

## Novel 4-Oxo-1,4-dihydroquinoline-3-carboxamide Derivatives as New CB<sub>2</sub> Cannabinoid Receptors Agonists: Synthesis, Pharmacological Properties and Molecular Modeling

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Recent data indicated that the CB<sub>2</sub> cannabinoid receptor constitutes an attractive drug target due to its potential functional role in several physiological and pathological processes. A set of 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives, characterized by the presence of some important structural requirements exhibited by other classes of cannabinoid ligands, such as an aliphatic or aromatic carboxamide group in position 3, and an alkyl or benzyl group in position 1, was synthesized and assayed to measure their respective affinity for both human CB<sub>1</sub> and CB<sub>2</sub> cannabinoid receptors. The results indicate that these 3-carboxamido-quinolones derivatives exhibited a CB<sub>2</sub> receptor selectivity, particularly derivatives **28–30**, and **32R**. Moreover, in the [<sup>35</sup>S]-GTPγS binding assay, all the compounds behaved as CB<sub>2</sub> receptor agonists. Molecular modeling studies showed that compound **30** interacts with the CB<sub>2</sub> receptor through a combination of hydrogen bond and aromatic/hydrophobic interactions. In conclusion, 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives constitute a new class of potent and selective CB<sub>2</sub> cannabinoid receptors agonists.

### Introduction

Hashish and marijuana, two preparations derived from the Indian hemp *Cannabis Sativa* L., have been used since immemorial time for their medicinal and psychoactive properties. Despite the isolation of Δ<sup>9</sup>-tetrahydrocannabinol (Δ<sup>9</sup>-THC, Chart 1) as the major psychoactive component of *Cannabis Sativa* L.<sup>1</sup> in the 1960s, the molecular targets of Δ<sup>9</sup>-THC were only discovered in the late 1980s, with on one hand the help of a synthetic radioligand [<sup>3</sup>H]-CP-55,940<sup>2</sup> (Chart 1) allowing suitable cannabinoid receptor binding assays and, on the other hand, the characterization of the biochemistry and the molecular biology of cannabinoid receptors, which led to two subtypes of G-protein coupled receptors named CB<sub>1</sub><sup>3</sup> and CB<sub>2</sub><sup>4</sup> cannabinoid receptors. They differ in sequences, tissue localization, and, to some extent, signal transduction mechanisms. In addition, generation of knock-out mice models where either one subtype or two subtypes genes have been deleted suggest the putative existence of additional cannabinoid receptors.<sup>5</sup>

The cannabinoid CB<sub>1</sub> receptor is nowadays extensively studied due to its implication both in the therapeutic and psychoactive effects of cannabinoids in the central nervous system. Transduction mechanisms of cannabinoid CB<sub>1</sub> receptors involve inhibition of cAMP production through inhibition of adenylate cyclase,<sup>6</sup> inhibition of calcium influx,<sup>7,8</sup> activation of potassium channels,<sup>9</sup> and activation of the MAP kinase pathway.<sup>10</sup>

Promising selective cannabinoid CB<sub>1</sub> receptor antagonists such as rimonabant or SLV-319 are currently under investigation in clinical human studies for the treatment of obesity and the

associated metabolic syndrome.<sup>11</sup> On the contrary, Δ<sup>9</sup>-THC and nabilone are currently marketed to reduce emesis and/or prevent cachexia in AIDS or cancer patients.<sup>12</sup> Other therapeutic applications currently studied include multiple sclerosis, neuropathic, and cancer pain.

Despite the discovery by sequence homology of the CB<sub>2</sub> cannabinoid receptor in the same years, the physiological as well as putative therapeutic potential of this receptor largely remains unexplored. However, recent data indicate that CB<sub>2</sub> cannabinoid receptors participate in the control of peripheral pain,<sup>13,14</sup> inflammation,<sup>15</sup> and cancer proliferation.<sup>16</sup> It also has an antifibrogenic role in the liver.<sup>17</sup> Moreover, the recent discovery of the presence of the CB<sub>2</sub> cannabinoid receptors in the brain microglial cells<sup>18,19</sup> gave a rationale for prevention of Alzheimer's disease pathology by cannabinoid agents. Indeed, it was recently shown that CB<sub>2</sub> cannabinoid receptor agonists might provide neuroprotection by blockade of microglial activation.<sup>20</sup>

Both types of cannabinoid receptors are sensitive to pertussis toxin, suggesting their predominant coupling to G<sub>i</sub>-type proteins allowing the use of [<sup>35</sup>S]-GTPγS assay<sup>21</sup> to characterize the functionality of cannabinoid ligands. Cannabinoid receptors are activated by endocannabinoids, long-chain polyunsaturated fatty acids where the carboxylate group is either amidated by ethanolamine or esterified by glycerol.<sup>22</sup> The two major representatives are anandamide (*N*-arachidonylethanolamine) and 2-AG (2-arachidonoylglycerol) (Chart 1), respectively.

On a pharmacological point of view, up to now, the cannabinoid ligands might be divided into three major categories: (a) compounds which are not, or poorly, selective for one cannabinoid receptor subtype such as classical cannabinoids (Δ<sup>9</sup>-THC and related compounds), nonclassical cannabinoids (e.g., CP-55,940 and derivatives), and aminoalkylindoles (e.g., WIN-55,212-2); (b) compounds which are selective for the cannabinoid CB<sub>1</sub> receptor subtype such as some biarylpyrazoles (e.g., SR-141716A) and many other diverse heterocyclic structures

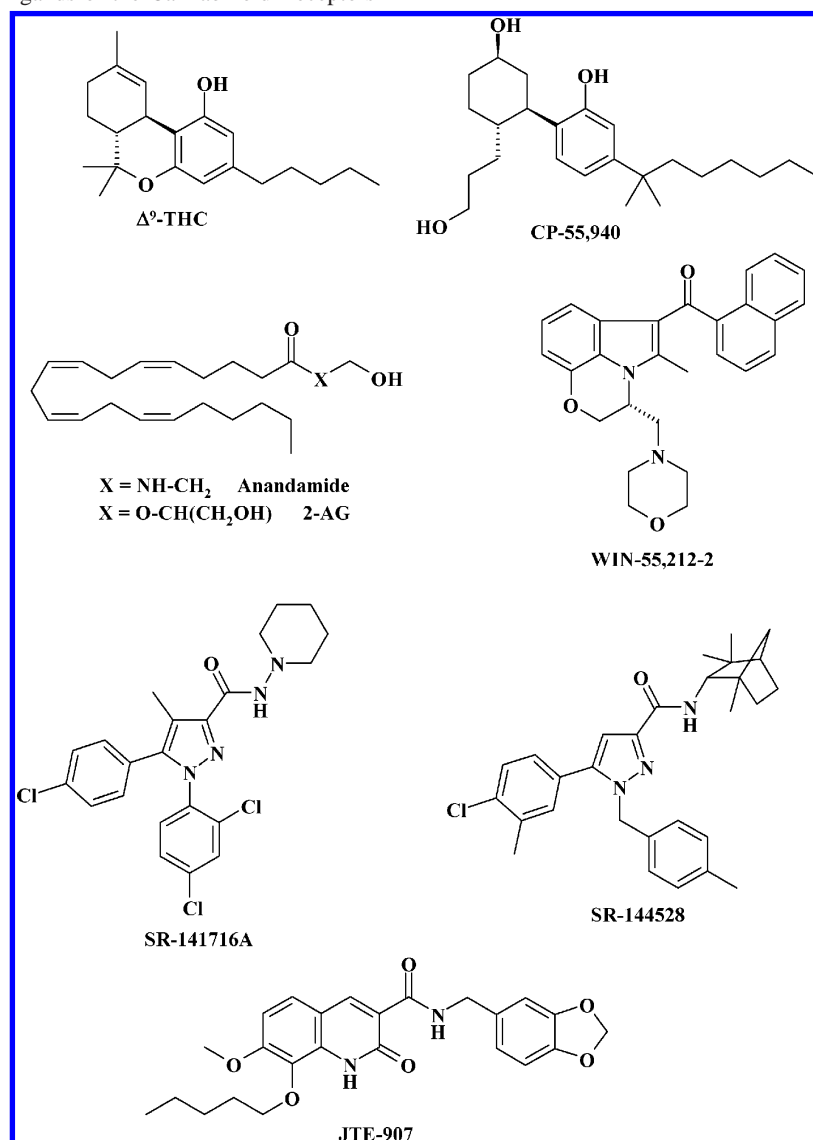
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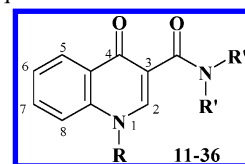
**Chart 1.** Representative Ligands of the Cannabinoid Receptors

