4-Oxo-1,4-dihydropyridines as Selective CB₂ Cannabinoid Receptor Ligands: Structural Insights into the Design of a Novel Inverse Agonist Series


1 Université Lille-Nord de France, Institut de Chimie Pharmaceutique Albert Lespagnol, EA 2692, IFR 114, 3 Rue du Professeur Laguesse, BP 83, 59006 Lille Cedex, France. 2 Université Catholique de Louvain, 73 Avenue E. Mounier UCL-CMFA (7540), B-1200 Bruxelles, Belgium. 3 Université Lille-Nord de France, Faculté de Pharmacie, Laboratoire de Chimie Thérapeutique, EA 1643, 14 Rue du Professeur Laguesse, BP 83, 59006 Lille Cedex, France, and Physiopathologie des Maladies Inflammatoires de l’Intestin, Université Lille-Nord de France, Amphi J & K, U795, IFR 114, Boulevard du Professeur Leclercq, 59045 Lille Cedex, France. * Both authors contributed equally to this work.

Received March 4, 2010

Growing evidence shows that CB₂ receptor is an attractive therapeutic target. Starting from a series of 4-oxo-1,4-dihydropyridine-3-carboxamide as selective CB₂ agonists, we describe here the medicinal chemistry approach leading to the development of CB₂ receptor inverse agonists with a 4-oxo-1,4-dihydropyridine scaffold. The compounds reported here show high affinity and potency at the CB₂ receptor while showing only modest affinity for the centrally expressed CB₁ cannabinoid receptor. Further, we found that the functionality of this series is controlled by its C-6 substituent because agonists bear a methyl or a tert-butyl group and inverse agonists, a phenyl or 4-chlorophenyl group, respectively. Finally, in silico studies suggest that the C-6 substituent could modulate the conformation of W6.48 known to be critical in GPCR activation.

Introduction

The CB₂ receptor belongs to the class A of G protein-coupled receptors superfamily. It is one of the components of the endocannabinoid system, which is a physiological system composed of cannabinoid receptors, CB₁ and CB₂, their endogenous ligands, named endocannabinoids, and the biotransformation enzymes involved in the synthesis, degradation, and cellular uptake of these endocannabinoids. This system plays a key role in numerous biological processes and is involved in maintaining homeostasis. Cannabinoids exhibit pharmacological effects in a large spectrum of diseases and disorders. Thus, in the past years, investigations were aimed at designing new synthetic molecules that target cannabinoid receptors. One of the main challenges for such compounds is to be as much as possible devoid of central nervous system (CNS) side effects. Because these undesirable effects are thought to be CB₁ receptor-mediated, the main strategy to avoid them is to develop CB₂ selective ligands. The high expression of CB₂ receptor in immune tissues and cells, both in periphery and in the CNS as well as the enhancement of its expression following inflammatory insults suggests that CB₂ receptor selective ligands might be effective in modulating inflammation. These observations were confirmed by the lack of immunomodulation induced by cannabinoids in CB₂ knockout mice. The CB₂ receptor exerts a critical role in the immune system by modulating cytokines release and immune cells migration. Besides, recent studies have emphasized the major role of CB₂ receptors in pathologies where an inflammatory component is involved, including Alzheimer disease, inflammatory bowel disease, or multiple sclerosis. Other studies indicate that CB₂ receptors could be involved in alleviating pain and could provide protection from bone loss.

This therapeutic potential has prompted the development of several CB₂ receptor selective ligands, either as agonists or as antagonists/inverse agonists (Chart 1). Among the selective agonists, classical cannabinoids and aminoalkylindoles have been extensively studied, whereas (1-(2-morpholin-4-yl-ethyl)-1H-indol-3-yl)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H-pyran [35S]-(3-[35S]thio)guanosine-5′-O-(5′-thiotriphosphate); Eₘₐₓ, maximum efficacy; TM, transmembrane domain; EL, extracellular loop; rmsd, root-mean-square deviation; LHMDS, lithium hexamethyldisilazane; DMF, N,N-dimethylformamide; DMS, dimethylsulfoxide; EtOAc, ethyl acetate; EtOH, ethanol; AcOH, acetic acid; DMSO, dimethylsulfoxide; DIPEA, N,N-diisopropylethylamine; HOBt, 1-hydroxybenzotriazole; HBTU, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DPPA, diphenylphosphoryl azide; t-BuOK, potassium tert-butoxide; t-BuOH, tert-butyl alcohol; rt, room temperature; TLC, thick-layer chromatography.
recently, other compounds have been disclosed such as the iminopyrazole \(N-(5\text{-}\text{tert}\text{-}\text{butyl}-2\text{-}\text{cyclopropylmethyl}-1\text{-}\text{methyl}-1,2\text{-}\text{dihydropyrazol}-3\text{-}\text{ylidene})\text{-}2\text{-}\text{fluoro}-3\text{-}(\text{trifluoromethyl})\text{-}\text{benzamide} \) (3, CBS-0550)\(^{39}\) and the pyrimidine derivative \(2\text{-}[(2,4\text{-}\text{dichlorophenyl})\text{amino}]\text{-}N\text{-}[(\text{tetrahydro}-2\text{-H}\text{-}\text{pyran}-4\text{-}\text{yl})\text{methyl}]\text{-}4\text{-}(\text{trifluoromethyl})\text{-}5\text{-}\text{pyrimidinecarboxamide} \) (4, GW842166X)\(^{30}\), which was chosen as a clinical candidate for the treatment of inflammatory pain. As for selective antagonists/ inverse agonists, much fewer compounds have been described.

The first to be discovered and the most widely used is the 1,5-diarylpyrazole, \(5\text{-} (4\text{-}\text{chloro}-3\text{-}\text{methylphenyl})\text{-}1\text{-}[(4\text{-}\text{methylph-}
\text{enyl})\text{methyl}]\text{-}N\text{-}[(15,4R,6S)\text{-}1,5,5\text{-}\text{trimethyl-6-bicyclo[2,2,1]-}
\text{heptyl}])\text{-}\text{pyrazol-3-carboxamide} \) (5, SR144528)\(^{31}\). This compound as well as the 2-quinolinone derivative \(N\text{-}[(\text{benzo}[1,3]\text{-}
\text{dioxol}-5\text{-}\text{ethyl})\text{-}7\text{-}\text{methoxy}-2\text{-oxo-8-pentyloxy}-1,2\text{-}\text{dihydro-
quinoline-3-carboxamide} \) (6, JTE907) received attention due to their anti-inflammatory properties.\(^{32}\) More recently, a new class of CB\(_2\) selective inverse agonists based on a triaryl bis-sulfone scaffold has been described.\(^{33\text{-}35}\) This class is represented by \(N\text{-}[(S)\text{-}[4\text{-}(4\text{-}\text{methoxy}-2\text{-}[(4\text{-}\text{methoxyphenyl})\text{ sulfonyl}]\text{phenyl})\text{-}sulfonyl]phenyl]ethyl)methanesulfonamide \) (7, Sch225336), which was shown to block the recruitment of leucocytes in vivo.\(^{36}\) Taken together, the available data strongly support CB\(_2\) ligands as modulators of inflammation.

However, it is essential to better understand the pharmacology of CB\(_2\) receptor ligands because, for instance, anti-inflammatory properties have been described for both agonists and inverse agonists. Similar trends were observed concerning studies on bone physiology. Indeed, some authors suggested that blocking the CB\(_2\) receptor protects from bone loss in ovariectomized mice\(^{37\text{-}38}\) and others showed the same effect using the highly selective CB\(_2\) receptor agonist \(\{4\text{-}[4\text{-}(1,1\text{-}\text{dimethylheptyl})-2,6\text{-}\text{dimethoxy-phenyl}]\text{-}6,6\text{-}\text{dimethyl-
benzene-2-\text{-}em-2-yl}\text{-}methanol} \) (HU-308, structure not shown).\(^{25\text{-}39}\)

In this context, our groups previously described the synthesis, pharmacological characterization, and structure–activity relationships of selective CB\(_2\) ligands based on a 4-oxo-1,4-dihydroquinoline-3-carboxamide scaffold (e.g., 8)\(^{40\text{-}41}\). The structure–activity relationship studies highlighted a significant correlation between affinity and/or selectivity toward the CB\(_2\) receptor and structural features such as an aliphatic moiety, especially an adamantyl substituent, on the C-3 carboxamide group as well as a n-pentyl chain in N-1 position. Concerning the functionality of these ligands, most of the compounds behaved as selective CB\(_2\) agonists and, more surprisingly, small changes in the position of the substituents around the heterocycle resulted in modifications of the compounds functionality.\(^{31}\)

In light of these considerations, we sought to find new CB\(_2\) selective ligands. Our efforts to identify such compounds allowed us to describe novel series of 4-oxo-1,4-dihydropyrindines and 4-thioxo-1,4-dihydropyridines (general structure shown in Chart 2) that were found to be potent and selective CB\(_2\) receptor ligands. We also report here the identification of a key substituent on the 4-oxo-1,4-dihydropyridine scaffold responsible for a functionality switch within this series of compounds.

