Tricyclic Pyrazole-Based Compounds as Useful Scaffolds for Cannabinoid CB₁/CB₂ Receptor Interaction

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Abstract: Cannabinoids comprise different classes of compounds, which aroused interest in recent years because of their several pharmacological properties. Such properties include analgesic activity, bodyweight reduction, the antiemetic effect, the reduction of intraocular pressure and many others, which appear correlated to the affinity of cannabinoids towards CB₁ and/or CB₂ receptors. Within the search aiming to identify novel chemical scaffolds for cannabinoid receptor interaction, the CB₁ antagonist/inverse agonist pyrazole-based derivative rimonabant has been modified, giving rise to several tricyclic pyrazole-based compounds, most of which endowed of high affinity and selectivity for CB₁ or CB₂ receptors. The aim of this review is to present the synthesis and summarize the SAR study of such tricyclic pyrazole-based compounds, evidencing, for some derivatives, their potential in the treatment of neuropathic pain, obesity or in the management of glaucoma.

Keywords: tricyclic pyrazoles; structure-activity relationship; CB₂ agonist; CB₁ agonist; CB₁ neutral antagonist; anti-nociceptive activity; glaucoma; obesity

1. Introduction

Cannabinoids are a class of different chemical compounds (Figure 1), including the endocannabinoids (produced naturally in the body by humans and animals such as anandamide and 2-arachidonoylglycerol), the phytocannabinoids (derived from Cannabis, exemplified by Δ⁹-tetrahydrocannabinol, the major psychoactive component of Cannabis sativa, commonly known as marijuana), and the synthetic cannabinoids (produced chemically by human, comprising a wide array of chemical entities, i.e., the pyrazole-based compounds SR141716A and SR144528 and the indole based compound WIN-55,212-2) [1–3]. The physiological and behavioral effects of cannabinoids appear directly correlated to their affinity towards two different classes of specific receptors: CB₁ receptors located predominantly in the central nervous system [4], and CB₂ receptors which are mostly found in peripheral tissues [5]. In the brain, the CB₁ receptors are abundantly expressed in the hippocampus, cerebellum and striatum [6,7]. Among the peripheral tissues wherein the CB₁ receptors have been found, the enteric nervous system [8], testis, urinary bladder, vas deferens can be mentioned [9]. CB₁ receptor mRNA and protein have been furthermore identified in the rat and human eye, both in the retina and in the iris, and in the ciliary body [10]. CB₂ receptors are located in the marginal zones of the spleen, tonsils, immune cells [9,11] and to a much lesser extent in CNS [12]. Furthermore, CB₂ receptor mRNA was expressed in the adult rat retina, including the somas of retinal ganglion cells [13].

During the last years, several ligands endowed with high affinities and subtype selectivity for both receptors were identified. Such ligands were proposed as potential therapeutic targets for the treatment of several diseases, including neuropathic pain [14], cancer [15,16] and osteoporosis [17].

Non-selective CB₁/CB₂ receptor agonists are the constituents of some approved medicines, i.e., Sativex®, Cesamet®, Marinol®. Sativex contains approximately equal...
amounts of ∆⁹-tetrahydrocannabinol and the non-psychoactive phytocannabinoid cannabidiol, and is prescribed for neuropathic pain relief in adults with multiple sclerosis and as an adjunctive analgesic treatment for adult patients in advanced cancer [18]. Furthermore, the relevance of CB receptors as an emerging target of pharmacotherapy is documented also by the discovery of mixed CB₁/CB₂ receptor agonists as antiglaucoma agents [19].

The only cannabinoid receptor antagonist approved as a medicine to-date is the CB₁ antagonist/inverse agonist SR141716A (rimonabant, Acomplia®, Figure 1). This compound has been developed for the treatment of obesity and related metabolic risk factors [20]. However, it was soon withdrawn for its serious psychiatric disorders including anxiety, depression and suicidal tendency. Although most pharmaceutical companies were deterred from developing a drug that displayed rimonabant-like CB₁ receptor antago-
mistent/inverse agonist activity for the management of any disorders, this compound still remains an extremely valuable lead for the design of new ligands for CB receptors interaction [21,22]. Within this frame, the 4-alkyl-5-arylpyrazole skeleton of rimonabant has been modified, leading to benzocycloalkylpyrazole-based tricyclic systems of general formula I-IV (Figure 2). Modifications carried out on such scaffolds, allowed to accede to further pyrazole-based tricyclic systems of general formula V-XVII (Figure 2). Fine tuning of these tricyclic systems (vide infra) allowed to identify hundreds of molecules, most of which endowed with high affinity and selectivity for CB1 or CB2 receptors. In this review, pyrazole-based tricyclic compounds I-XVII are divided into four main groups according to the size of the central ring connected to the pyrazole one. If a five-membered ring is connected to the pyrazole, the compound belongs to (5,5)-condensed pyrazole derivatives, and if the connected ring is a six-, seven- or eight-membered ring, the compound belongs to (5,6)-, (5,7)- or (5,8)-condensed pyrazole derivatives. Here, we briefly present the synthesis and the bioactivities of pyrazole-based tricyclic compounds I-XVII investigated by us and other groups. Furthermore, this review surveys chemical and biological literature of some miscellaneous molecules featuring tricyclic pyrazole structure closely related to compounds I-XVII, investigated for cannabinoid receptor affinity.

![Figure 2. Design of cannabinoid CB1/CB2 receptor ligands.](image)

2. (5,5)-Condensed Pyrazole Derivatives

2.1. 1,4-dihydroindeno(1,2-c)pyrazole-Based Derivatives

The first (5,5)-condensed pyrazole derivatives synthesized by us for cannabinoid receptors interaction are those of general formula I (Figure 2) featuring the 1,4-dihydroindeno(1,2-c)pyrazole core. These compounds were designed as rigid analogs of CB1 antagonist/inverse agonist SR141716A (rimonabant), endowed with a high affinity for CB1 receptors and good
selectivity over CB$_2$ receptors. It has been proposed that minimization of the flexibility of the lead compound through the introduction of structural constraints could have some impact on cannabinoid receptor interaction, maybe allowing the ligand to bind with high affinity and selectivity to its receptor. Accordingly, the first series of 1,4-dihydroindeno(1,2-c)pyrazole derivatives have been synthesized and evaluated for their in vitro binding affinities for CB$_1$ and CB$_2$ receptors. Most of these compounds are N-piperidine-carboxamides that differ for R substituent (Cl, F, I, CH$_3$, OCH$_3$), whereas G, quite often, maintained the 2,4-Cl-phenyl substitution pattern of rimonabant [23].

