Journal of Medicinal Chemistry

ARTICLE

pubs.acs.org/jmc

Investigations on the 4-Quinolone-3-carboxylic Acid Motif. 4. Identification of New Potent and Selective Ligands for the Cannabinoid Type 2 Receptor with Diverse Substitution Patterns and Antihyperalgesic Effects in Mice

Serena Pasquini,[†] Maria De Rosa,[†] Valentina Pedani,[†] Claudia Mugnaini,[†] Francesca Guida,[‡] Livio Luongo,[‡] Maria De Chiaro,[‡] Sabatino Maione,[‡] Stefania Dragoni,[§] Maria Frosini,[§] Alessia Ligresti,[∥] Vincenzo Di Marzo,[∥] and Federico Corelli*[,†]

[†]Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via A. Moro, 53100 Siena, Italy

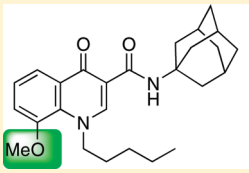
[‡]Department of Experimental Medicine—Section of Pharmacology "L. Donatelli", Second University of Naples, Via S. Maria di Costantinopoli 16, 80138 Naples, Italy

[§]Dipartimento di Neuroscienze, Sezione di Farmacologia, Università degli Studi di Siena, Via A. Moro, 53100 Siena, Italy

[∥]Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Via dei Campi Flegrei 34, 80078 Pozzuoli (Naples), Italy

Supporting Information

ABSTRACT: Experimental evidence suggests that selective CB2 receptor modulators may provide access to antihyperalgesic agents devoid of psychotropic effects. Taking advantage of previous findings on structure−activity/selectivity relationships for a class of 4-quinolone-3-carboxamides, further structural modifications of the heterocyclic scaffold were explored, leading to the discovery of the 8-methoxy derivative **4a** endowed with the highest affinity *and* selectivity ever reported for a CB2 ligand. The compound, evaluated in vivo in the formalin test, behaved as an inverse agonist by reducing at a dose of 6 mg/kg the second phase of the formalin-induced nocifensive response in mice.



CB1, K_i > 10,000 nM
CB2, K_i = 0.6 nM
SI > 16,666

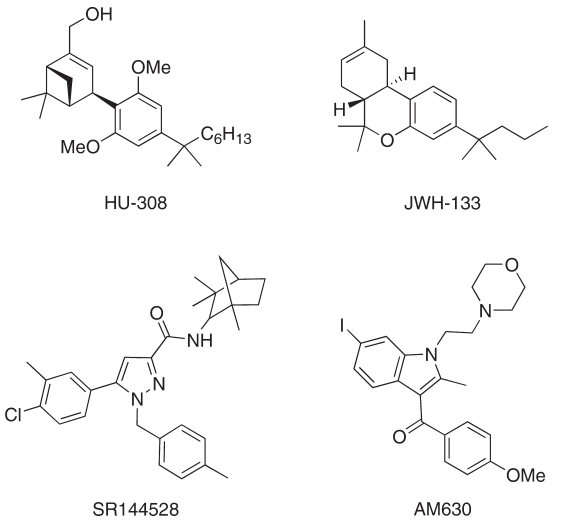
INTRODUCTION

Research in medicinal chemistry during the last years has led to the identification of a spectrum of compounds with different affinities and selectivities for cannabinoid type 1 (CB1) and type 2 (CB2) receptors, compounds that have been essential in characterizing the role of cannabinoid receptors in the body.[1] Differences in receptor distribution and signal transduction mechanisms[2] are likely to account for the relative absence of CNS side effects induced by CB2 ligands. These considerations suggest that novel pharmacotherapies selectively targeting CB2 receptors may have considerable therapeutic potential.[3] Accordingly, significant medicinal chemistry efforts have been directed to the characterization of selective CB2 agonists (Chart 1), leading to the identification of compounds eliciting antinociceptive effects in models of acute pain, persistent inflammatory pain, postoperative pain, cancer pain, and neuropathic pain.[4] In recent years, a number of reports have suggested that CB2 inverse agonists/antagonists (Chart 1) may possess antiinflammatory activity, being able to inhibit carrageenan-induced paw edema in mice, to reduce leukocytes trafficking, and to impair the migration of cells toward cannabinoid agonists.[5] The CB2 receptor is also an emerging target in osteoporosis disease modification,[6] as it has been reported to regulate bone mass, but conflicting results have been reported with regard to its effects on bone resorption and osteoclast function. In fact, in one study carried out in the mouse, it has been demonstrated that CB2-selective antagonist/inverse agonist AM630[7] (Chart 1) inhibited osteoclast formation and activity in vitro, whereas the CB2-selective agonists JWH133[8] and HU308[9] stimulated osteoclast formation.[10] However, a more recent study performed in human cells has shown that AM630 instead enhances osteoclast activity.[11] Ofek and coworkers have reported that activation of CB2 stimulates osteoblast proliferation and bone marrow derived colony-forming units osteoblastic and that selective and nonselective CB2 agonists are mitogenic in MC3T3 E1 and newborn mouse calvarial osteoblastic cultures.[12]

Very recently we have described a family of 6-substituted-4-quinolone-3-carboxamide derivatives displaying high CB2 receptor affinity and selectivity over CB1 receptor. Some of these compounds, such as **1**[13] and **2**[14] (Chart 2), were demonstrated to act as agonists or inverse agonists, respectively, in functional activity assays, depending on the aliphatic or aromatic nature of the 6-substituent. On the other hand, a three-dimensional

Received: April 20, 2011
Published: June 27, 2011

Chart 1. Representative CB2-Selective Agonists (HU-308, JWH-133) and Inverse Agonists/Antagonists (SR144528, AM630)



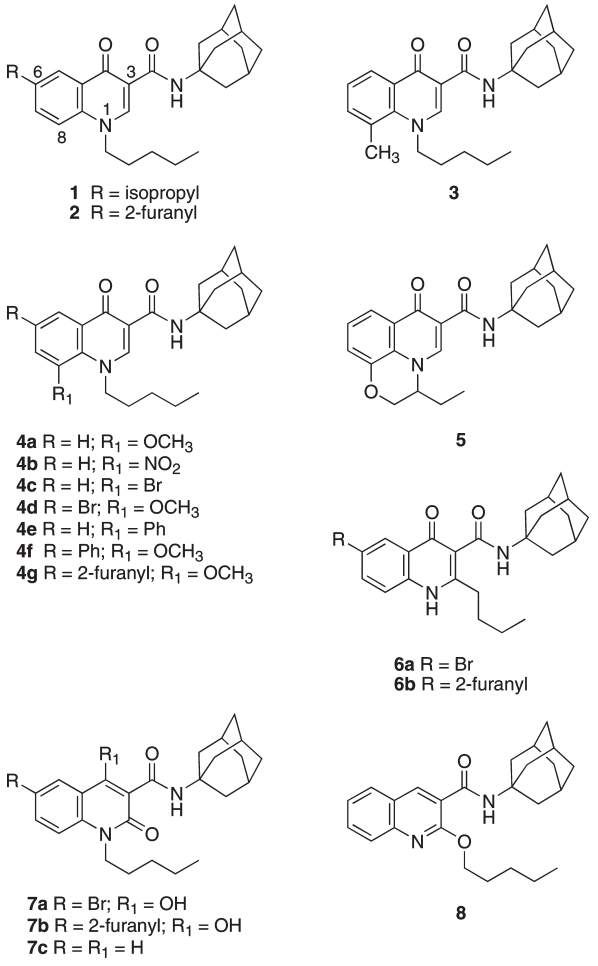
quantitative structure−selectivity relationship (3D-QSSAR) analysis performed on a large set of known CB1 and CB2 ligands resulted in the rational design of the 8-methyl-4-quinolone-3-carboxamide **3** as a high affinity and very selective CB2 receptor ligand.[15]

Starting from these achievements, we first synthesized some 8-substituted-4-quinolone-3-carboxamide derivatives **4a**−**c**,**e** and subsequently 6,8-disubstituted quinolones **4d**,**f**−**g** to explore the possibility of obtaining a more pronounced effect in terms of CB2 affinity and selectivity through double substitution. Then, treasuring the indications delivered by the molecular modeling studies, compounds **6a** and **6b**, exhibiting an aliphatic chain at position 2 and a free NH group at position 1, were investigated, together with the tricyclic derivative **5**. These structural modifications were expected to improve the hydrophilic/hydrophobic balance of the compounds and to provide useful information on the role of the aliphatic chain in the receptors binding. Finally, 6-substituted-4-hydroxy-2-quinolone-3-carboxamides **7a** and **7b** ($pK_a < 5$)[16] were prepared by considering the possibility of converting them into the corresponding sodium salts. For comparison purposes, the 2-quinolone and 2-alkoxyquinoline derivatives **7c** and **8** were also synthesized.