such as triazoles,<sup>23</sup> thiazoles,<sup>24</sup> imidazoles,<sup>24,25</sup> imidazolidinediones,<sup>26</sup> 2-thioxoimidazolidinones,<sup>27</sup> and pyridines<sup>28</sup> that have been recently reported; and (c) a few compounds that are selective for the CB<sub>2</sub> cannabinoid receptor subtype such as some biarylpyrazoles (e.g., SR-144528), 1,2-dihydroquinoline-3-carboxamide (JTE-907, Chart 1),<sup>29</sup> 1,8-naphthyridines,<sup>30</sup> and triaryl bis-sulfones.<sup>31</sup>

At present, new potent CB<sub>2</sub>-selective cannabinoid receptor ligands are important to understand some of the physiological effects of cannabinoids, such as their immunosuppressive, anti-inflammatory, and antinociceptive activities.<sup>32–37</sup> For instance, it was very recently shown that low doses of Δ<sup>9</sup>-THC could reduce atherosclerosis in mice by acting at the CB<sub>2</sub> cannabinoid receptor.<sup>38</sup> Finally but not least, cannabinoid agonists that selectively target CB<sub>2</sub> cannabinoid receptors should be devoid of psychoactive effects.

Along this line, the present paper describes the synthesis and the pharmacological properties of a set of 27 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives, with various substitutions on the heterocyclic nucleus, as illustrated in Chart 2.

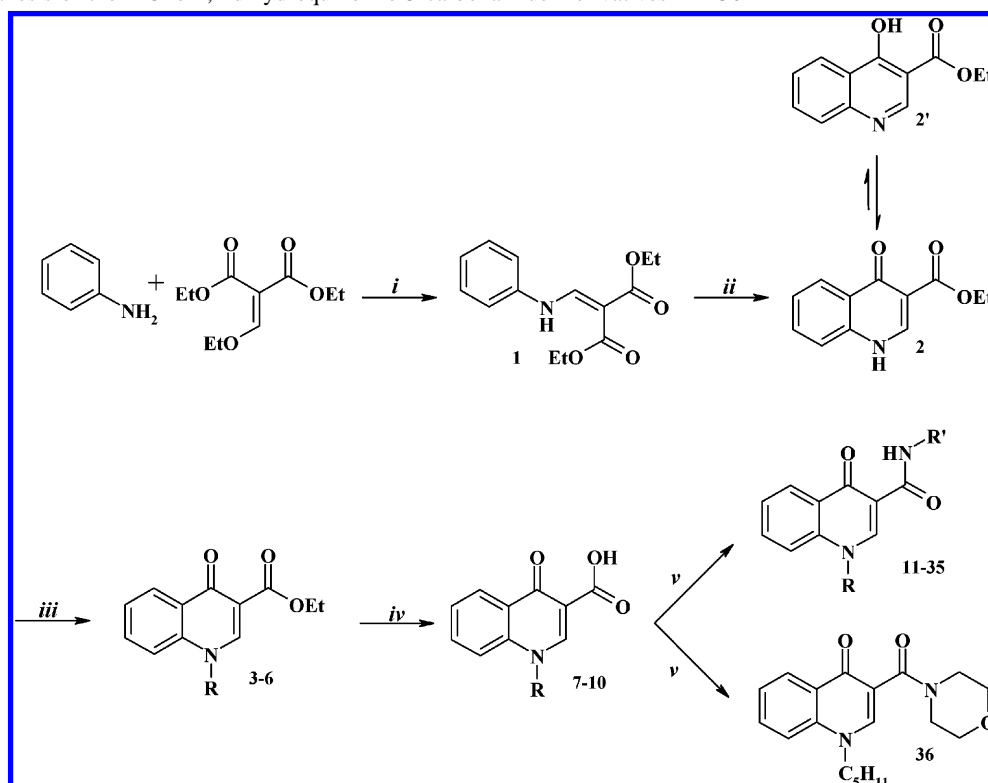
Aliphatic or aromatic carboxamide groups in position 3 have been selected on the basis of other cannabinoid pharmacophores—such as those present both in the quinolone derivative JTE-907 and in the biarylpyrazole SR-144528—and a hydrophobic

**Chart 2.** General Structure of 4-Oxo-1,4-dihydroquinoline-3-carboxamide Derivatives **11–36**

substituent in position 1 as in the aminolakylindole derivatives.<sup>39</sup> They were tested in competitive binding assays toward both human CB<sub>1</sub> (hCB<sub>1</sub>) and CB<sub>2</sub> (hCB<sub>2</sub>) cannabinoid receptors expressed in CHO cells<sup>40</sup> and were found selective for the CB<sub>2</sub> cannabinoid receptor subtype. For compounds exhibiting a *K<sub>i</sub>* value lower than 1 μM, [<sup>35</sup>S]-GTPγS binding assays were performed to determine their functionality.<sup>32</sup> Additionally, overlay of WIN-55,212-2, CP-55,940, and **30** allowed us to study their structural similarities. Docking was finally performed to elucidate the mode of interaction of compound **30** with a CB<sub>2</sub> cannabinoid receptor model.

## Results

**Chemistry.** The synthetic routes to obtain the target 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives **11–36** are outlined in Scheme 1. All substituents are summarized in Table

**Scheme 1.** Synthesis of the 4-Oxo-1,4-dihydroquinoline-3-carboxamide Derivatives 11–36<sup>a</sup>

<sup>a</sup> Reagents and conditions: (i), 100 °C, 91%; (ii) Ph–O–Ph, reflux, 77%; (iii) R–X, NaH, DMF, 90 °C, 50–93%; (iv) NaOH, EtOH, 100 °C, 70–76%; (v) R'–NH<sub>2</sub> or morpholine, PyBROP, PS–HOBt (HL), DIEA, DMF, rt, 14–97%.

1. Compounds **11–36** were obtained by a coupling reaction between selected amines and 4-oxo-1,4-dihydroquinoline-3-carboxylic acids **7–10** obtained in three steps following Gould–Jacobs' procedure.<sup>41,42</sup>

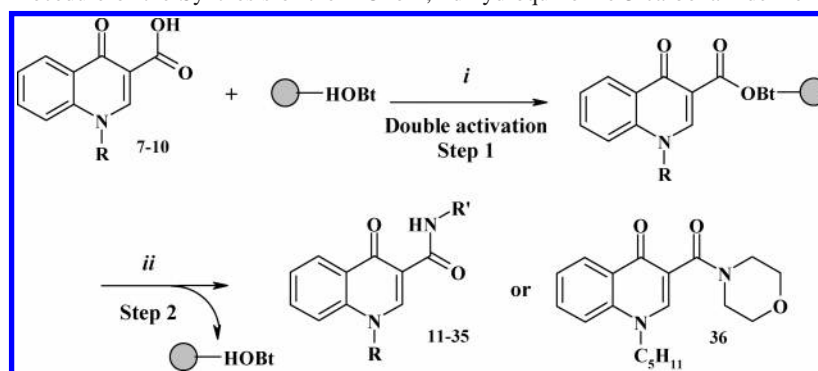
To afford the 4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester **2**, extreme conditions (i.e., reflux of **1** at ~ 255 °C in diphenyl ether<sup>43</sup>) were required to induce cyclization. In this process, as observed by <sup>1</sup>H NMR, the 4-quinolone form **2**, and not its 4-hydroxy-quinoline tautomeric form **2'**, was obtained. *N*-alkylation of **2** in anhydrous DMF with appropriate alkyl bromides or benzylbromide in the presence of sodium hydride gave the 1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl esters **3–6**.<sup>44</sup> Hydrolysis in 10% aqueous NaOH yielded the corresponding 1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acids **7–10**.<sup>45</sup>

To obtain some chemical diversity among 1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxamides, a solid phase procedure was set up using a previously reported polystyrene-supported HOBt as coupling reagent.<sup>46,47</sup> The reaction performed on a Quest 205 synthesizer according to the general reaction pathway outlined in Scheme 2 generated compounds **11–36**. This is a two-step procedure: (i) formation of the polymer bound activated ester of the carboxylic acid (**7–10**) using bromotrispyrrolidinophosphonium hexafluorophosphate (PyBROP); (ii) release of the target amide in solution by addition of the amine. Optimization of both steps was performed to obtain a solution of amide free of byproducts. The best conditions for activation were found to be 2 × 3 h activating time step in DMF. The coupling step was achieved in 24 h with 0.9 equiv of the amine in a DMF solution. The target compounds **11–36** were purified either by recrystallization or by preparative thick-layer chromatography. The *N*(1) alkylation was confirmed by 2D <sup>1</sup>H NMR (ROESY) as evidenced by the presence of cross-peaks between H(2) and N–CH<sub>2</sub> protons.