Compounds I were synthesized starting from the appropriate ketones I (Scheme 1) which were α-acylated by Claisen reaction, furnishing 1,3-diketoesters 2 as a tautomeric equilibrium shifted towards the alkenylidene structure (2'). The cyclization with appropriate hydrazines in refluxing EtOH gave the tricyclic dihydroindeno(1,2-c)pyrazole esters 3, which were hydrolyzed with KOH, affording the corresponding acids 4. Target compounds I were synthesized by condensation of acids 4, previously activated to acyl chlorides with SOCl$_2$, with the appropriate amines.

![Scheme 1. General synthetic route for the preparation of compounds I.](image)

If not otherwise stated, the four-step general synthetic route for I compounds, with minor changes, was employed for the preparation of all compounds of general formula II-XVII, starting from the appropriate ketones and using the appropriate hydrazines and amines.

Our first SAR study revealed that several compounds incorporating the planar 1,4-dihydroindeno(1,2-c)pyrazole scaffold displayed very high in vitro binding affinity for CB$_2$ receptors comparable to, or exceeding, that of SR144528 (Figure 1), identified as the first highly potent and selective ligand for the CB$_2$ receptors. Representative derivatives are I$_{AaX}$, I$_{AhX}$, I$_{AfX}$, I$_{BaX}$ (Figure 3), compound I$_{AhX}$ emerging for its high affinity for CB$_2$ receptors and exceptional selectivity over CB$_1$ receptor (CB$_1$/CB$_2$ selectivity ratio of 9811).

Our results prompted us to investigate new 1,4-dihydroindeno(1,2-c)pyrazoles (I), which were obtained modifying the N-carboxamide moiety (Q) and the aryl substitution of the lead compounds I$_{AaX}$ and I$_{AhX}$ [24]. In general, our SAR study showed that the CB$_1$ receptor affinities of all the investigated compounds were lower than their CB$_2$ receptor affinities, evidencing the suitability of 1,4-dihydroindeno(1,2-c)pyrazole architecture to generate ligands for CB$_2$ receptor interaction. Representative compounds of this series are I$_{DhX}$, I$_{EhX}$, I$_{FhX}$, I$_{GhX}$ (Figure 4), all displaying high CB$_2$ affinity and reasonable selectivity over CB$_1$ receptors, even if lower than that elicited by compounds I$_{AaX}$ and I$_{AhX}$. 
Figure 3. Structure of representative 1,4-dihydroindeno(1,2-c)pyrazoles (I). Affinities at CB₁ and CB₂ receptors were assessed by competition of (³H)-CP 55,940 in mouse cerebellum membranes and in mouse spleen homogenates, respectively [23]. Permission has been obtained and there is no copyright issue.

Figure 4. Structure of representative 1,4-dihydroindeno(1,2-c)pyrazoles (I) obtained by modification of carboxamide moiety of compound IAhX. Affinities at CB₁ and CB₂ receptors were assessed by competition of (³H)-CP 55,940 in mouse cerebellum membranes and in mouse spleen homogenates, respectively [24].

In vitro CB₂ intrinsic activity evaluation assay, based on the determination of P-ERK 1/2 increasing expression in human promyelocytic leukemia HL-60 cells exposed to the compounds to be assayed, highlighted agonist activity toward CB₂ receptors for derivatives IDhX, IEhX and for prototype IAaX, IAhX. In particular, the tested compounds significantly increased P-ERK 1/2 expression, reaching the maximum effect at the concentration of 10 nM with the following values: +61.3 ± 12.4% (IDhX), +125.0 ± 35.8% (IEhX), +65.0 ± 18.5% (IAaX), +83.0 ± 4.2% (IAhX) versus vehicle. Overall, the effect of tested compounds as CB₂ agonists was specific as it was blocked by the CB₂ receptor antagonist SR144528 [24].

By pursuing our interest in expanding SAR studies around 1,4-dihydroindeno(1,2-c)pyrazole core, especially in the light of the potential of CB₂ ligands for the treatment of immune disorders or as anti-nociceptive agents [14,25], novel derivatives have been designed, making use of molecular hybridization based on scaffold hopping [26]. In particular, based upon the putative interacting sites and structural features of selective CB₂ antagonist SR144528 (Figure 1), i.e., N₁-benzyl group, the C₃ carboxamide moiety, and the substitution of the C₅ phenyl ring [26,27], it was postulated that the introduction of such pharmacophoric elements in the tricyclic core of compound IAhX might provide new CB₂ ligands with potential therapeutic value. Briefly, different synthesized compounds were monoterpane derivatives incorporating bulky groups in the carboxamide moiety (i.e., fenchyl-, bornyl-, isopinocampheyl-, myrtyl-, menthyl-), bearing a 6-CH₃,7-Cl or 6-Cl,7-CH₃ substitution pattern at the aryl ring of 1,4-dihydroindeno(1,2-c)pyrazole core. Some adamantane derivatives were also investigated, together with compounds incorporating simple cycloalkyl motifs at the carboxamide moiety. Compounds IAjX, ILjX, IHjX, IJjX, IKjX, IDjX, IDKX, ILHX (Figure 5) are representatives of these third series of I compounds. As described above for representative compounds depicted in Figures 3 and 4, our SAR study carried out on a third series of molecules, evidenced for
1,4-dihydroindeno(1,2-c)pyrazole-based molecules preferential affinity for CB2 receptors. However, the introduction of a chlorine atom, as well as its exchange with the methyl group in all-new hybrid compounds seemed to play a modest role in lowering the levels of CB2-affinity as compared to the reference compounds IaAx and IahX.

