Synthesis and SAR Studies of 2-Oxoquinoline Derivatives as CB2 Receptor Inverse Agonists

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Received September 6, 2005

The highly CB2 selective cannabinoid receptor inverse agonist, 7-methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid *N*-benzo[1,3]dioxol-5-ylmethyl)amide (JTE-907; **9b**), served as the lead compound for investigating the structure−activity relationships of its analogues and in the search for more potent and effective CB2 receptor inverse agonists. A series of aromatic amides of 7-methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic acid **6** was synthesized, and the CB2 receptor activities of the compounds were determined by a [^{35}S]GTP$_\gamma$S-binding assay using membranes of CHO cells stably transfected with the human CB2 receptor. As a result, all the compounds were defined as full CB2 receptor inverse agonists, and additionally, except for two 3,4-dihydroxyphenylalkylamides, they were found to be equally potent as SR144528.

Introduction

The peripheral cannabinoid receptor CB2 was molecularly identified in 1993 by Munro et al.,[1] and their work in determining the distribution pattern of CB2 was subsequently complemented by Galiègue et al.[2] The two signaling pathways utilized by the CB2 receptor are inhibition of adenylate cyclase through activation of the $G_{i/o}$ protein[3] and activation of mitogen-activated protein (MAP) kinase resulting in induction of the growth-related gene *Krox*-24 expression.[4]

The immunomodulatory properties of the cannabinoids present in the plant *Cannabis sativa* have been proposed to be mediated via the CB2 receptor. This hypothesis is supported by the abundant expression of CB2 in cells of the immune system, such as those in spleen, tonsils, and thymus, natural killer cells, T cells, and B cells.[2] Therefore, modification of immune functions by CB2 receptor ligands has become a focus of particular interest. In drug development, the mainly peripheral distribution of CB2 suggests that CB2-selective ligands will not have cannabinoid-related psychoactive side effects, i.e., drugs can be selectively targeted at the peripheral cannabinoid receptor.[5] The CB2 selective ligands developed so far have recently been reviewed by Raitio et al. [6]

This study stems from the Japan Tobacco invention of the CB2 selective inverse agonist **9b** (JTE-907), which binds to the CB2 receptor with nanomolar affinity (K_i 35.9 nM) and possesses antiinflammatory properties in vivo.[7] The concept of inverse agonism can be explained with the prevailing two-state receptor model theory, according to which the G-protein coupled receptors may fluctuate between active (*R**) and inactive (*R*) states. Inverse agonists possess higher affinity to the *R* form, agonists to *R** and neutral agonists bind with similar affinity to both receptor states. Therefore, inverse agonists decrease the proportion of *R** in the receptor population, and this results in reduced constitutive activation of G-proteins by receptors in the absence of agonist.[8,9] **9b** contains several functionalities which might be important for its action. Based on the affinity data presented in the patent,[10] the selected backbone structure was considered as one of the most potential starting points for nontraditional cannabinoid receptor ligand development with a special focus on CB2 selectivity.

In this study we have improved the previously published synthesis pathway[10] for compound **6** (see Scheme 1) and coupled it with a series of phenylalkylamines carrying variable substitution patterns to produce a series of amides. Also the effect of inversion of the amide bond has been studied, since we also prepared the **9b** inverse amide **9a** (Scheme 2). CB2 receptor activities of these compounds were determined by using a functional assay monitoring G protein activation, assessed by [^{35}S]GTP$_\gamma$S binding to Chinese hamster ovary (CHO) cell membranes stably expressing the human CB2 receptor (hCB2). Our previous study has established that the CB2 receptor is constitutively active in this model and therefore allows detection of agonist, neutral antagonist, and inverse agonist ligand activities.[11] As far as we are aware, these results have permitted us to derive the first structure−activity relationships of 2-oxoquinoline derivatives as CB2 receptor inverse agonists.