**Chemistry**

The synthesis of \(N3\text{-}(1\text{-adamantyl})\text{-6-methyl-1-pentyl-4-oxo-1,4-dihydropyridine-3-carboxamide} \) (compound 11), outlined in Scheme 1, was performed using a methodology adapted from the literature.\(^{42\text{-}44}\) The commercially available 4-hydroxy-6-methyl-2-pyrone was reacted with \(N,N\text{-dimethylformamide} \) dimethyl acet in mild conditions to give 9, which, when treated with n-pentylamine under alkaline conditions followed by acidification gave the carboxylic acid 10. Finally, amidation was accomplished with 1-aminoadamantane hydrochloride under peptide coupling conditions to give the target amide 11.

The synthesis of the 6-\text{-}tert\text{-}butyl, 6-phenyl or 6-(4-chlorophenyl) substituted 4-oxo-1,4-dihydropyridine-3-carboxamides and 4-thioxo-1,4-dihydropyridine-3-carboxamides 17–36 is...
described in Scheme 2. Structures of intermediate compounds 13–16 as well as target amides 17–40 are summarized in Table 1. The 4-oxo-1,4-dihydropyridine scaffold was prepared from ethyl acetoacetate as previously described.44 Enaminone 12 was obtained by reaction of ethyl acetoacetate with the N,N-dimethylformamide/dimethylsulfate adduct (DMF/DMS) in combination with triethylamine. Subsequent deprotonation of 12 with lithium hexamethyldisilazane (LHMDS) in the presence of the appropriate acyl chloride at −70 °C followed by acidification at room temperature led directly to pyran-4-one 13a in good yields from the corresponding 4-oxo-1,4-dihydropyridines 14a by acidification at room temperature led directly to pyran-4-one 14d. Aminolysis in acidic conditions afforded 4-oxo-1,4-dihydropyridine 15 as the novel compound exhibiting a specific displacement superior to 60% either for hCB1 or the hCB2 cannabinoid receptor were used in these experiments. All compounds were first screened at 10 μM concentration for their affinity toward both cannabinoid receptors. The inhibition constant (Ki) values were then determined for compounds exhibiting a specific displacement superior to 60% either for hCB1 or the hCB2 (Table 2), and the selectivity index (CB2 vs CB1) was calculated whenever possible. We also investigated their functionality at the CB2 receptor using a guanosine-5'-O-(3-[35S]triphosphate ([35S]GTPγS) binding assay and hCB1–CHO cells membranes, as previously described.46 This assay constitutes a functional measure of the interaction of the receptor and the G-protein, the first step in activation of the G-protein coupled receptors. In this system, neutral antagonists do not affect [35S]-GTPγS binding, while agonists and inverse agonists respectively increase and decrease nucleotide binding. The functionality of reference cannabinoid agonists 1, WIN-55,212-2 and CP-55,940 as well as the inverse agonist 5 were determined. Half-maximal effective concentration (EC50) and maximum efficacy (Emax) values of reference and original compounds are summarized in Table 3.

### Pharmacology

The affinities of the newly synthesized compounds 11, 17–41, and 43 were determined by a competitive radioligand displacement assay using [3H]-CP55,940 and [3H]-SR141716A as radioligands for human CB2 cannabinoid receptor (hCB2) and human CB1 cannabinoid receptor (hCB1), respectively, as previously described.45 Membranes from Chinese hamster ovary (CHO) cells expressing either the hCB1 or the hCB2 cannabinoid receptor were used in these experiments. All compounds were first screened at 10 μM concentration for their affinity toward both cannabinoid receptors. The inhibition constant (Ki) values were then determined for compounds exhibiting a specific displacement superior to 60% either for hCB1 or the hCB2 (Table 2), and the selectivity index (CB2 vs CB1) was calculated whenever possible. We also investigated their functionality at the CB2 receptor using a guanosine-5'-O-(3-[35S]triphosphate ([35S]GTPγS) binding assay and hCB1–CHO cells membranes, as previously described.46 This assay constitutes a functional measure of the interaction of the receptor and the G-protein, the first step in activation of the G-protein coupled receptors. In this system, neutral antagonists do not affect [35S]-GTPγS binding, while agonists and inverse agonists respectively increase and decrease nucleotide binding. The functionality of reference cannabinoid agonists 1, WIN-55,212-2 and CP-55,940 as well as the inverse agonist 5 were determined. Half-maximal effective concentration (EC50) and maximum efficacy (Emax) values of reference and original compounds are summarized in Table 3.

### Structure–Affinity/Activity Relationships

The starting point of our investigation was the lead compound 8, which was described by our groups as a potent selective CB2 agonist (Ki = 16.4 nM). In the present work, we removed the condensed benzene ring of 4-oxo-1,4-dihydropyridine core. A set of four compounds was first synthesized on the basis of our previously reported work (11, 17, 25, and 32).40 These compounds possessed some features in common, like the N-1 pentyl chain and a carboxamidoadamantyl...
moiety at position 3, but differed by their substituents at position 6 of the heterocycle (i.e., methyl, tert-butyl, phenyl, and 4-chlorophenyl). All of these 6-substituted analogues were selective, displaying high affinity at the CB2 receptor and low or no affinity at CB1 receptor. Compound 11, with a methyl at position 6, exhibits a $K_i$ value at CB2 receptor (20 nM) of the same magnitude as the starting compound 8 and was found to be highly selective ($K_i$ at CB1 > 3000). A similar result was

