Investigations on the 4-Quinolone-3-carboxylic Acid Motif. 2. Synthesis and Structure−Activity Relationship of Potent and Selective Cannabinoid-2 Receptor Agonists Endowed with Analgesic Activity in Vivo[⊥]

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Quinolone-3-carboxamides **11** bearing at position 5, 6, 7, or 8 diverse substituents such as halides, alkyl, aryl, alkoxy, and aryloxy groups differing in their steric/electronic properties, were prepared. The new compounds were tested in vitro for CB1 and CB2 receptor affinity in comparison with the reference compounds rimonabant and SR144528. The tested compounds exhibited CB2 affinity in the range from 55.9 to 0.8 nM and CB1 affinity in the range from >10 000 to 5.3 nM, with selectivity indeces [K_i(CB1)/K_i(CB2)] varying from >2666.6 to 1.23. On the basis of the structure−selectivity relationship developed, the presence of a substituent at C6/C8 or C7 well accounts for the high or low CB2 selectivity, respectively. Compound **11c**, characterized by high CB2 affinity and selectivity, showed analgesic activity in the formalin test of acute peripheral and inflammatory pain in mice as a result of selective CB2 agonistic activity.

Introduction

The progress achieved over the past 15 years in understanding the action mechanisms of cannabinoids has revived the therapeutic interest in these substances.[1] On the basis of several controlled clinical trials, it is possible to affirm that cannabinoids possess interesting therapeutic potential in a number of pathological conditions, such as pain, immunosuppression, peripheral vascular disease, appetite enhancement or suppression, and locomotor disorders.[2] Currently, cannabinoid type 1 receptor (CB1) antagonists/inverse agonists are evaluated for obesity, metabolic disorders, smoking cessation, and alcohol abuse.[3,4] Rimonabant (**1**) (Chart 1) is a potent and selective antagonist for the CB1 that was launched in Europe in 2006 by Sanofi-Aventis for the treatment of obesity and associated risk factors.[5,6] On the other hand, selective cannabinoid type 2 receptor (CB2) ligands may be used to treat pain,[7] inflammation,[8] osteoporosis,[9] CB2-expressing malignant gliomas,[10] tumors of immune origin,[11] and immunological disorders such as multiple sclerosis.[12] Among the different therapeutic strategies designed to interfere with the deleterious processes involved in the pathology of multiple sclerosis, the use of cannabinoid compounds is currently one of the most promising ones.[13,14] Growing literature describes a beneficial role of cannabinoids, and more specifically CB2 selective agonists such as **2** (JWH-133),[2] in Alzheimer's disease, the most common form of dementia, by blocking β-amyloid peptide-induced activation of microglial cells.[15] Since CB2 appears to be almost exclusively expressed by microglia, blockade of its activation may be attained by CB2 agonists with no overt psychoactivity. All these findings may set the basis for the use of CB2 agonists as a therapeutic approach for the treatment of multiple sclerosis and the prevention of neurodegenerative disorders, such as Alzheimer's disease. CB2 activation can also produce analgesic effects devoid of psychotropic activity, and importantly, the CB2-specific ligand **3** (GW842166X)[16] is under clinical development for the treatment of inflammatory pain. Other representatives of CB2 selective agonists are, for example, compounds **4** (HU308), **5** (AM1241), **6** (GW405833), and **7** (JWH-015), while the pyrazole derivative **8** (SR144528) is a CB2 selective antagonist/inverse agonist.[2]

Very recently, 1,8-naphthyridine-4(1*H*)-on-3-carboxamide derivatives,[17] such as **9**, and 4-oxo-1,4-dihydroquinoline-3-carboxamide derivatives[18] (for example, **10**) have been described as structurally diverse ligands endowed with high affinity and selectivity toward CB2 (Chart 2). Our interest in the quinolone chemistry[19] led us to choose compound **10** as the prototype for the development of a new family of CB2 selective agonists. This compound is characterized by a 1-adamantyl substituent on the carboxamide nitrogen and a *n*-pentyl substituent at the N-1 position. It was demonstrated that whatever the carboxamide substituent, the *n*-pentyl group is the preferred N-1 side chain.[18] However, the effects on CB2 affinity and/or selectivity of modifications on the benzene portion of the quinolone ring have not been explored in great detail so far, as only few examples of such modified quinolone carboxamides have been recently reported.[17,20] In light of these considerations and following our previous findings on CB1/CB2 ligands,[21] we decided to investigate the effect on cannabinoid receptor activity/selectivity of decoration of the quinolone nucleus with various substituents, such as halides, alkyl, aryl, alkoxy, and aryloxy groups differing in their respective steric/electronic properties as well as their position on the condensed benzene ring. The N-1 pentyl chain

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Chart 1. CB1 and CB2 Receptors Selective Ligands

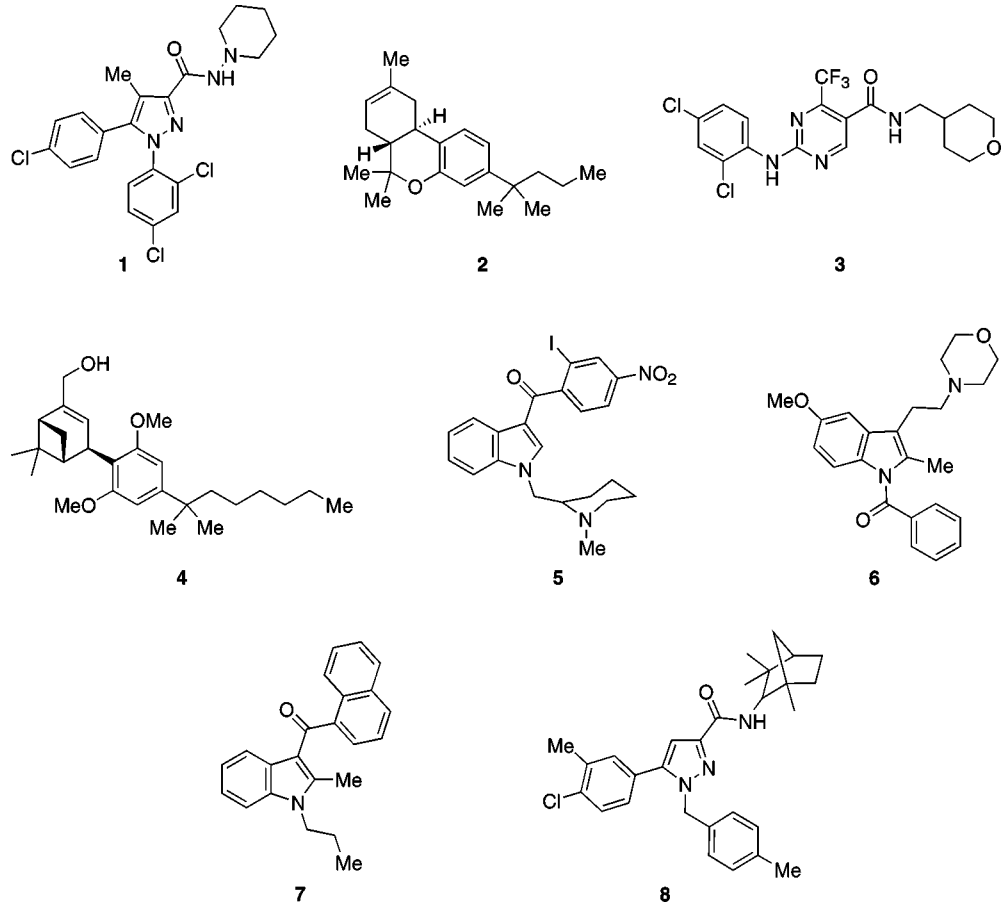
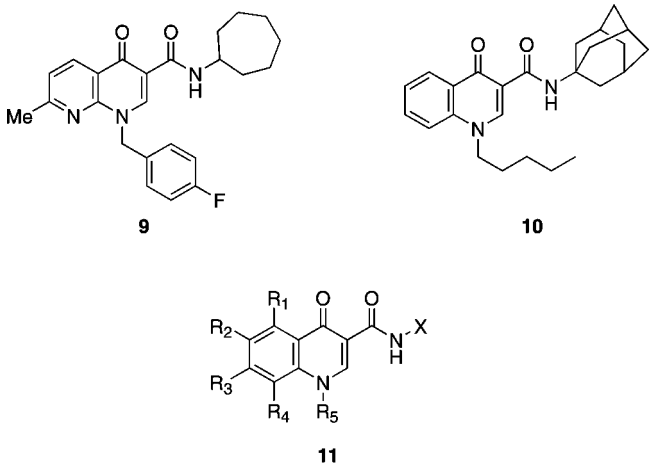