**Table 1.** Structures and Percentages of Displacement of [<sup>3</sup>H]-SR-141716A and [<sup>3</sup>H]-CP-55,940, Respectively, by Compounds **8–36** (10 μM) on hCB<sub>1</sub> and hCB<sub>2</sub> Cannabinoid Receptors<sup>a</sup>

			% of displacement	
compd	R'	R	CB <sub>1</sub> –R	CB <sub>2</sub> –R
<b>8</b>		<i>n</i> -pentyl	<20	<15
<b>11</b>	4-methoxyphenyl	<i>n</i> -butyl	<20	50.5 ± 6.9
<b>12</b>	4-methoxyphenyl	<i>n</i> -pentyl	<20	60.1 ± 7.6
<b>13</b>	4-methoxyphenyl	<i>n</i> -hexyl	<10	28.2 ± 8.0
<b>14</b>	1-naphthyl	<i>n</i> -butyl	45.3 ± 7.4	85.4 ± 6.3
<b>15</b>	1-naphthyl	<i>n</i> -pentyl	65.9 ± 6.8	90.6 ± 1.3
<b>16</b>	1-naphthyl	<i>n</i> -hexyl	53.3 ± 7.4	80.0 ± 6.6
<b>17</b>	benzyl	<i>n</i> -butyl	33.6 ± 6.5	44.9 ± 6.7
<b>18</b>	benzyl	<i>n</i> -pentyl	50.5 ± 5.5	66.7 ± 8.1
<b>19</b>	benzyl	<i>n</i> -hexyl	37.4 ± 4.0	54.6 ± 6.5
<b>20</b>	4-methoxybenzyl	<i>n</i> -pentyl	<10	51.7 ± 4.3
<b>21</b>	2-naphthyl	<i>n</i> -pentyl	<10	63.9 ± 4.1
<b>22</b>	2-phenylethyl	<i>n</i> -pentyl	64.8 ± 5.1	98.2 ± 2.9
<b>23</b>	3-phenylpropyl	<i>n</i> -pentyl	32.8 ± 4.8	85.5 ± 6.0
<b>24</b>	3,4-dichlorophenyl	<i>n</i> -pentyl	<10	68.1 ± 3.0
<b>25</b>	4-cyanophenyl	<i>n</i> -pentyl	49.6 ± 3.3	79.4 ± 6.0
<b>26</b>	4-biphenyl	<i>n</i> -pentyl	<20	<20
<b>27</b>	2-(benzo[1,3]dioxol-5-yl)ethyl	<i>n</i> -pentyl	43.6 ± 2.0	88.7 ± 1.7
<b>28</b>	1-adamantyl	<i>n</i> -pentyl	59.0 ± 3.9	104.0 ± 3.9
<b>29</b>	2-adamantyl	<i>n</i> -pentyl	37.8 ± 3.3	103.8 ± 1.8
<b>30</b>	1-(3,5-dimethyl)adamantyl	<i>n</i> -pentyl	54.6 ± 2.4	95.6 ± 5.4
<b>31</b>	1-(3,5-dimethyl)adamantyl	benzyl	32.5 ± 1.4	86.3 ± 2.1
<b>32</b>	( <i>RS</i> )-1-phenylethyl	<i>n</i> -pentyl	68.3 ± 3.1	101.1 ± 3.2
<b>33</b>	( <i>RS</i> )-1-(2-naphthyl)ethyl	<i>n</i> -pentyl	<10	82.9 ± 3.7
<b>34</b>	( <i>RS</i> )-1-(1-naphthyl)ethyl	<i>n</i> -pentyl	35.5 ± 3.7	98.9 ± 4.1
<b>35</b>	( <i>RS</i> )-1-(1,2,3,4-tetrahydro-naphthyl)	<i>n</i> -pentyl	79.7 ± 4.1	96.5 ± 4.0
<b>36</b>		<i>n</i> -pentyl	<10	36.5 ± 3.4

<sup>a</sup> Mean ± SEM of at least four experiments performed in duplicate.

**Scheme 2.** Solid-Phases Procedure of the Synthesis of the 4-Oxo-1,4-dihydroquinoline-3-carboxamide Derivatives 11–36<sup>a</sup>

<sup>a</sup> Reagents and conditions: (i) PyBrOP, PS–HOBt (HL), DIEA, DMF, rt; (ii) R'–NH<sub>2</sub> or morpholine, DMF, rt.

**Table 2.** Affinities of Selected Derivatives and of Reference Compounds (SR-144528, WIN-55,212-2, CP-55,940, JWH-133, and HU-210) at the hCB<sub>2</sub> Cannabinoid Receptor<sup>a</sup>

compd	hCB <sub>2</sub> cannabinoid receptor K <sub>i</sub> (nM)
14	455 ± 63
15	371 ± 34
16	844 ± 78
18	> 1000
22	201 ± 28
23	> 1000
24	> 1000
25	772 ± 72
27	426 ± 39
28	16.4 ± 1.5
29	13.4 ± 1.2
30	15.8 ± 1.4
31	664 ± 62
32	70.8 ± 9.1
32R	37.1 ± 3.4
32S	784 ± 71
33	> 1000
33R	584 ± 54
33S	> 5000
34	174 ± 16
34R	125 ± 12
34S	> 1000
35	60.2 ± 5.5
SR-144528	51.7 ± 4.8
WIN-55,212-2	9.1 ± 0.8
CP-55,940	15.4 ± 1.4
JWH-133	20.3 ± 2.6
HU-210	7.3 ± 0.9

<sup>a</sup> The K<sub>i</sub> values were obtained from nonlinear analysis of competition curves using [<sup>3</sup>H]-CP-55,940 as radioligand. Data are the mean ± SEM of at least four experiments performed in duplicate.

**Pharmacology.** The 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives (**11–36**) were screened at 10 μM concentrations for their affinity and selectivity toward the hCB<sub>1</sub> and hCB<sub>2</sub> cannabinoid receptors in a competitive binding experiment as previously described.<sup>48</sup> Membranes from Chinese hamster ovarian (CHO) cells expressing either the hCB<sub>1</sub> or the hCB<sub>2</sub> cannabinoid receptors were used in these experiments. [<sup>3</sup>H]-SR-141716A and [<sup>3</sup>H]-CP-55,940 at concentrations of 1 nM were used as radioligands for the hCB<sub>1</sub> and the hCB<sub>2</sub> cannabinoid receptor, respectively. The results expressed as the displacement percentages of the radioligand from its binding site are summarized in Table 1. The K<sub>i</sub> values were then determined for compounds exhibiting a specific displacement superior to 60% either for the cannabinoid hCB<sub>2</sub> (Table 2) or the hCB<sub>1</sub> receptors (Table 3). The selectivity ratio is presented in the Table 3 when K<sub>i</sub> values have been obtained for both receptors. All together these results indicated that the 4-oxo-

**Table 3.** Affinities of Selected Derivatives (Compounds **15**, **29**, **32R**, and **35**) and of Reference Cannabinoid (SR-141716A) at the hCB<sub>1</sub> Cannabinoid Receptor<sup>a</sup>

compd	hCB <sub>1</sub> cannabinoid receptor K <sub>i</sub> (nM)	selectivity ratio CB <sub>2</sub> versus CB <sub>1</sub>
15	4083 ± 375	11
29	1925 ± 179	143
32R	1154 ± 108	31
35	1045 ± 96	17
SR-141716A	5.37 ± 0.6	

<sup>a</sup> The K<sub>i</sub> values were obtained from nonlinear analysis of competition curves using [<sup>3</sup>H]-SR-141716A as radioligand. Data are the mean ± SEM of at least four experiments performed in duplicate.

