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Research paper

Tricyclic pyrazoles. Part 8. Synthesis, biological evaluation and modelling of tricyclic pyrazole carboxamides as potential CB_2 receptor ligands with antagonist/inverse agonist properties

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ABSTRACT

Previous studies have investigated the relevance and structure-activity relationships (SARs) of pyrazole derivatives in relation with cannabinoid receptors, and the series of tricyclic 1,4-dihydroindeno[1,2-*c*]pyrazoles emerged as potent CB_2 receptor ligands. In the present study, novel 1,4-dihydroindeno[1,2-*c*]pyrazole and 1*H*-benzo[*g*]indazole carboxamides containing a cyclopropyl or a cyclohexyl substituent were designed and synthesized to evaluate the influence of these structural modifications towards CB_1 and CB_2 receptor affinities. Among these derivatives, compound **15** (6-cyclopropyl-1-(2,4-dichlorophenyl)-*N*-(adamantan-1-yl)-1,4-dihydroindeno[1,2-*c*]pyrazole-3-carboxamide) showed the highest CB_2 receptor affinity (K_i = 4 nM) and remarkable selectivity (K_iCB_1/K_iCB_2 = 2232), whereas a similar affinity, within the nM range, was seen for the fenchyl derivative (compound **10**: K_i = 6 nM), for the bornyl analogue (compound **14**: K_i = 38 nM) and, to a lesser extent, for the aminopiperidine derivative (compound **6**: K_i = 69 nM). Compounds **10** and **14** were also highly selective for the CB_2 receptor (K_iCB_1/K_iCB_2 > 1000), whereas compound **6** was relatively selective (K_iCB_1/K_iCB_2 = 27). The four compounds were also subjected to GTPγS binding analysis showing antagonist/inverse agonist properties (IC_{50} for compound **14** = 27 nM, for **15** = 51 nM, for **10** = 80 nM and for **6** = 294 nM), and this activity was confirmed for the three more active compounds in a CB_2 receptor-specific in vitro bioassay consisting in the quantification of prostaglandin E2 release by LPS-stimulated BV2 cells, in the presence and absence of WIN55,212-2 and/or the investigated compounds. Modelling studies were also conducted

Abbreviations: Δ^9-THC, Δ^9-Tetrahydrocannabinol; 2-AG, 2-Arachidonoylglycerol; CB_1, cannabinoid receptor type 1; CB_2, cannabinoid receptor type 2; CNS, Central nervous system; TRPV1, transient receptor potential vanilloid-1 channel; PPAR, peroxisome proliferator-activated receptor; GTPγS, guanosine 5′-*O*-[gamma-thio]triphosphate; LPS, lipopolysaccharide; DDQ, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; *h*CB_2, human CB_2 receptor; FC, flash chromatography.

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with the four compounds, which conformed with the structural requirements stated for the binding of antagonist compounds to the human CB_2 receptor.

1. Introduction

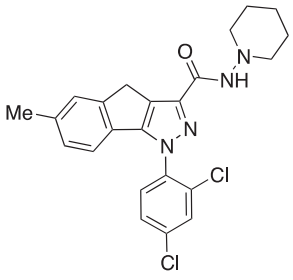
Derivatives of *Cannabis sativa*, commonly known as marijuana and hashish, have been known due to their medical and recreational properties for hundreds of years [1]. Despite the active constituents responsible for these properties in *C. sativa* being identified in the 60 s, in particular Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive component of *Cannabis* (see **1** in Fig. 1), the biochemical and physiological bases underlying the effects of cannabinoids were identified only in the 90 s. These studies derived in the identification of cannabinoid receptors [2,3], endogenous ligands *N*-arachidonoylethanolamine (anandamide) **2** [4] and 2-arachidonoylglycerol (2-AG) **3** [5] (Fig. 1), and the enzymatic machinery for their biosynthesis and hydrolysis [6], as necessary steps leading to the understanding of the mechanisms by which plant-derived cannabinoids affect our mind and body.

To date, two G-protein-coupled seven transmembrane receptors, namely cannabinoid receptor type-1 (CB_1) and cannabinoid receptor type-2 (CB_2), have been identified. CB_1 receptors are mainly expressed in areas of the brain that control movement, motor coordination, sensory perception, learning and memory, reward and emotions, preferentially located on numerous neuronal subpopulations, so they appear to be responsible for most of the central effects of cannabinoids [7]. They are also present outside the central nervous system (CNS) in numerous peripheral tissues [2]. CB_2 receptors are concentrated in cells and tissues of the immune system (e.g. spleen, macrophages, tonsils, B cells and natural killer cells, monocytes, neutrophils and T cells) [3], but they have been recently identified in the brain in healthy conditions (with a more restricted distribution compared to CB_1 receptors) and, in particular, in the damaged brain after different cytotoxic stimuli [8]. Specifically, CB_2 receptors were identified in microglial cells, astrocytes and in certain subpopulations of neurons making this receptor an interesting target for the treatment of neurological diseases [9].