Chart 2. CB2 Selective Quinolones



R_1 = H, MeO
R_2 = H, F, Br, CF_3, *i*-Pr, Ph, PhS, 3-Cl-4-F-PhO, 4-MeO-styryl
R_3 = H, Cl, MeO, EtO, 1-pyrrolyl, 1-pyrrolidinyl, PhS, PhSO, $PhSO_2$
R_4 = H, Me, MeO
R_5 = 1-pentyl, 1-pentenyl, 1-butenyl, allyl
X = 1-adamantyl, (*R*)-1-phenylethyl

and adamantyl group were either retained or replaced with other lipophilic groups, such as prop-1-en-3-yl, but-1-en-4-yl, pent-1-en-5-yl, or (*R*)-1-phenylethyl, to obtain the new quinolonecarboxamides of general structure **11**.[22]

Chemistry

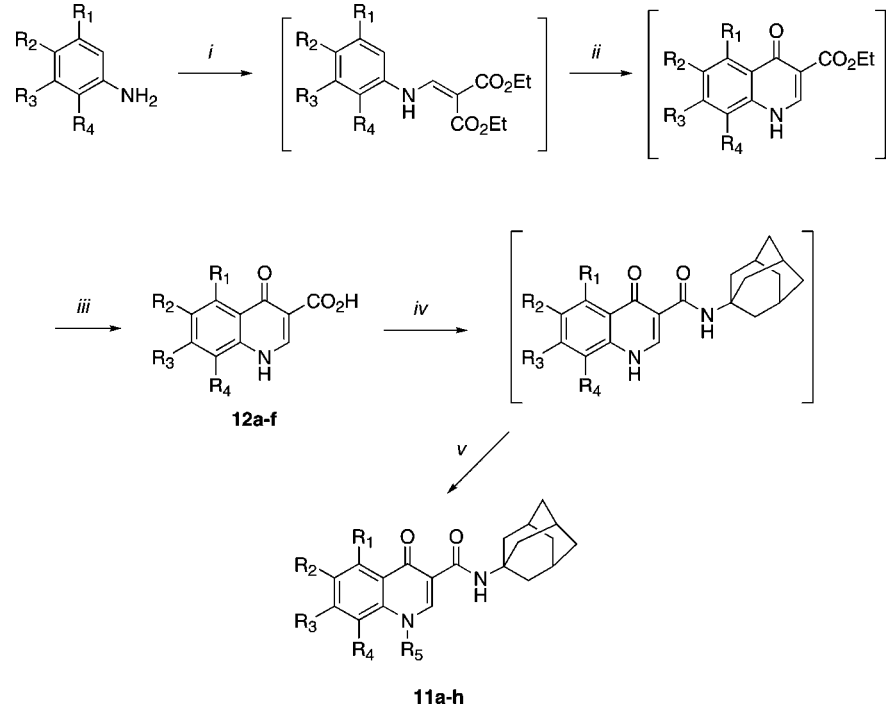
The synthesis of the target compounds **11a**−**v** is highlighted in Schemes 1−4. Initially, in order to achieve in a fast way some chemical diversity, a parallel synthesis approach was performed using a carousel reaction station. Six commercially available or easily accessible anilines[23] were condensed with diethyl ethoxymethylenemalonate (EMME)[a] according to the Gould−Jacobs reaction.[24] The intermediate enaminoesters were directly cyclized in refluxing diphenyl ether, and the quinolone esters were hydrolyzed by adding 10% aqueous NaOH to the reaction mixture and by heating at reflux temperature for 4 h. Acids were precipitated by adding HCl to the reaction mixture and collected by filtration. In this way it was possible to prepare six 4-oxo-1,4-dihydroquinoline-3-carboxylic acids (**12a**−**f**) in a single step in good overall yield (23−40%) (Scheme 1, Table 1).

Acids **12a**−**f** were subjected to coupling reaction with 1-aminoadamantane using 1-hydroxybenzotriazole (HOBt) and *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) as coupling reagents in the presence of diisopropylethylamine (DIPEA) in DMF to give the corresponding amides, which in turn were directly reacted with the appropriate alkyl halide and potassium carbonate in the same solvent. The final compounds **11b**−**h** were obtained in a satisfactory overall yield of 32−50%, whereas **11a** was obtained in low yield (7%), probably because of some steric hindrance toward the approaching electrophile exerted by the methyl substituent at position 8.

Other final compounds **11i**−**m** were prepared by a linear, step-by-step synthesis (Scheme 2). Thus, esters **13a**[25] and **13b**[26]

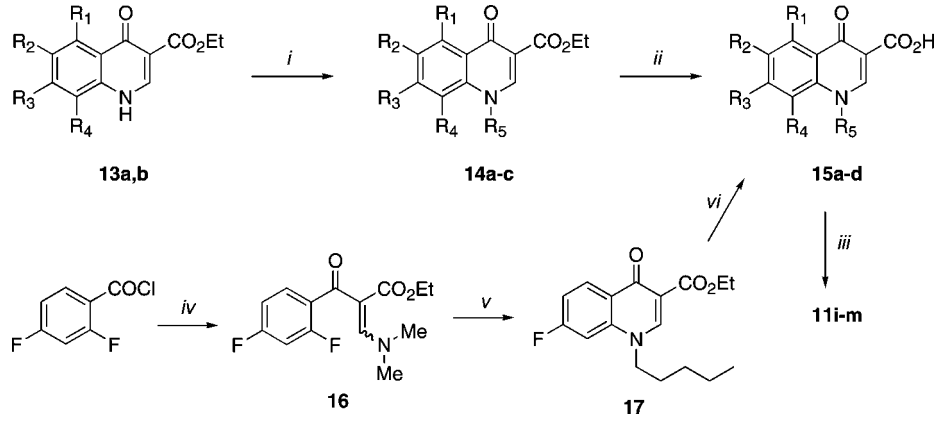
[a] Abbreviations: EMME, diethyl ethoxymethylenemalonate; HOBt, 1-hydroxybenzotriazole; HBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIPEA, diisopropylethylamine; SI, selectivity index.