Results and Discussion

Chemistry. The synthesis pathway for **6** is presented in Scheme 1. Nitration of isovanillin **1** was carried out with nitronium tetrafluoroborate[12] since the typical nitration reagent (HNO_3/H_2SO_4) used in the patent[10] only gave **2** as a side product with approximately 10% yield. The phenolic hydroxyl group of **2** was alkylated with pentylbromide by using the Williamson ether synthesis[10] with satisfactory yields. The reduction of the nitro group with iron powder in EtOAc/AcOH/H_2O-mixture with a catalytic amount of HCl[13] was found to be a rapid and efficient method to reduce the nitro group prior to the aldehyde group. Reaction of **4** with dimethylmalonate in the presence of piperidine and AcOH using EtOH as solvent produced the transesterified 2-oxoquinoline as an ethyl ester **5** at high yield. Hydrolyzation of **5** in aqueous EtOH/2M HCl yielded the directly crystallized carboxylic acid **6** with high purity and at relatively high yield.[14]

As presented in Scheme 2, compound **9a**, the inverse amide of **9b**, was prepared from the acid chloride of **6** with concentrated NH_4OH in dioxane.[15] The amide was subjected to

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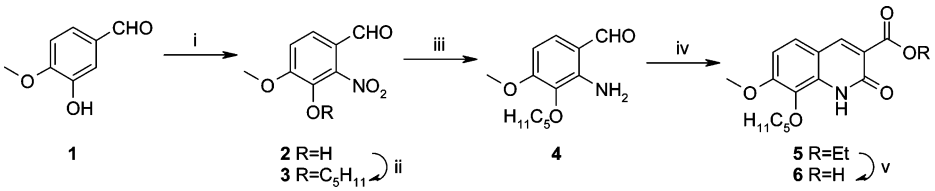
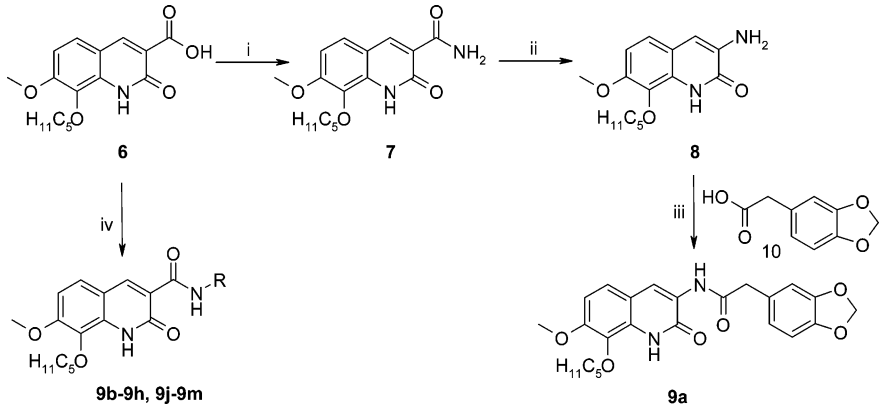
10.1021/jm050879z CCC: $33.50
Published on Web 02/22/2006

Scheme 1[a]



[a] Reagents:(i) NO_2BF_4, DCM; (ii) 1-bromopentane, K_2CO_3, DMF; (iii) Fe-powder, concentrated HCl, EtOH/AcOH/water; (iv) dimethyl malonate, piperidine, AcOH, EtOH; (v) 2 M HCl, EtOH.

Scheme 2[a]



[a] Reagents: (i) Concentrated NH_4OH, dioxane; (ii) Br_2, NaOH, water; (iii) (1) **10**, $SOCl_2$, toluene; (2) Et_3N, DCM; (iv) (1) $SOCl_2$, toluene; (2) R-NH_2, Et_3N, DCM.

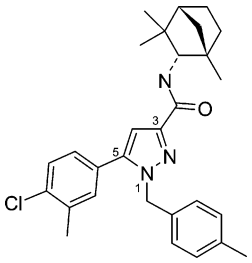


Figure 1. The chemical structure of SR144528, a full inverse agonist at the CB2 receptor.

bromine-catalyzed Hofmann rearrangement under alkaline conditions[16] yielding decarbonylated **8**, which was reacted with 3,4-(methylenedioxy)phenylacetyl chloride, prepared from the corresponding carboxylic acid **10** with $SOCl_2$, to produce compound **9a**. To yield amides **9b**−**m** (except for **9i**, for the synthesis of which see below), the carboxylic acid **6** was converted to the corresponding acid chloride, which was then reacted with the appropriate amines in the presence of triethylamine.[17] Compound **9i** was synthesized from **9f** by using the same reduction procedure as in the preparation of **4**. The final products as well as most of the intermediates were purified with flash chromatography using suitable solvent systems.