**Table 1. Structures of the Newly Synthesized Compounds 13–40**

<table>
<thead>
<tr>
<th>compd</th>
<th>R</th>
<th>R'</th>
<th>R''</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
</tr>
</thead>
<tbody>
<tr>
<td>13a</td>
<td>tert-butyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>O</td>
</tr>
<tr>
<td>13b</td>
<td>phenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>O</td>
</tr>
<tr>
<td>13c</td>
<td>4-chlorophenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>O</td>
</tr>
<tr>
<td>13d</td>
<td>3-chlorophenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>O</td>
</tr>
<tr>
<td>13e</td>
<td>2-chlorophenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>O</td>
</tr>
<tr>
<td>13f</td>
<td>4-methylphenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>O</td>
</tr>
<tr>
<td>13g</td>
<td>4-methoxyphenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>O</td>
</tr>
<tr>
<td>14a</td>
<td>tert-butyl</td>
<td>pentyl</td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>N</td>
</tr>
<tr>
<td>14b</td>
<td>phenyl</td>
<td>ethyl</td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>N</td>
</tr>
<tr>
<td>14c</td>
<td>phenyl</td>
<td>butyl</td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>N</td>
</tr>
<tr>
<td>14d</td>
<td>phenyl</td>
<td>pentyl</td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>N</td>
</tr>
<tr>
<td>14e</td>
<td>phenyl</td>
<td>hexyl</td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>N</td>
</tr>
<tr>
<td>14f</td>
<td>phenyl</td>
<td>phenyl</td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>N</td>
</tr>
<tr>
<td>14g</td>
<td>4-chlorophenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>N</td>
</tr>
<tr>
<td>14h</td>
<td>3-chlorophenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>N</td>
</tr>
<tr>
<td>14i</td>
<td>2-chlorophenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>N</td>
</tr>
<tr>
<td>14j</td>
<td>4-methylphenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>N</td>
</tr>
<tr>
<td>14k</td>
<td>4-methoxyphenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOEt</td>
<td>N</td>
</tr>
<tr>
<td>15</td>
<td>phenyl</td>
<td>pentyl</td>
<td></td>
<td>S</td>
<td>COOEt</td>
<td>N</td>
</tr>
<tr>
<td>16a</td>
<td>tert-butyl</td>
<td>pentyl</td>
<td></td>
<td>O</td>
<td>COOH</td>
<td>N</td>
</tr>
<tr>
<td>16b</td>
<td>phenyl</td>
<td>ethyl</td>
<td></td>
<td>O</td>
<td>COOH</td>
<td>N</td>
</tr>
<tr>
<td>16c</td>
<td>phenyl</td>
<td>butyl</td>
<td></td>
<td>O</td>
<td>COOH</td>
<td>N</td>
</tr>
<tr>
<td>16d</td>
<td>phenyl</td>
<td>pentyl</td>
<td></td>
<td>O</td>
<td>COOH</td>
<td>N</td>
</tr>
<tr>
<td>16e</td>
<td>phenyl</td>
<td>hexyl</td>
<td></td>
<td>O</td>
<td>COOH</td>
<td>N</td>
</tr>
<tr>
<td>16f</td>
<td>phenyl</td>
<td>phenyl</td>
<td></td>
<td>O</td>
<td>COOH</td>
<td>N</td>
</tr>
<tr>
<td>16g</td>
<td>4-chlorophenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOH</td>
<td>N</td>
</tr>
<tr>
<td>16h</td>
<td>3-chlorophenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOH</td>
<td>N</td>
</tr>
<tr>
<td>16i</td>
<td>2-chlorophenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOH</td>
<td>N</td>
</tr>
<tr>
<td>16j</td>
<td>4-methylphenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOH</td>
<td>N</td>
</tr>
<tr>
<td>16k</td>
<td>4-methoxyphenyl</td>
<td></td>
<td></td>
<td>O</td>
<td>COOH</td>
<td>N</td>
</tr>
<tr>
<td>16l</td>
<td>phenyl</td>
<td>pentyl</td>
<td></td>
<td>S</td>
<td>COOH</td>
<td>N</td>
</tr>
<tr>
<td>17</td>
<td>tert-butyl</td>
<td>pentyl</td>
<td>1-adamantyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>18</td>
<td>tert-butyl</td>
<td>pentyl</td>
<td>cyclohexyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>19</td>
<td>phenyl</td>
<td>ethyl</td>
<td>(R,S)-1-(adamantyl)ethyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>20</td>
<td>phenyl</td>
<td>pentyl</td>
<td>(R,S)-1-(adamantyl)ethyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>21</td>
<td>phenyl</td>
<td>phenyl</td>
<td>(R,S)-1-(adamantyl)ethyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>22</td>
<td>phenyl</td>
<td>pentyl</td>
<td>1-(adamantyl)methyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>23</td>
<td>phenyl</td>
<td>pentyl</td>
<td>1-(3,3-dimethyl)adamantyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>24</td>
<td>phenyl</td>
<td>butyl</td>
<td>1-adamantyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>25</td>
<td>phenyl</td>
<td>pentyl</td>
<td>1-adamantyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>26</td>
<td>phenyl</td>
<td>hexyl</td>
<td>1-adamantyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>27</td>
<td>phenyl</td>
<td>pentyl</td>
<td>cyclohexyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>28</td>
<td>phenyl</td>
<td>pentyl</td>
<td>(R)-1-(1,2,3,4-tetrahydronaphthyl)</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>29</td>
<td>phenyl</td>
<td>pentyl</td>
<td>(S)-1-(1,2,3,4-tetrahydronaphthyl)</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>30</td>
<td>phenyl</td>
<td>pentyl</td>
<td>piperidin-1-yl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>31</td>
<td>phenyl</td>
<td>pentyl</td>
<td>3-(trifluoromethyl)phenyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>32</td>
<td>4-chlorophenyl</td>
<td></td>
<td>1-adamantyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>33</td>
<td>4-chlorophenyl</td>
<td></td>
<td>cyclohexyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>34</td>
<td>4-chlorophenyl</td>
<td></td>
<td>3-(trifluoromethyl)phenyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>35</td>
<td>3-chlorophenyl</td>
<td></td>
<td>1-adamantyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>36</td>
<td>2-chlorophenyl</td>
<td></td>
<td>1-adamantyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>37</td>
<td>4-methylphenyl</td>
<td></td>
<td>1-adamantyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>38</td>
<td>4-methoxyphenyl</td>
<td></td>
<td>1-adamantyl</td>
<td>O</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>39</td>
<td>phenyl</td>
<td>pentyl</td>
<td>1-adamantyl</td>
<td>S</td>
<td>CONH</td>
<td>N</td>
</tr>
<tr>
<td>40</td>
<td>phenyl</td>
<td>pentyl</td>
<td>cyclohexyl</td>
<td>S</td>
<td>CONH</td>
<td>N</td>
</tr>
</tbody>
</table>
obtained for compound 17 bearing a tert-butyl group. Replacing the methyl or tert-butyl groups by a phenyl resulted in a 5-fold enhancement of the affinity (e.g., 25 with a $K_i$ value of 4 nM), while selectivity was not altered. Introducing a chlorine atom in the para position of the phenyl ring resulted in a decrease in both selectivity and CB2 affinity (compare compounds 25 and 32). When considering their functionality, we noticed that compound 11 dose-dependently increased the $[^{35}S]$-GTPyS binding up to 148% with an EC$_{50}$ value of 5.5 nM, which means that this compound behaves as a partial agonist, whereas compound 17 behaves as a potent full agonist increasing $[^{35}S]$-GTPyS binding to the same extent as CP-55,940 (EC$_{50}$ = 12.2 nM and $E_{max}$ = 212%). More surprisingly, introducing a phenyl or a 4-chlorophenyl group in place of methyl or tert-butyl shifted the functionality from agonist to inverse agonist because both compounds 25 and 32 decreased $[^{35}S]$-GTPyS binding with $E_{max}$ values of 39% and 42%, respectively. Because the functionality switch observed within this series of compounds is an interesting feature, we decided to investigate whether the change in functionality we observed when replacing the C-6 tert-butyl substituent with a phenyl or 4-chlorophenyl substituent was dependent on the phenyl substituent. Therefore we synthesized four additional compounds characterized by differently substituted phenyls (35–38) and evaluated their affinity and functionality. These compounds show a similar affinity for the CB2 receptor when compared to 25, although they are marginally less selective (Table 2). When looking at their functionality, they too behave as inverse agonists decreasing $[^{35}S]$-GTPyS binding ($E_{max}$ values of 75 ± 2%, 88 ± 1%, and 75 ± 5% for 35, 36,

![Scheme 3](https://example-image-url.com)

**Scheme 3**

> Reagents and conditions: (i) DPPA, t-BuOK, t-BuOH, reflux, 12 h, 38%; (ii) (a) 5N HCl, isopropyl alcohol, rt, 14 h, (b) 10% NaOH, 95%; (iii) (a) cyclohexyl carboxylic acid, HOBt, HBTU, DIPEA, DMF, rt, 3 h, (b) 38, rt, 12 h, 38%.