1,4-dihydroquinoline-3-carboxamide derivatives (**11–36**) are selective for the CB<sub>2</sub> cannabinoid receptors.

The question of the 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives functionality was investigated by using a [<sup>35</sup>S]-GTPγS binding assay.<sup>49</sup> 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. It is a useful tool to distinguish between agonists (increasing the nucleotide binding), inverse agonists (decreasing the nucleotide binding), and neutral antagonists (not affecting the nucleotide binding). The reference cannabinoid agonists JWH-133, HU-210, WIN-55,212-2, and CP-55,940 as well as the inverse agonist SR-144528 were assayed in a [<sup>35</sup>S]-GTPγS binding stimulation assay at the hCB<sub>2</sub> cannabinoid receptors. Results expressed as percentages of the basal stimulation (set at 100%) are summarized in Table 4. In this model, agonists at 10 μM give a [<sup>35</sup>S]-GTPγS binding stimulation from 128 to 149% of basal stimulation, while the inverse agonist SR-144528 abolishes it to 22%. 4-Oxo-1,4-dihydroquinoline-3-carboxamide derivatives **14–16**, **18**, **22–25**, and **27–35** were challenged in this assay and gave values from 117 up to 137% of basal stimulation indicating that these compounds act as agonists at the hCB<sub>2</sub> cannabinoid receptors. To further investigate the agonistic properties, the potency and efficacy of compounds **29**, **30**, **32R**, and **35** were determined (Table 5). These compounds dose-dependently increased the [<sup>35</sup>S]-GTPγS binding, exhibiting EC<sub>50</sub> values of the same magnitude as the respective K<sub>i</sub> values. For instance, compounds **30** and **32R** exhibited EC<sub>50</sub> values of 14.1 and 16.8 nM, respectively.

**Structure–Affinity Relationships.** Preliminary screening results showed that the first 3-carboxamidoquinolone derivatives studied displayed affinity for the cannabinoid receptors and therefore could constitute a suitable template in the design of new cannabinoid derivatives. In addition, a selectivity profile for the hCB<sub>2</sub> subtype was observed. To define the correct profile for binding to the hCB<sub>2</sub> cannabinoid receptor, we decided to



**Table 4.** [<sup>35</sup>S]-GTPγS Binding Stimulation Assays (10 μM) of Selected Compounds and Reference Cannabinoid Ligands (SR-144528, WIN-55,212-2, CP-55,940, JWH-133, and HU-210) for the hCB<sub>2</sub> Cannabinoid Receptors<sup>a</sup>

compd	hCB <sub>2</sub> cannabinoid receptor [ <sup>35</sup> S]-GTPγS specific binding (% of basal)
<b>14</b>	131.8 ± 3.5**
<b>15</b>	123.7 ± 3.8**
<b>16</b>	123.9 ± 2.4**
<b>18</b>	128.6 ± 2.3**
<b>22</b>	135.7 ± 2.5**
<b>23</b>	133.7 ± 2.2**
<b>24</b>	116.5 ± 0.8**
<b>25</b>	133.1 ± 1.1**
<b>27</b>	136.5 ± 1.3**
<b>28</b>	125.6 ± 2.5**
<b>29</b>	129.6 ± 1.1**
<b>30</b>	130.7 ± 1.8**
<b>31</b>	121.0 ± 2.0**
<b>32</b>	130.5 ± 1.0**
<b>32R</b>	123.6 ± 1.4**
<b>33</b>	122.5 ± 1.7**
<b>33R</b>	122.3 ± 2.1**
<b>34</b>	116.8 ± 1.0**
<b>34R</b>	119.2 ± 0.8**
<b>35</b>	123.1 ± 1.1**
SR-144528	21.6 ± 2.7**
WIN-55,212-2	127.8 ± 2.3**
CP-55,940	145.6 ± 1.8**
JWH-133	148.0 ± 2.5**
HU-210	135.0 ± 1.4**

<sup>a</sup> Results are expressed as the percentages of stimulation of [<sup>35</sup>S]-GTPγS binding (basal value set at 100%). Data are the mean ± SEM of three experiments performed in duplicate. Statistical significance was assessed by one-way ANOVA followed by a Dunnett post-test (\**P* < 0.05 and \*\**P* < 0.01).

**Table 5.** Determination of the Potency and Efficacy of Compounds **29**, **30**, **32R**, **35**, SR-144528, WIN-55,212-2, CP-55,940, JWH-133, and HU-210 at the hCB<sub>2</sub> Cannabinoid Receptors

compd	EC <sub>50</sub> (nM)	E <sub>max</sub> (%)
<b>29</b>	24.8 ± 3.4	126.0 ± 1.0
<b>30</b>	14.1 ± 5.6	124.1 ± 0.8
<b>32R</b>	16.8 ± 4.1	128.7 ± 1.2
<b>35</b>	157.4 ± 54	123.5 ± 1.5
SR-144528	2.1 ± 1.1	21.6 ± 2.7
WIN-55,212-2	24.6 ± 1.7	126.2 ± 1.5
CP-55,940	6.1 ± 2.1	145.6 ± 2.98
JWH-133	145.6 ± 3.0	149.0 ± 3.2
HU-210	4.1 ± 1.3	135.1 ± 3.4

<sup>a</sup> E<sub>max</sub> results are expressed as the percentages of stimulation of [<sup>35</sup>S]-GTPγS binding (basal value set at 100%). Data are the mean ± SEM of three experiments performed in duplicate. The EC<sub>50</sub> values were obtained from nonlinear analysis of [<sup>35</sup>S]-GTPγS binding curves. Data are the mean ± SEM of at least three experiments performed in duplicate.

investigate structure modifications on quinolone nucleus either by introducing various alkyl side chains on the N<sub>1</sub> nitrogen or by varying the substitution of the amide nitrogen.

A first set of nine compounds was synthesized (**11–19**). These compounds encompassed an N<sub>1</sub>-alkyl side chain (butyl, pentyl or hexyl) and a 4-methoxyphenyl, 1-naphthyl, or benzyl carboxamido substituent. In these series, the 1-naphthyl derivatives showed the highest affinity and, regardless of the nature of the carboxamido substituent, the *n*-pentyl group proved to be more potent than the *n*-butyl or *n*-hexyl. The highest hCB<sub>2</sub> cannabinoid receptor affinity was found for compound **15** (*K*<sub>i</sub> = 371 nM). Therefore, to continue our investigation, we decided to keep the *n*-pentyl residue constant and develop a library of compounds with various 3-carboxamido substituents.

The hCB<sub>2</sub> receptor binding data (Table 2) revealed that the affinity is quite sensitive to the 3-carboxamido group modifica-

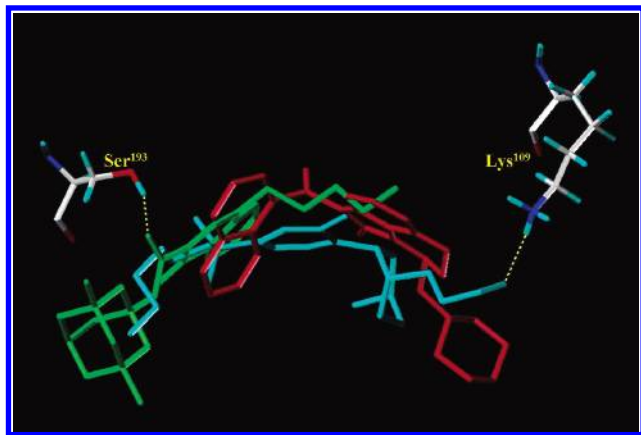
tions. Indeed, replacement of 1-naphthyl (**15**) by the 2-naphthyl isomer (**21**) resulted in a decreased affinity. However, replacement of the benzyl group (**18**) by 4-methoxybenzyl (**20**) and replacement of the 4-methoxyphenyl group (**12**) by 4-cyanophenyl (**25**) or 3,4-dichlorophenyl (**24**) did not elicit a clear effect on affinity. Therefore, electronic effects on the aromatic moiety did not influence the affinity for the cannabinoid CB<sub>2</sub> receptor.