CHEMISTRY

The synthesis of the final compounds **4**−**8** was accomplished as depicted in Schemes 1−4. Commercially available anilines **9a**−**d** and 2-(1-pentyloxy)aniline (**9e**) (see Supporting Information) were converted into the corresponding *N*-(adamantan-1-yl)-1,4-dihydro-4-oxoquinoline-3-carboxamide derivatives **10a**−**e** in 7−37% overall yield according to synthetic procedures previously set up for similar compounds (Scheme 1).[13,14] Intermediates **10a**−**d** were reacted with 1-iodopentane in the presence of potassium carbonate to provide the final quinolones **4a**−**d** in a yield of 57−74% (23% for **4b**). Compound **10c** was subjected to Suzuki cross-coupling reaction with phenylboronic acid to give **11**, which proved to be resistant to *N*-alkylation using 1-iodopentane. Therefore, the final derivative **4e** was prepared from **4c** via Suzuki reaction. Analogously, compounds **4f** and **4g** were obtained from **4d** using phenylboronic acid and 2-furanboronic acid, respectively.

Chart 2. Lead Compounds 1−3 and General Structure of Variously Substituted 4-Quinolone-3-carboxamide Derivatives 4−8

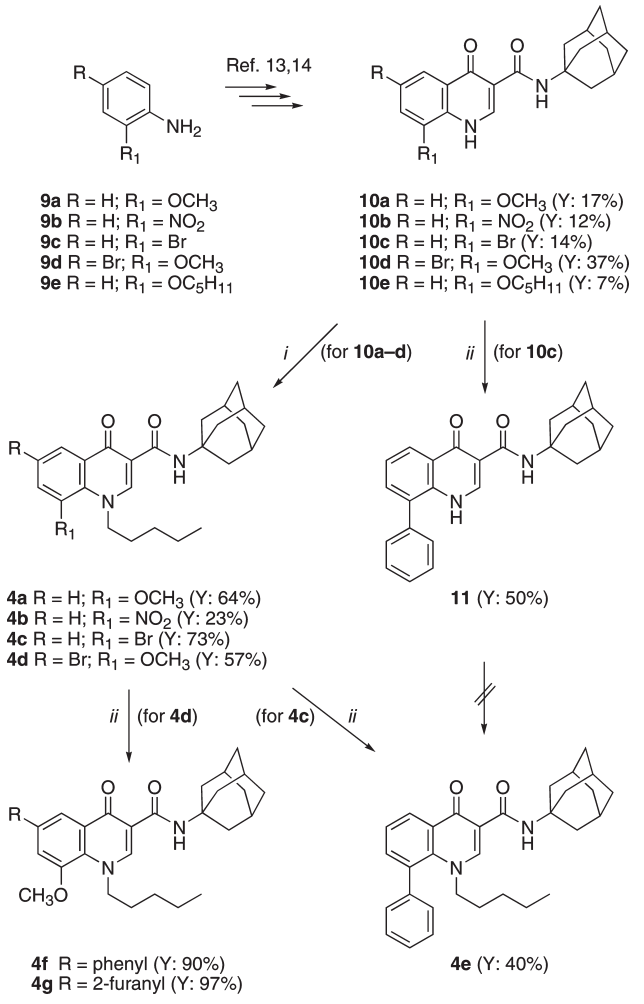


The 1,4-oxazino[2,3,4-*ij*]quinoline **5** was synthesized in a different way, according to a modified Grohe−Heitzer procedure (Scheme 2).[13] Treatment of 2,3-difluorobenzoyl chloride with 3-dimethylaminopropenoic acid ethyl ester led to the enamino ketone **12**, which was subsequently reacted with 2-amino-1-butanol to yield the quinolone ester **14** via the intermediate **13**. Taking advantage of the ease of displacement of the fluorine substituent, the tricyclic acid **15** was obtained by treating **14** with potassium fluoride/potassium carbonate[17] in DMF at 150 °C, followed by alkaline hydrolysis of the ester group. The product **15** so obtained was subjected to coupling with 1-aminoadamantane to deliver the amide **5** in 8% overall yield (from 2,3-difluorobenzoyl chloride).

Both 2-substituted 4-quinolones **6a**,**b** and 4-hydroxy-2-quinolones **7a**,**b** were synthesized from 5-bromoisatoic anhydride (Scheme 3), which was condensed with either methyl 3-oxoheptanoate[18] to give **16** or diethyl malonate,[16] after alkylation to **17**, to yield the 4-hydroxy-2-quinolone derivative **18**. Compound **16** was converted into the corresponding adamantanyl amide **6a** by the hydrolysis/amidation sequence, while the amide **7a** was obtained in quantitative yield by direct aminolysis of **6b** with 1-aminoadamantane in refluxing toluene. Compounds **6a** and **7a** in turn provided the 6-(2-furanyl)-quinolones **6b** and

dx.doi.org/10.1021/jm200476p |*J. Med. Chem.* 2011, 54, 5444–5453

Scheme 1. Synthesis of 4-Quinolone-3-carboxamide Derivatives 4a−g and 11[a]



[a] Reagents and conditions: (i) pentyl iodide, K_2CO_3, DMF, 90 °C, 4 h; (ii) arylboronic acid, $Pd(OAc)_2$, PPh_3, 2 M Na_2CO_3, DME, EtOH, MW, 150 °C, 10 min.

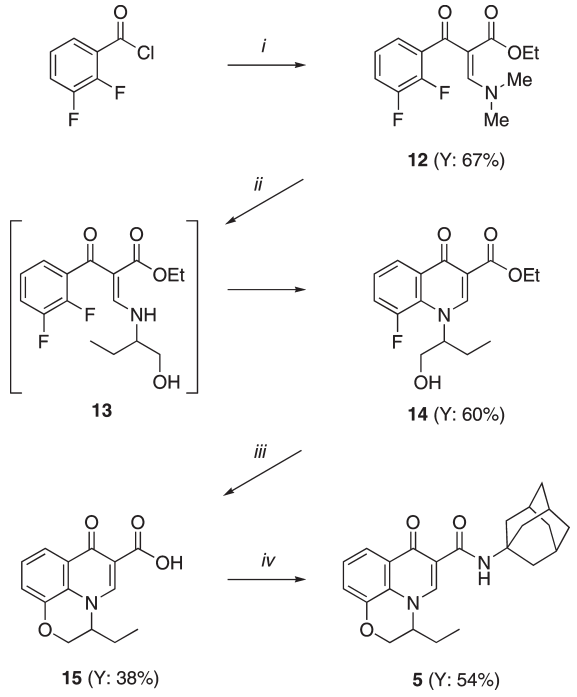
7b, respectively, through Pd-catalyzed reaction with 2-furanboronic acid.

Finally, 2-oxo-1,2-dihydroquinoline-3-carboxylic acid (**19**)[19] was converted into the *N*-adamantyl amide **20** (Scheme 4), which on treatment with 1-iodopentane and potassium carbonate afforded compounds **7c** and **8** in 53% and 33% yield, respectively, after chromatographic separation.

PHARMACOLOGY

With the exception of compounds **4c**, **4e**, **8**, and **10c**, which proved to be insoluble under the test conditions, all the newly synthesized quinolones were screened, in a competitive binding experiment, for their affinity and selectivity toward the human recombinant CB1 and CB2 receptors. The tested compounds were evaluated in parallel with SR144528[20] and rimonabant[21] as CB2 and CB1 reference ligands, respectively, as previously described.[13,14] The results, in terms of binding affinities for the two receptors (K_i values), are reported in Table 1.

Scheme 2. Synthesis of 2*H*-[1,4]-Oxazino[2,3,4-*ij*]quinoline-6-carboxamide Derivative 5[a]



[a] Reagents and conditions: (i) ethyl 3-dimethylaminoacrylate, Et_3N, toluene, 100 °C, 4 h; (ii) (1) 2-aminobutanol, EtOH, Et_2O, rt, 2 h, (2) K_2CO_3, DMF, 90 °C, 3 h; (iii) (1) KF, K_2CO_3, DMF, 150 °C, 3 h, (2) 10% NaOH, reflux, 1 h; (iv) 1-aminoadamantane, HOBt, HBTU, DIPEA, DMF, rt, 3 h.