Table 2. Affinities ($K_i$ values) of Compounds 11, 17–41, 43, and Reference Compounds (5, WIN-55,212-2, CP-55,940, and 1) towards hCB$_1$ and hCB$_2$ Cannabinoid Receptors

<table>
<thead>
<tr>
<th>Compd</th>
<th>$K_i$ (nM)</th>
<th>$K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>&gt;3000</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>17</td>
<td>&gt;3000</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>18</td>
<td>&gt;3000</td>
<td>29 ± 3</td>
</tr>
<tr>
<td>19</td>
<td>&gt;3000</td>
<td>414 ± 61</td>
</tr>
<tr>
<td>20</td>
<td>368 ± 84</td>
<td>14.3 ± 2.1</td>
</tr>
<tr>
<td>21</td>
<td>&gt;3000</td>
<td>&gt;3000</td>
</tr>
<tr>
<td>22</td>
<td>626 ± 92</td>
<td>23.3 ± 1.9</td>
</tr>
<tr>
<td>23</td>
<td>454 ± 55</td>
<td>6.6 ± 0.7</td>
</tr>
<tr>
<td>24</td>
<td>&gt;1000</td>
<td>18.4 ± 1.3</td>
</tr>
<tr>
<td>25</td>
<td>592 ± 97</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>26</td>
<td>596 ± 75</td>
<td>13.5 ± 0.9</td>
</tr>
<tr>
<td>27</td>
<td>929 ± 131</td>
<td>10.6 ± 1.1</td>
</tr>
<tr>
<td>28</td>
<td>131 ± 20</td>
<td>10.1 ± 1.1</td>
</tr>
<tr>
<td>29</td>
<td>&gt;1000</td>
<td>369 ± 41</td>
</tr>
<tr>
<td>30</td>
<td>&gt;3000</td>
<td>92 ± 7</td>
</tr>
<tr>
<td>31</td>
<td>&gt;3000</td>
<td>25.8 ± 3</td>
</tr>
<tr>
<td>32</td>
<td>&gt;1000</td>
<td>79 ± 7</td>
</tr>
<tr>
<td>33</td>
<td>&gt;1000</td>
<td>78 ± 9</td>
</tr>
<tr>
<td>34</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
</tr>
<tr>
<td>35</td>
<td>51.2 ± 6</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>36</td>
<td>34.4 ± 6</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>37</td>
<td>134 ± 19</td>
<td>13.2 ± 1.6</td>
</tr>
<tr>
<td>38</td>
<td>384 ± 56</td>
<td>91.1 ± 14</td>
</tr>
<tr>
<td>39</td>
<td>&gt;1000</td>
<td>18.8 ± 2.3</td>
</tr>
<tr>
<td>40</td>
<td>&gt;1000</td>
<td>48 ± 4</td>
</tr>
<tr>
<td>41</td>
<td>&gt;3000</td>
<td>209 ± 33</td>
</tr>
<tr>
<td>42</td>
<td>&gt;3000</td>
<td>51 ± 4</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>51.7 ± 4.8</td>
</tr>
<tr>
<td>WIN-55,212-2</td>
<td>ND</td>
<td>9.1 ± 0.8</td>
</tr>
<tr>
<td>CP-55,940</td>
<td>ND</td>
<td>15.4 ± 1.4</td>
</tr>
<tr>
<td>1</td>
<td>ND</td>
<td>20.3 ± 2.6</td>
</tr>
</tbody>
</table>

$^a$ The results are expressed as mean ± SEM of at least four experiments performed in duplicate. Basal constitutive activity of the receptor has been set at a value of 100%. $E_{max}$ values between 100% and 200% indicated that the compound behaves as a partial agonist. $E_{max}$ values around 200% indicated that the compound behaves as a full agonist. $E_{max}$ values under 100% indicated that the compound behaves as an inverse agonist.

Table 3. Potency (EC$_{50}$) and Maximal Stimulation ($E_{max}$) of Selected Compounds and Reference Ligands on hCB$_2$ Cannabinoid Receptor

<table>
<thead>
<tr>
<th>Compd</th>
<th>EC$_{50}$ (nM)</th>
<th>$E_{max}$ (%) $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>5.5 ± 1</td>
<td>148 ± 2</td>
</tr>
<tr>
<td>17</td>
<td>12.2 ± 2.5</td>
<td>212 ± 3</td>
</tr>
<tr>
<td>18</td>
<td>7.8 ± 3.5</td>
<td>135 ± 3</td>
</tr>
<tr>
<td>20</td>
<td>6.5 ± 1.5</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>22</td>
<td>10.9 ± 2.8</td>
<td>37 ± 1</td>
</tr>
<tr>
<td>24</td>
<td>5.8 ± 1.1</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>25</td>
<td>7.4 ± 0.9</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>26</td>
<td>3.2 ± 1.6</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>27</td>
<td>8.7 ± 0.9</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>29</td>
<td>1.9 ± 0.5</td>
<td>62 ± 2</td>
</tr>
<tr>
<td>30</td>
<td>1.9 ± 0.4</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>32</td>
<td>60 ± 16</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>33</td>
<td>26 ± 6</td>
<td>48 ± 2</td>
</tr>
<tr>
<td>34</td>
<td>14 ± 4</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>36</td>
<td>23 ± 6</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>37</td>
<td>22 ± 4</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>38</td>
<td>158 ± 42</td>
<td>42 ± 3</td>
</tr>
<tr>
<td>39</td>
<td>17 ± 2</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>40</td>
<td>27 ± 3</td>
<td>45 ± 1</td>
</tr>
<tr>
<td>41</td>
<td>164 ± 28</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>43</td>
<td>25 ± 3</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>5</td>
<td>1.8 ± 0.9</td>
<td>21.6 ± 2.7</td>
</tr>
<tr>
<td>WIN-55,212-2</td>
<td>24.8 ± 1.6</td>
<td>207.1 ± 10.1</td>
</tr>
<tr>
<td>CP-55,940</td>
<td>6.1 ± 2.1</td>
<td>230.5 ± 13.7</td>
</tr>
<tr>
<td>1</td>
<td>145.6 ± 3</td>
<td>201.4 ± 7.5</td>
</tr>
</tbody>
</table>

$^a$ The results are expressed as mean ± SEM of nonlinear analysis of competition curves using [1H]-SR141716A and [1H]-CP-55,940 as radioligands for hCB$_1$ and hCB$_2$ cannabinoid receptors, respectively, and are expressed as mean ± SEM of at least four experiments performed in duplicate.

$^b$ Basal constitutive activity of the receptor has been set at a value of 100%.
and 37, respectively), although the 4-methoxyphenyl substituted 4-oxo-1,4-dihydropyridine behaves as a partial agonist ($E_{\text{max}} = 148 \pm 4\%$). Taken together, these observations suggest that the C-6 substituent of the 4-oxo-1,4-dihydropyridine ring appears to be crucial for the control of functionality at CB$_2$ receptor and also modulates affinity, efficacy, and selectivity.

Because derivative 25 combines a good affinity with the highest selectivity, we decided to retain the C-6 phenyl moiety and to develop a series of CB$_2$ inverse agonists with various N-1 and 3-carboxamido substituents. Therefore, the replacement of the n-pentyl chain by n-ethyl, n-butyl, or n-hexyl groups resulted in moderate to strong reduction of the affinity (see compounds 19, 24, 26) while introducing a phenyl (compound 21) completely abolished the CB$_2$ affinity. Taken together, these data, in accordance with our previous work, clearly emphasize that the affinity toward the CB$_2$ receptor is very sensitive to N-1 substituent, with n-pentyl chain being the preferred one.

Next, we investigated the modification of the amide substituent because it seems to affect affinity toward both cannabinoid receptors. It was shown for the 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives, we introduced a chiral center on the carboxamide function in order to assess the effect of stereoselectivity on the affinity, selectivity, and functionality of our inverse agonist series. Therefore, we prepared two compounds characterized by the 1-(1,2,3,4-tetrahydronaphthyl) moiety, 28 represents the (R) enantiomer and 29 the (S) enantiomer. The eutomer (compound 28) of this novel series exhibited more than 30-fold higher affinity for the CB$_2$ receptor than the distomer (compound 29). In accordance with our earlier work, a stereoselectivity is observed with the (R) enantiomer exhibiting a better affinity and efficacy than the (S) enantiomer. Furthermore, compound 28 as well as compound 27 are the most potent compounds of our series, with EC$_{50}$ values of 1.9 nM.

As expected, the 3-carboxamido substituent is an important parameter for the affinity, efficacy, and selectivity. However, and opposite to what we found for the C-6 position, this modification did not affect the functionality because, regardless the carboxamido substituent, all the compounds retained their respective functionality.

When looking at the amide link of 27, we found that the reverse amide 43 has 5-fold lower affinity as compared to the amide analogue. In a similar manner, the carbamate intermediate 41, which can be regarded as an analogue of compound 43 wherein its cyclohexyl moiety is replaced by a tert-butoxy group, displayed a lower affinity with a $K_i$ value of 209 nM at CB$_2$ and no affinity at CB$_1$.