Figure 5. Structure of representative 1,4-dihydroindeno(1,2-c)pyrazoles (I) designed making use of molecular hybridization based on scaffold hopping. Affinities at CB1 and CB2 receptors were assessed by competition of (3H)-CP 55,940 in mouse cerebellum membranes and in mouse spleen homogenates, respectively [26].

According to previous data obtained for compounds IaAx, IahX, IDhX and IEhX, analogs IIJX, IDjX and IHkX, endowed with the highest affinity for CB2 receptors, exhibited CB2 agonism activity in in vitro model based on the determination of P-ERK 1/2 increasing expression in HL-60 cells, with maximum values reached at 125 nM for compounds IIJX (+54.5 ± 12.1%) and IDjX (+82.5 ± 19.1%), and at 50 nM for compound IHkX (+46.4 ± 15.6%) versus vehicle. Furthermore, the effect of such tested compounds on P-ERK 1/2 expression was counteracted by the reference CB2 antagonist AM630, suggesting the correspondence between the detected effect and CB2 modulation.

The obtained data confirmed that the flattening of 1,4-dihydroindeno(1,2-c)pyrazole core is important to assure agonist rather than antagonist activity, with respect to not condensed and more flexible analogs such as the antagonist SR144528.

Within this frame, a series of compounds incorporating the 1,4-dihydroindeno(1,2-c)pyrazole scaffold (I), sharing the N1-benzyl group and bulky groups in the carboxamide moiety (i.e., IOkW, Figure 2) were claimed by Sanofi-Aventis, but no specific biological activity was presented [28]. However, the compounds of the invention were generally described as potent and selective CB2 receptor antagonists with Ki values < 5 × 10−7 M. Antagonistic properties of such compounds have been demonstrated by the results in the models for inhibition of adenylate cyclase induced by forskolin, although no specific examples or data were disclosed.

Furthermore, 1,4-dihydroindeno(1,2-c)pyrazoles featuring a cyclopropyl or cyclohexyl building block in C6 position were investigated by us, in order to evaluate the effect of cycloalkyl moiety in place of methyl group both on cannabinoid receptor binding and activity [29]. The most interesting compounds coming from this fourth series are depicted in Figure 6. Whereas, these analogs provided further insight regarding the structural features for CB2 affinity and selectivity, our data evidenced that the introduction of a
cyclopropyl moiety in most of the synthesized compounds seemed to play a modest role in lowering the CB2 receptor affinity, especially if compared to compound LAhX. Interestingly, intrinsic activity for compounds LAlX, LIlX, LlAlX, LIHlX, evaluated by GTPγS binding assay, showed antagonist/inverse agonist properties (IC50 for compound LAlX = 294 nM, for LIHlX = 80 nM, for LIlX = 27 nM and for LIHlX = 51 nM).

Independently, other groups investigated the biological properties of some 1,4-dihydroindeno(1,2-c)pyrazoles synthesized by our group. In particular, within the search aiming to characterize the molecular pharmacology of the most widely used CB2 receptor ligands, it was reported for compound LAhX, namely Gp-1a, an affinity for the CB2 receptors markedly different from that reported by our group (Ki CB1 = 426 ± 0.08 nM, Ki CB2 = 20.9 ± 0.23 nM; CB1/CB2 selectivity ratio = 20), using (3H)CP55,940 and mouse brain and spleen as source for CB1 and CB2 receptors, respectively [30]. Furthermore, in contrast to our results, the same authors reported for Gp-1a inverse agonist properties on CB2 receptors in GTPγS assay and resulted not active in hCB2 receptor pERK assay [30]. Indeed, the first two series of 1,4-dihydroindeno(1,2-c)pyrazole-based compounds synthesized by our group [23,24], including Gp-1a, were described in a patent application providing methods and pharmaceutical compositions for reducing the serum level of immunoglobulin IgE in an animal or human subject [31]. In particular, it was reported the CB2 agonist Gp-1a attenuated the serum levels of total IgE from BALB/e mice and the co-treatment with the CB2 antagonist SR144528 reversed the Gp-1a effect. Therefore, the patent provided a method for modulating this type of antibody for the treatment of immune system-related conditions such as allergy, hay fever and the like. Biological literature concerning Gp-1a pointed out the relevance of such a compound as a useful pharmacological tool to ascertain in more detail the role of CB2 receptors in physiopathological conditions [32–36].

Furthermore, the pharmacological relevance of 1,4-dihydroindeno(1,2-c)pyrazole-based compounds emerged also from the significant anti-nociceptive activity of the potent and selective CB2 agonist LDhX, namely NESS400, in Spared Nerve Injury (SNI) neuropathic mice, by alleviating both mechanical allodynia and thermal hyperalgesia [37].

2.2. Benzofuro(3,2-c)pyrazol-Based Derivatives (V)

Further condensed (5,5)-pyrazole derivatives were designed by us making use of bioisosteric replacement as drug design approach to obtain novel planar tricyclic scaffold for cannabinoid receptor interaction [38]. Following this approach, a new series of 1,4-dihydroindeno(1,2-c)pyrazole analogs, namely benzofuro(3,2-c)pyrazoles (V, Figure 2), containing an oxygen atom at position 4 instead of a methylene unit, were designed. The structure of representative compounds is depicted in Figure 7. These compounds were previously described by Neuroscienze Pharmaness S.C.A.R.L in a patent application claiming specific composition of microemulsions of pharmaceutical compounds. Indeed,
the patent covers a more extensive series of tricyclic pyrazoles belonging both to (5,5) as well as (5,6) and (5,7) series [39].

![Figure 7](image.png)

**Figure 7.** Structure of representative benzofuro(3,2-c)pyrazol-based derivatives (V). Affinities at CB1 and CB2 receptors were assessed by competition of \(^{3}H\)-CP 55,940 in mouse cerebellum membranes and in mouse spleen homogenates, respectively [38].