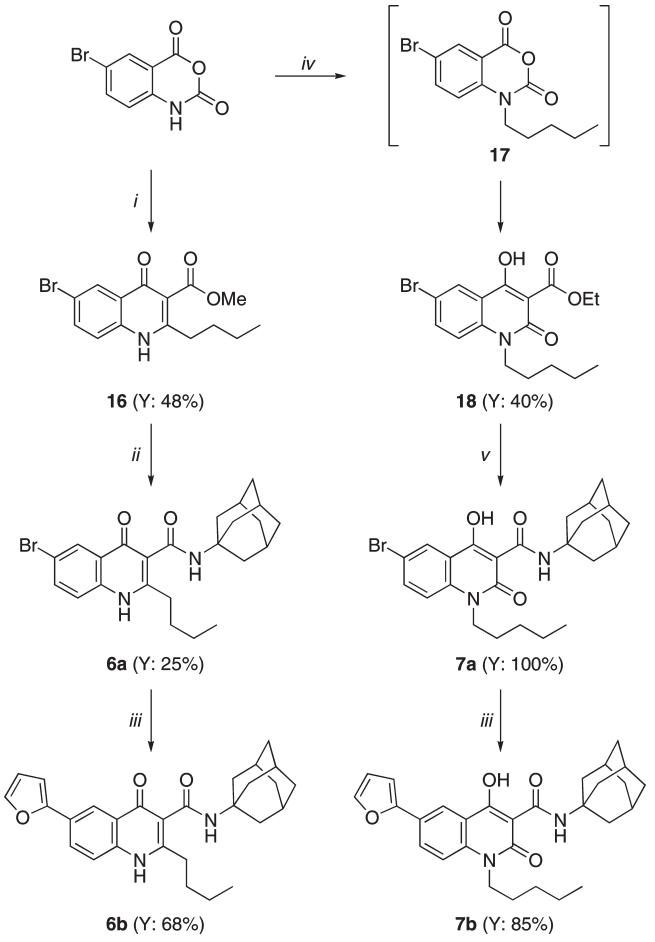
Cytotoxicity assays were also performed to establish the effect of selected quinolone-3-carboxamides on cell proliferation in vitro, using human hepatoblastoma (Hep-G2) cells via MTT assay.

Furthermore, in order to exclude the possibility that these molecules could indirectly activate CB1 and CB2 receptors, through inhibition of endocannabinoid inactivation by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), four compounds, i.e. **4a**, **4g** (the ligands endowed with highest affinity and selectivity for CB2), **5**, and **7a** (the ligand endowed with the lowest CB2 affinity), were selected for enzymatic assays, for which rat brain FAAH and COS-7 cell MAGL activities were used.[22]

Finally, the in vivo activity of the same compounds was evaluated in 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 min before injection of formalin, mice received intraperitoneal (ip) administration of vehicle or of either of the four compounds (1, 3, or 6 mg/kg), alone or in combination with either the selective CB2 antagonist, 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl](4-methoxyphenyl)methanone (AM630) (1 mg/kg, ip), administered 5 min before the compound.[23] The results are presented in Figures 1−4.

dx.doi.org/10.1021/jm200476p | *J. Med. Chem.* 2011, 54, 5444–5453

Scheme 3. Synthesis of Quinolone-3-carboxamide Derivatives 6a,b and 7a,b[a]



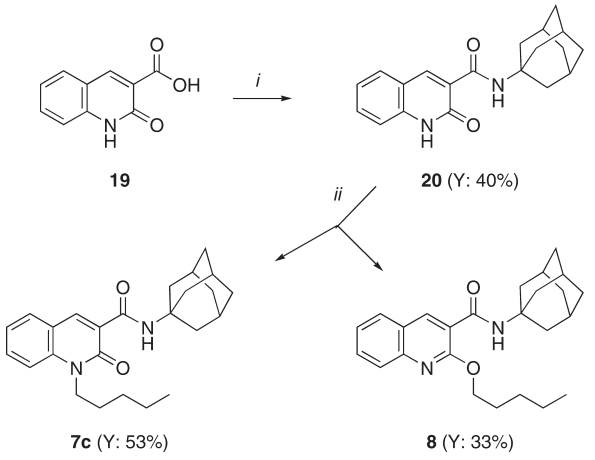
[a] Reagents and conditions: (i) methyl 3-oxoheptanoate, NaOH, 1,4-dioxane, reflux, 48 h; (ii) (1) 10% NaOH, reflux, 1 h, (2) 1-aminoadamantane, HOBt, HBTU, DIPEA, DMF, rt, 30 h; (iii) furanboronic acid, $Pd(OAc)_2$, PPh_3, 2 M Na_2CO_3, DME, EtOH, MW, 150 °C, 10 min; (iv) 1. pentyl iodide, DIPEA, DMF, 80 °C, 18 h, (2) diethyl malonate, sodium *tert*-butoxide, DMF, 100 °C, 2 h; (v) 1-aminoadamantane, toluene, reflux, 1 h.

RESULTS AND DISCUSSION

In Vitro Pharmacology and SAR. All the tested compounds showed moderate to very high affinity toward the CB2 receptor, with K_i values in the range 430−0.2 nM, except for **6a** and **10a**, which proved to be completely inactive on both receptors subtypes. Only compounds **4b**, **7b**, **7c**, and **10e** displayed significant CB1 affinity. According to this binding profile, the selectivity index (SI), calculated as $K_i(CB1)/K_i(CB2)$ ratio, for tested compounds varied widely, going from >16666 (for **4a**) to 4.3 (for **10e**).

In the series of 8-substituted quinolones, the best results in terms of both CB2 affinity and selectivity were obtained with the introduction of electron-donating groups, such as methyl (compound **3**)[15] or methoxy group (compound **4a**), while electron-withdrawing groups, such as nitro, were responsible for significant CB1 affinity as well, resulting in a loss of CB2 selectivity (compound **4b**). Unexpectedly, the double substitution at

Scheme 4. Synthesis of Compounds 7c and 8[a]



[a] Reagents and conditions: (i) 1-aminoadamantane, HOBt, HBTU, DIPEA, DMF, rt, 3 h; (ii) pentyl iodide, K_2CO_3, DMF, 90 °C, 4 h.

positions 6 and 8 of the quinolone nucleus was not beneficial. In fact, starting from the 8-methoxy derivative **4a**, eliciting excellent values of affinity (K_i 0.6 nM) and selectivity (SI > 16666) toward the CB2 receptor, the introduction of a bromine atom (compound **4d**) or a (hetero)aromatic moiety (compounds **4f** and **4g**) led to a loss of CB2 affinity.

1-Unsubstituted compounds **6a** and **10a** showed the lowest receptor affinity within the series in line with a previuos report describing the importance of the *N*1-nitrogen substitution for quinolones to bind at the CB2 receptor.[24] However, when additional lipophilic substituents were introduced in other positions of the bicyclic scaffold, moderate (**6b**, **11**) to significant (**10e**) CB2 affinity was restored, although in the latter case associated with poor selectivity.

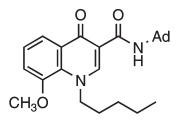
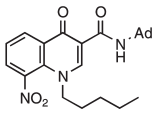
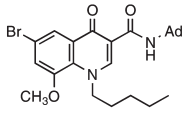
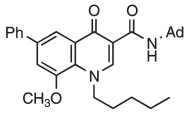
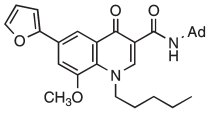
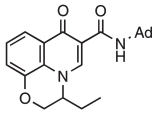
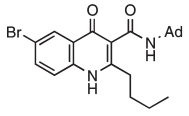
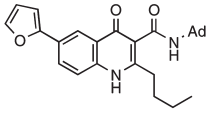
Reducing the conformational freedom of the *N*1-pentyl chain, as in compound **5**, did not abolish the binding capability, although **5** exhibited 10-fold lower CB2 receptor affinity and 100-fold lower selectivity compared to the lead compound **2**.

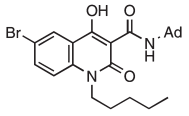
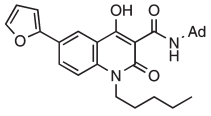
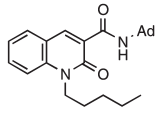
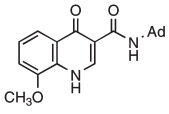
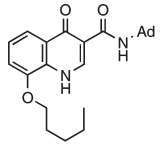
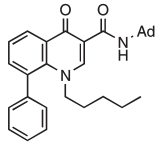
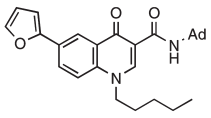
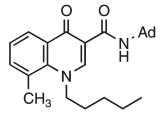
Isomeric 2-quinolone derivatives **7a**, **7b**, and **7c** all proved to be potent CB2 ligands, with **7c** displaying highest CB2 affinity among the tested compounds, including the reference compound **2**, although with markedly reduced selectivity. These findings, which are in agreement with the results recently reported for structurally related 1-substituted-1,8-naphthyridin-4(1*H*)-on-3-carboxamide derivatives,[25] deserve further attention as they represent a good starting point for the development of a novel class of CB2 ligand based on the 2-quinolone scaffold. Furthermore, we show here that at least four of the novel compounds, i.e. **4a**, **4g**, **5**, and **7a**, are devoid of any potential "indirect" agonist activity at cannabinoid receptors, exerted by prolonging the lifespan of endocannabinoids because these compounds did not inhibit anandamide or 2-AG degradation by FAAH or MAGL, respectively, at up to a 10 μM concentration.