We also introduced a thioke in place of the carbonyl of 4-oxo-1,4-dihydropyridine core, leading to compounds 39 and 40. Albeit this frequent substitution has already been proved to affect the affinity or functionality of some cannabinoid ligands, we found here that this modification resulted in a 5-fold reduction of the affinity and had no effect on the functionality.

**In Silico Insights from an Inactive State Model of the CB$_2$ Receptor**

The inactive state of the human CB$_2$ apo-receptor was built from the homologous crystal template of the human $\beta_2$-adrenergic receptor (Figure 1), as specified in the Experimental Section. Thus, the modeled CB$_2$ receptor should be in its inactive state because the human $\beta_2$-adrenergic receptor template used was cocrystallized with a high-affinity inverse agonist.

![Figure 1. Sequence alignment between the model human CB$_2$ receptor and the crystal template sequences. This JalView graphical representation shows the alignment between the whole CB$_2$ model sequence and the partial sequence (without N-terminal, C-terminal, and IL3 regions) of the X-ray $\beta_2$-adrenergic receptor (PDB 2RH1). The identical conserved amino acids and relevant CB$_2$ features Ser4.53, Ser4.57, and Tyr5.58 are respectively displayed as blue and red overlined residues. The TM domains deduced from crystal template are annotated in the “TM pred” section.](image-url)
As illustrated in Figure 2, the resulting ligand binding site is restricted by transmembrane domain (TM) III, IV, V, VI, and VII, as well as extracellular loops (EL) 2 and 3. The resulting docking poses of both compounds 17 and 25 revealed a consensual binding mode, as the five best ones were superimposed within a 1.5 Å root-mean-square deviation (rmsd) (Figure 3). Both compounds 17 and 25 are anchored by hydrogen bonds with Tyr5.39 (Tyr190) and Phe183 backbone of EL2. The pentyl chains spread out in a lipophilic pocket defined by Phe91, Phe94, Phe106, Ile110, Val113, and Leu182. The adamantyl groups fit in the extracellular region particularly EL2, whereas the C-6 tert-butyl or phenyl substituents of compounds 17 and 25, respectively, orient toward the bottom of the pocket defined by an aromatic cage including Phe3.36 (Phe117), Trp5.43 (Trp194), and Trp6.48 (Trp258). Trp6.48 is included in the CWXP pattern of helix 6 and is strictly conserved among the class A GPCRs. Trp6.48 and adjacent side chains have been shown to undergo conformational transitions as a “rotamer toggle switch” during the activation of rhodopsin\textsuperscript{52–54} and \(\beta\)-adrenergic receptors.\textsuperscript{55} This “rotamer toggle switch” has also been shown to be critical for the activation of the CB\(_2\) receptor with specific contacts between Phe3.36 and Trp6.48 in the inactive state, which are broken during the activation, leading to a \(\chi_1\) rotamer switch (F3.36 trans(180°)/W6.48 g\(+(-60°)) \rightarrow (F3.36 g\+, W6.48\) trans).\textsuperscript{56} As observed in the CB\(_2\) receptor bound to the compound 25 (Figure 3A), Phe3.36 and Trp6.48 form a \(\pi-\pi\) face-to-face interaction, whereas the C-6 phenyl substituent of compound 25 establishes edge-to-face aromatic interactions with Trp6.48. During the MD simulation, the placement of both ligands was examined by monitoring the distance between the C-6 substituents of the ligands and the side chain of Trp6.48 (Figure 4). Taking into account the distance between the closest carbon atoms in the minimized complex at \(t = 0\), the simulation shows that the distance implying the C-6 phenyl group of compound 25 tends to converge toward a distance of 4 Å, whereas the distance between the C-6 tert-butyl substituent of compound 17 and the side chain of Trp6.48 does not converge and varies between 5 and 8 Å. The preservation of the aromatic cluster between the C-6 substituent of compound 25 and Trp6.48 is also depicted by monitoring the receptor−ligand binding energy during the MD simulation (Figure 4). Indeed, the binding energy of the complex with compound 25 ranges from \(-65\) to \(-55\) kcal/mol and is thus more stabilizing.
the functionality from agonist to inverse agonist. Conversely, because replacing an alkyl group by a phenyl group switched controlling the functionality of this series of compounds heterocycle strongly impacts on both affinity and function-
ing assays showed that the nature of substituents around the ligands based on a 4-oxo-1,4-dihydropyridine scaffold. Bind-

Conclusion

We have synthesized a series of selective CB2 receptor ligands based on a 4-oxo-1,4-dihydropyridine scaffold. Binding assays showed that the nature of substituents around the heterocycle strongly impacts on both affinity and functionality. We have identified the C-6 substituent as crucial in controlling the functionality of this series of compounds because replacing an alkyl group by a phenyl group switched the functionality from agonist to inverse agonist. Conversely,

we demonstrate that substituents at N-1 and C-3 position are crucial for affinity but not for functionality. Using a β2-
adrenergic receptor-based CB2 receptor model, we suggest that the phenyl at C-6 confers the inverse agonist profile by blocking the χ₁ torsion of Trp6.48 side chain in its inactive conformation. Overall, the data presented here show that the 4-oxo-1,4-dihydropyridine ring is a highly effective scaffold for the design of new CB2 receptor ligands. Moreover, the novel selective CB2 ligands reported here will be useful tools for characterizing the functions of CB2 receptor.

Experimental Section

Chemistry. All commercial reagents and solvents were used without further purification. Analytical thin-layer chromatography was performed on precoated Kieselgel 60F254 plates (Merck); the spots were located by UV (254 and 366 nm) and/or with iodine. Silica gel 60 230–400 mesh purchased from Merck was used for column chromatography. Alumina silica gel (neutral) 150 mesh purchased from Sigma Aldrich was used for column chromatography for purification of compound 13c. Preparative thick-layer chromatography (TLC) was performed using silica gel from Merck, the compounds were extracted from the silica using CHCl₃/MeOH (8:2, v/v). All melting points were determined with a Büchi 535 capillary apparatus and remain uncorrected. ¹H NMR spectra were obtained using a Bruker 300 MHz spectrometer, chemical shifts (δ) are expressed in ppm relative to tetramethylsilane used as an internal standard, J values are in hertz, and the splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. All compounds were analyzed by HPLC-MS on a HPLC combined with a Surveyor MSQ (Thermo Electron) equipped with an APCI-source. All tested compounds showed a purity of >96% in APCI⁺ mode.

3-(Dimethylaminomethyl)-6-methyl-4-oxo-2-pyone 9 was prepared according to a procedure already described.⁴²,⁴³ Compound 9 crystallizes in a mixture of toluene/cyclohexane (2:8, v/v).

The ethyl 2-[(dimethylamino)methylene]-4-oxopyran-3-carboxylate and ethyl 4-oxo-6-phenyl-4-oxopyran-3-carboxylate) were obtained using the procedures previously described⁴⁴ with minor modifications. In our case, pyran-4-ones 13a—g were unstable on silica gel and could not be purified even by flash chromatography. We found that pyran-4-

Figure 4. Analysis of molecular dynamics simulations. Binding energy between 17 and 25 with the CB2 receptor was plotted (A) as well as the distance between the less buried carbon atom of Trp258 (W258) side chain and the more buried carbon atom of the ligand (B).

Figure 4. Analysis of molecular dynamics simulations. Binding energy between 17 and 25 with the CB2 receptor was plotted (A) as well as the distance between the less buried carbon atom of Trp258 (W258) side chain and the more buried carbon atom of the ligand (B). than the complex, including compound 17, for which the binding energy ranges from -55 to -45 kcal/mol.

Even though the duration of the simulation is not long enough to observe a conformational switch of Trp6.48 χ₁ torsion from the g(+)(-60°) toward its trans conformation (180°), these results suggest that the phenyl substituent at the C-6 position of compound 25 confers the inverse agonist profile by stabilizing the χ₁ torsion of Trp6.48 side chain in its inactive g(+) conformation and thus could prevent its transition toward the trans conformation thought to be essential for the receptor activation.