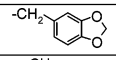
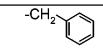
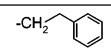
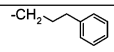
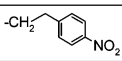
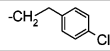
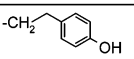
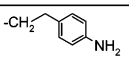
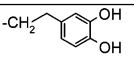
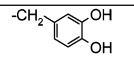
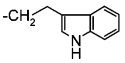
Structure−**Activity Relationship.** The CB2 receptor activities of the compounds were determined by using the [^{35}S]GTP$_\gamma$S binding assay with membranes of stably hCB2-transfected CHO cells. We have previously shown that the human CB2 receptor is constitutively active in this particular assay, allowing potency and efficacy determinations for the entire spectrum of CB2 receptor ligands, including full (CP-55,940) and partial (WIN55212-2) agonists, neutral antagonists (WIN55212-3) and inverse agonists (SR144528, Figure 1).[11] In this system, agonists typically stimulate [^{35}S]GTP$_\gamma$S binding responses by 2−3-fold, the neutral antagonist WIN55212-3 per se has no effect and the inverse agonist SR144528 inhibits [^{35}S]GTP$_\gamma$S basal binding by 20−40%.[11] The biological data are summarized in Table 1.

None of the compounds **9a**−**m** stimulated G protein activity via the CB2 receptor but instead inhibited basal [^{35}S]GTP$_\gamma$S binding in CB2 expressing but not in control CHO cell membranes. The responses were compared to that of 1 μM SR144528, which corresponds to the maximal inhibitory effect (I_{max}) of SR144528. Accordingly, relative I_{max} values as percentages of SR144528 I_{max} were produced (Table 1). Furthermore, statistical analysis of the I_{max}-values did not reveal any differences between compounds **9a**−**m** and SR144528. Consequently, as SR144528 is commonly accepted to be a full inverse agonist of the CB2 receptor, compounds **9a**−**m** were likewise concluded to evoke full CB2 receptor inverse agonist responses in the assay employed in our study.

First, we synthesized compound **9c**, in which the benzo[1,3]-dioxole ring of **9b** was replaced with a phenyl ring. This modification affected neither efficacy nor potency. Consequently, compounds **9d** and **9e** with ethylene and propylene linkers between the amide bond and the phenyl ring, respectively, were synthesized. As a result, 100-fold increases in potency without any losses of efficacy were achieved with both **9d** and **9e** when compared to **9c** (Table 1).

Compound **9d** being less lipophilic than **9e** was chosen as the template for studying the effects of phenyl ring para-substitution on CB2 activity. Accordingly, the *p*-nitro-substituted **9f** was found to be significantly less potent than **9d** (Table 1) whereas none of the chloro, hydroxyl, or amino substituents (in **9g**, **9h**, and **9i**, respectively) caused any significantly notable chances in I_{max} or in IC_{50} values when compared to **9d.** The preliminary results from docking **9f** to our rhodopsin based CB2 model[18] (data not shown) indicated that the negatively charged nitro group might repulsively interact with the amino acids at the entrance of the receptor binding cavity, which has been suggested to carry a negative charge.[19] In addition, loss of phenyl ring electron density to the electron-withdrawing nitro group might partly explain the potency decrease through diminishing

Table 1. CB2 Receptor Activities of the Synthesized Compounds[a]