We also synthesized 2-phenylethyl (**22**), 3-phenylpropyl (**23**), 4-biphenyl (**26**), and 2-(benzo[1,3]dioxol-5yl)ethyl (**27**) derivatives in order to evaluate the importance of hydrophobic character in the 3-position, since 1-naphthyl (**15**) revealed a good affinity for CB<sub>2</sub> receptor (*K*<sub>i</sub> = 371 nM). The best result was found with **22** (*K*<sub>i</sub> = 204 nM) when the phenyl group was spaced from the 3-carboxamidoquinolone ring by an ethyl link. Replacement of this ethyl link by methyl or propyl homologues led to a decreased affinity (*K*<sub>i</sub> values > 1000 nM) that was even more markedly observed in the 4-biphenyl derivative (**26**, <20% displacement at 10 μM). Compound **27**, in which the phenethyl substituent of **22** is replaced by a 2-(benzo[1,3]dioxol-5yl)ethyl, showed about 2-fold less affinity (*K*<sub>i</sub> = 426 nM).

Nonaromatic compounds with different adamantyl substituents (**28**, **29**, **30**, **31**) were also investigated. These moieties are widely found in the structure of cannabinoid ligands<sup>11,50</sup> with antagonistic properties. They can also generate hydrophobic interactions. These modifications induced a marked improvement in affinity (*K*<sub>i</sub> values of 16.4 nM and 13.4 nM for **28** and **29**, respectively). Interestingly, in the [<sup>35</sup>S]-GTPγS binding assay, these compounds kept their agonistic properties. Enhancement of adamantyl hydrophobicity by addition of two methyl groups (in position 3 and 5) did not elicit a marked effect on affinity as shown by compound **30** (*K*<sub>i</sub> = 15.8 nM). Compound **31** in which the *n*-pentyl side chain is replaced by a benzyl showed about 44-fold less affinity (*K*<sub>i</sub> = 664 nM). Replacement of the adamantyl substituent by a morpholino group, yielding compound **36**, resulted in a decreased affinity.

The cannabinoid hCB<sub>2</sub> receptor binding data reported herein suggested that the hydrophobicity of the carboxamido substituent appears to be as important as the aromatic character. In addition, the poor affinity of the 3-carboxylic acid intermediate **8** (<15% displacement at 10 μM) underlines the importance of the amide substitution. The carboxamido group is most likely located in a large hydrophobic cluster. To study the pocket, for the aromatic carboxamide compounds, a steric constraint by modulation of **22** using its 1-phenylethyl isomer (**32**) was introduced. The steric constraint imposed by a methyl group on the methylene spacer induced a particular orientation of the aromatic group. In this series, we also replaced the phenyl moiety by other aromatic nuclei: 1- and 2-naphthyl (**33** and **34**). Compounds **32–34** were first tested as racemates, as shown in Table 2. Introduction of this pseudo-rigidification led to an increase of the hCB<sub>2</sub> cannabinoid receptor affinity as illustrated by compounds **32** and **18** (*K*<sub>i</sub> values of 70.8 and >1000 nM, respectively). Modification of the aromatic group, in compounds **33** (*K*<sub>i</sub> > 1000 nM) and **34** (*K*<sub>i</sub> = 174 nM), resulted in a decreased affinity. Compound **35**, corresponding to a constrained analogue of **32**, showed an affinity of the same magnitude (*K*<sub>i</sub> values of 60.2 nM and 70.8 nM for **35** and **32**, respectively).

The enantiopure forms of **32–34**, respectively noted **32–34R** and **32–34S**, synthesized starting from the corresponding enantiopure amine were also tested, as summarized in Table 2. In all cases, the *R* enantiomers exhibited about 10-fold higher affinity than the *S* enantiomers. The highest affinity was found in the eutomer **32R** (*K*<sub>i</sub> = 37.1 nM). The distomer **32S** with an affinity of 776 nM highlighted that this chiral ligand binds



**Figure 1.** Receptor-based alignment results of WIN-55,212-2 (red), CP-55,940 (cyan), and **30** (green).

stereoselectively to the *h*CB<sub>2</sub> receptor. Introduction of this pseudo-rigidification induced an improvement of affinity for both of the two receptor subtypes.

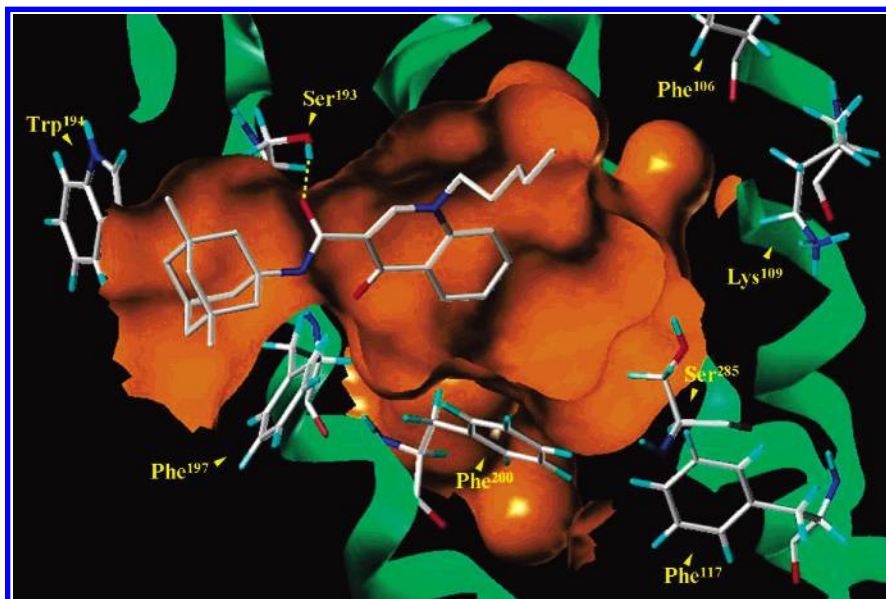
**Superposition and Docking Studies.** Bovine rhodopsin was used for homology modeling of the CB<sub>2</sub> cannabinoid receptor as it is the only currently solved G-protein coupled receptor tridimensional structure. However, its crystallographic structure has been determined in a dark-adapted conformation which is probably an inactive form. Therefore, besides pure homology modeling, a more elaborated construction of the CB<sub>2</sub> cannabinoid receptor was required to approach its putative active conformation. Previous works performed on the CB<sub>1</sub> cannabinoid receptor have shown that transmembrane domains 3 and 6 (TM 3 and TM 6) should undergo a rotation around their axis to render the effect of an agonist binding.<sup>51</sup> We followed these conclusions to build a model of CB<sub>2</sub> cannabinoid receptor able to bind the 3-carboxamido-4-quinolone agonists. TM3 was rotated by 20° anticlockwise when seen from the extracellular side. TM6 was also rotated until Cys<sup>257</sup> pointed toward the pocket, resulting in a 30° anticlockwise rotation. The geometry of this final model was optimized until convergence to a 0.01 kcal/mol·Å gradient with the backbone hydrogen bonds constrained and subsequently checked to verify its structural validity. The ligands were then docked in a pocket defined as a sphere of 15 Å around Lys<sup>98</sup> using the Gold software.