Experimental Section

Chemistry. All commercial reagents and solvents were used without further purification. Analytical thin-layer chromatography was performed on precoated Kieselgel 60F254 plates (Merck); the spots were located by UV (254 and 366 nm) and/or with iodine. Silica gel 60 230–400 mesh purchased from Merck was used for column chromatography. Alumina silica gel (neutral) 150 mesh purchased from Sigma Aldrich was used for column chromatography for purification of compound 13c. Preparative thick-layer chromatography (TLC) was performed using silica gel from Merck, the compounds were extracted from the silica using CHCl₃/MeOH (8:2, v/v). All melting points were determined with a Büchi 535 capillary apparatus and remain uncorrected. ¹H NMR spectra were obtained using a Bruker 300 MHz spectrometer, chemical shifts (δ) are expressed in ppm relative to tetramethylsilane used as an internal standard, J values are in hertz, and the splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. All compounds were analyzed by HPLC-MS on a HPLC combined with a Surveyor MSQ (Thermo Electron) equipped with an APCI-source. All tested compounds showed a purity of >96% in APCI⁺ mode.

3-(Dimethylaminomethyl)-6-methyl-4-oxo-2-pyone 9 was prepared according to a procedure already described.⁴²,⁴³ Compound 9 crystallizes in a mixture of toluene/cyclohexane (2:8, v/v).

The ethyl 2-[(dimethylamino)methylene]-3-oxobutanate 12, pyran-4-ones 13a (ethyl 6-tert-butyl-4-oxo-4H-pyran-3-carboxylate) and 13b (ethyl 4-oxo-6-phenyl-4H-pyran-3-carboxylate) and pyridin-4-one 14f (ethyl 4-oxo-1,6-diphenyl-1,4-dihydropyridine-3-carboxylate) were obtained using the procedures previously described⁴⁴ with minor modifications. In our case, pyran-4-ones 13a—g were unstable on silica gel and could not be purified even by flash chromatography. We found that pyran-4-

Figure 4. Analysis of molecular dynamics simulations. Binding energy between 17 and 25 with the CB2 receptor was plotted (A) as well as the distance between the less buried carbon atom of Trp258 (W258) side chain and the more buried carbon atom of the ligand (B).

Figure 4. Analysis of molecular dynamics simulations. Binding energy between 17 and 25 with the CB2 receptor was plotted (A) as well as the distance between the less buried carbon atom of Trp258 (W258) side chain and the more buried carbon atom of the ligand (B). than the complex, including compound 17, for which the binding energy ranges from -55 to -45 kcal/mol.

Even though the duration of the simulation is not long enough to observe a conformational switch of Trp6.48 χ₁ torsion from the g(+)(-60°) toward its trans conformation (180°), these results suggest that the phenyl substituent at the C-6 position of compound 25 confers the inverse agonist profile by stabilizing the χ₁ torsion of Trp6.48 side chain in its inactive g(+) conformation and thus could prevent its transition toward the trans conformation thought to be essential for the receptor activation.

Conclusion

We have synthesized a series of selective CB2 receptor ligands based on a 4-oxo-1,4-dihydropyridine scaffold. Binding assays showed that the nature of substituents around the heterocycle strongly impacts on both affinity and functionality. We have identified the C-6 substituent as crucial in controlling the functionality of this series of compounds because replacing an alkyl group by a phenyl group switched the functionality from agonist to inverse agonist. Conversely,
Ethyl 1-Alkyl-4-aryl-4-oxo-1,4-dihydropyridine-3-carboxylate (14a). A solution of pyrone ester (13a) (3 g, 13.4 mmol), n-pentylamine (3.1 mL, 26.7 mmol) in EtOH (60 mL) and acetic acid (AcOH) (40 mL) was refluxed for 4 h. The mixture was cooled to room temperature and the solvents were distilled off to leave a brown oil. Water was added and the product was extracted with CHCl₃. The crude oil was chromatographed on silica gel (dichloromethane/methanol 9:1, v/v); yellow oil (57%). 1H NMR (CDCl₃) δ 8.56 (s, 1H), 7.70 (d, 2H, J = 9.0 Hz), 7.0.0 (d, 2H, J = 9.0 Hz), 6.77 (s, 1H), 4.40 (q, 2H, J = 7.0 Hz), 3.88 (s, 3H), 1.39 (t, 3H, J = 7.0 Hz) LC-MS (APCI⁺) m/z 294.1 (MH⁺).  

Ethyl 4-Oxo-6-phenyl-1,4-dihydropyridine-3-carboxylate (14b). Purification by silica gel chromatography (dichloromethane/ethanol 1:1); beige solid (77%); mp 159 °C. 1H NMR (CDCl₃) δ 8.4 (s, 1H), 7.50 (m, 5H), 6.07 (s, 1H), 4.22 (q, 2H, J = 6.9 Hz), 3.81 (q, 2H, J = 7.4 Hz), 1.26 (t, 3H, J = 6.9 Hz), 1.04 (t, 3H, J = 7.4 Hz) LC-MS (APCI⁺) m/z 272.1 (MH⁺).  

Ethyl 4-Oxo-1-pentyl-6-phenyl-1,4-dihydropyridine-3-carboxylate (14d). Purification by silica gel chromatography (dichloromethane/ethyl acetate 1:1, v/v); brown oil (71%). 1H NMR (CDCl₃) δ 8.22 (s, 1H), 7.48 (m, 3H), 7.32 (m, 2H), 6.42 (s, 1H), 4.39 (q, 2H, J = 7.2 Hz), 3.74 (t, 2H, J = 7.6 Hz), 1.52 (m, 2H), 1.35 (t, 3H, J = 7.2 Hz), 1.22 (m, 2H), 0.75 (t, 3H, J = 7.4 Hz) LC-MS (APCI⁺) m/z 300.2 (MH⁺).  

Ethyl 4-Oxo-1-hexyl-6-phenyl-1,4-dihydropyridine-3-carboxylate (14e). Purification by silica gel chromatography (dichloromethane/ethyl acetate 1:1, v/v); brown oil (68%). 1H NMR (CDCl₃) δ 8.22 (s, 1H), 7.41 (m, 5H), 6.08 (s, 1H), 4.19 (q, 2H, J = 6.9 Hz), 3.93 (t, 2H, J = 7.3 Hz), 1.26–1.02 (m, 11H), 0.73 (t, 3H, J = 7.1 Hz) LC-MS (APCI⁺) m/z 314.2 (MH⁺).  

Ethyl 6-(4-Chlorophenyl)-4-oxo-1-pentyl-1,4-dihydropyridine-3-carboxylate (14f). Purification by silica gel chromatography (dichloromethane/methanol 9:1, v/v); yellow oil (75%). 1H NMR (CDCl₃) δ 8.22 (s, 1H), 7.50 (t, 2H, J = 8.8 Hz), 7.30 (d, 2H, J = 8.8 Hz), 6.40 (s, 1H), 4.40 (q, 2H, J = 7.1 Hz), 1.45 (t, 3H, J = 7.1 Hz) LC-MS (APCI⁺) m/z 348.2 (MH⁺).  

Ethyl 6-(3-Chlorophenyl)-4-oxo-1-pentyl-1,4-dihydropyridine-3-carboxylate (14h). Purification by silica gel chromatography (dichloromethane/methanol 9:1, v/v); yellow oil (63%). 1H NMR (CDCl₃) δ 8.22 (s, 1H), 7.48 (m, 2H), 7.34 (s, 1H), 7.24 (2H, J = 7.6 Hz), 6.41 (s, 1H), 4.40 (q, 2H, J = 7.1 Hz), 3.72 (t, 2H, J = 7.7 Hz), 1.55 (m, 2H), 1.40 (t, 3H, J = 7.1 Hz), 1.18 (m, 4H), 0.82 (t, 3H, J = 7.0 Hz) LC-MS (APCI⁺) m/z 348.1 (MH⁺).  