Compound	R	Relative I_{max} (% SR144528-I_{max} ± S.E.M.)	-logIC_{50}± S.E.M.	Remaining CB1 agonist activity (%) stimulated by HU-210 (10nM)	
				1 μM	10 μM
SR144528	-	100±3	8.5±0.1	77±1 [b]	53±1 [c]
9a	-	60±5	7.7±0.4	93±1	75±1
9b		80±11	7.6±0.4	94±2	78±0
9c		75±11	7.2±0.4 [d]	94±0	79±1
9d		76±6	9.2±0.4	87±1	61±1
9e		82±7	9.1±0.3	98±1	59±1
9f		61±10	7.0±0.7 [d]	87±0	65±1
9g		104±6	8.3±0.2	95±1	64±1
9h		90±8	8.0±0.4	92±1	68±1
9i		97±6	8.5±0.3	91±1	74±4
9j		93±20	6.4±0.5 [d,e]	93±1	63±1
9k		124±16	5.4±0.3 [d,e,f]	93±2	85±1
9l		82±5	7.9±0.2	87±1	82±1
9m		79±10	7.6±0.4	94±1	72±1

[a] The I_{max} values are presented as percentages of the I_{max} of SR144528. CB1 antagonist activities were determined by antagonizing HU-210 (10 nM) response as described in Experimental Section. Values are from at least three independent experiments performed in duplicate. [b] Significantly higher inhibition ($P < 0.05$) when compared to **9a**-**9m**. [c] Significantly higher inhibition ($P < 0.05$) when compared to **9a**-**9m** except for **9d**, **9e** and **9j**. [d] Significantly lower potency ($P < 0.05$) when compared to **9d**. [e] Significantly lower potency ($P < 0.05$) when compared to SR144528. [f] Significantly lower potency ($P < 0.05$) when compared to **9b**.

aromatic stacking interactions between the ligand and aromatic amino acids of the receptor.

Another phenyl ring substitution pattern affecting potency was the catechol group, whose potency-decreasing effect was shown by comparing compound **9j** with **9d**. Both catechol group bearing derivatives **9j** and **9k** were also shown to be significantly less potent than SR144528 (and **9k** also less potent than **9b**), while all of the other synthesized compounds possessed potencies similar to that of SR144528 (Table 1). The low potency of **9j** and **9k** could be accounted for by the ionization of one of the catechol hydroxy groups and moreover with intramolecular hydrogen bond formation providing stabilization of the negative charge. The possible potency-lowering mechanisms based on the negative charge have been discussed above in the case of the nitro group and are also likely to be valid for the catechol derivatives.

To confirm the engagement of the CB2 receptor in mediating the response, compounds **9a**−**m** were further tested at a 10 μM concentration in native CHO cell membranes (data not shown). Thereby, the inverse agonist responses were shown to be CB2-mediated as none of the compounds showed any activity in the control cells. All the synthesized compounds were also tested at 10 μM concentration for their CB1 agonist activity and at 1 μM and 10 μM concentrations for their ability to antagonize agonist (10 nM HU-210) response in rat cerebellar membranes. No CB1 agonist activities were detected in these studies (data not shown). At 1 μM concentration, maximum 13% decreases in 10 nM HU-210-induced CB1 agonist activity were detected, whereas for SR144528 a significantly higher (23%) decrease was measured (Table 1). The data therefore suggests that compounds **9a**−**m** seem to be more CB2 selective than SR144528. At 10 μM concentration compounds **9a**−**m** blocked 15−41% of the HU-210 response (Table 1). Considering the IC_{50} values of compounds **9a**−**m** as CB2 inverse agonists and their low ability to cause CB1 response at 1 μM concentration, we conclude that these compounds, excluding the less potent **9j** and **9k**, are at least 10−1000-fold CB2 selective.

Conclusions

We have synthesized a series of CB2 receptor ligands by replacing the piperonylamide end of **9b** with a variety of aromatic amide structures. In the [^{35}S]GTP$_\gamma$S-binding assay, all the compounds elicited full CB2 inverse agonist responses similar to SR144528. Therefore, we conclude that the changes we made to the structure of **9b** did not affect efficacy. However, differences in potency were observed. The unsubstituted phenylethylamide **9d** showed significantly higher potency than the benzylamide **9c** and was therefore chosen as the probe for examination of para-substitution on CB2 activity. As a result, only nitro substitution (**9f**), but not chloro, hydroxy, and amino substitutions (**9g**, **9h**, and **9i**, respectively), decreased potency when compared to **9d**. Additionally, the catechol group bearing amides **9j** and **9k** both exhibited lower potencies than SR144528.