In general, our SAR study revealed high CB2 receptor affinity for the new compounds, with values qualitatively similar with those of compound IDhX, as well as those of related analogs incorporating the 1,4-dihydroindenophen(1,2-c)pyrazole skeleton. In contrast, a comparison of \(K_{i}\) values of compound IAhX and that of the analog VAhX revealed two orders of magnitude decreased affinity for VAhX. Compounds VlhX and VMhX, featuring a bornyl and isopinocamphyl moiety at the carboxamide portion, exhibited the best CB2 cannabinoid binding profiles among all synthesized derivatives. CB2 functional assay carried out on HL-60 cells, based on the evaluation of P-ERK1/2 expression induced by cannabinoid ligands, evidenced agonism behavior for all synthesized compounds [38], with calculated maximum values, in most cases, exceeding that of the agonist WIN-55,212-2 (i.e., VAhX: dose 50 nM, 205 ± 12%; VDhX, dose 50 nM, 196 ± 20%; VLhX: dose 10 nM, 189 ± 11%; VMhX: dose 5 nM, 205 ± 6%; WIN-55,212-2: dose 75 nM, 190 ± 17% versus vehicle). Overall, the effect of tested compounds as CB2 agonists was specific as it was blocked by the CB2 receptor antagonist AM630 [38].

2.3. 1,4-Dihydrothieno(2',3'-4,5)cyclopenta(1,2-c)pyrazole-Based Derivatives (VII) and 1,4-Dihydrothieno(3',2'-4,5)cyclopenta(1,2-c)pyrazole-Based Derivatives (X)

To further investigate the versatility of (5,5)-pyrazole condensed tricyclic derivatives in the development of CB2 ligands as therapeutic agents, our attention was focused on the benzene ring of the tricyclic indenopyrazole scaffold (I), by replacing it with a thiophene ring, giving rise to the novel dihydrothienocyclopentapyrazole architecture which fine-tuning furnished novel derivatives with general formula VII (Figure 2). It was postulated that bioisosteric replacement benzene/thiophene could be an efficient strategy to develop novel CB2 selective ligands and maybe provide further insight concerning structural features for cannabinoid receptor interaction [40]. In our SAR study, we planned to investigate the effect of changing the size of carboxamide moiety (Q), of methyl shifting from C6 to C7 of the tricyclic platform, as well as the effect related to the replacement of the N1-dichlorophenyl group (X) with p-methylbenzyl moiety (Z) on cannabinoid receptor affinity. The structure of representative compounds is depicted in Figure 8. The major term, compound VIIAhX, displayed a high affinity for CB2 receptors, even if 62-fold decreased with respect to the reference compound IAhX with selectivity ratio \(K_{i}\) \(CB_{1}/K_{i}\) \(CB_{2} = 191\). A similar trend was observed in several thienocyclopentapyrazole compounds (Figure 8) with the exception of VIINhX, which exhibited a mixed binding profile, reaching \(K_{i}\) values of 6.5 and 5.1 nM for CB1 and CB2, respectively. All these compounds profiled as full agonists at CB2 receptors in an assay based on the determination of P-ERK 1/2 increasing expression in HL-60 cells [40].
Within this framework, a series of 1,4-dihydrothieno(3′,2′-4,5)cyclopenta(1,2-c)pyrazole-based derivatives with general formula X (Figure 2) were investigated. Representative compounds were described by Neuroscienze Pharmaness S.C.A.R.L in a patent application encompassing a more extensive series of condensed tricyclic pyrazoles [41]. According to binding data, shifting the sulfur atom of the 1,4-dihydrothieno(2′,3′-4,5)cyclopenta(1,2-c)pyrazole structural template from position 5 to 7 induced a significant impact on cannabinoid receptor affinity. In general, most of the investigated compounds exhibited a preferential affinity for CB₂ receptors, with different compounds showing mixed and high CB₁/CB₂ cannabinoid receptor affinities (XZH, XNH, XHhX, XHz, XhX, XgX, Figure 9).

Amongst the compounds claimed in the patent application, derivatives XZH, XNH, XHhX were investigated to evaluate their intrinsic activity as agonist or antagonist on CB₁ receptors in an ex-vivo model based on the use of the vas deferens. According to the behavior of WIN-55,212-2 and other CB₁ ligands in mice vas deferens isolated organ assay [42], compounds XZH, XNH, XHhX showed agonist activity, with XZH being the most effective in inhibiting the contractions induced by an electric stimulus compared to the basal value, as evidenced by the reported dose-response curve [41].

Compounds XZH, XNH, XHhX were also investigated for their ability to reduce intraocular pressure (IOP) which is considered a prominent risk factor for glaucoma development and progression [43]. The first study highlighting the relevance of CB₁/CB₂ agonists for the treatment of glaucoma was conducted in 1971, reporting that smoking marijuana significantly lowered the IOP [44]. Very positive results were reported also for WIN-55,212-2, which is the prototype of the aminoalkylindole class of synthetic cannabinoids, activating both CB₁ and CB₂ receptors, albeit with a proclivity for the CB₂ receptors. In particular, in normotensive rabbits, a single dose of WIN-55,212-2, either topical or systemic, significantly reduced IOP without apparent ocular toxicity, most likely through effects on CB₁ receptors [45]. Subsequent studies showed a reduction of IOP in glaucomatous rats after local and chronic administration of WIN-55,212-2, without adverse effects [46]. These findings are consistent with another experiment showing that WIN-55,212-2 decreased IOP in human glaucoma resistant to conventional therapies [47]. Further studies have demonstrated increased aqueous outflow after exposure to the CB₂ agonist JWH015, suggesting a beneficial function derived from CB₂ receptor activation in the treatment of ocular diseases such as glaucoma [48]. Neuroprotective properties of cannabinoids have been demonstrated in CNS neurodegenerative diseases with different mechanisms [49].
Several studies have shown in the retina that CB1 agonists (Δ⁹-tetrahydrocannabinol and cannabidiol) protected ganglion cells from glutamate-excitotoxicity and ischemia caused by increased IOP [50,51]. Although all these data are promising, an important issue for the clinical potential of cannabinoids as anti-glaucoma agents has been cardiovascular and psychotropic side effects mediated by systemic and brain cannabinoid receptor activation [52–54]. Additionally, short duration of action of cannabinoids on IOP reduction (i.e., the duration of action after smoking marijuana is only 3–4 h) is another issue that has to be overcome in the application of these compounds in treating glaucoma. Actually, standard therapeutic options in treating glaucoma include a few IOP-lowering drugs as prostaglandin analogs, β-adrenoreceptor antagonists, α₂-adrenoreceptor agonists, carbonic anhydrase inhibitors, and cholinergic agonists [55]. When medical therapy failed to lower IOP, laser or surgical interventions are extremely considered in order to prevent disease progression toward blindness.