Ethyl 6-(2-Chlorophenyl)-4-oxo-1-pentyl-1,4-dihydropyridine-3-carboxylate (14i). Purification by silica gel chromatography (dichloromethane/methanol 9:1, v/v); yellow oil (45%). 1H NMR (CDCl₃) δ 8.10 (s, 1H), 7.17 (m, 4H), 6.12 (s, 1H), 4.10 (q, 2H, J = 7.1 Hz), 3.78 (t, 2H, J = 7.7 Hz), 1.32 (m, 2H), 1.12 (t, 3H, J = 7.1 Hz), 0.86 (m, 4H), 0.51 (t, 3H, J = 7.0 Hz) LC-MS (APCI⁺) m/z 348.1 (MH⁺).  

Ethyl 6-(4-Methoxyphenyl)-4-oxo-1-pentyl-1,4-dihydropyridine-3-carboxylate (14j). Purification by silica gel chromatography (dichloromethane/methanol 9:1, v/v); yellow oil (75%). 1H NMR (CDCl₃) δ 8.02 (s, 1H), 6.98 (q, 2H, J = 6.9 Hz), 6.10 (s, 1H), 4.06 (q, 2H, J = 7.1 Hz), 3.57 (t, 2H, J = 7.4 Hz), 2.11 (s, 3H), 1.27 (2H, J = 7.3 Hz), 1.07 (t, 3H, J = 7.1 Hz), 0.82 (m, 4H), 0.48 (t, 3H, J = 6.9 Hz) LC-MS (APCI⁺) m/z 328.2 (MH⁺).  

Ethyl 6-(4-Methoxypyphenyl)-4-oxo-1-pentyl-1,4-dihydropyridine-3-carboxylate (14k). Purification by silica gel chromatography (dichloromethane/methanol 9:1, v/v); yellow oil (57%). 1H NMR (CDCl₃) δ 8.15 (s, 1H), 7.20 (d, 2H, J = 8.8 Hz), 6.90 (d, 2H, J = 8.8 Hz), 6.31 (s, 1H), 4.29 (q, 2H, J = 7.1 Hz), 3.72 (t, 2H, J = 7.6 Hz), 3.79 (s, 3H), 1.52 (m, 2H), 1.34 (3H, J = 7.1 Hz), 1.06 (m, 4H), 0.72 (t, 3H, J = 6.7 Hz) LC-MS (APCI⁺) m/z 344.2 (MH⁺).  

Ethyl 1-Pentyl-6-phenyl-1-thioxo-1,4-dihydropyridine-3-carboxylate (15). A mixture of 14d (2.6 g, 8.3 mmol) and phosphorus pentasulfide (3.7 g, 16.6 mmol) was refluxed for 12 h in pyridine (80 mL). After cooling to room temperature, the solvent was removed under reduced pressure and the residue was partitioned in H₂O–EtOAc. The organic layer was washed with brine, dried, and concentrated under reduced pressure to leave a crude oil. The sample was purfied by silica gel chromatography (cyclohexane/ethanol ace 9:1, v/v) to give an orange oil (2.46 g, 90%). 1H NMR (DMSO-d₆) δ 8.22 (s, 1H), 7.54 (m, 5H), 7.06 (s, 1H), 4.26 (q, 2H, J = 7.0 Hz), 3.85 (t, 2H, J = 7.6 Hz), 1.45 (m, 2H), 1.28 (t, 3H, J = 7.0 Hz), 1.03–0.98 (m, 4H), 0.70 (t, 3H, J = 6.7 Hz) LC-MS (APCI⁺) m/z 330.8 (MH⁺).  

General Procedure for the Preparation of Carboxylic Acids (16a–h). Esters 14a–g and 15 were dissolved in ethanol and 2.5 N NaOH (v/v). The mixture was refluxed for 6 h. After cooling to room temperature, EtOH was removed under reduced pressure and the residue was dissolved in water and washed with EtOAc. The aqueous phase was acidified (1 N HCl, pH 2) and extracted with EtOAc. The combined organic extracts were washed with water and brine, dried over MgSO₄, and concentrated under reduced pressure to afford essentially pure carboxylic acids 16a–h.
1-Hexyl-4-oxo-6-phenyl-1,4-dihydropyridine-3-carboxylic Acid (16e). Purification by silica gel chromatography (dichloromethane/ethyl acetate 1:1, v/v); white powder (67%); mp 119 °C. 

1-Hexyl-4-oxo-6-phenyl-1,4-dihydropyridine-3-carboxylic Acid (16g). White powder (94%); mp 182 °C. 

1-Hexyl-4-oxo-6-phenyl-1,4-dihydropyridine-3-carboxylic Acid (16h). Beige powder (72%); mp 119 °C. 

1-Hexyl-4-oxo-6-phenyl-1,4-dihydropyridine-3-carboxylic Acid (16i). Yellow solid (66%); mp 153 °C. 

1-Hexyl-4-oxo-6-phenyl-1,4-dihydropyridine-3-carboxylic Acid (16j). White solid (85%); mp 73 °C. 

General Procedure for the Preparation of Carboxamides (11 and 17). A solution of carboxylic acid 10a and 16a in dry DMF were added N,N-diisopropylethylamine (DIEA) (3 equiv) and the coupling agents 1-hydroxybenzotriazole (HOBt) (0.5 equiv), 2-([1H-benzo[d]azol-1-yl]-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (1.5 equiv). The resulting mixture was stirred at room temperature until thin-layer chromatography showed the starting material to be consumed (ca. 3 h). The appropriate amine (1.5 equiv) was then added, and the solution was stirred at room temperature for 12 h. The solvent was removed under reduced pressure and the residue taken up in water and extracted with CH₂Cl₂. The organic phase was washed with saturated aqueous NaHCO₃ solution, with 1 N aqueous HCl, and water. The organic extract was dried over MgSO₄ and concentrated in vacuo to a brown oil. The crude material was purified by TLC using the appropriate eluent (dichloromethane/methanol 9:1, v/v) and re-crystallized in heptane or acetone (except for compound 30) to afford the titled compounds (11 and 17-40).
0.71 (t, 3H, J = 6.8 Hz). LC-MS (APCI<sup>+</sup>) m/z 453.3 (M<sup>+</sup>).

6-(4-Chlorophenyl)-N3-cyclohexyl-4-oxo-1-pentyl-1,4-dihydropyridine-3-carboxamide (33). White powder (32%); mp 157°C. ¹H NMR (DMSO-d<sub>6</sub>) δ 8.17 (s, 1H), 7.63 (d, 2H, J = 8.7 Hz), 7.15 (d, 2H, J = 8.7 Hz), 6.60 (s, 1H), 5.50 (m, 4H), 4.34 (s, 1H), 2.13 (m, 4H), 1.70 (m, 4H), 0.70 (t, 3H, J = 6.8 Hz). LC-MS (APCI<sup>+</sup>) m/z 453.2 (M<sup>+</sup>).

N3-(1-Adamantyl)-6-(3-chlorophenyl)-1-pentyl-4-oxo-1,4-dihydropyridine-3-carboxamide (35). Beige solid (62%); mp 178°C. ¹H NMR (CDCl<sub>3</sub>) δ 10.31 (s, 1H), 8.17 (s, 1H), 7.61 (d, 2H, J = 8.7 Hz), 7.55 (d, 2H, J = 8.7 Hz), 6.29 (s, 1H), 3.92 (t, 2H, J = 7.6 Hz), 3.82 (m, 1H), 1.69 (m, 6H), 1.42 (m, 2H), 1.05 (m, 4H), 0.71 (t, 3H, J = 6.8 Hz). LC-MS (APCI<sup>+</sup>) m/z 432.3 (M<sup>+</sup>),

N3-(1-Adamantyl)-6-(4-methylphenyl)-1-pentyl-4-oxo-1,4-dihydropyridine-3-carboxamide (37). White solid (73%); mp 210°C. ¹H NMR (CDCl<sub>3</sub>) δ 10.19 (s, 1H), 8.50 (s, 1H), 7.30 (d, 2H, J = 8.4 Hz), 7.22 (d, 2H, J = 8.1 Hz), 6.43 (s, 1H), 3.78 (t, 2H, J = 7.6 Hz), 2.43 (s, 3H), 2.16 (m, 1H), 1.72 (m, 6H), 1.58 (m, 2H), 1.13 (m, 4H), 0.79 (t, 3H, J = 6.8 Hz). LC-MS (APCI<sup>+</sup>) m/z 443.3 (M<sup>+</sup>),