Cytotoxicity Evaluation. Cytotoxicity assays were performed to establish the effect of selected quinolone-3-carboxamides on cell proliferation in vitro. Because many drugs are cleared from the body through the liver, this organ is a good choice for target organ toxicity assessment. Consequently, the cytotoxicity of compounds **3**, **4a**, **4d**, **4f**, **4g**, **5**, **6b**, **7a**, **7b**, **10e**, and **11** was evaluated toward human hepatoblastoma (Hep-G2) cells via the MTT assay. The compounds were tested at a single

dx.doi.org/10.1021/jm200476p | *J. Med. Chem.* 2011, 54, 5444–5453

Table 1. CB1 and CB2 Receptor Affinity Values for Compounds 4−7, 10, and 11[a]

compd[b]	CB1[c,e]	CB2[d,e]	
	K_i[f] (nM)	K_i (nM)	S.I.[g]
 **4a**	> 10000	0.6	> 16666
 **4b**	200	1.0	200
 **4d**	> 10000	22.9	> 437
 **4f**	> 10000	430	23
 **4g**	> 10000	8.5	> 1176
 **5**	1000	6.9	145
 **6a**	> 10000	1180	> 8
 **6b**	> 10000	133	> 75

compd[b]	CB1[c,e]	CB2[d,e]	
	K_i[f] (nM)	K_i (nM)	S.I.[g]
 **7a**	> 10000	29.4	> 340
 **7b**	270	15.0	18
 **7c**	31.3	0.2	157
 **10a**	> 10000	3443	> 2.9
 **10e**	260	60.0	4.3
 **11**	> 10000	220	45
 **2**[h]	> 10000	0.7	> 14286
 **3**[h]	> 10000	4.9	>2040
SR144528[h,i]	>2820	5.4	>522
Rimonabant[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] Ad = adamantan-1-yl. [c] CB1: human cannabinoid type 1 receptor. [d] CB2: human cannabinoid type 2 receptor. [e] 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-hydroxy-propyl)-cyclohexanol ([^{3}H]CP-55,940). [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] SI: selectivity index for CB2, calculated as K_i(CB1)/K_i(CB2) ratio. [h] The binding affinities of reference compounds were evaluated in parallel with the test compounds under the same conditions. [i] CB2 reference compound. [j] CB1 reference compound.

dx.doi.org/10.1021/jm200476p |*J. Med. Chem.* 2011, 54, 5444–5453

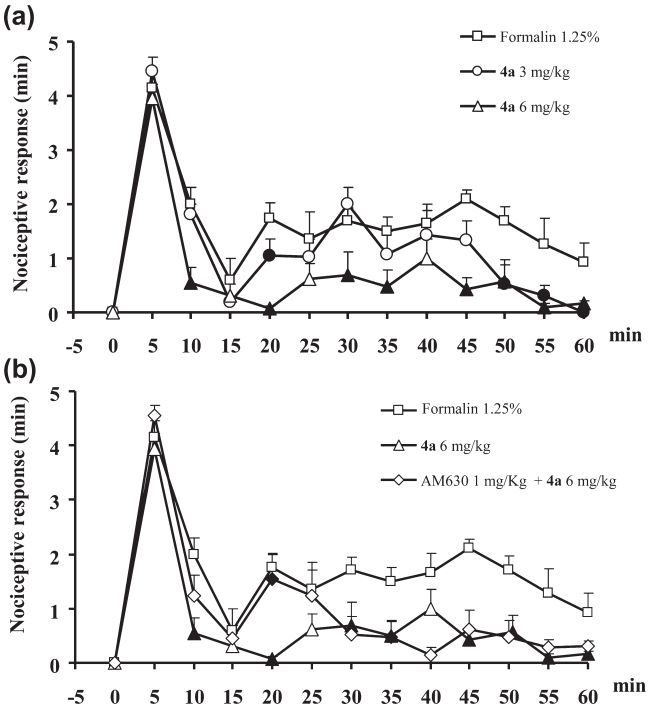


Figure 1. Effect of **4a** (3−6 mg/kg, ip) alone (A) or in combination, at the dose of 6 mg/kg, with AM630 (1 mg/kg, ip) (B) in the formalin test in mice. The total time of the nociceptive response was measured every 5 min and expressed as the total time of the nociceptive responses in min. Results are mean ± SEM (n = 8−10 for each group). In (A), filled symbols denote statistically significant differences vs formalin. In (B), filled symbols denote statistically significant differences ($P < 0.05$) vs **4a**. One-way analysis of variance followed by a Tukey−Kramer multiple comparisons test was used for data analysis.

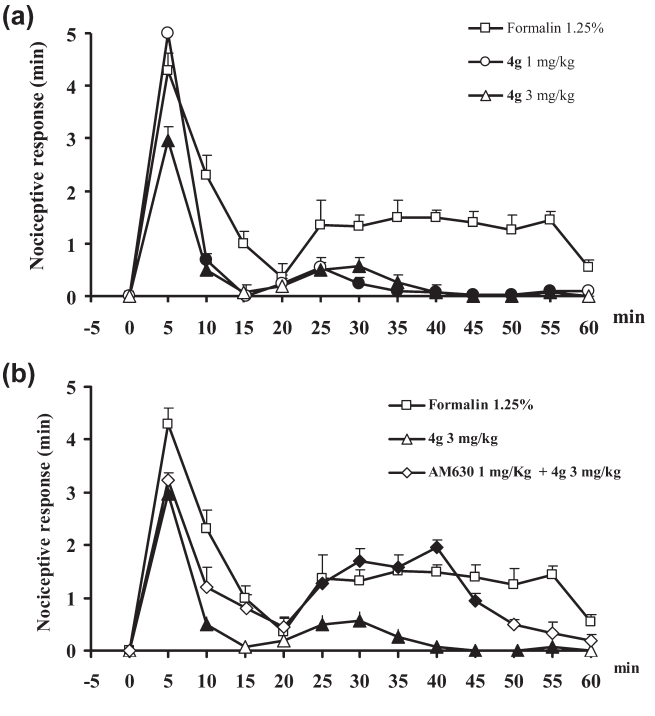


Figure 2. Effect of **4g** (1−3 mg/kg, ip) alone (A) or in combination, at the dose of 3 mg/kg, with AM630 (1 mg/kg, ip) (B) in the formalin test in mice. The total time of the nociceptive response was measured every 5 min and expressed as the total time of the nociceptive responses in min. Results are mean ± SEM (n = 8−10 for each group). In (A), filled symbols denote statistically significant differences vs formalin. In (B), filled symbols denote statistically significant differences ($P < 0.05$) vs **4g**. One-way analysis of variance followed by a Tukey−Kramer multiple comparisons test was used for data analysis.

concentration of 1 μM, i.e. about 1−3 orders of magnitude higher than their K_i toward CB_2 receptors. Results show that all the derivatives exhibit very low or no cytotoxicity, the cell viability being always above 95% after a 72 h treatment (see Supporting Information).

In Vivo Pharmacology. Compounds **4a**, **4g**, **5**, and **7a** exhibited antinociceptive activity in the formalin test in mice. The compound with highest affinity and selectivity for CB2, **4a**, was surprisingly the less efficacious in this test, a dose of 6 mg/kg being necessary in order to observe a reduction of the second (and, to a much lesser extent, the first) phase of the formalin-induced nocifensive response (Figure 1A). This might suggest that **4a** might behave as an inverse agonist, rather than an agonist, at CB2 receptors. Indeed, CB2 inverse agonists exert activity in the formalin test, particularly on the second phase, only at relatively high doses.[13,23] This behavior differs from that of potent CB2 agonists, which reduce both phases of the formalin response at doses usually proportional to their corresponding K_i values. In agreement with its potential activity as inverse agonist, the effect of **4a** was not significantly reversed by the CB2 antagonist AM630 (Figure 1B). The two compounds with similar affinity for CB2 receptors, **4g** and **5**, exhibited different efficacies in the formalin test, the former being very potent (with maximal effect being reached already at the 1 mg/kg dose) and efficacious also on the first phase of the nocifensive response (Figure 2A), the latter instead showing activity only at the higher tested dose (3 mg/kg) (Figure 3A). The effect of **4g** was strongly reduced by AM630 (Figure 2B), thus indicating that this compound might indeed act as a potent and selective CB2 agonist. Conversely, the effects of **5** were only partly attenuated by AM630 (Figure 3B), thus suggesting for this compound a potential dual action at CB2 and other targets (including CB1). Finally, compound **7a** also exhibited antihyperalgesic effects against both the first and second phase of the formalin response, although only at the highest dose tested of 3 mg/kg (Figure 4A). These effects were strongly antagonized by AM630 (Figure 4B), thus suggesting also for this compound a selective CB2 agonist-like activity, although less potent than that observed with **4g**, in agreement with the relative affinities of the two compounds for CB2, measured here (K_i = 8.5 and 29.4 nM, respectively, see Table 1). In conclusion, these in vivo data, while confirming that 4-quinolone-3-carboxamide CB2 ligands produce antihyperalgesic effects in vivo, suggest for compounds **4a**, **4g**, **5**, and **7a** different tentative functional activities as a: selective CB2 inverse agonist, potent and selective CB2 agonist, weaker but still selective CB2 agonist, and nonselective CB2 agonist, respectively. Clearly, appropriate in vitro functional assays will have to be carried out in order to confirm these predictions.