Using this receptor model, docking of the 3-carboxamido-4-quinolone derivatives was performed, followed by energy minimization. Nonselective CB<sub>2</sub> ligands, WIN-55,212-2 and CP-55,940, were used as template molecules, using the information obtained in mutants receptors to delineate the binding site. The resulting receptor-based alignments of WIN-55,212-2, CP-55,940, and **30** are illustrated in Figure 1. These results suggested that the 4-oxo-1,4-dihydroquinoline derivatives interact in the same region of the CB<sub>2</sub> receptor as WIN-55,212-2 and CP-55,940. However, the CB<sub>2</sub> cannabinoid receptor residues involved in the interactions with the ligands slightly differ between WIN-55,212-2 and CP-55,940 and derivative **30**.

Compound **30** was also docked in the cannabinoid CB<sub>2</sub> cannabinoid receptor active site, as shown in Figure 2. Aromatic residues are abundant in the region encompassed by transmembrane regions 3 to 5 (TM 3–TM 5), particularly near the extracellular side of the cannabinoid receptor. The hydrophobic *N*(1) alkyl side chain is perfectly positioned to interact with hydrophobic residues Ile<sup>110</sup>, Cys<sup>175</sup>, Pro<sup>178</sup>, and Leu<sup>182</sup>. The amide oxygen atom in **30** forms a hydrogen bond with Ser<sup>193</sup>. The quinolone nucleus is in interaction with aromatic or hydrophobic residues Pro<sup>168</sup>, Thr<sup>173</sup>, Leu<sup>182</sup>, Leu<sup>196</sup>, and Phe<sup>200</sup>. The 1-(3,5-dimethyl)adamantyl carboxamido substituent fits well in a pocket formed by various lipophilic or aromatic residues Phe<sup>183</sup>, Pro<sup>184</sup>, Pro<sup>187</sup>, Phe<sup>197</sup>, and Leu<sup>269</sup>.

## Conclusion

In light of these results, this series of 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives represents a new class of heterocyclic derivatives, acting as potent CB<sub>2</sub>-selective receptor ligands. The [<sup>35</sup>S]-GTPγS binding data revealed the CB<sub>2</sub> cannabinoid receptor agonistic properties of these derivatives. Best affinity values were obtained with compounds **28–30**, bearing an adamantyl-carboxamido substituent. Compounds containing aromatic moieties on the amide resulted in a decreased, albeit notable, affinity for the *h*CB<sub>2</sub> cannabinoid receptors, as shown by **15**, **22**, and **27**. The introduction of a steric constraint induced a marked improvement in affinity. Moreover, interactions of **32R**, **33R**, and **34R** with the CB<sub>2</sub> cannabinoid receptor are highly stereoselective. Whatever the carboxamido substituent, the preferred *N*<sub>1</sub> side chain was the *n*-pentyl one. Finally, molecular modeling of **30** obtained either by superim-



**Figure 2.** Compound **30** docked in the putative active site of the CB<sub>2</sub> receptor. Hydrogen bond is colored in yellow.



position or by docking indicated that these derivatives may share to some extent the binding mode of WIN-55,212-2.

## Experimental Section

**Chemistry.** All commercial reagents and solvents were used without further purification. Analytical thin-layer chromatography was performed on precoated Kieselgel 60F<sub>254</sub> plates (Merck); the spots were located by UV (254 and 366 nm) and/or with iodine; R<sub>f</sub> values are given for guidance. Silica gel 60 230–400 mesh purchased from Merck was used for column chromatography. Preparative thick-layer chromatography was performed using silica gel from Merck, and the compounds were extracted from silica gel by the following solvent system: CH<sub>2</sub>Cl<sub>2</sub>/MeOH 70:30. All melting points were determined with a Büchi 535 capillary apparatus and remain uncorrected. <sup>1</sup>H NMR spectra were obtained using a Brücker 300 MHz spectrometer, chemical shifts (δ) were expressed in ppm relative to tetramethylsilane used as an internal standard, *J* values are in hertz, and the splitting patterns were designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. IR spectra were determined with a Brücker Vector 22 spectrometer on a germanium crystal. APCI<sup>+</sup> (atmospheric pressure chemical ionization) mass spectra were obtained on an LC-MS system Thermo Electron Surveyor MSQ. Optical rotations ([α]<sub>D</sub>) were measured on a Perkin-Elmer 343 polarimeter. Specific rotations are given as deg/dm; the concentration values are reported as g/mL of the specified solvent and were recorded at 25 °C. Elemental analyses were performed by the “Service Central d’Analyses” at the CNRS, Vernaison (France).

**2-Phenylaminomethylene-malonic Acid Diethyl Ester (1).** A mixture of 2-ethoxymethylene-malonic acid diethyl ester (9.30 mL, 46 mmol) and aniline (4.20 mL, 46 mmol) was heated at 100 °C for 4 h. Petroleum ether (100 mL) was added, and the resulting solution was cooled in an ice bath. The resulting precipitate was collected by filtration, washed with petroleum ether, and recrystallized from petroleum ether to provide 11.02 g (91%) of compound **1** as a white solid: mp 54–55 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>).

**4-Oxo-1,4-dihydro-quinoline-3-carboxylic Acid Ethyl Ester (2).** Phenyl ether (40 mL) was heated under stirring at 240 °C. The malonic acid diethyl ester (**1**) (9.50 g, 36 mmol) was slowly added, and the resulting mixture was refluxed for 4 h. After the mixture was cooled at room temperature, the resulting precipitate was collected by filtration, washed with petroleum ether, and recrystallized from DMF to provide 6.02 g (77%) of compound **2** as a white solid: mp >250 °C; IR; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>).

**General Procedure for the Preparation of 1-Alkyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic Acid Ethyl Ester (3–6).** NaH 60% (0.22 g, 5.52 mmol) was added to a mixture of quinoline carboxylic acid ethyl ester **2** (1.00 g, 4.60 mmol) and anhydrous DMF (30 mL) under stirring at room temperature. The appropriate alkyl bromide (5.52 mmol) was added, and the mixture was stirred at 90 °C for 3 h (**3–4**) and 6 h (**5–6**). The solvent was removed under reduced pressure, and the residue subsequently dissolved in ethyl acetate. The precipitate was eliminated by filtration, and the organic layer was concentrated under reduced pressure and finally purified by flash chromatography, using dichloromethane/ethyl acetate 5:5 as eluent.

**1-Butyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic Acid Ethyl Ester (3).** Orange oil (0.72 g, 57%); IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>).

**4-Oxo-1-pentyl-1,4-dihydro-quinoline-3-carboxylic Acid Ethyl Ester (4).** Orange oil (1.23 g, 93%); IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>).

**1-Hexyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic Acid Ethyl Ester (5).** Orange oil (0.69 g, 50%); IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>).

**1-Benzyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic Acid Ethyl Ester (6).** Beige solid (1.06 g, 75%); mp 120–121 °C; IR; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>).

**General Procedure for the Preparation of 1-Alkyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic Acid (7–10).** The appropriate quinoline-3-carboxylic acid ethyl ester **3–6** (4.13 mmol) was refluxed for 3 h in a mixture of aqueous 10% sodium hydroxide (5 mL) and ethyl alcohol (5 mL). After cooling, the solution was

adjusted to pH 4 with aqueous 10% hydrochloric acid. The resulting precipitate was collected by filtration, washed with H<sub>2</sub>O, and recrystallized from diisopropyl ether.

**1-Butyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic Acid (7).** White solid (0.71 g, 70%); mp 165–166 °C; IR; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>).

**4-Oxo-1-pentyl-1,4-dihydro-quinoline-3-carboxylic Acid (8).** White solid (0.81 g, 76%); mp 135–137 °C; IR; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>).

**1-Hexyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic Acid (9).** White solid (0.67 g, 75%); mp 150–151 °C; IR; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>).

**1-Benzyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic Acid (10).** White solid (0.86 g, 75%); mp 175–176 °C; IR; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>).

**General Procedure for the Preparation of N3-aryl-1-alkyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (11–36).** To a solution of PybrOP (1.5 mmol) in 3 mL of dry DMF were added at room temperature compounds **7–10** and diisopropylethylamine (3.0 mmol). The preswollen resin (0.75 g) in dry DMF was treated with the above mixture at room temperature for 3 h, after which time the resin was washed three times with dry DMF and three times with dichloromethane. The same activation procedure was repeated a second time. The appropriate amine (0.67 mmol) dissolved in dry DMF was reacted with the polymer-bound activated ester for 24 h at room temperature. The supernatant was then separated from the resin by filtration and the polymer beads washed three times with dry DMF and three times with dichloromethane. The combined solutions were concentrated and, the residue was purified either by crystallization or preparative TLC.