N3-(1-Adamantyl)-6-(4-methoxymethylphenyl)-1-pentyl-4-oxo-1,4-dihydropyridine-3-carboxamide (38). White solid (73%); mp 144°C. ¹H NMR (CDCl<sub>3</sub>) δ 10.19 (s, 1H), 8.50 (s, 1H), 7.24 (d, 2H, J = 8.7 Hz), 7.01 (d, 2H, J = 8.7 Hz), 6.43 (s, 1H), 3.88 (s, 3H), 3.79 (t, 2H, J = 7.6 Hz), 2.16 (m, 9H), 1.70 (m, 8H), 1.14 (m, 4H), 0.80 (t, 3H, J = 6.8 Hz). LC-MS (APCI<sup>+</sup>) m/z 449.3 (M<sup>+</sup>),

N3-(1-Adamantyl)-1-pentyl-6-phenyl-4-thioxo-1,4-dihydropyridine-3-carboxamide (39). Yellow solid (56%); mp 163°C. ¹H NMR (CDCl<sub>3</sub>) δ 11.41 (s, 1H), 8.75 (s, 1H), 7.60 (s, 1H), 7.54 (m, 3H), 7.34 (m, 2H), 3.88 (t, 2H, J = 7.7 Hz), 2.22 (m, 9H), 1.75–1.56 (m, 8H), 1.13 (m, 4H), 0.79 (t, 3H, J = 7.0 Hz). LC-MS (APCI<sup>+</sup>) m/z 435.3 (M<sup>+</sup>),

N3-(Cyclohexyl)-1-pentyl-6-phenyl-4-thioxo-1,4-dihydropyridine-3-carboxamide (40). Yellow solid (59%); mp 119°C. ¹H NMR (CDCl<sub>3</sub>) δ 11.53 (d, 1H, J = 7.3 Hz), 8.76 (s, 1H), 7.60 (s, 1H), 7.53 (m, 3H), 7.35 (m, 2H), 4.09 (m, 1H), 3.91 (t, 2H, J = 7.7 Hz), 1.97 (m, 2H), 1.80–1.59 (m, 10H), 1.13 (m, 4H), 0.82 (t, 3H, J = 6.8 Hz). LC-MS (APCI<sup>+</sup>) m/z 383.3 (M<sup>+</sup>),

tert-Butyl 4-Oxo-1-pentyl-6-phenyl-1,4-dihydropyridin-3-yl carbonate (41). To a solution of carboxylic acid 16d (0.4 g, 1.4 mmol) in tert-butyl alcohol (20 mL) were added under nitrogen atmosphere, potassium tert-butoxide (0.17 g, 1.7 mmol) and diphenylphosphoryl azide (0.36 mL, 1.7 mmol). The mixture was refluxed for 12 h, cooled to room temperature, and diluted with EtOAc. The organic phase was washed with saturated aqueous NaHCO<sub>3</sub> solution and brine, dried over MgSO<sub>4</sub>, and concentrated under reduced pressure to a yellowish oil. The latter was purified by silica gel chromatography (dichloromethane/methanol 95:5 v/v) to afford pure 41 (0.17 g, 38%) as a yellow oil. ¹H NMR (DMSO-d<sub>6</sub>) δ 8.9 (s, 1H), 7.74 (s, 1H), 7.56–7.43 (m, 7H), 7.12 (s, 1H), 5.12 (s, 1H), 3.81 (t, 2H, J = 7.6 Hz), 1.48 (m, 11H), 1.03 (m, 4H), 0.69 (t, 3H, J = 6.8 Hz). LC-MS (APCI<sup>+</sup>) m/z 357.1 (M<sup>+</sup>),

Amino-1-pentyl-2-phenyl-1H-pyrindine-4-one (42). To a solution of 5N hydrochloric acid in isopropyl alcohol (20 mL) was added the carbamate 41 (0.1 g, 0.28 mmol). The mixture was stirred at room temperature for 14 h and then concentrated under reduced pressure. The resulting solid was solubilized in water and washed with EtOAc. The aqueous phase was alkalized with 10% NaOH and extracted with EtOAc. The organic phase was dried over MgSO<sub>4</sub> and the solvent removed under reduced pressure to afford pure 42 as a white solid (0.068 g, 95%); mp 98°C. ¹H NMR (DMSO-d<sub>6</sub>) δ 7.48–7.38 (m, 5H), 7.19 (s, 1H), 5.82 (s, 1H), 4.67 (s, 2H), 3.67 (t, 2H, J = 7.3 Hz), 1.46 (m, 2H), 1.04 (m, 4H), 0.72 (t, 3H, J = 6.7 Hz). LC-MS (APCI<sup>+</sup>) m/z 257.1 (M<sup>+</sup>),

Pharmacology. hCB<sub>1</sub> and hCB<sub>2</sub> membranes of CHO cells were purchased from PerkinElmer. Fatty acid free bovine serum albumin (BSA) was purchased from Sigma Chemical Co. (St. Louis, MO). WIN-55,212-2 was purchased from RBI (Natick, MA), HU-210 and CP-55,940 from Tocris (Bristol, UK), and SR141716A and 5 were kindly donated by Sanofi Recherche (Montpellier, France). [³H]-SR141716A (52 Ci/mol) was from Amersham (Roosendaal, The Netherlands). [³H]-CP-55,940 (101 Ci/mol) was from NEN Life Science (Zaventem, Belgium), and HU-210 was from Tocris (Bristol, UK). Glass fiber filters were purchased from Whatman (Maidstone, UK), while Aqu alumina was from PerkinElmer (Schaebers, The Netherlands). [³H]-GTPyS (1173 Ci/mmol) was from Amersham (Roosendaal, The Netherlands).

Competition Binding Assay. Stock solutions of the compounds were prepared in DMSO and further diluted (100×) with the binding buffer to the desired concentration. Final DMSO concentrations in the assay were less than 0.1%. The
competitive binding experiments were performed as described earlier. Briefly [3H]-SR141716A (1 nM) or [3H]-CP-55,940 (1 nM) as radioligands for the hCB1 and the hCB2 cannabinoid receptors respectively, were added to 40 μg of membranes resuspended in 0.5 mL (final volume) binding buffer (50 mM Tris-HCl, 3 mM MgCl2, 1 mM EDTA, 0.5% bovine serum albumine, pH 7.4). After 1 h at 30 °C, the incubation was stopped and the solutions were rapidly filtered through 0.5% PEl pretreated GF/B glass fiber filters on a M-48T Brandell cell harvester and washed twice with 5 mL of ice-cold binding buffer without serum albumin. The radioactivity on the filters was counted as mentioned above. Assays were performed in triplicate. The nonspecific binding activity of the receptor has been set at a value of 100%; reported values were calculated from the IC50 , based on the Cheng-Prus- 

Data Analysis. IC50 and EC50 values were determined by nonlinear regression analysis performed using the GraphPad prism 4.0 program (GraphPad Software, San Diego). The Kᵢ values were calculated from the IC50, based on the Cheng–Prus-off equation: \( \text{Ki} = \text{IC50}/(1 + L/K_d) \). Statistical significance of [35S]-GTPyS assay results was assessed using a one-way ANOVA followed by a Dunnett post-test.

Homology Modeling of the Inactive Human CB2 Apo-Receptor. Sequence homology rates are very comparable between each GPCR crystal and CB2 sequences (44%, 46%, 44%, and 46% in conserved pattern and 40%, 37%, 33%, and 39% in whole sequence) in bovine rhodopsin, turkey β1-adrenergic receptor, human β2-adrenergic receptor, and α2A adenosine receptor, respectively). However, the two serine residues 4.53 (161) and 4.57 (165), known as critical for the binding of 5.58 were simultaneously conserved only in the sequences of the β-adrenergic receptors. The human β2-adrenergic receptor (PDB 2RH1) was selected as the crystal template for homology modeling because it has the advantage of conserving the tyrosine residue in position 5.58 (209) of the critical TM V instead of an alanine because it has the advantage of conserving the tyrosine residue. This work was financially supported by a grant from the Nord-Pas-de-Calais Regional Council and University of Lille 2.

References