CONCLUSIONS

In our continuing effort to explore structure−affinity relationship for quinolones binding at cannabinoid receptors, we have described herein the synthesis and pharmacological evaluation of new compounds characterized by diverse substitution patterns of

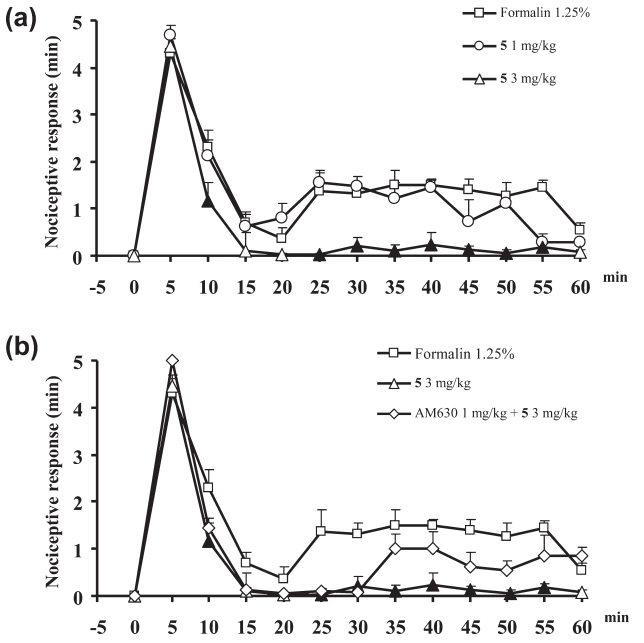


Figure 3. Effect of **5** (1−3 mg/kg, ip) alone (A) or in combination, at the dose of 3 mg/kg, with AM630 (1 mg/kg, ip) (B) in the formalin test in mice. The total time of the nociceptive response was measured every 5 min and expressed as the total time of the nociceptive responses in min. Results are mean ± SEM (n = 8−10 for each group). In Figure (A), filled symbols denote statistically significant differences vs formalin. In (B), filled symbols denote statistically significant differences ($P < 0.05$) vs **5**. One-way analysis of variance followed by a Tukey−Kramer multiple comparisons test was used for data analysis.

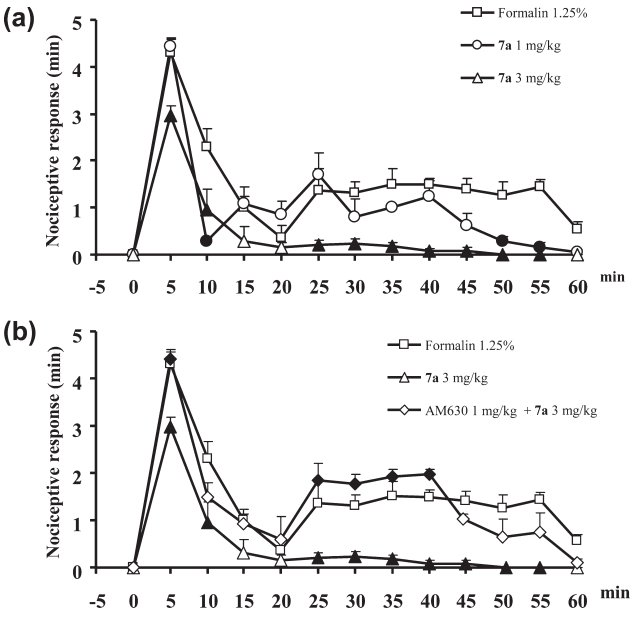


Figure 4. Effect of **7a** (1−3 mg/kg, ip) alone (A) or in combination, at the dose of 3 mg/kg, with AM630 (1 mg/kg, ip) (B) in the formalin test in mice. The total time of the nociceptive response was measured every 5 min and expressed as the total time of the nociceptive responses in min. Results are mean ± SEM (n = 8−10 for each group). In (A), filled symbols denote statistically significant differences vs formalin. In (B), filled symbols denote statistically significant differences ($P < 0.05$) vs **7a**. One-way analysis of variance followed by a Tukey−Kramer multiple comparisons test was used for data analysis.

the bicyclic core. In particular, 8-substituted 4-quinolone-3-carboxamides possessing high affinity for the human CB2 receptor at nanomolar or subnanomolar concentration and selectivity index values as high as >16000 were identified. Thus, some of these compounds proved to be very potent toward the human CB2 receptor, while no affinity for the human CB1 receptor could be measured at all. Because of the relevance of the selectivity issue in the cannabinoid area,[26] these results seem to hold some promise with regard to the possibility that truly selective CB2 ligands can be developed. Moreover, other compounds belonging to structurally related chemical classes, namely 2-quinolones (compound **7c**) and 4-hydroxy-2-quinolones (compound **7b**), demonstrated high CB2 affinity although accompanied with less receptor selectivity. However, it is worth reminding that **4a** possesses CB2 affinity and selectivity approximately 8-fold and 1800-fold, respectively, higher than the 8-unsubstituted parent compound.[13] Therefore, considering the crucial effect frequently exerted by appropriate substituents at positions 6 and/or 8 of quinolones in increasing CB2 receptor affinity and selectivity, compound **7c** emerges as a specially interesting candidate for further structural optimization.

When assayed in vivo in the formalin test of analgesia in mice, compounds **4a**, **4g**, **5**, and **7a** elicited antihyperalgesic effects, **4g** resulting more potent than the other tested compounds. On the basis of the results obtained in the formalin assay in the absence and in the presence of the CB2 antagonist AM630, a functional profile of CB2 agonist (inverse agonist for **4a**) could be inferred for these compounds. In any case, the analgesic effects should be ascribed to direct activity at CB2 receptor, as no inhibitory activity of FAAH and MAGL by the test compounds could be demonstrated.

EXPERIMENTAL SECTION

Chemistry. Reagents were purchased from commercial suppliers and used without further purification. Anhydrous reactions were run under a positive pressure of dry N_2. Merck Silica Gel 60 was used for flash chromatography (23−400 mesh). IR spectra were recorded on a Perkin-Elmer BX FT-IR system using $CHCl_3$ as the solvent or a Nujol dispersion. ^{1}H NMR and ^{13}C NMR were recorded at 200 and 50 MHz, respectively, on a Bruker AC200F spectrometer and at 400 and 100 MHz on a Bruker Advance DPX400. Chemical shifts are reported relative to tetramethylsilane at 0.00 ppm. Mass spectral (MS) data were obtained using 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 or in negative mode scanning over the mass range of 105−1500. Melting points were determined on a Gallenkamp apparatus and are uncorrected. Microwave irradiations were conducted using a CEM Discover Synthesis Unit (CEM Corporation, Matthews, NC). 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. The chemical purity of the target compounds was determined using the following conditions: an Agilent 1100 series LC/MSD with a Lichrocart 125-4 Lichrospher 100 RP-18 (4.6 mm × 100 mm, 5 μm) reversed phase column; method: 86% (v/v) of MeOH in H_2O, isocratic, flow rate of 1 mL/min, UV detector, 254 nm. The purity of each compound was ≥95% in either analysis.

Synthesis of Quinolones 4a−d by Alkylation Reaction. *General Procedure.* A mixture of the appropriate quinolone derivative **10a−d** (1 mmol), 1-iodopentane (0.4 mL, 2.8 mmol), and solid K_2CO_3 (386 mg, 2.8 mmol) in dry DMF (1 mL) was heated under N_2 atmosphere at 90 °C for 4 h and then poured into an ice−water mixture. The precipitated *N*-alkylquinolone was extracted with DCM and

purified by flash chromatography on silica gel eluting with DCM/MeOH (97:3).

*Example: N-(Adamantan-1-yl)-8-methoxy-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (**4a**).* Prepared in 64% yield from **10a**. White solid; mp 153−154 °C (EtOH). ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.91 (s, 1H), 8.54 (s, 1H), 8.10−8.05 (m, 1H), 7.34−7.26 (m, 1H), 7.10−7.05 (m, 1H), 4.43 (t, J = 7.5 Hz, 2H), 3.90 (s, 3H), 2.08−1.99 (m, 12H), 1.80−1.59 (m, 5H), 1.32−1.19 (m, 4H), 0.83 (t, J = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.9, 163.6, 149.9, 149.6, 130.5, 130.2, 125.0, 119.1, 114.1, 112.3, 59.8, 56.2, 51.5, 41.8, 36.5, 31.1, 29.5, 28.6, 22.2, 13.9. IR ($CHCl_3$): ν 1655 cm^{-1}. MS (ESI): m/z 423 $[M + H]^+$. Anal. ($C_{26}H_{34}N_2O_3$) C, H, N.