Scheme 1[a]



[a] For R_1-R_5, see Table 1. Reagents and conditions: (i) diethyl ethoxymethylenemalonate, 120 °C, 4 h; (ii) diphenyl ether, reflux, 16 h; (iii) 10% NaOH, reflux, 4 h, then HCl; (iv) 1-aminoadamantane, HOBt, HBTU, DIPEA, DMF, room temp, 20 h; (v) alkyl halide, KI, K_2CO_3, DMF, 90 °C, 20 h.

Scheme 2[a]



[a] For R_1-R_5, see Table 1. Reagents and conditions: (i) see (v) in Scheme 1; (ii) 10% NaOH, reflux, 2 h, then HCl; (iii) see (iv) in Scheme 1; (iv) ethyl 3-dimethylaminoacrylate, Et_3N, toluene, 100 °C, 4 h; (v) 1-pentylamine, K_2CO_3, DMF, 100 °C, 2 h; (vi) 6 N HCl, reflux, 7 h.

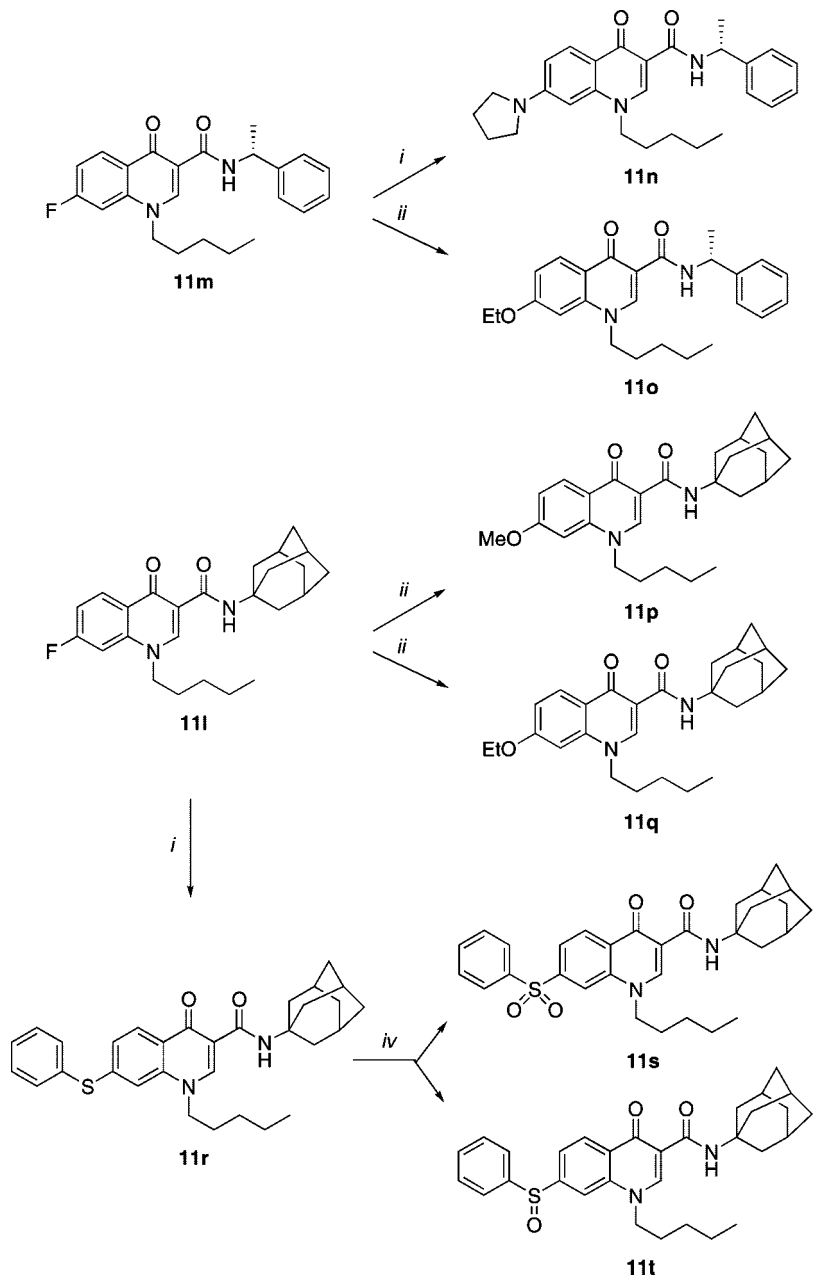
were first N-alkylated to **14a**−**c**, which were then hydrolyzed to the corresponding acids **15a**−**c**. Finally, coupling reaction of **15a**−**c** with 1-aminoadamantane under the usual conditions provided amides **11i**−**k**.

7-Fluoro-4-oxo-1-(1-pentyl)-1,4-dihydroquinoline-3-carboxylic acid (**15d**) was synthesized in a different way, according to a modified Grohe−Heitzer procedure.[27] Treatment of 2,4-difluorobenzoyl chloride with 3-dimethylaminopropenoic acid ethyl ester led to the enamino ketone **16** that was subsequently reacted with *n*-pentylamine to yield the quinolone ester **17**. Because of the high reactivity toward nucleophiles exhibited by the fluorine atom at position 7 (para to the carbonyl group), the ester **17** could not be subjected to hydrolysis under basic conditions but was conveniently hydrolyzed using 6 N HCl. The product **15d** so obtained was transformed into amides **11l**,**m** by coupling with 1-aminoadamantane and (*R*)-1-phenylethylamine.

Taking advantage of the ease of displacement of their fluorine substituent, amides **11l**,**m** were reacted with different nucleophiles to enhance the chemical diversity within this family of 4-oxoquinoline-3-carboxamides. Accordingly, when treated with pyrrolidine or sodium ethoxide, **11m** afforded **11n** and **11o** in 38% and 61% yield, respectively (Scheme 3). Similarly, amide **11l** yielded the alkoxy derivatives **11p** (60%) and **11q** (50%) by reaction with sodium methoxide or sodium ethoxide, respectively. Displacement of fluorine by thiophenol converted **11l** into **11r**, which in turn was oxidized using Oxone in 1,4-dioxane/water to give an approximately 1:1 mixture of the corresponding sulfone **11s** and sulfoxide **11t**, which were separated by column chromatography on silica gel.

Finally, the bromoamide **11g** was further modified through Suzuki coupling using phenylboronic acid and *trans*-(4-methoxyphenyl)vinylboronic acid under microwave irradia-

Scheme 3[a]



[a] Reagents and conditions: (i) pyrrolidine or thiophenol, K_2CO_3, DMF, 100 °C, 6 h; (ii) MeONa or EtONa, THF, 50 °C, 16 h; (iii) Oxone, 1,4-dioxane, H_2O, room temp, 24 h.

tion.[28] Compounds **11u** and **11v** were obtained in reasonable yield (30 and 45%, respectively) (Scheme 4).