Experimental Section

Chemistry. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance instrument operating at 500.1 and 125.8 MHz, respectively. Chemical shifts are reported as δ values (ppm) relative to an internal standard of tetramethylsilane (TMS). Electrospray ionization mass spectra were determined on Finnigan MAT LCQ quadrupole ion

trap mass spectrometer. Elemental analyses were performed on ThermoQuest CE instrument (EA 1110 CHNS-O).

All chemicals and solvents were of commercial quality and were used without further purification. Most intermediate and end products were purified by flash chromatography using 30−60 μm silica gel and an appropriate eluent.

Procedure A: Preparation of a Carboxylic Acid Chloride from the Parent Carboxylic Acid. To a solution of carboxylic acid (1.0 mmol) in anhydrous toluene (10−20 mL) was added thionyl chloride (3.5 mmol), and the reaction mixture was refluxed under argon for 3 h. The mixture was allowed to cool to room temperature, and the solvent was evaporated. Anhydrous toluene (10 mL) was added, evaporation repeated, and the residue dried under high vacuum.

Procedure B: Coupling of a Carboxylic Acid Chloride and an Amine under Basic Conditions. The acid chloride was added dropwise in anhydrous dichloromethane (DCM) at 0 °C to a solution of the amine (1.5 mmol) and triethylamine (1.5−3.5 mmol) in anhydrous DCM (10−20 mL). The ice bath was removed, and the reaction mixture stirred at room temperature under argon for 1−2 h. Saturated NaCl (20−30 mL) was added to the mixture, and the aqueous layer was extracted with DCM. The combined organic layers were washed with saturated NaCl, dried over Na_2SO_4, and purified by flash chromatography.

3-Hydroxy-4-methoxy-2-nitrobenzaldehyde (2). Isovanillin **1** (5.0 g, 32.86 mmol) and nitronium tetrafluoroborate (6.55 g, 49.29 mmol) were reacted in anhydrous DCM (100 mL) at −20 °C for 20 h. Water (30 mL) was added dropwise, and the mixture was allowed to reach room temperature. The combined diethyl ether extracts (200 mL) were washed with water (2 × 25 mL) and saturated NaCl (50 mL), dried over Na_2SO_4, and purified by flash chromatography using petroleum ether/EtOAc 1:1 as eluent to yield a yellow solid product (4.1 g, 63%, purity ca. 90%), which was used without further purification. Typical yields varied from 40% to 81%. ^{1}H NMR (DMSO).

4-Methoxy-2-nitro-3-pentyloxybenzaldehyde (3). 1-Bromopentane (4.4 mL, 35.51 mmol) was added dropwise to a solution of **2** (2.0 g, 10.14 mmol) and potassium carbonate (4.91 g, 35.51 mmol) in anhydrous DMF (40 mL), and the reaction mixture was stirred vigorously under argon at 100 °C for 1 h followed with filtration. The combined organic layers from extractions with EtOAc:/hexane 1:1 (3 × 30 mL) were washed with water (2 × 20 mL) and saturated NaCl (20 mL), dried over Na_2SO_4, and purified by flash chromatography, eluting with a gradient from petroleum ether/EtOAc 6:1 to petroleum ether/EtOAc 1:1, yielding **3** as a yellow oil (1.8 g, 66%). Typical yields varied from 62% to 79%. ^{1}H NMR (DMSO).

2-Amino-4-methoxy-3-pentyloxybenzaldehyde (4). Iron powder (0.65 g, 11.6 mmol) and concentrated HCl (0.2 mL) were added to the solution of **3** (1.05 g, 3.7 mmol) in EtOH/AcOH/water 2:2:1 (25 mL). The reaction mixture was refluxed for 15 min and then stirred at room temperature for 40 min, filtered, and extracted with EtOAc (3 × 50 mL). The organic layer was washed with saturated $NaHCO_3$ (3 × 30 mL) and saturated NaCl (40 mL), dried over Na_2SO_4, and then purified by flash chromatography using petroleum ether/EtOAc 10:1 as eluent to produce **4** as an oily product (800 mg, 91%). Typical yields varied from 73% to 91%. ^{1}H NMR ($CDCl_3$).