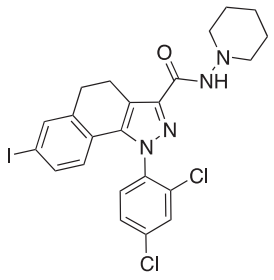
Recent data indicate that endocannabinoid spectrum is more complex that initially thought, being the transient receptor potential vanilloid-1 channels (TRPV1) [10] or the peroxisome proliferator-activated receptors (PPARs) [11] considered new targets for the action of endocannabinoids and related signaling lipids. Particularly, PPARs are a group of nuclear receptor proteins constituted by different isoforms (α, β/δ and γ), which are involved in regulation of cellular differentiation [12], energy metabolism [13] and inflammation [14], so that they may mediate some of the biological effects of endocannabinoids and of some specific plant-derived cannabinoids too.

Given the ubiquitous distribution in the body of endocannabinoids and related lipids, their receptors and the enzymes involved in their metabolism, drugs acting on this modulatory system appear to have therapeutic potential in a number of pathological conditions, including obesity and metabolic syndrome [15], mood and anxiety disorders [16], neuropathic pain [17], multiple sclerosis [18], neurodegenerative disorders [19–21], as well as in atherosclerosis [22], myocardial infarction [23], cancer [24], glaucoma [25] and osteoporosis [26]. Thus, in recent years, investigations were aimed to the design of new synthetic molecules targeting endocannabinoid-related receptors and enzymes that provide advantages over the already existing compounds, mainly plant-derived and endogenous cannabinoids, e.g. selectivity for a specific target, agonist *versus* antagonist/inverse agonist activity, better water solubility, peripherally-restricted action, effects as allosteric modulators, and others [27].

In our previously published studies [28–31], we described the preliminary structure-activity relationships (SARs) of different tricyclic compounds typified by a 1,4-dihydroindeno[1,2-*c*] pyrazole and 4,5-dihydro-1*H*-benzo[*g*]indazole structures endowed with interesting cannabinoid binding profiles. Among these, the 1-(2,4-dichlorophenyl)-6-methyl-*N*-piperidin-1-yl-1,4-dihydroindeno [1,2-*c*]pyrazole-3-carboxamide (compound **4**) showed the best selectivity towards CB_2 receptor compared to CB_1 receptor (K_iCB_1/K_iCB_2 = 9810) [30], whereas the 7-iodo-4,5-dihydro-1*H*-benzo[*g*] indazole analogue (compound **5**) exhibited a moderate CB_1 receptor selectivity (K_iCB_2/K_iCB_1 = 262) [29] (Fig. 2). From these studies, we pointed out that changes in the size and shape of the tricyclic

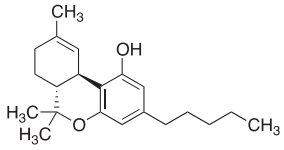


4
K_i CB_1 = 363 ± 30 nM
K_i CB_2 = 0.037 ± 0.003 nM
CB_2 selectivity = 9810

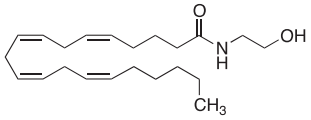


5
K_i CB_1 = 4.11 ± 0.22 nM
K_i CB_2 = 1079 ± 44 nM
CB_1 selectivity = 262

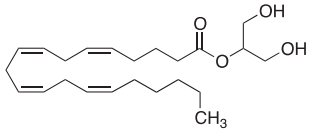
Fig. 2. Tricyclic cannabinoid receptor ligands 4 and 5 (see details in references [28–30]).



1
(Δ^9-THC)
K_i CB_1 = 41 nM
K_i CB_1 = 36 nM



2
(Anandamide)
K_i CB_1 = 89 nM
K_i CB_1 = 371 nM



3
(2-AG)
K_i CB_1 = 472 nM
K_i CB_1 = 1400 nM

Fig. 1. Δ^9-THC and the major endogenous ligands for cannabinoid receptors.