Biology

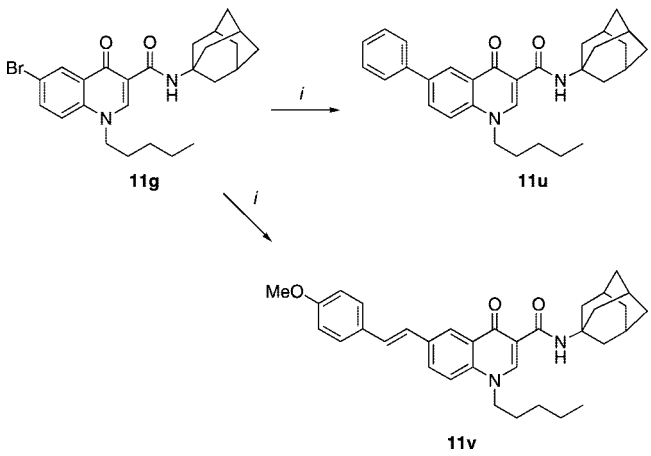
The binding affinities (K_i values) of compounds **11** for human recombinant CB1 and CB2 receptors are reported in Table 2. Tested compounds were evaluated in parallel with **8** (Chart 1) and **1** as CB2 and CB1 reference compounds, respectively, as previously described.[29]

The functional activity of compound **11c** was assessed using the formalin test of acute peripheral and inflammatory pain in mice. Formalin injection induces a biphasic stereotypical nocifensive behavior. Nociceptive responses are divided into an early, short lasting first phase (0−7 min) caused by a primary afferent discharge produced by the stimulus, followed by a quiescent period and then a second, prolonged phase (15−60 min) of tonic pain. Fifteen minutes before injection of formalin, mice received intraperitoneal (ip) administration of vehicle or **11c** (1 or 3 mg/kg), alone or in combination with the selective CB2 antagonist, 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone (AM630) (3 mg/kg, ip), administered 5 min before the compound. The results are presented in Figure 1.

Results and Discussion

Out of the 22 synthesized quinolone carboxamides **11**, compounds **11p** and **11q** elicited solubility issues that prevented their biological testing as DMSO/water solution in the cannabinoid receptor binding assay, while amides **11a**−**o**,**r**−**v** proved to be high affinity CB2 ligands, with K_i values ranging from 55.9 nM (**11v**) to 0.8 nM (**11l**) (Table 2). Under the conditions of our assay, compound **10**, taken as a representative of

Scheme 4[a]



[a] Reagents and conditions: (i) phenylboronic acid or *trans*-2-(4-methoxyphenyl)vinylboronic acid, $Pd(OAc)_2$, Ph_3P, Na_2CO_3, DME, EtOH, microwave, 150 °C, 10 min.

quinolone CB2 agonist, showed K_i = 4.7 nM instead of the reported value of 16.4 nM,[18] while the CB2-selective reference ligand **8** showed K_i = 5.4 nM. With regard to the CB1 affinity, K_i values spanned 4 orders of magnitude in the range from >10000 nM (**11a**,**j**,**u**) to 5.3 nM (**11r**), with **1** (used as the CB1-selective reference ligand) displaying K_i = 12.0 nM. As a consequence of the high variability of affinity for both CB1 and CB2 receptors, the CB2 selectivity index (SI), calculated as K_i(CB1)/K_i(CB2) ratio, for compounds **11a**−**o**,**r**−**v** also varied widely, from >2666.6 to 1.2. In no case was the SI value lower than 1; that is, no compound exhibited a reversed selectivity (higher affinity for CB1 than for CB2).

The nature of the 3-carboxamido substituent seems to affect both receptor affinity and selectivity, since the highly lipophilic adamantylamido substituent confers higher affinity and selectivity for the CB2 receptor with respect to its 1-phenylethylamido counterpart (compare, for instance, **11l** and **11m**) and the two completely unselective compounds **11m** and **11o** are both 1-phenylethylamido derivatives. Coming to the effects of the N1 alkyl chain, affinity increased in the order 1-butenyl < 1-pentyl < 1-pentenyl, while no substantial effect on selectivity was noticed (compare **11d**, **11g**, and **11f**). While this result could be expected on the basis of the findings of Stern et al.,[18] no definitive explanation for the better affinity and selectivity profile of the *N*-allyl derivative **11j**, with respect to the corresponding *N*-pentyl analogue **11i**, can yet be given.

To best discuss the effects of substitution on the condensed benzene ring, the issues of CB2 affinity and selectivity should be distinguished. Thus, several substituents with different steric and electronic properties are compatible with high CB2 affinity, since seven compounds (**11l**, **11s**, **11r**, **11h**, **11k**, **11t**, and **11u**) displayed a K_i value lower than that of the unsubstituted compound **10**, and even the less active compound **11v** was still a nanomolar affinity ligand with a K_i value of 55.9 nM. On the other hand, a preliminary structure−selectivity relationship emerged, with CB2 selectivity depending on the substitution pattern on the aromatic ring. In fact, when the quinolone carboxamides are ranked in order of decreasing SI value, it can be seen that the 12 more selective derivatives **11u**, **11j**, **11a**, **11b**, **11c**, **11h**, **11d**, **11f**, **11g**, **11v**, **11e**, and **11i** (2666.6 ≥ SI ≥ 39.9) are all substituted at least at C6 or C8, followed by **11k** (SI = 38.8) bearing substituents at both C6 and C7, whereas the compounds **11l**, **11n**, **11t**, **11s**, **11r**, **11m**, and **11o** (38.1 ≥ SI ≥ 1.2) substituted only at C7, along with the unsubstituted compound **10** (SI = 9.0), all belong to the group of the less selective ligands. The effect on selectivity of C5 or C8 substitution cannot be yet defined, since the C5 or C8 substituted compounds **11a**, **11i**, and **11j** also present substituents at other positions on the benzene ring. On the basis of these observations, the possibility for a substituent at the C6/C8 positions on the quinolone ring of **11**, regardless of its electronic properties, to occupy defined regions of the space inside CB2 receptor well accounts for the ability of the given ligand to selectively bind to this receptor subtype. That does not mean that substitution at C7 position is detrimental for CB2 affinity, since some of the 7-substituted quinolone carboxamides, such as **11l**, **11s**, **11r**, and **11t**, are among those with the highest affinity at CB2 receptor. This finding is in line with those reported by Stern et al.,[18] who demonstrated that the introduction of a chlorine atom at position 7 of the quinoline template has a favorable effect on CB2 affinity. On the other hand, moving the substituent from C6 to C7 markedly enhances also CB1 affinity, thus giving rise to compounds endowed with lower selectivity (compare, for instance, **11h** and **11r**). Such a result is difficult to be compared with Manera's findings, regarding the increased or decreased CB2 selectivity for their 7-chloro or 7-methyl substituted quinolones, respectively. However, it is considered that compounds of general structure **11** here described differ from those reported by Manera et al.[17] (such as **9**), being characterized by different substituents at positions 1 and 3 of the bicyclic nucleus.

When tested for functional activity, compound **11c**, one of the most potent and CB2-selective ligands in binding assays, dose-dependently inhibited the second phase of the formalin-induced nocifensive response in mice (Figure 1A). The effect was maximal with the 3 mg/kg dose, the action of which was significantly attenuated by the CB2 antagonist AM630 (1 mg/kg) (Figure 1B). These findings suggest that **11c** is able to exert analgesic activity in vivo by acting as a CB2 agonist.

Conclusions

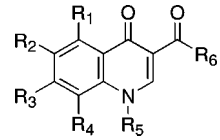
The results of our preliminary screening program on substituted quinolone-3-carboxamides led to the identification of cannabinoid receptor ligands characterized by high affinity and selectivity. In in vitro assays, some of the new compounds exhibited affinity and selectivity values for the CB2 subtype that are even higher than those of the CB2-specific ligand **8**, used as the reference compound. Such a biochemical profile is strongly dependent upon the presence of a substituent at the C6 position of the quinolone ring, while the nature of the substituent itself is far less crucial. The activity shown by derivative **11c** in the formalin-induced nocifensive response in mice suggests that quinolone-3-carboxamides possess agonist properties at the CB2 receptor and might be considered as potential analgesic agents. On the other hand, ligands with high CB1 affinity, though substantially devoid of receptor selectivity, were identified as well, possessing a C7-substituent on the quinolone nucleus. Also in this case, some of the compounds showed CB1 affinity superior to that of **1**, used in our tests as the CB1 reference standard.