Figure 9. Structure of representative 1,4-dihydrothieno(3′,2′-4,5)cyclopenta(1,2-c)pyrazole-based derivatives (X). Affinities at CB₁ and CB₂ receptors were assessed by competition of (³H)-CP 55,940 in mouse cerebellum membranes and in mouse spleen homogenates, respectively [41].

According to unmet medical need, compounds X₁hZ, X₁NhZ, X₁hX, exhibiting high and mixed CB₁/CB₂ receptor affinities, with a proclivity for CB₂ subtype, and endowed of CB₁ agonist properties, according to the CB receptor profile of the reference cannabinoidergic compound WIN-55,212-2, by using the animal model of old DBA/2J mice, were investigated for their ability to reduce IOP [41]. Compounds X₁hZ, X₁NhZ, X₁hX and WIN-55,212-2, which was used as reference compound, were dispersed in the commercial emulsion Tocrisolve™ and applied to the eye of old DBA/2J mice at a concentration of 100 µg or 50 µg. The obtained results, reported in Table 1, showed that commercial emulsion Tocrisolve™ (20, 40 µL) had no effect on the IOP. Furthermore, at the dose of 100 µg, all tested compounds were effective in reducing eye pressure as the reference compound WIN-55,212-2, while only X₁hZ, at the dose of 50 µg was more effective in reducing IOP.
than the reference compound. What is claimed in the patent application was pharmaceutical compositions comprising emulsions or microemulsions may be useful to avoid systemic side effects of such cannabinoids. The preliminary results reported in the patent application [41] for compound XlhZ could pave the way for the development of novel cannabinergic compounds as anti-glaucoma agents.

Table 1. Intraocular pressure (IOP) variation in old DBA/2J mice after administration of compounds XlhZ, XNhZ, XHhX and WIN-55,212-2 as reference compounds. The results are expressed as percent decrease of the IOP with respect to the animal basal IOP value [41].

| Time of Administration (Minutes) | Carrier 20 µL | XlhZ 50 µg | XNhZ 100 µg | XHhX 50 µg | WIN-55,212-2 50 µg |
|---------------------------------|--------------|------------|-------------|------------|-------------------|
| 30                              | 0.1          | 15.9       | 23.9        | 2.9        | 16.6              |
| 60                              | 0.1          | 15.0       | 22.8        | 2.2        | 14.0              |
| 90                              | 0.2          | 12.6       | 19.6        | 0.8        | 11.2              |
| 120                             | 0.0          | 11.7       | 21.7        | 1.5        | 21.7              |

2.4. 1,4-Dihydropyrazolo(3,4-a)pyrrolizine-Based Derivatives (XV)

Continuing with our interest in expanding SAR studies on cannabinoid receptors, and taking into consideration the binding profile of compounds IAAx and IAhx, a new tricyclic pyrazole scaffold, namely 1,4-dihydropyrazolo(3,4-a)pyrrolizine XV, was designed on the basis of bioisosteric replacement approach (benzene/pyrrole) [56]. Representative compounds are depicted in Figure 10. Surprisingly, none of the new compounds exhibited a high affinity for CB2 receptors, with Ki values above 314 nM. Negligible affinity was also determined for CB1 receptors, with XVHhX reaching the Ki value of 142 nM.

Figure 10. Structure of representative 1,4-dihydropyrazolo(3,4-a)pyrrolizine-based derivatives (XV). Affinities at CB1 and CB2 receptors were assessed by competition of (3H)-CP 55,940 in mouse whole-brain membranes and in CHO cell membranes transfected with hCB2 receptors [56].

3. (5,6)-Condensed Pyrazole Derivatives
3.1. 4,5-Dihydro-1H-benzo(g)indazole-Based Derivatives (II)

The first (5,6)-condensed pyrazole derivatives synthesized by us are those of general formula II (Figure 2), featuring the 4,5-dihydro-1H-benzo(g)indazole scaffold which is a homologue of 1,4-dihydroindeno(1,2-c)pyrazole one (compounds I). Representative compounds are depicted in Figure 11 [57]. Most of such compounds were described by Sanofi-Aventis in a patent application as cannabinoid CB1 receptor antagonists, but no biological data were reported [58].
Interestingly, as evidenced by $K_i$ values of compounds IIAbX and IIAiX with respect to compounds IAaX and IAhX, increasing the carbocyclic central ring size by one methylene unit (from (5,5)- to (5,6)-condensed pyrazole derivatives) involved a marked loss of affinity for CB2 receptors, a significant increase in CB1 affinity, and a consequent loss of CB2 selectivity. A similar trend was exhibited by other derivatives incorporating such 4,5-dihydro-1H-benzo(g)indazole scaffold (i.e., II AeX and II BbX). The SAR study carried out on the two homologous series I and II, prompted us to suppose that to achieve high binding affinity to CB1 receptors and CB1 over CB2 selectivity it is important for the tricyclic system to be non-planar. To evaluate the functional profile, several compounds were assayed for the capability to affect gastrointestinal transit in mice, making use of the upper gastrointestinal test which is based on the determination of the intestinal length traveled by a non-absorbable marker as a consequence of the active compound administration. From this test, compound IIAbX was able to induce a dose-dependent gastrointestinal motility increase, as well as compound IIiX. This effect was markedly reversed by the in vivo administration of CP-55,940, suggesting for the series of 4,5-dihydro-1H-benzo(g)indazole-3-carboxamides antagonistic profile for CB receptors [57].

