoarthritis pain model with a comparable efficacy to morphine (3 mg/Kg subcutaneously) when measuring the paw withdrawal threshold (PWT). The pyrazole carboxamide 116 exhibited therapeutic efficacy in the CFA-induced hyperalgesia model of inflammatory pain at 1 h post-dosing at 1, 3, 10 and 30 mg/Kg p.o. doses. Furthermore, 116 demonstrated efficacy in models of neuropathic pain (10 mg/Kg p.o.).

21. ALLERGAN

Allergan Inc. claims pyridazine derivatives as CB2 agonists useful in the reduction of symptoms related to pain and inflammation in the international patent application WO097553 (2011) [128]; the 4-chloro-5-[[2-(dimethylamino)ethyl]methylamino]-2-(3,3,5,5-tetramethylcyclohexyl) pyridazin-3(2H)-one (117) Fig. (58) showed affinity and selectivity for CB2 receptors with an IC50 value of 20 nM (IC50CB1 = 2630 nM; CB2 selectivity ratio, CB1/CB2 = 132), and an EC50 value of 330 nM in mouse isolated vas deferens (MVD) bioassay.

22. ELY LILLY

In WO123482 (2011) [129] aryl-piperazinyl-purine compounds acting as CB2 receptors agonists have been reported by Ely Lilly. Notably, the 8-(2-chlorophenyl)-6-(4-ethylpiperazin-1-yl)-2-methyl-9-[2-(methylsulfonyl)ethyl]-
Further, even if the mechanism and site of action involved in CB2-mediated analgesia remain unexplained, the therapeutic utility of selective CB2 agonists as useful analgesic agents for different kind of pain, will be determined through their evaluation in human clinical trials as this represents essential information required for a definitive answer regarding their efficacy in the management of pain.

CONFLICT OF INTEREST
None of the authors have a conflict of interest to declare.

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Fig. (59). 8-(2-Chlorophenyl)-6-(4-ethylpiperazin-1-yl)-2-methyl-9-[2-(methylsulfonyl)ethyl]-9H-purine hydrochloride (118) and N-(2-[8-(2-chlorophenyl)-6-(4-ethylpiperazin-1-yl)-2-methyl-9H-purin-9-yl]ethyl)acetamide hydrochloride (119).

23. CURRENT & FUTURE DEVELOPMENTS

The large amount of filed and granted patents on CB2 receptor modulators with special reference to the treatment of pain by different companies and research groups from academia, is an indication of the continuous interest to the identification of CB2 receptor agonists as potential analgesic drugs candidates for the pharmaceutical market; however, nowadays no CB2 receptor agonists have been marketed for the treatment of pain.

In this review we have selected and updated recent patents that focus on new developments in the CB2 receptor field, particularly highlighting those aspects which concern their use in the treatment of pain.

From these patents it is possible to identify some compounds which possess high affinity and selectivity for CB2 receptors on CB2 receptors, together with good oral bioavailability and good metabolic stability.

Several selective CB2 receptor agonists exhibited analgesic activity in different preclinical models of acute, inflammatory and neuropathic pain; therefore these compounds could be useful tools to assess the biological consequences of CB2 receptor activation in animals.

9H-purine hydrochloride (118) exhibited an EC50CB2 value of 17.2 nM in the GTPyS binding assay (EC50CB2 = 5560 nM) and a human and rat KCNQ2 of 28.4 and 48.7 nM, respectively, in the binding assay using [1H]-CP-55,940 as radiolabelled probe.

Compounds of this patent are shown to be useful in the treatment of pain, in particular migraine: authors assess that the N-(2-[8-(2-chlorophenyl)-6-(4-ethylpiperazin-1-yl)-2-methyl-9H-purin-9-yl]ethyl)acetamide hydrochloride (119) Fig. (59) significantly reduce pain in an animal model of dural plasma protein extravasation (PPE) with an extravasation ratio of 1.18 at a single oral dose of 10 mg/Kg, measured two hours after administration.
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