The structure−affinity/selectivity relationships emerging from our study suggest that the quinolone carboxamide scaffold can be optimized through selected chemical modifications aimed at designing either CB1 or CB2 selective ligands.

Experimental Section

Chemistry. Reagents were purchased from commercial suppliers and used without further purification. Anhydrous reactions were run under (a positive pressure) dry N_2. Merck silica gel 60 was

Table 1. Structure of the New Compounds **11**−**15**



compd	R_1	R_2	R_3	R_4	R_5	R_6
11a	H	H	Cl	Me	1-pentyl	1-adamantylamino
11b	H	CF_3	H	H	1-pentyl	1-adamantylamino
11c	H	*i*-Pr	H	H	1-pentyl	1-adamantylamino
11d	H	Br	H	H	but-1-en-4-yl	1-adamantylamino
11e	H	3-Cl-4-F-PhO	H	H	1-pentyl	1-adamantylamino
11f	H	Br	H	H	pent-1-en-5-yl	1-adamantylamino
11g	H	Br	H	H	1-pentyl	1-adamantylamino
11h	H	PhS	H	H	1-pentyl	1-adamantylamino
11i	MeO	H	H	MeO	1-pentyl	1-adamantylamino
11j	MeO	H	H	MeO	allyl	1-adamantylamino
11k	H	F	pyrrol-1-yl	H	1-pentyl	1-adamantylamino
11l	H	H	F	H	1-pentyl	1-adamantylamino
11m	H	H	F	H	1-pentyl	(*R*)-1-phenylethylamino
11n	H	H	pyrrolidin-1-yl	H	1-pentyl	(*R*)-1-phenylethylamino
11o	H	H	EtO	H	1-pentyl	(*R*)-1-phenylethylamino
11p	H	H	MeO	H	1-pentyl	1-adamantylamino
11q	H	H	EtO	H	1-pentyl	1-adamantylamino
11r	H	H	PhS	H	1-pentyl	1-adamantylamino
11s	H	H	$PhSO_2$	H	1-pentyl	1-adamantylamino
11t	H	H	PhSO	H	1-pentyl	1-adamantylamino
11u	H	Ph	H	H	1-pentyl	1-adamantylamino
11v	H	(*E*)-4-MeO-styryl	H	H	1-pentyl	1-adamantylamino
12a	H	H	Cl	Me	H	OH
12b	H	CF_3	H	H	H	OH
12c	H	*i*-Pr	H	H	H	OH
12d	H	Br	H	H	H	OH
12e	H	3-Cl-4-F-PhO	H	H	H	OH
12f	H	PhS	H	H	H	OH
13a	MeO	H	H	MeO	H	OEt
13b	H	F	pyrrol-1-yl	H	H	OEt
14a	MeO	H	H	MeO	1-pentyl	OEt
14b	MeO	H	H	MeO	allyl	OEt
14c	H	F	pyrrol-1-yl	H	1-pentyl	OEt
15a	MeO	H	H	MeO	1-pentyl	OH
15b	MeO	H	H	MeO	allyl	OH
15c	H	F	pyrrol-1-yl	H	1-pentyl	OH
15d	H	H	F	H	1-pentyl	OH

used for flash chromatography (23−400 mesh). ^{1}H NMR and ^{13}C NMR were recorded at 200 and 50 MHz, respectively, on a Brucker AC200F spectrometer and at 400 and 100 MHz on a Brucker Advance DPX400. Chemical shifts are reported relative to tetramethylsilane at 0.00 ppm. Mass spectral (MS) data were obtained using an Agilent 1100 LC/MSD VL system (G1946C) with a 0.4 mL/min flow rate using a binary solvent system of 95:5 methanol/water. UV detection was monitored at 254 nm. Mass spectra were acquired either in positive mode or in negative mode scanning over the mass range of 105−1500. Melting points were determined on a Gallenkamp apparatus and are uncorrected. Elemental analyses were performed on a Perkin-Elmer PE 2004 elemental analyzer, and the data for C, H, and N are within 0.4% of the theoretical values. Microwave irradiations were conducted using a CEM Discover Synthesis Unit (CEM Corp., Matthews, NC).

Parallel Synthesis of Acid Derivatives 12a−f. Six anilines (1 mmol) were added into six round-bottom flasks of a carousel reactor, and diethyl ethoxymethylemalonate (1 mmol) was added. The reaction mixtures were heated at 120 °C for 4 h and cooled to room temperature. Ph_2O (1 mL) was added to each vessel, and the reaction mixtures were heated at reflux temperature for 16 h. After the mixture was cooled to room temperature, 10% aqueous NaOH was added to each flask and the reaction mixtures were refluxed for 4 h. After the mixture was cooled, concentrated HCl was added to the reaction mixtures, allowing the precipitation of the five acid derivatives, which were collected by filtration, washed with water, then petroleum ether, and recrystallized from DMF.

Example. 4-Oxo-6-(trifluoromethyl)-1,4-dihydroquinoline-3-carboxylic Acid (12b). 12b was prepared by parallel synthesis from 4-(trifluoromethyl)aniline in 25% yield. White solid; mp >280 °C (dec). ^{1}H NMR (200 MHz, DMSO-d_6): δ 17.09 (s, 1H), 8.83 (s, 1H), 8.44 (s, 1H), 7.81−7.75 (m, 2H). MS (ESI): *m*/*z* 258 $[M + H]^+$, 280 $[M + Na]^+$. IR (nujol): ν 3406, 1643, 1619 cm^{-1}. Anal. ($C_{11}H_6F_3NO_3$) C, H, N.

Parallel Synthesis of Amides 11a−h. Acids **12a−f** (1 mmol) and DMF (3 mL) were placed in different vessels of a Syncore reactor under a nitrogen atmosphere. HOBt (1 mmol), HBTU (2 mmol), DIPEA (1.5 mmol), and 1-aminoadamantane (1.2 mmol) were added to the solutions, and the reaction mixtures were stirred at room temperature for 30 min. Further DIPEA (1.5 mmol) was thereafter added, and the reaction mixtures were stirred at room temperature for 20 h. K_2CO_3 (5 mmol), the appropriate alkyl halide (5 mmol), and KI (5 mmol) were added to every mixture, which was heated at 90 °C for 20 h. Each reaction mixture was poured into ice and extracted with AcOEt. The organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. The crude residues were purified by chromatography using light petroleum ether/AcOEt (5:1 to 1:1) to afford the pure amides.