Synthesis of Quinolones 4e−g, 6b, 7b, and 11 by Suzuki Coupling. *General Procedure.* A 5 mL process vial was charged with the appropriate bromo derivative **4c**, **4d**, **6a**, **7a**, or **10c** (1 mmol), the appropriate boronic acid (5 mmol), $Pd(OAc)_2$ (22.5 mg, 0.1 mmol), PPh_3 (78.6 mg, 0.3 mmol), 2 M Na_2CO_3 (2 mL, 4 mmol), EtOH (1 mL), and DME (4 mL). The vessel was sealed under air and exposed to microwave heating for 10 min at 150 ⊠C. The reaction mixture was thereafter cooled down to room temperature, diluted with AcOEt, and filtered through a short plug of Celite. The solution was washed with brine, dried over anhydrous sodium sulfate, and evaporated. The residue was purified by flash chromatography on silica gel.

*Example: N-(Adamantan-1-yl)-6-(furan-2-yl)-8-methoxy-4-oxo-1-pentyl-1,4-dihydroquinoline-3-carboxamide (**4g**).* Prepared in 97% yield from **4d**. White solid; mp 202−203 °C (EtOH). ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.50 (s, 1H), 8.58 (s, 1H), 8.38 (d, J = 1.4 Hz, 1H), 7.49−7.45 (m, 2H), 6.81 (d, J = 3.3 Hz, 1H), 6.51−6.49 (m, 1H), 4.50 (t, J = 7.6 Hz, 2H), 4.02 (s, 3H), 2.16−2.09 (m, 9H), 1.79−1.70 (m, 8H), 1.34−1.30 (m, 4H), 0.88 (t, J = 6.5 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 175.9, 163.8, 152.6, 150.5, 149.6, 142.6, 128.0, 113.9, 112.1, 109.6, 106.5, 59.9, 56.42, 51.7, 41.8, 36.8, 31.2, 29.6, 28.6, 22.3, 13.9. IR (Nujol): ν 1501, 1556, 1666, 3112 cm^1. MS (ESI): m/z 489 $[M + H]^+$, 511 $[M + Na]^+$. Anal. ($C_{30}H_{36}N_2O_4$) C, H, N.

3-Ethyl-3,7-dihydro-7-oxo-2*H*-[1,4]oxazino[2,3,4-*ij*]quinoline-6-carboxylic Acid (15). KF (88 mg, 1.5 mmol) and K_2CO_3 (415 mg, 3 mmol) were added to a solution of **14** (307 mg, 1 mmol, for its preparation see Supporting Information) in dry DMF (2.7 mL), and the mixture was heated to 150 °C for 3 h under a nitrogen atmosphere. After cooling, the reaction is diluted with water and extracted with DCM (3 × 5 mL). The organic phase was washed with brine, dried, and evaporated to afford a residue, which was hydrolyzed with 10% NaOH (33 mL) by refluxing for 1 h. After cooling to rt, 37% HCl was added to pH 2 and the mixture was extracted with DCM (3 × 10 mL). After usual workup of the organic solution, a residue was obtained, which was filtered through a short column of silica gel (9:1 DCM/MeOH) to give the pure compound **15** (142 mg, 55%) as a white solid; mp 209−212 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 14.53 (s, 1H), 8.73 (s, 1H), 8.00−7.95 (m, 1H), 7.47−7.29 (m, 2H), 4.57−4.50 (m, 1H), 4.39−4.32 (m, 2H), 2.00−1.77 (m, 2H), 1.04 (t, J = 7.1 Hz, 3H). IR ($CHCl_3$): ν 1616, 1718 cm^{-1}. MS (ESI): m/z 260 $[M + H]^+$, 282 $[M + Na]^+$. Anal. ($C_{14}H_{13}NO_4$) C, H, N.

Synthesis of Compounds 5, 6a, and 20 by Amidation Reaction. *General Procedure.* The appropriate carboxylic acid (2 mmol) was dissolved in DMF (5 mL). HOBt (270 mg, 2 mmol), HBTU (1.72 g, 4 mmol), DIPEA (152 μL, 3 mmol), and 1-aminoadamantane (360 mg, 2.4 mmol) were added to the solution, and the reaction mixture was stirred at room temperature for 30 min. Further DIPEA (152 μL, 3 mmol) was thereafter added, and the reaction mixture was stirred at room temperature for further 4 h. The reaction mixture was poured into ice, and the solid precipitated was collected by filtration and washed with water and petroleum ether. Recrystallization from EtOH gave the pure amide.

*Example: N-(Adamantan-1-yl)-3-ethyl-3,7-dihydro-7-oxo-2H-[1,4]-oxazino[2,3,4-ij]quinoline-6-carboxamide (**5**).* Prepared in 54% yield from **15** as a light-brown solid; mp 218−219 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 10.61 (s, 1H), 8.62 (s, 1H), 8.02−8.00 (m, 1H), 7.34−7.30 (m, 1H), 7.20−7.15 (m, 1H), 4.51 (d, J = 12 Hz, 1H), 4.29−4.25 (m, 1H), 4.19 (t, J = 9.2 Hz, 1H), 2.16−2.11 (m, 6H), 2.09−1.83 (m, 3H), 1.76−1.70 (m, 8H), 1.03 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 177.0, 158.8, 144.1, 142.2, 133.9, 127.0, 122.2, 121.3, 118.8, 111.8, 68.9, 63.7, 40.9, 37.9, 36.6, 25.8, 21.0, 11.9. IR ($CHCl_3$): ν 1654, 1728 cm^{-1}. MS (ESI): m/z 393 $[M + H]^+$, 415 $[M + Na]^+$. Anal. ($C_{24}H_{28}N_2O_3$) C, H, N.

Methyl 6-Bromo-2-butyl-1,4-dihydro-4-oxoquinoline-3-carboxylate (16). Solid NaOH (25 mg, 0.6 mmol) and methyl 3-oxoheptanoate (978 μL, 6 mmol) were added to a solution of 5-bromoisatoic anhydride (1 g, 4 mmol) in 1,4-dioxane (6 mL). The mixture was refluxed for 48 h, then cooled to room temperature and concentrated under reduced pressure. The residue was taken up into water and AcOEt, and the organic layer was dried and evaporated. Purification by column chromatography on silica gel (3:1 AcOEt/PE) furnished **16** (660 mg, 47%) as a white solid; mp 222−223 °C. ^{1}H NMR (200 MHz, DMSO-d_6) δ 11.08 (bs, 1H), 8.26 (d, J = 2.4 Hz 1H), 7.73 (dd, J_1 = 2.4 Hz, J_2= 8.7 Hz, 1H), 7.52 (d, J = 8.7 Hz, 1H), 3.77 (s, 3H), 2.71 (t, J = 7.8 Hz, 2H), 1.74−1.62 (m, 2H), 1.42−1.26 (m, 2H), 0.87 (t, J = 7.3 Hz, 3H). IR ($CHCl_3$): ν 1634, 1720 cm^{-1}. MS (ESI): m/z 339 $[M + H]^+$. Anal. ($C_{15}H_{16}BrNO_3$) C, H, N.

***N*-(Adamantan-1-yl)-6-bromo-2-butyl-1,4-dihydro-4-oxoquinoline-3-carboxamide (6a).** Prepared in 25% overall yield from **16** by ester hydrolysis followed by amidation reaction according to the procedures described for the preparation of **15** and **5**, respectively. White solid; mp 172−173 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 10.40 (bs, 1H), 9.30 (s, 1H), 8.38 (d, J = 2.0 Hz, 1H), 7.58 (dd, J_1 = 2.0, J_2 = 8.8 Hz, 1H), 7.34 (d, J = 8.8 Hz, 1H), 3.65 (q, J = 7.2 Hz, 1H), 3.04 (t, J = 8.0 Hz, 2H), 2.10 (s, 5H), 2.02 (s, 2H), 1.63−1.55 (m, 5H), 1.54−1.48 (m, 2H), 1.20−1.12 (m, 4H), 0.66 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 176.2, 165.6, 158.6, 137.0, 135.6, 129,0, 126.9, 119.4, 118.4, 113.6, 58.7, 52.3, 42.0, 36.7, 33.9, 31.8, 29.7, 22.8, 18.6, 13.9. IR (Nujol): ν 1616, 1650 cm^{-1}. MS (ESI): m/z 458 $[M + H]^+$. Anal. ($C_{24}H_{29}BrN_2O_2$) C, H, N.