3.2. 4,5-Dihydro-1H-thieno(2,3-g)indazole-Based Derivatives (VIII) and 4,5-Dihydro-1H-thieno(3,2-g)indazole-Based Derivatives (XI)

A number of compounds sharing a 4,5-dihydro-1H-thieno(2,3-g)indazole scaffold (VIII), which is a homolog of the previously-described 1,4-dihydrothieno(2′,3′,4,5)cyclopenta(1,2-c)pyrazole one (VII) were claimed by Neuroscienze Pharmaness S.C.A.R.L. [59]. The structure of representative compounds and CB1/CB2 receptor affinity values are reported in Figure 12. According to binding data, most compounds exhibited a potent and mixed CB1/CB2 binding profile, with a proclivity for CB1 receptors. The thieno(2,3-g)indazole-based derivative VIII AeX resulted in the most selective for CB1 receptors between all reported compounds. The disclosed compound VIII AeX, namely NESS038C6, highlighted CB1 antagonism behavior by both in isolated organ assays and in vivo tests based on rat intestinal motility (data not shown). Chronic treatment with NESS038C6 in C57BL/6N diet-induced obesity (DIO) mice determined a significant reduction of weight which was comparable to that detected in DIO mice treated with SR141716 [60]. The Neuroscienze Pharmaness S.C.A.R.L. patent [59] encompasses also a series of isomeric compounds incorporating the 4,5-dihydro-1H-thieno(3,2-g)indazole scaffold XI (i.e., XIAbX, Figure 2) and others, but not cannabinoid binding receptor affinity was reported.
Figure 12. Structure of representative 4,5-dihydro-1H-thieno(2,3-g)indazole-based derivatives (VIII). Affinities at CB₁ and CB₂ receptors were assessed by competition of (³H)-CP 55,940 in mouse cerebellum membranes and in mouse spleen homogenates, respectively [59].

3.3. 1,5-Dihydroisothiochromen(4,3-c)pyrazole-Based Derivatives (XIII) and 1,4-Dihydrothiochromeno(4,3-c)pyrazole-Based Derivatives (XIV)

A series of derivatives featuring a 1,5-dihydroisothiochromeno(4,3-c)pyrazole- and 1,4-dihydrothiochromeno(4,3-c)pyrazole-scaffolds, XIII and XIV, respectively (i.e., XIIIbbX or XIVbbX, Figure 2) were claimed by Sanofi-Aventis for cannabinoid receptor interaction, but no biological activity was presented [58].

3.4. 4,5-Dihydro-1H-Pyrazolo(4,3-g)indolizine-Based Derivatives (XVI)

To further extend SAR study on cannabinoid receptors, and driven by the negligible results on CB₁/CB₂ receptor affinity of 1,4-dihydropyrazolo(3,4-a)pyrrolizines (XV), homologue 4,5-dihydro-1H-pyrazolo(4,3-g)indolizinones (XVI, Figure 2) have been synthesized [56]. Representative compounds are depicted in Figure 13. According to binding data reported for compounds XVIbbX, XVICbX, XVIDbX with respect to compounds XVIAaX, XVIAhX, XVITaX, XVITbX, the expansion of the central ring size by a methylene unit led to conformational changes that promote the affinity for CB₁ receptors and improve CB₂/CB₁ selectivity ratio, with XVIICbX reaching reasonable affinity for CB₁ receptors (Kᵢ CB₁ = 81 nM) and the highest CB₂/CB₁ selectivity ratio (>12).
Figure 13. Structure of representative 4,5-dihydro-1H-pyrazolo(4,3-g)indolizine-based derivatives (XVI). Affinities at CB₁ and CB₂ receptors were assessed by competition of (³H)-CP 55,940 in mouse whole-brain membranes and in CHO cell membranes transfected with hCB₂ receptors [56].

4. (5,7)-Condensed Pyrazole Derivatives

4.1. 1,4,5,6-Tetrahydrobenzo(6,7)cyclohepta(1,2-c)pyrazole-Based Derivatives (III)

The intriguing SAR study carried out on ligands with the 1,4-dihydroindeno(1,2-c)pyrazole core (i.e., IAaX, IAhX) endowed with very high binding affinity for CB₂ receptors in comparison to homologous ligands having 4,5-dihydro-1H-benzo(g)indazoles (i.e., IIAbX, IIAiX) exhibiting higher CB₁ binding affinity, prompted us to design and synthetize a new homologous series of general formula III, incorporating 1,4,5,6-tetrahydrobenzo(6,7)-cyclohepta(1,2-c)pyrazole scaffold, for cannabinoid receptor interaction [61]. Representative compounds are depicted in Figure 14. Compound IIIAcX, namely NESS0327, was first claimed by Sanofi-Aventis, but no biological data was presented in the patent [58].

Figure 14. Structure of representative 1,4,5,6-tetrahydrobenzo(6,7)cyclohepta(1,2-c)pyrazole-based derivatives (III). Affinities at CB₁ and CB₂ receptors were assessed by competition of (³H)-CP 55,940 in mouse whole-brain membranes and in CHO cell membranes transfected with hCB₂ receptors [61].