Example. *N*-(Adamant-1-yl)-7-chloro-8-methyl-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (11a). 11a was prepared by parallel synthesis from **12a** in 7% yield. Light-yellow oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.29 (s, 1H), 8.51 (s, 1H), 7.88 (d, J = 9.0 Hz, 1H), 7.51 (d, J = 9.0 Hz, 1H), 4.12 (t, J = 6.8 Hz, 2H), 2.8 (s, 3H), 2.15−1.96 (m, 8H), 1.92−1.89 (m, 2H), 1.73−1.63 (m, 7H),

Table 2. CB1 and CB2 Receptor Affinity Values for Compounds **11a−v**[a]

compd	K_i[f] (nM) CB1[b,d]	CB2[c,d]	SI[e]
11a	>10000	25.5	>392.7
11b	2520	11.6	216.7
11c	1220	6.3	194.3
11d	2680	23.8	112.7
11e	739	16.1	46.0
11f	696	9.4	74.4
11g	996	14.3	69.4
11h	640	3.4	189.3
11i	2100	52.6	39.9
11j	>10000	9.2	>1091.7
11k	139.8	3.6	38.8
11l	30.5	0.8	38.1
11m	14.3	10.7	1.3
11n	160	17.1	9.4
11o	29.7	24.2	1.2
11p	NT[g]	NT[g]	
11q	NT[g]	NT[g]	
11r	5.3	1.6	3.3
11s	6.1	0.9	6.8
11t	28.0	3.5	8.0
11u	>10000	3.8	>2666.6
11v	2630	55.9	47.0
10[h]	42.1	4.7	9.0
8[h,i]	>2820	5.4	>522.2
1[h,j]	12.0	790	0.015

[a] Data represent mean values for at least three separate experiments performed in duplicate and are expressed as K_i (nM). [b] CB1: human cannabinoid type 1 receptor. [c] CB2: human cannabinoid type 2 receptor. [d] For both receptor binding assays, the new compounds were tested using membranes from HEK cells transfected with either the CB1 or CB2 receptor and [^{3}H]-(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol ([^{3}H]CP-55,940). [e] SI: selectivity index for CB2, calculated as K_i(CB1)/K_i(CB2) ratio. [f] K_i: "Equilibrium dissociation constant", that is, the concentration of the competing ligand that will bind to half the binding sites at equilibrium in the absence of radioligand or other competitors. [g] NT: not tested because of solubility problems. [h] The binding affinities of reference compounds were evaluated in parallel with compounds**11** under the same conditions. [i] SR144528, CB2 reference compound. [j] Rimonabant, CB1 reference compound.

1.53−1.35 (m, 4H), 0.96 (t, J = 6.9 Hz, 3H). MS (ESI): *m*/*z* 441 [M + H]$^+$, 464 [M + Na]$^+$. Anal. ($C_{26}H_{33}ClN_2O_2$) C, H, N.

Synthesis of 4-Oxo-1,4-dihydroquinoline-3-carboxamides 11i−m by Amidation Reaction. General Procedure. To a solution of the appropriate acid derivative **15a−d** (1 mmol) in DMF (5 mL), HBTU (2 mmol) was added followed by HOBt (1 mmol), DIPEA (1.5 mmol), and 1-aminoadamantane or (*R*)-1-phenylethylamine (1.2 mmol). The mixture was stirred at room temperature under N_2 for 30 min, and further DIPEA (1.5 mmol) was added. The reaction mixture was stirred at room temperature for 1 h, poured into water, and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The crude residue was recrystallized from EtOH, with the exception of **11k**, which was triturated with diethyl ether.

Example. *N*-(Adamant-1-yl)-7-fluoro-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (11l). 11l was prepared from **15d** and 1-aminoadamantane in 76% yield. Pink solid; mp 197.5−200.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.76 (s, 1H), 8.65 (s, 1H), 8.49−8.42 (m, 1H), 7.15−7.07 (m, 2H), 4.09 (t, J = 7.5 Hz, 2H), 2.11−2.05 (m, 10H), 1.97−1.78 (m, 2H), 1.74−1.66 (m, 5H), 1.33−1.19 (m, 4H), 0.85 (t, J = 6.7 Hz, 3H). MS (ESI): *m*/*z* 411 [M + H]$^+$, 433 [M + Na]$^+$. IR ($CHCl_3$): 1659, 1605 cm^{-1}. Anal. ($C_{25}H_{31}FN_2O_2$) C, H, N.

Synthesis of 1-Alkyl-4-oxo-1,4-dihydroquinoline Derivatives 14a−c by Alkylation Reaction. General Procedure. To a mixture of the quinolone derivative **13a**,**b** (1 mmol) and K_2CO_3 (2.8 mmol) in dry DMF (2 mL) under a nitrogen atmosphere, the appropriate alkyl iodide (2.8 mmol) or alkyl bromide along with KI (2.8 mmol each) was added. The reaction mixture was heated at 90 °C for 18 h and then poured into ice−water. The resulting *N*-alkylquinolone was collected by filtration and recrystallized from DMF or extracted in CH_2Cl_2 and purified by flash chromatography on silica gel eluting with CH_2Cl_2/MeOH (98:2).

Example. Ethyl 5,8-Dimethoxy-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxylate (14a). 14a was prepared in 41% yield from **13a**. White solid, mp 121−122 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.17 (s, 1H), 7.05 (d, J = 8.9 Hz, 1H), 6.76 (d, J = 8.9 Hz, 1H), 4.38−4.28 (m, 4H), 3.87 (s, 3H), 3.85 (s, 3H), 1.77−1.63 (m, 2H), 1.35 (t, J = 7.0 Hz, 3H), 1.28−1.20 (m, 4H), 0.86 (t, J = 6.2 Hz, 3H). MS (ESI): *m*/*z* 348 [M + H]$^+$, 370 [M + Na]$^+$. IR (nujol): ν 1656, 1615 cm^{-1}. Anal. ($C_{19}H_{25}NO_5$) C, H, N.

Synthesis of 7-Substituted-4-oxo-1,4-dihydroquinoline-3-carboxamides 11n and 11r. General Procedure. K_2CO_3 (3 mmol) and pyrrolidine (7.6 mmol) or thiophenol (3 mmol) were added to a solution of **11m** or **11l** (1 mmol), respectively, in DMF (8 mL) under a nitrogen atmosphere. The reaction mixture was heated at 100 °C for 6 h, cooled to room temperature, and poured into water. The solid precipitate was collected by filtration, washed with light petroleum ether, and recrystallized from EtOH.

Example. *N*-(Adamant-1-yl)-4-oxo-1-pentyl-7-(phenylthio)-1,4-dihydroquinoline-3-carboxamide (11r). 11r was prepared from **11l** and thiophenol in 58% yield. White solid; mp 164.5 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.86 (s, 1H), 8.60 (s, 1H), 8.33 (d, J = 8.6 Hz, 1H), 7.57−7.52 (m, 2H), 7.50−7.41 (m, 3H), 7.24−7.23 (m, 1H), 7.19 (s, 1H), 3.92 (t, J = 7.7 Hz, 2H), 2.14−2.08 (m, 8H), 1.76−1.57 (m, 9H), 1.32−1.10 (m, 4H), 0.86 (t, J = 6.8 Hz, 3H). MS (ESI): *m*/*z* 501 [M + H]$^+$, 523 [M + Na]$^+$. IR (nujol): ν 1659, 1602 cm^{-1}. Anal. ($C_{31}H_{36}N_2O_2S$) C, H, N.

Synthesis of 7-Substituted-4-oxo-1,4-dihydroquinoline-3-carboxamides 11o−q. General Procedure. A solution of **11l** or **11m** (0.14 mmol) in dry THF (3 mL) was added to a solution of freshly prepared MeONa or EtONa (0.28 mmol) under a nitrogen atmosphere. The reaction mixture was heated at 50 °C for 16 h and neutralized using 1 N HCl. The mixture was concentrated under reduced pressure and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to dryness. The crude residue was purified by chromatography using light petroleum ether:AcOEt (1:1) as eluent.