Ethyl 6-Bromo-1,2-dihydro-4-hydroxy-2-oxo-1-pentylquinoline-3-carboxylate (18). A solution of 5-bromoisatoic anhydride (400 mg, 1.65 mmol), 1-iodopentane (239 μL, 1.82 mmol), and DIPEA (575 μL, 3.3 mmol) in DMF (5 mL) was heated to 80 °C for 18 h while monitoring the progress of the reaction by TLC (98:2 DCM/MeOH). Diethyl malonate (500 μL, 3.3 mmol) and a solution of sodium *tert*-butoxide in DMF, prepared by adding NaH (87 mg, 3.6 mmol) to dry *tert*-butanol (315 μL, 3.3 mmol) in DMF (1.5 mL), were added. After heating to 100 °C for 2 h, the reaction mixture was poured into ice and 37% HCl was added until pH 2 is reached. Extraction with DCM, followed by the usual workup of the organic phase, and purification by silica gel chromatography (99:1 DCM/MeOH) of the solid residue gave **18** (240 mg, 40% overall yield from 5-bromoisatoic anhydride); mp 84−85 °C (EtOH). ^{1}H NMR (200 MHz, $CDCl_3$): δ 17.01 (s, 1H), 8.32 (d, J = 2.0 Hz, 1H), 7.70 (dd, J_1 = 2,0 Hz, J_2 = 8.8 Hz, 1H), 7.18 (d, J = 8.8 Hz, 1H), 4.21 (t, J = 7.0 Hz, 2H), 4.15 (t, J = 7.2 Hz, 2H), 1.74−1.62 (m, 2H), 1.42−1.02 (m, 7H), 0.90 (t, J = 7.3 Hz, 3H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.5, 170.5, 158.8, 139.4, 137.0, 128.2, 115.9, 114.6, 98.5, 62.6, 42.5, 29.1, 27.1, 22.5, 14.2, 14.0. IR ($CHCl_3$): ν 1640, 1721 cm^{-1}. MS (ESI): m/z 383 $[M + H]^+$. Anal. ($C_{17}H_{20}BrNO_4$) C, H, N.

***N*-(Adamantan-1-yl)-6-bromo-1,2-dihydro-4-hydroxy-2-oxo-1-pentylquinoline-3-carboxamide (7a).** A solution of **18** (500 mg, 1.4 mmol) and 1-aminoadamantane (420 mg, 27.6 mmol) in toluene (100 mL) was refluxed with azeotropical removal of water during 1 h. Solvent was evaporated and the residue was filtered through a short pad of silica gel (DCM as eluent) to give the title compound **7a**

(630 mg, 100%) as a white solid; mp 191−192 °C. ^{1}H NMR (200 MHz, $CDCl_3$): δ 17.68 (s, 1H), 8.30 (d, J = 2.1 Hz, 1H), 7.68 (dd, J_1 = 2.1 Hz, J_2 = 8.7 Hz, 1H), 7.15 (d, J = 8.7 Hz, 1H), 4.14 (t, J = 7.1 Hz, 2H), 2.13 (s, 9H), 1.70−1.56 (m, 8H), 1.42−1.36 (m, 4H), 0.90 (t, J = 7.2 Hz, 3H). ^{13}C (100 MHz, $CDCl_3$): δ 171.3, 170.3, 163.1, 152.3, 143.6, 138.3, 136.0, 128.0, 115.9, 115.0, 112,5, 97.8, 72.6, 52.6, 45.9, 42.3, 41.4, 36.6, 36.3, 36.2, 29.7, 29.4, 29.0, 27.3, 13.9. IR ($CHCl_3$): ν 1632, 1724 cm^{-1} MS (ESI): m/z 488 $[M + H]^+$. Anal. ($C_{25}H_{31}BrN_2O_3$) C, H, N.

Binding Assays. CB1 and CB2 receptor binding assays were performed exactly as described previously,[13,14] using membranes of cells overexpressing the human recombinant CB1 or CB2 receptors.

Enzymatic Assays. The inhibitory activity of varying concentrations (0.1, 1, 5, and 10 μM) of the compounds on the hydrolysis of [^{14}C]-anandamide and [^{14}C]-2-arachidonoyl-glycerol by rat brain FAAH-like and COS-7 cell MAGL-like enzymatic activities was assessed as described previously.[22]

Formalin Test. The experimental procedures applied in the formalin test were approved by the Animal Ethics Committee of the Second University of Naples. Animal care was in compliance with the IASP and European Community guidelines on the use and protection of animals in experimental research (EC L358/1 18/12/86). All efforts were made to minimize animal suffering and to reduce the number of animals used. Formalin injection induces a biphasic stereotypical nocifensive behavior.[27] 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. Mice received formalin (1.25% in saline, 30 μL) in the dorsal surface of one side of the hindpaw. 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 hindpaws. Lifting, favoring, licking, shaking, and flinching of the injected paw were recorded as nociceptive responses. The duration of those mentioned noxious behaviors were monitored by an observer blind to the experimental treatment for periods of 0−10 min (early phase) and 20−60 min (late phase) after formalin administration. Results are expressed as means ± SEM. Significant differences between groups were evaluated by using analysis of variance followed by the Dunnett's test. The version of the formalin test we applied is based on the fact that a correlational analysis showed that no single behavioral measure can be a strong predictor of formalin or drug concentrations on spontaneous behaviors.[28] Consistently, we considered that a simple sum of time spent licking plus elevating the paw, or the weighted pain score, is in fact superior to any single (lifting, favoring, licking, shaking, and flinching) measure (r ranging from 0.75 to 0.86).[29] Treatments: groups of 8−10 animals per treatment were used with each animal being used for one treatment only. Mice received intraperitoneal vehicle (20% DMSO in 0.9% NaCl) or different doses of before mentioned compounds.

Cytotoxicity Studies: Cells and Cell Culture. Human hepatocellular carcinoma HepG2 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum at 37 °C under a humidified atmosphere containing 5% carbon dioxide. Until reached 70% confluence in tissue culture flasks, the cells were trypsinized with buffered saline solution containing 0.25% trypsin. After that, the cells were plated in 96-well cultural plate at a density of 1×10^5 cells per well and allowed to attach for 24 h. Then, stock solutions (1×10^{-3} M in DMSO) of the test compounds were diluted with medium and immediately added into the wells in order to obtain 1 μM final concentration. After 72 h of incubation, the cells were analyzed for their viability.

Cell Viability (MTT Assay). Cell viability was investigated by using standard protocols of the method based on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Briefly, at the end of 72 h of incubation time, Hep-G2 cells were added with 20 μL/well of sterile filtered MTT stock solution (5 mg/mL in PBS, pH 7.4) and incubated for additional 4 h at 37 °C to allow the yellow dye to be transformed into blue crystals. Then, the MTT solution was carefully aspirated, and formazan was extracted from the cells with 200 μL/well of DMSO. The optical density was measured by 96-well ELISA plate reader at a wavelength of 540 nm. Cell viability (%) was calculated as $([A]_{test}/[A]_{control}) \times 100$. Wells containing cell culture medium without test compounds were used as control. Data are reported as mean ± SEM (n = 6).

■ ASSOCIATED CONTENT

Supporting Information. Additional synthetic, spectroscopic/analytical, and biological data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +39 0577 234308. Fax: +39 0577 234333. E-mail: corelli@unisi.it.

■ ACKNOWLEDGMENT

The authors from the University of Siena gratefully acknowledge financial support from Siena Biotech SpA and from the University of Siena (PAR Progetti 2006). The authors from CNR, Pozzuoli, are very grateful to Marco Allarà for technical assistance.

■ ABBREVIATIONS USED

CB1, cannabinoid type-1; CB2, cannabinoid type-2; DIPEA, diisopropylethylamine; 3D-QSSAR, three-dimensional quantitative structure−selectivity relationship; DCM, dichloromethane; DME, 1,2-dimethoxyethane; DMF, dimethylformamide; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; HOBt, 1-hydroxybenzotriazole; HBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; SI, selectivity index; TEA, triethylamine

■ REFERENCES

(1) Di Marzo, V. Targeting the endocannabinoid system: to enhance or reduce?. *Nature Rev. Drug Discovery* **2008**, *7*, 438–455.

(2) Di Marzo, V.; De Petrocellis, L. Plant, synthetic, and endogenous cannabinoids in medicine. *Annu. Rev. Med.* **2006**, *57*, 553–574.

(3) Pacher, P.; Mechoulam, R. Is lipid signaling through cannabinoid 2 receptors part of a protective system?. *Prog. Lipid Res.* **2011**, *50*, 193–211.

(4) (a) Guindon, J.; Hohmann, A. G. Cannabinoid CB2 receptors: a therapeutic target for the treatment of inflammatory and neuropathic pain. *Br. J. Pharmacol.* **2008**, *153*, 319–334. (b) Whiteside, G. T.; Lee, G. P.; Valenzano, K. J. The role of the cannabinoid CB2 receptor in pain transmission and therapeutic potential of small molecule CB2 receptor agonists. *Curr. Med. Chem.* **2007**, *14*, 917–936.