In general, all the investigated compounds exhibited preferential affinity for CB₁ receptors, with NESS0327, endowed of Kc, CB₁ = 4.2 nM, Kc, CB₂ = 55.7 nM and Kc, CB₂/Kc, CB₁ selectivity ratio = 13.26. Slightly different values for CB₁ receptors, possibly due to different receptor matrix, were reported for IIIAcX by two other independent groups: Stoit et al. Kc, CB₁ = 126 nM [62] and Zhang et al. 18.4 nM [63] using (³H)-CP 55,940 and hCB₁ receptor cloned in CHO cells or membranes isolated from a HEK-293 expression system, respectively. Compound IIIcX, bearing the bulky myrtanil substituent, showed the best CB₁ receptor affinity which was equivalent to that exhibited by IIIAcX. Moving the chlorine atom from position 8 to 9 of the myrtanil-based derivative IIIcX to give IIIdX induced a decrease of CB₁ receptor affinity with a concurrent loss of selectivity. CB₂ receptor intrinsic activity of selected derivatives was evaluated through in vitro tests based on the determination of phosphorylated ERK 1/2 (P-ERK 1/2) expression in mouse neuroblastoma...
N1E-115 cell line. According to rimonabant, compounds IIIAcX and IIIBcX didn’t affect P-ERK expression in N1E-115 cells in the concentration range 1 nM-10μM; P-ERK 1/2 (% of vehicle): vehicle, 100 \( \div \) 10; rimonabant (1 μM): 110 \( \div \) 8; IIIAcX (1 μM): 111 \( \div \) 12; IIIBcX (1 μM): 95 \( \div \) 9. Furthermore, these compounds inhibited the P-ERK 1/2 expression up-regulation induced by the reference cannabinoïd agonist WIN-55,212-2; vehicle: 185 \( \div \) 12; rimonabant (1 μM): 108 \( \div \) 7; IIIAcX (1 μM): 115 \( \div \) 13; IIIBcX (1 μM): 103 \( \div \) 9. In contrast, the myrtanol-based derivatives IIIEx and IIIydX enhanced P-ERK 1/2 expression in N1E-115 cells, highlighting CB1 receptor agonism profile for both compounds [61]. The pharmacological relevance of 1,4,5,6-tetrahydrobenzo(6,7)cyclohepta(1,2-

4.2. 4,5-Dihydro-1H-benzo(2,3)oxepino(4,5-c)pyrazole-Based Derivatives (VI)

Endocannabinoids are orexigenic factors promoting appetite via CB1 receptor activation. This finding provided the rationale for the development of CB1 antagonist/inverse agonist rimonabant for obesity treatment and its metabolic complications. However, rimonabant side effects responsible for its withdrawal were principally related to both activities on SNC and to inverse agonism profile [70,71]. Therefore, new strategies have been proposed for the development of more safe anti-obesity agents, such as the identification of peripherally restricted CB1 receptor antagonists [71] as well as neutral CB1 receptor antagonists or CB1 allosteric modulators [72]. Within this frame, it was envisioned that bioisosteric modification of 1,4,5,6-tetrahydrobenzo(6,7)cyclohepta(1,2-c)pyrazole core (III) might offer new templates for CB1 receptor interaction. Thus, a series of 4,5-dihydro-1H-benzo(2,3)oxepino(4,5-c)pyrazoles (VI, Figure 2), containing an oxygen atom at position 6 in place of a methylene unit, were designed and synthesized. Such bioisosteric methylene/oxygen replacement might give access to CB1 ligands with increased polar surface area and decreased lipophilicity, which were considered critical parameters to influence the blood-brain permeability. Representative compounds are VIAcX, VIDcX, VIICX (Figure 15), all exhibiting nanomolar/near nanomolar affinity for CB1 receptors [73].

Within this frame, a series of compounds sharing a 4,5-dihydro-1H-benzo(2,3)oxepino(4,5-c)pyrazole core, including VIAcX, were claimed by Cadila Healthcare Limited, but no binding data for cannabinoïd receptors were presented [74]. However, the compounds of the invention were generally said, in the cAMP accumulation model, to antagonizes the WIN-55,212-2 inhibition of forskolin-induced cAMP accumulation in hCB1 CHO cells. Furthermore, VIAcX, and other representative compounds have been shown to reduce, in the sucrose solution intake rat model, the sucrose solution consumption [74,75]. In our hands,
compound VI\textsubscript{AcX}, namely NESS06SM, emerged for its nanomolar CB\textsubscript{1} receptor affinity and good selectivity with respect to CB\textsubscript{2} one \((K_i \text{CB}_1 = 10.25 \text{ nM}, K_i \text{CB}_2 > 5000 \text{nM})\). Evaluation of intrinsic activity carried out on NESS06SM highlighted a neutral antagonist profile both in \((\text{35}^S)\text{GTP} \gamma \text{S} \) assay and in isolated organ assays (mouse vas deferens) \([76]\). In silico parameters \((c\text{LogP}, t\text{PSA}, \text{log BB})\), compared with III\textsubscript{AcX} (NESS0327) and other already known CB\textsubscript{1} antagonists (i.e., rimonabant), suggested that NESS06SM exhibited sparing BBB permeability. Moreover, chronic treatment with NESS06SM resulted in the reduction of body weight and cardiovascular risk factor improvement in C57BL/6N diet-induced obesity (DIO) mice fed with a fat diet. Furthermore, in contrast to rimonabant, the chronic treatment of NESS06SM did not change mRNA expression of both monoaminergic transporter and neurotrophins, highly related to anxiety and mood disorders \([76]\). Interestingly, the co-treatment of NESS06SM has been shown to reduce food intake and weight gain, mitigate the side effects induced by chronic administration of the atypical antipsychotic olanzapine, without altering the positive effects of olanzapine on behavior \([77]\).

As expected, evaluation of the intrinsic activity of VID\textsubscript{cX}, namely SM-11, evidenced CB\textsubscript{1} antagonist activity, both in vitro test (P-ERK 1/2 expression in N1E-115 cells) and in isolated organs (mouse vas deferens). Behavioral studies highlighted that dose-dependently SM-11 decreased food intake in rats by 15–20%. Moreover, the i.v. administration of SM-11 fully and readily antagonized the effect of the agonist WIN-55,212-2 on the activity of ventral tegmental area (VTA) dopamine neurons projecting to the nucleus accumbens cells, confirming its antagonist profile. Furthermore, this data supported that SM-11 can lessen the hedonic aspect of food thus promoting bodyweight reduction \([78]\).