Example. (*R*)-7-Ethoxy-4-oxo-1-pentyl-*N*-(1-phenylethyl)-1,4-dihydroquinoline-3-carboxamide (11o). 11o was prepared from **11m** and EtONa in 61% yield. White solid; mp 128.2 °C (from EtOH). ^{1}H NMR (400 MHz $CDCl_3$): δ 10.50 (d, J = 6.8 Hz, 1H), 8.61 (s, 1H), 8.37 (d, J = 9.1 Hz, 1H), 7.36−7.14 (m, 5H), 7.0−6.97 (m, 1H), 6.76 (s, 1H), 5.24 (t, J = 7.0 Hz, 1H), 4.12−4.05 (m, 4H), 1.80−1.73 (m, 2H), 1.53 (d, J = 6.8 Hz, 3H), 1.42 (t, J = 6.8 Hz, 3H), 1.31−1.27 (m, 4H), 0.87−0.78 (m, 3H). MS (ESI): *m*/*z* 407 [M + H]$^+$, 429 [M + Na]$^+$. IR (nujol): ν 1667, 1603 cm^{-1}. Anal. ($C_{25}H_{30}N_2O_3$) C, H, N.

Synthesis of Compounds 11s and 11t. To a solution of Oxone (1.1 g, 1.8 mmol) in H_2O (2 mL), a solution of **3r** (28 mg, 0.06 mmol) in 1,4-dioxane (2 mL) was added. The reaction mixture was stirred at room temperature for 20 h and extracted with CH_2Cl_2. The organic layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. The crude residue was purified by chromatography using light petroleum ether/AcOEt (2:1) as eluent to provide compounds **11s** (9.5 mg, 33% yield) and **11t** (11 mg, 37% yield) as white solids.

***N*-(Adamant-1-yl)-4-oxo-1-pentyl-7-(phenylsulfonyl)-1,4-dihydroquinoline-3-carboxamide (11s).** Mp 215.6 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.36 (s, 1H), 8.78 (s, 1H), 8.60 (d, J = 8.7 Hz, 1H), 8.17 (s, 1H), 7.99−7.96 (m, 2H), 7.82 (d, J = 8.7 Hz, 1H), 7.57−7.54 (m, 3H), 4.26 (t, J = 7.5 Hz, 2H), 2.14−2.02 (m, 10H), 1.88−1.81 (m, 2H), 1.80−1.70 (m, 5H), 1.37 (m, 4H), 0.92−0.84 (m, 3H). MS (ESI): *m*/*z* 555 [M + Na]$^+$. IR ($CHCl_3$): ν 1716, 1659 1598 cm^{-1}. Anal. ($C_{31}H_{36}N_2O_4S$) C, H, N.

***N*-(Adamant-1-yl)-4-oxo-1-pentyl-7-(phenylsulfinyl)-1,4-dihydroquinoline-3-carboxamide (11t).** Mp 102−103 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.73 (s, 1H), 8.78 (s, 1H), 8.50 (d, J = 8.1 Hz, 1H), 8.02 (s, 1H), 7.68−7.34 (m, 6H), 4.27 (t, J = 7.2 Hz, 2H), 2.14−2.09 (m, 10H), 1.90−1.81 (m, 2H), 1.80−1.71 (m, 5H), 1.37

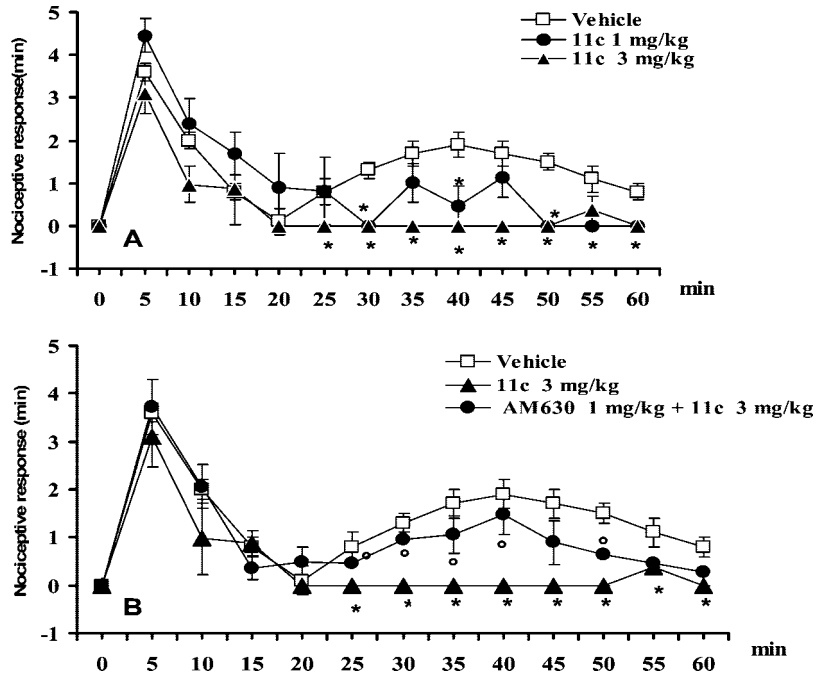


Figure 1. (A) Analgesic effect of **11c** in the formalin test in mice. Each point represents the mean ± standard error of the mean (SEM) of 8−10 animals per group. Asterisks denote values that were significantly different from vehicle ($P < 0.05$), as assessed using two-way ANOVA followed by the Bonferroni's test. (B) Effect of the CB2 antagonist 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-y l](4-methoxyphenyl)methanone (AM630) on the analgesic effect of **11c**. Asterisks denote values that were significantly different from vehicle ($P < 0.05$), whereas circles denote values that were significantly different from **11c** only, as assessed using two-way ANOVA followed by the Bonferroni's test.

(m, 4H), 0.92−0.86 (m, 3H). MS (ESI): *m/z* 517 $[M + 1]^+$, 539 $[M + Na]^+$. IR ($CHCl_3$): ν 1722, 1660, 1598 cm^{-1}. Anal. ($C_{31}H_{36}N_2O_3S$) C, H, N.

Synthesis of Compounds 11u and 11v by Suzuki Reaction. General Procedure. To a solution of **11g** (1 mmol) in DME (4 mL), $Pd(OAc)_2$ (0.1 mmol), PPh_3 (0.3 mmol), the appropriate boronic acid (0.5 mmol), EtOH (1 mL), and 1 N Na_2CO_3 (2 mL) were added. The mixture was irradiated with microwaves at 150 °C for 10 min, then filtered through a plug of Celite. The filtrate was washed with H_2O, brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. The solid residue was purified by flash chromatography using CH_2Cl_2 as eluent.

Example. *N*-(Adamant-1-yl)-4-oxo-1-pentyl-6-phenyl-1,4-dihydroquinoline-3-carboxamide (11u). 11u was prepared from **11g** and phenylboronic acid in 45% yield. Yellow oil. ^1H NMR (200 MHz, $CDCl_3$): δ 9.97 (s, 1H), 8.76−8.74 (m, 2H), 7.96 (dd, $J_1 = 1.8$ Hz, $J_2 = 8.8$ Hz, 1H), 7.72−7.67 (m, 2H), 7.57 (d, $J = 8.9$ Hz, 1H), 7.51−7.32 (m, 3H), 4.23 (t, $J = 7.4$ Hz, 2H), 2.18−2.10 (m, 8H), 1.95−1.78 (m, 2H), 1.72−1.62 (m, 7H), 1.40−1.27 (m, 4H), 0.91 (t, $J = 6.8$ Hz, 3H). MS (ESI): *m/z* 469 $[M + H]^+$, 491 $[M + Na]^+$. IR ($CHCl_3$): ν 3248, 1657, 1601 cm^{-1}. Anal. ($C_{31}H_{36}N_2O_2$) C, H, N.