**N3-(4-Methoxyphenyl)-1-butyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (11).** Recrystallization from diisopropyl ether, yellow solid (57 mg, 24%); mp 129–130 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 351 (MH<sup>+</sup>). Anal. (C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O<sub>3</sub>) C, H, N.

**N3-(4-Methoxyphenyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (12).** Recrystallization from diisopropyl ether, yellow solid (34 mg, 14%); mp 134–135 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 365 (MH<sup>+</sup>). Anal. (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>3</sub>) C, H, N.

**N3-(4-Methoxyphenyl)-1-hexyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (13).** Recrystallization from diisopropyl ether, yellow solid (130 mg, 51%); mp 119–120 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 379 (MH<sup>+</sup>). Anal. (C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>O<sub>3</sub>) C, H, N.

**N3-(1-Naphthyl)-1-butyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (14).** Recrystallization from ethyl acetate, white solid (90 mg, 36%); mp 174–175 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 371 (MH<sup>+</sup>). Anal. (C<sub>24</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(1-Naphthyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (15).** Recrystallization from ethyl acetate, white solid (184 mg, 71%); mp 184–185 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 385 (MH<sup>+</sup>). Anal. (C<sub>25</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(1-Naphthyl)-1-hexyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (16).** Recrystallization from ethyl acetate, white solid (81 mg, 30%); mp 158–159 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 399 (MH<sup>+</sup>). Anal. (C<sub>26</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(Benzyl)-1-butyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (17).** Recrystallization from *n*-heptane, white solid (70 mg, 31%); mp 165–166 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 335 (MH<sup>+</sup>). Anal. (C<sub>21</sub>H<sub>22</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(Benzyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (18).** Recrystallization from *n*-heptane, white solid (143 mg, 61%); mp 170–171 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 349 (MH<sup>+</sup>). Anal. (C<sub>22</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(Benzyl)-1-hexyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (19).** Recrystallization from *n*-heptane, white solid (102 mg, 42%); mp 176–177 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 363 (MH<sup>+</sup>). Anal. (C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(4-Methoxybenzyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (20).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, yellow oil (140 mg, 55%); IR; LC-MS (APCI<sup>+</sup>) *m/z* 379 (MH<sup>+</sup>). Anal. (C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>O<sub>3</sub>) C, H, N.

**N3-(2-Naphthyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (21).** Recrystallization from ethyl acetate, white solid (129 mg, 50%); mp 162–163 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 385 (MH<sup>+</sup>). Anal. (C<sub>25</sub>H<sub>24</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(Phenethyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (22).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, yellow oil (188 mg, 77%); IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 363 (MH<sup>+</sup>). Anal. (C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(3-Phenylpropyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (23).** Purified by TLC eluting from dichloromethane/methyl alcohol 95:5, white solid (162 mg, 64%); mp 78–79 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 377 (MH<sup>+</sup>). Anal. (C<sub>24</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(3,4-Dichlorophenyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (24).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, white solid (201 mg, 74%); mp 175–176 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 404 (MH<sup>+</sup>). Anal. (C<sub>21</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>Cl<sub>2</sub>) C, H, N.

**N3-(4-Cyanophenyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (25).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, white solid (111 mg, 46%); mp 119–120 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 360 (MH<sup>+</sup>). Anal. (C<sub>22</sub>H<sub>21</sub>N<sub>3</sub>O<sub>2</sub>) C, H, N.

**N3-(4-Biphenyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (26).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, white solid (108 mg, 39%); mp 153–154 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 411 (MH<sup>+</sup>). Anal. (C<sub>27</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(2-(Benzo[1,3]dioxol-5-yl)ethyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (27).** Purified by TLC eluting from dichloromethane/methyl alcohol 95:5, white solid (121 mg, 44%); mp 85–86 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 407 (MH<sup>+</sup>). Anal. (C<sub>24</sub>H<sub>26</sub>N<sub>2</sub>O<sub>4</sub>) C, H, N.

**N3-(1-Adamantyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (28).** Recrystallization from *n*-heptane, white solid (144 mg, 55%); mp 184–185 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 393 (MH<sup>+</sup>). Anal. (C<sub>25</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(2-Adamantyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (29).** Recrystallization from *n*-heptane, white solid (118 mg, 45%); mp 180–181 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 393 (MH<sup>+</sup>). Anal. (C<sub>25</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(1-(3,5-Dimethyl)adamantyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (30).** Recrystallization from *n*-heptane, white solid (190 mg, 67%); mp 164–165 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 421 (MH<sup>+</sup>). Anal. (C<sub>27</sub>H<sub>36</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**N3-(1-(3,5-Dimethyl)adamantyl)-4-oxo-1-benzyl-1,4-dihydroquinoline-3-carboxamide (31).** Recrystallization from *n*-heptane, white solid (221 mg, 70%); mp 174–175 °C; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 441 (MH<sup>+</sup>). Anal. (C<sub>29</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**(RS)-N3-(1-Phenylethyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (32).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, white oil (188 mg, 77%); [α]<sub>D</sub><sup>25</sup> = 0°, *c* = 0.01, CH<sub>2</sub>Cl<sub>2</sub>; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 363 (MH<sup>+</sup>). Anal. (C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**(R)-N3-(1-Phenylethyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (32R).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, white oil (237 mg, 97%); [α]<sub>D</sub><sup>25</sup> = –95°, *c* = 0.01, CH<sub>2</sub>Cl<sub>2</sub>; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 363 (MH<sup>+</sup>). Anal. (C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**(S)-N3-(1-Phenylethyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (32S).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, white oil (234 mg, 96%); [α]<sub>D</sub><sup>25</sup> = +95°, *c* = 0.01, CH<sub>2</sub>Cl<sub>2</sub>; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 363 (MH<sup>+</sup>). Anal. (C<sub>23</sub>H<sub>26</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**(RS)-N3-(1-(2-Naphthyl)ethyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (33).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, white oil (220 mg, 79%); [α]<sub>D</sub><sup>25</sup> = 0°, *c* = 0.01, CH<sub>2</sub>Cl<sub>2</sub>; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 413 (MH<sup>+</sup>). Anal. (C<sub>27</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**(R)-N3-(1-(2-Naphthyl)ethyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (33R).** Purified by TLC eluting from cyclo-

hexane/ethyl acetate 6:4, white oil (203 mg, 73%); [α]<sub>D</sub><sup>25</sup> = –151°, *c* = 0.01, CH<sub>2</sub>Cl<sub>2</sub>; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 413 (MH<sup>+</sup>). Anal. (C<sub>27</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**(S)-N3-(1-(2-Naphthyl)ethyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (33S).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, white oil (239 mg, 86%); [α]<sub>D</sub><sup>25</sup> = +151°, *c* = 0.01, CH<sub>2</sub>Cl<sub>2</sub>; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 413 (MH<sup>+</sup>). Anal. (C<sub>27</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**(RS)-N3-(1-(1-Naphthyl)ethyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (34).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, white oil (150 mg, 54%); [α]<sub>D</sub><sup>25</sup> = 0°, *c* = 0.01, CH<sub>2</sub>Cl<sub>2</sub>; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 413 (MH<sup>+</sup>). Anal. (C<sub>27</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**(R)-N3-(1-(1-Naphthyl)ethyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (34R).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, white oil (228 mg, 82%); [α]<sub>D</sub><sup>25</sup> = –200°, *c* = 0.01, CH<sub>2</sub>Cl<sub>2</sub>; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 413 (MH<sup>+</sup>). Anal. (C<sub>27</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**(S)-N3-(1-(1-Naphthyl)ethyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (34S).** Purified by TLC eluting from cyclohexane/ethyl acetate 6:4, white oil (217 mg, 78%); [α]<sub>D</sub><sup>25</sup> = +200°, *c* = 0.01, CH<sub>2</sub>Cl<sub>2</sub>; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 413 (MH<sup>+</sup>). Anal. (C<sub>27</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**(RS)-N3-(1-(1,2,3,4-Tetrahydronaphthyl)-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (35).** Purified by TLC eluting from cyclohexane/ethyl acetate 7:3, white oil (143 mg, 55%); [α]<sub>D</sub><sup>25</sup> = 0°, *c* = 0.01, CH<sub>2</sub>Cl<sub>2</sub>; IR; <sup>1</sup>H NMR (CDCl<sub>3</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 389 (MH<sup>+</sup>). Anal. (C<sub>25</sub>H<sub>28</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**3-(Morpholino-4-carbonyl)-1-pentyl-1,4-dihydroquinoline-4-one (36).** Purified by TLC eluting from *n*-heptane/ethyl acetate 6:4, white oil (177 mg, 80%); IR; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>); LC-MS (APCI<sup>+</sup>) *m/z* 329 (MH<sup>+</sup>). Anal. (C<sub>25</sub>H<sub>32</sub>N<sub>2</sub>O<sub>2</sub>) C, H, N.