### 4.3. 1,4,5,6-Tetrahydrothieno(2′,3′-6,7)cyclohepta(1,2-c)pyrazole-Based Derivatives (IX) and 1,4,5,6-Tetrahydrothieno(3′,2′-6,7)cyclohepta(1,2-c)pyrazole-Based Derivatives (XII)

A series of derivatives incorporating a 1,4,5,6-tetrahydrothieno(2′,3′-6,7)cyclohepta(1,2-c)pyrazole- and 1,4,5,6-tetrahydrothieno(3′,2′-6,7)cyclohepta(1,2-c)pyrazole-scaffolds IX and XII, respectively, (i.e., IX\textsubscript{AcX} or XII\textsubscript{AcX}, Figure 2) were claimed by Neuroscienze Pharmaness S.C.A.R.L. for cannabinoid receptor interaction, but no biological activity was presented \([59]\).

### 4.4. 1,4,5,6-Tetrahydropyrazolo(3,4-c)pyrrolo(1,2-a)azeidine-Based Derivatives (XVII)

A series of 1,4,5,6-tetrahydropyrazolo(3,4-c)pyrrolo(1,2-a)azeidine-based derivatives (XVII, Figure 2) has been investigated for cannabinoid receptor interaction \([56]\). Representative compounds are depicted in Figure 16, all devoid of affinity for CB\textsubscript{2} receptors and endowed of negligible affinity for CB\textsubscript{1} receptors.
Figure 16. Structure of representative 1,4,5,6-tetrahydropyrazolo(3,4-c)pyrrolo(1,2-a)azepine-based derivatives (XVII). Affinities at CB₁ and CB₂ receptors were assessed by competition of (³H)-CP 55,940 in mouse whole-brain membranes and in CHO cell membranes transfected with hCB₂ receptors [56].

5. (5,8)-Condensed Pyrazole Derivatives

4,5,6,7-Tetrahydro-1H-benzo(7,8)cyclooct(1,2-c)pyrazole-Based Derivatives (IV)

Zhang et al. described the synthesis and biological evaluation of a series of conformationally constrained analogs of rimonabant [63]. Within this frame, they synthesized both compound IIIAcX from our lab and IVAdX (Figure 17) which is the only pyrazole-based tricyclic compound known belonging to (5,8)-condensed derivatives, featuring the 4,5,6,7-tetrahydro-1H-benzo(7,8)cycloocta(1,2-c)pyrazole core. The compound, endowed of lower affinity for CB₂ than CB₁ receptors, exhibited a similar trend with respect to homologous compounds IIAbX and IIIAcX. However, increasing the carbocyclic central ring size by one methylene unit, i.e., from (5,7)- to (5,8)-condensed pyrazole derivatives, involved a marked loss of affinity for CB₁ receptors.

Figure 17. Structure of 4,5,6,7-tetrahydro-1H-benzo(7,8)cycloocta(1,2-c)pyrazole derivative IVAdX. Affinities at CB₁ and CB₂ receptors were assessed by competition of (³H)-CP 55,940 in HEK-293 and CHO-K1 cell membranes, respectively [63].

6. Miscellaneous Derivatives

Other pyrazole-based tricyclic derivatives, featuring the pyrazolo(5,1-f)(1,6)naphthyridine core (Figure 18), were investigated for cannabinoid receptor interaction [79].
The pyrazolo(5,1-f)(1,6)naphthyridine derivatives were synthesized making use of AgOTf and proline-cocatalyzed multicomponent methodology (Scheme 2), starting from the appropriate o-alkynylaldehydes 9, p-toluenesulfonyl hydrazide (PTSH) and ethyl pyruvate, to gave key pyrazole-esters 10 which, hydrolyzed with NaOH, afforded the corresponding acids 11. Target compounds were synthesized by condensation of acids 11, previously activated with pivaloyl chloride, with the appropriate amines. The o-alkynylaldehydes 9 were obtained from 2-bromonicotinaldehyde 8 and appropriate alkynes which were reacted under the conventional Sonogashira conditions.

Compounds 5–7 exhibited affinity levels for CB2 receptors in the near nM range (Kᵢ: 33–67 nM) with a good degree of selectivity for CB2 receptors compared to CB1. According to in vitro assays based on the effects of forskolin-stimulated cAMP levels in human CB2 CHO cells, compounds 5–7 exhibited antagonist/inverse agonist properties [79].

7. Conclusions

In the last decades, the CB1 antagonist/inverse agonist rimonabant has been considered an extremely valuable lead for the design of new ligands for CB receptors interaction, with potential therapeutic value. In the context of this review, the introduction of structural constraints in rimonabant, the use of medicinal chemistry approaches as homologation or bioisosterism, gave access to several compounds most of which belong to (5,5), (5,6) and (5,7)-condensed tricyclic pyrazole derivatives. Here we have summarized the extensive SAR studies carried out on such compounds, allowing us to identify different ligands endowed with high affinity and selectivity for CB1 or CB2 receptors. In particular, the phar-
macological relevance of 1,4-dihydroindeno(1,2-c)pyrazole-based I compounds, belonging to (5,5) tricyclic pyrazole derivatives, emerged from the significant anti-nociceptive activity of the potent and selective CB₂ agonist DIX, by alleviating both mechanical allodynia and thermal hyperalgesia in Spared Nerve Injury (SNI) neuropathic mice. The interest of ligands belonging to (5,5) tricyclic pyrazole derivatives emerged also for their potential as anti-glaucoma agents, for the ability to reduce intraocular pressure (IOP). Compound XIHZ, featuring the 1,4-dihydrothieno(3′,2′-c)-4,5)cyclopenta(1,2-c)pyrazole core and exhibiting high and mixed CB₁/CB₂ receptor affinities, effectively reduced intraocular pressure in the animal model of old DBA/2J mice, providing a potential alternative to the use of WIN-55,212-2 and smoking marijuana, known for their IOP lowering properties. SAR study pointed out the relevance of ligands belonging to (5,7) tricyclic pyrazole derivatives, for their anti-obesity potential. In this frame, the selective CB₁ ligand VIAX, featuring the 4,5-dihydro-1H-benzo(2,3)oxepino(4,5-c)pyrazole core, exhibiting neutral antagonist expression of the cloned cDNA. Nature 1990, 346, 561–564. [CrossRef] [PubMed]

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