(5) Mackie, K.; Ross, R. A. CB2 cannabinoid receptors: new vistas. *Br. J. Pharmacol.* **2008**, *153*, 177–178and references cited therein.

(6) Allen, J. G.; Fotsch, C.; Babij, P. Emerging targets in osteoporosis disease modification. *J. Med. Chem.* **2010**, *53*, 4332–4353.

(7) Ross, R. A.; Brockie, H. C.; Stevenson, L. A.; Murphy, V. L.; Templeton, F.; Makriyannis, A.; Pertwee, R. G. Agonist−inverse agonist characterization at CB1 and CB2 cannabinoid receptors of L759633, L759656, and AM630. *Br. J. Pharmacol.* **1999**, *126*, 665–672.

(8) Huffman, J. W.; Liddle, J.; Yu, S.; Aung, M. M.; Abood, M. E.; Wiley, J. L.; Martin, B. R. 3-(1′,1′-Dimethylbutyl)-1-deoxy-Delta8-THC

and related compounds: synthesis of selective ligands for the CB2 receptor. *Bioorg. Med. Chem.* **1999**, *7*, 2905–2914.

(9) Hanus, L.; Breuer, A.; Tchilibon, S.; Shiloah, S.; Goldenberg, D.; Horowitz, M.; Pertwee, R. G.; Ross, R. A.; Mechoulam, R.; Fride, E. HU-308: a specific agonist for CB2, a peripheral cannabinoid receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 14228–14233.

(10) Idris, A. I.; Sophocleous, A.; Landao-Bassonga, E.; van't Hof, R. J.; Ralston, S. H. Regulation of bone mass, osteoclast function, and ovariectomy-induced bone loss by the type 2 cannabinoid receptor. *Endocrinology* **2008**, *149*, 5619–5626.

(11) Rossi, F.; Siniscalco, D.; Luongo, L.; De Petrocellis, L.; Bellini, G.; Petrosino, S.; Torella, M.; Santoro, C.; Nobili, B.; Di Marzo, V.; Maione, S. The endovanilloid/endocannabinoid system in human osteoclasts: Possible involvement in bone formation and resorption. *Bone* **2009**, *44*, 476–484.

(12) Ofek, O.; Attar-Namdar, M.; Kram, V.; Dvir-Ginzberg, M.; Mechoulam, R.; Zimmer, A.; Frenkel, B.; Shohami, E.; Bab, I. CB2 cannabinoid receptor targets mitogenic Gi protein-cyclin D1 axis in osteoblasts. *J. Bone Miner. Res.* **2011**, *26*, 308–316.

(13) Pasquini, S.; Botta, L.; Semeraro, T.; Mugnaini, C.; Ligresti, A.; Palazzo, E.; Maione, S.; Di Marzo, V.; Corelli, F. 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. *J. Med. Chem.* **2008**, *51*, 5075–5084.

(14) Pasquini, S.; Ligresti, A.; Mugnaini, C.; Semeraro, T.; Cicione, L.; De Rosa, M.; Guida, F.; Luongo, l.; De Chiaro, M.; Cascio, M. G.; Bolognini, D.; Marini, P.; Pertwee, R.; Maione, S.; Di Marzo, V.; Corelli, F. Investigations on the 4-Quinolone-3-carboxylic Acid Motif. 3. Synthesis, Structure−Affinity Relationships and Pharmacological Characterization of 6-Substituted 4-Quinolone-3-carboxamides as Highly Selective Cannabinoid-2 Receptor Ligands. *J. Med. Chem.* **2010**, *53*, 5915–5928.

(15) Brogi, S.; Corelli, F.; Di Marzo, V.; Ligresti, A.; Mugnaini, C.; Pasquini, S.; Tafi, A. Three-Dimensional Quantitative Structure−Selectivity Relationships (3D-QSSR) Analysis Guided Rational Design of a Highly Selective Ligand for the Cannabinoid Receptor 2. *Eur. J. Med. Chem.* **2011**, *46*, 547–555.

(16) Jansson, K.; Fristedt, T.; Olsson, A.; Svensson, B.; Jönsson, S. Synthesis and Reactivity of Laquinimod, a Quinoline-3-carboxamide: Intramolecular Transfer of the Enol Proton to a Nitrogen Atom as a Plausible Mechanism for Ketene Formation. *J. Org. Chem.* **2006**, *71*, 1658–1667.

(17) Egawa, H.; Miyamoto, T.; Matsumoto, J. A new synthesis of 7*H*-Pyrido[1,2,3-*de*][1,4]benzoxazine derivatives including an antibacterial agent, ofloxacin. *Chem. Pharm. Bull.* **1986**, *34*, 4098–4102.

(18) Mai, A.; Rotili, D.; Tarantino, D.; Ornaghi, P.; Tosi, F.; Vicidomini, C.; Sbardella, G.; Nebbioso, A.; Miceli, M.; Altucci, L.; Filetici, P. Small-molecule inhibitors of histone acetyltransferase activity: identification and biological properties. *J. Med. Chem.* **2006**, *49*, 6897–6907.

(19) Ukrainets, I. V.; Davidenko, A. A.; Mospanova, E. V.; Sidorenko, L. V.; Svechnikova, E. N. 4-Hydroxy-2-quinolones. 176. 4-*R*-2-Oxo-1,2-dihydroquinoline-3-carboxylic acids. Synthesis, physicochemical, and biological properties. *Chem. Heterocycl. Compd.* **2010**, *46*, 559–568.

(20) Rinaldi-Carmona, M.; Barth, F.; Millan, J.; Derocq, J. M.; Casellas, P.; Congy, C.; Oustric, D.; Sarran, M.; Bouaboula, M.; Calandra, B.; Portier, M.; Shire, D.; Breliere, J. C.; Le Fur, G. L. SR144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 644–650.

(21) Rinaldi-Carmona, M.; Barth, F.; Héaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Néliat, G.; Caput, D.; Ferrara, P.; Soubrié, P.; Brelière, J. C.; Le Fur, G. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett.* **1994**, *350*, 240–244.

(22) Bisogno, T.; Burston, J. J.; Rai, R.; Allarà, M.; Saha, B.; Mahadevan, A.; Razdan, R. K.; Wiley, J. L.; Di Marzo, V. Synthesis and pharmacological activity of a potent inhibitor of the biosynthesis of the endocannabinoid 2-arachidonoylglycerol. *ChemMedChem* **2009**, *4*, 946–950.

(23) Cascio, M. G.; Bolognini, D.; Pertwee, R. G.; Palazzo, E.; Corelli, F.; Pasquini, S.; Di Marzo, V.; Maione, S. In vitro and in vivo pharmacological characterization of two novel selective cannabinoid CB2 receptor inverse agonists. *Pharmacol. Res.* **2010**, *61*, 349–354.

(24) Stern, E.; Muccioli, G.; Bosier, B.; Hamtiaux, L.; Millet, R.; Poupaert, J. H.; Hénichart, J. P.; Depreux, P.; Goossens, J. F.; Lambert, D. M. Pharmacomodulations around the 4-oxo-1,4-dihydroquinoline-3-carboxamides, a class of potent CB2-selective cannabinoid receptor ligands: consequences in receptor affinity and functionality. *J. Med. Chem.* **2007**, *50*, 5471–5484.

(25) Manera, C.; Saccomanni, G.; Adinolfi, B.; Benetti, V.; Ligresti, A.; Cascio, M. G.; Tuccinardi, T.; Lucchesi, V.; Martinelli, A.; Nieri, P.; Masini, E.; Di Marzo, V.; Ferrarini, P. L. Rational Design, Synthesis, and Pharmacological Properties of New 1,8-Naphthyridin-2(1*H*)-on-3-Carboxamide Derivatives as Highly Selective Cannabinoid-2 Receptor Agonists. *J. Med. Chem.* **2009**, *52*, 3644–3651.

(26) Pertwee, R. G.; Howlett, A. C.; Alexander, S. P. H.; Di Marzo, V.; Elphick, M. R.; Greasley, P. J.; Hansen, H. S.; Kunos, G.; Mackie, K.; Mechoulam, R.; Ross, R. A. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid Receptors and Their Ligands: Beyond CB1 and CB2. *Pharmacol. Rev.* **2010**, *62*, 588–631.

(27) Dubuisson, D.; Dennis, S. G. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. *Pain* **1977**, *4*, 161–174.

(28) Xu, J. J.; Diaz, P.; Astruc-Diaz, F.; Craig, S.; Munoz, E.; Naguib, M. Pharmacological characterization of a novel cannabinoid ligand, MDA19, for treatment of neuropathic pain. *Anesth. Analg.* **2010**, *111*, 99–109.

(29) Abbott, F. V.; Franklin, K. B.; Westbrook, R. F. The formalin test: scoring properties of the first and second phases of the pain response in rats. *Pain* **1995**, *60*, 91–102.