**Pharmacology.** Fatty acid free bovine serum albumin (BSA) was purchased from Sigma Chemical Co (St. Louis, MO). WIN-55,21-2 was purchased from RBI (Natick, MA), HU-210 and CP-55,940 were acquired from Tocris (Bristol, U.K.). SR-141716A and SR-144528 were kindly donated by Sanofi Recherche (Montpellier, France).

**Cell Culture and Preparation of hCB<sub>1</sub>- or hCB<sub>2</sub>-Transfected CHO Cell Membranes.** CHO cells stably transfected with the cDNA sequences encoding either the human CB<sub>1</sub> or the human CB<sub>2</sub> cannabinoid receptors were kindly donated by Dr. M. Detheux and Dr. P. Nokin, respectively (Euroscreen s.a., Gosselies, Belgium). Cells were grown in Ham's F12 nutrient mixture supplemented with 10% FBS, 2.5 μL/mL fungizone, 100 U/mL penicillin, 100 μg/mL streptomycin, and 400 μg/mL G418. Once at confluence, the cells were trypsinized and collected by centrifugation at 100g for 10 min. The following steps were performed on ice. The pellet was lysed in ice-cold 50 mM Tris-HCl, pH 7.4, and the homogenate was centrifuged at 15 000g for 10 min. The resulting pellet (membranes) was washed twice with the same solution under identical conditions. The protein content was determined as described by Bradford<sup>52</sup> using Coomassie Blue (Biorad, Belgium) with bovine serum albumin as standard.

**Competition Binding Assay.** [<sup>3</sup>H]-SR-141716A (52 Ci/mol) was purchased from Amersham (Roosendaal, The Netherlands) and [<sup>3</sup>H]-CP-55,940 (101 Ci/mol) from NEN Life Science (Zaventem, Belgium). Glass fiber filters were purchased from Whatman (Maidstone, U.K.), while Aqualuma was from PerkinElmer (Schaesberg, The Netherlands). 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%.

Under these conditions, using [<sup>3</sup>H]-SR-141716A, the *B*<sub>max</sub> value was 57 pmol/mg protein and the *K*<sub>d</sub> value was 1.13 (0.13 nM) for the hCB<sub>1</sub> cannabinoid receptor. Using [<sup>3</sup>H]-CP-55,940, the *B*<sub>max</sub> value was 57 pmol/mg protein and the *K*<sub>d</sub> value was 4.3 (0.13 nM) for the hCB<sub>2</sub> cannabinoid receptor.

The competitive binding experiments were performed using [<sup>3</sup>H]-SR-141716A (1 nM) or [<sup>3</sup>H]-CP-55,940 (1 nM) as radioligands for the hCB<sub>1</sub> and the hCB<sub>2</sub> cannabinoid receptor, respectively, at



30 °C in plastic tubes, and 40 µg of membranes per tube resuspended in 0.5 mL (final volume) of binding buffer (50 mM Tris-HCl, 3 mM MgCl<sub>2</sub>, 1 mM EDTA, 0.5% bovine serum albumine, pH 7.4). The test compounds were present at varying concentrations, and the nonspecific binding was determined in the presence of 10 µM HU-210. After 1 h the incubation was stopped, and the solutions were rapidly filtered through 0.5% PEI pretreated GF/B glass fiber filters on a M-48T Brandell cell harvester and washed twice with 5 mL ice-cold binding buffer without serum albumin. The radioactivity on the filters was measured in a Pharmacia Wallac 1410 β-counter using 10 mL of Aqualuma, after 10 s shaking and 3 h resting. Assays were performed at least in triplicate.

**[<sup>35</sup>S]-GTPγS Assays.** [<sup>35</sup>S]-GTPγS (1173 Ci/mmol) was purchased from Amersham (Roosendaal, The Netherlands). The binding experiments were performed at 30 °C in plastic tubes containing 40 µg of protein in 0.5 mL (final volume) of binding buffer (50 mM Tris-HCl, 3 mM MgCl<sub>2</sub>, 1 mM EDTA, 100 mM NaCl, 0.1% bovine serum albumin, pH 7.4) supplemented with 20 µM GDP. The test compounds were present at varying concentrations, and the nonspecific binding was measured in the presence of 100 µM Gpp(NH)p. The assay was initiated by the addition of [<sup>35</sup>S]-GTPγS (0.05 nM, final concentration). The tubes were incubated for 1 h. The incubations were terminated by the addition of 5 mL ice-cold washing buffer (50 mM Tris-HCl, 3 mM MgCl<sub>2</sub>, 1 mM EDTA, 100 mM NaCl). The suspension was immediately filtered through GF/B filters using a 48-well Brandell cell harvester and washed twice with the same ice-cold buffer. The radioactivity on the filters was counted as mentioned above. Assays were performed in triplicate.

**Data Analysis.** IC<sub>50</sub> and EC<sub>50</sub> values were determined by nonlinear regression analysis performed using the GraphPad prism 4.0 program (GraphPad Software, San Diego). The K<sub>i</sub> values were calculated from the IC<sub>50</sub>, based on the Cheng–Prusoff equation:  $K_i = IC_{50}/(1 + L/K_d)$ . Statistical significance of [<sup>35</sup>S]-GTPγS assay results was assessed using a one-way ANOVA followed by a Dunnett post-test.

**Molecular Modeling.** All the calculations have been carried out under the Sybyl 6.9 modeling software<sup>53</sup> running on Silicon Graphics Octane 2 workstations. The construction of a homology model of the CB<sub>2</sub> cannabinoid receptor was realized by aligning its sequence on the bovine rhodopsin (PIR entry 1O0BO)<sup>54</sup> with ClustalW<sup>55</sup> then transferring the 3D coordinates of the rhodopsin crystallographic structure (PDB entry 1U19)<sup>56</sup> with Jackal.<sup>57</sup> To build a model in a putative activated conformation, transmembrane domains 3 and 6 (TM3 and TM6) were rotated as described for the CB<sub>1</sub> cannabinoid receptor by McAllister and co-workers.<sup>51</sup> Three-dimensional models of WIN-55,212-2, CP-55,940, and compound **30** were built from a standard fragments library, and their geometry was subsequently optimized using the Tripos force field<sup>58</sup> including the electrostatic term calculated from Gasteiger and Hückel atomic charges. The method of Powell available in the Maximin2 procedure was used for energy minimization until the gradient value was smaller than 0.001 kcal/mol·Å. Flexible docking of the compounds into the receptor active site was performed using GOLD<sup>59</sup> software. For each compound, the most stable docking model was selected according to the best scored conformation predicted by the GoldScore<sup>59</sup> and X-Score<sup>60</sup> scoring functions. The complexes were energy-minimized using the Powell method available in the Maximin2 procedure with the Tripos force field and a dielectric constant of 4.0 until the gradient value reached 0.01 kcal/mol·Å.

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**Supporting Information Available:** 2D <sup>1</sup>H NMR (ROESY) data of **15**, elemental analysis results of **11–36**, and spectroscopic data for all compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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