Synthesis of 1-Alkyl-4-oxo-1,4-dihydroquinoline-3-carboxylic Acids 15a−c by Basic Hydrolysis. General Procedure. A suspension of ester **14a−c** (1 mmol) in 10% aqueous NaOH (10 mL) was refluxed for 2 h (until the suspension became a solution). After cooling at room temperature, the reaction mixture was acidified using concentrated HCl. The resulting precipitate was filtered and washed with water to give the corresponding quinolone-3-carboxylic acid, which was recrystallized from EtOH.

Example. 5,8-Dimethoxy-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxylic Acid (15a). 15a was prepared from **14a** in 86% yield. White solid; mp 209.7 °C. ^1H NMR (200 MHz, DMSO-d_6): δ 15.73 (s, 1H), 8.59 (s, 1H), 7.40 (d, $J = 9.0$ Hz, 1H), 6.97 (d, $J = 9.0$ Hz, 1H), 4.54 (t, $J = 7.5$ Hz, 2H), 3.87 (s, 3H), 3.78 (s, 3H), 1.68−1.50 (m, 2H), 1.03 (m, 4H), 0.81 (t, $J = 6.8$ Hz, 3H). MS (ESI): *m/z* 320 $[M + H]^+$, 342 $[M + Na]^+$. IR (nujol): ν 3378, 1728, 1630 cm^{-1}. Anal. ($C_{17}H_{21}NO_5$) C, H, N.

7-Fluoro-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxylic Acid (15d). A suspension of **17** (305 mg, 1 mmol) in EtOH (3.5 mL) and 6 N HCl (3.5 mL) was refluxed for 7 h. After the mixture was cooled to room temperature, the solid precipitate was filtered and recrystallized from EtOH to furnish **15d** (260 mg, 94%) as a light-pink solid; mp 148.3 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 14.7 (b, 1H), 8.73 (s, 1H), 8.61−8.53 (dd, $J_1 = 6.6$ Hz, $J_2 = 8.8$ Hz, 1H), 7.33−7.21 (m, 2H), 4.23 (t, $J = 7.3$ Hz, 2H), 1.95−1.88 (m, 2H), 1.55−1.39 (m, 4H), 0.96−0.89 (m, 3H). MS (ESI): *m/z* 278 $[M + H]^+$, 300 $[M + Na]^+$. IR ($CHCl_3$): ν 1722, 1615 cm^{-1}. Anal. ($C_{15}H_{16}FNO_3$) C, H, N.

Ethyl 2-(2,4-Difluorobenzoyl)-3-(dimethylamino)acrylate (16). To a solution of 2,4-difluorobenzoyl chloride (2.8 mL, 22.7 mmol) and Et_3N (4.8 mL, 34.1 mmol) in toluene, ethyl (dimethylamino)acrylate (3.2 g, 22.7 mmol) was added, and the reaction mixture was heated at 90 °C for 4 h. The solvent was removed at reduced pressure, and the crude solid was purified by chromatography using AcOEt/light petroleum ether (1:1 to 2:1) to afford **16** (4.29 g, 67% yield) as a yellow solid, which was recrystallized from EtOH. Mp >280 °C (dec). ^1H NMR (200 MHz $CDCl_3$): δ 7.73 (s, 1H), 7.66−7.55 (m, 1H), 6.92−6.82 (m, 1H), 6.79−6.69 (m, 1H), 3.97 (q, $J = 7.1$ Hz, 2H), 3.05 (b, 6H), 0.94 (t, $J = 7.1$ Hz, 3H). MS (ESI): *m/z* 284 $[M + H]^+$, 306 $[M + Na]^+$. IR ($CHCl_3$): ν 1725, 1612, 1234, 1220 cm^{-1}. Anal. ($C_{14}H_{15}F_2NO_3$) C, H, N.

Ethyl 7-Fluoro-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxylate (17). *n*-Pentylamine (1.7 mL, 15.2 mmol) was added dropwise to a solution of **16** (4.29 g, 15.2 mmol) in Et_2O (160 mL) and EtOH (69 mL). The reaction mixture was stirred at room temperature for 25 min, and the solvent was removed under reduced pressure. The residue was washed with 0.1 N HCl, dried under anhydrous Na_2SO_4, and evaporated to dryness. DMF (20 mL) and K_2CO_3 (3.5 g, 25.6 mmol) were added to the solid residue, and the mixture was heated at 90 °C for 1 h, then poured into ice−water. The precipitated solid was collected by filtration and recrystallized from EtOH to give **17** (3.88 g, 89% yield) as a yellow solid; mp 104−106.3 °C. ^1H NMR (200 MHz, $CDCl_3$): δ 8.57−8.49 (m, 1H), 8.43 (s,1H), 7.16−7.04 (m, 2H), 4.39 (q, $J = 7.2$ Hz, 2H), 4.08 (t, $J = 7.5$ Hz, 2H), 1.90−1.80 (m, 2H), 1.43−1.34 (m, 7H), 0.92 (t, $J = 6.6$ Hz,

3H). MS (ESI): *m/z* 306 $[M + H]^+$, 328 $[M + Na]^+$. IR ($CHCl_3$): ν 1726, 1689 cm^{-1}. Anal. ($C_{17}H_{20}FNO_3$) C, H, N.

CB1 and CB2 Receptors Binding Assays. The new compounds were evaluated in CB1 and CB2 receptors binding assays using membranes from HEK cells transfected with either the CB1 or CB2 receptor and [^{3}H]-(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol ([^{3}H]CP-55,940; K_d = 0.18 nM for CB1R and K_d = 0.31 nM for CB2R) as the high affinity ligand, as described by the manufacturer (Perkin-Elmer, Italy).[29] Stock solutions of compounds were daily prepared in DMSO with final DMSO concentration less than 0.1%. Displacement curves were generated by incubating drugs with [^{3}H]CP-55,940 (0.14 nM for CB1R and 0.084 nM for CB2R binding assay). In all cases, K_i values were calculated by applying the Cheng−Prusoff equation[30] to the IC_{50} values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compounds.

Formalin Test in Mice. Mice received formalin (1.25%) in the dorsal surface of one side of the hind paw. Each mouse was randomly assigned to one of the experimental groups (n = 8−10) and placed in a Plexiglas cage and allowed to move freely for 15−20 min. A mirror was placed at a 45° angle under the cage to allow full view of the hind paws. Lifting, favoring, licking, shaking, and flinching of the injected paw were recorded as nociceptive responses. Fifteen minutes before injection of formalin, mice received intraperitoneal vehicle (10% DMSO in 0.9% NaCl, 50 μL) or **11c** (1 or 3 mg/kg, ip) in the same volume solution, alone or in combination with the selective CB2 antagonist, AM630 (3 mg/kg, ip), administered 5 min before the compound. The total time of the nociceptive response was measured every 5 min and expressed as the total time of the nociceptive responses in min (mean ± SEM). Recording of nociceptive behavior commenced immediately after formalin injection and was continued for 60 min.

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Supporting Information Available: Synthetic and spectral data for compounds **11b**−**k**,**m**,**n**,**p**,**q**,**v**, **12a**,**c**−**f**, **14b**,**c**, **15b**,**c** and elemental analyses for compounds **11**−**17**. This material is available free of charge via the Internet at http://pubs.acs.org.

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