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid Ethyl Ester (5). Dimethyl malonate (5.16 mL, 45.15 mmol), piperidine (4.47 mL, 45.15 mmol), and AcOH (0.1 mL) were added to a solution of **4** (4.3 g, 18.06 mmol) in EtOH (80 mL), and the reaction mixture was refluxed under argon overnight. After being cooled to room temperature, the mixture was treated with water (50 mL) and extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with water (2 × 25 mL) and saturated NaCl (50 mL), dried over Na_2SO_4, and purified by flash chromatography eluting with a gradient from petroleum ether/EtOAc 2:1 to EtOAc, yielding **5** as white crystals (4.9 g, 85%). Typical yields varied from 80% to 85%. ^{1}H NMR (DMSO).

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid (6). To a solution of **5** (1.26 g, 3.78 mmol) in EtOH (40 mL) was added 2 M HCl (25 mL), and the mixture was stirred at 60 °C overnight, cooled, and filtered. The solids were dried under vacuum to give **6** as a white powder (950 mg, 82% yield, typically varying from 62% to 87%). ^{1}H NMR (DMSO).

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxamide (7). The acid chloride prepared from **6** (200 mg, 0.66 mmol) according to procedure A was added dropwise in dioxane (10 mL) to concentrated NH_4OH (11 mL). The reaction mixture was stirred at room temperature for 3.5 h and evaporated to dryness, and the residue was purified by flash chromatography eluting with a gradient from petroleum ether/EtOAc/concentrated NH_3 (aq) 100:100:1 to petroleum ether/EtOAc/concentrated NH_3 (aq) 100:200:1, yielding **7** as white crystals (170 mg, 85%). ^{1}H NMR ($CDCl_3$).

3-Amino-7-methoxy-8-pentyloxy-1*H*-quinolin-2-one (8). Compound **7** (140 mg, 0.46 mmol) was suspended with cold (0 °C) hypobromide solution (15 mL), prepared from bromine (0.25 mL, 4.88 mmol), NaOH (1.9 g, 47.51 mmol), and water (100 mL). The reaction mixture was heated to 65 °C within 75 min and extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water (2 × 20 mL) and saturated NaCl (15 mL) and dried over Na_2SO_4. The brown solid crude product was purified by flash chromatography using petroleum ether/EtOAc 3:1 as eluent to yield **8** a whitish solid product (50 mg, 39%). ^{1}H NMR (DMSO).

3,4-(Methylenedioxy)phenylacetyl Chloride. Prepared according to procedure A starting from 3,4-(methylenedioxy)phenyl acetic acid **10** (49 mg, 0.27 mmol). The reaction was assumed to proceed quantitatively, and the product was used without purification.

2-Benzo[1,3]dioxol-5-yl-*N*-(7-methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinolin-3-yl)acetamide (9a). Acid chloride **10** was added dropwise in anhydrous DCM (5 mL) to a solution of **8** (50 mg, 0.18 mmol) and triethylamine (0.038 mL, 0.27 mmol) in anhydrous DCM (3 mL) at 0 °C. The ice bath was removed, and the reaction mixture was stirred at room temperature for 36 h. Saturated NaCl (20 mL) was added to the mixture, the layers were separated, and the aqueous layer was extracted with DCM (3 × 30 mL). The combined organic layers were washed with saturated NaCl (20 mL), dried over Na_2SO_4, and purified by flash chromatography using petroleum ether/EtOAc 3:1 as eluent yielding **9a** as a yellow solid (30 mg, 38%). ^{1}H and ^{13}C NMR (DMSO); ESI-MS: m/z = 439 $(M + H)^+$. Anal. ($C_{24}H_{26}N_2O_6$·0.18hexane) C, H, N.

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid (Benzo[1,3]dioxol-5-ylmethyl)amide (9b). Prepared according to procedures A and B starting from **6** (300 mg, 0.98 mmol) and 3,4-methylenedioxybenzylamine (0.18 mL, 1.45 mmol). Flash chromatography with a gradient from hexane/EtOAc 3:1 to hexane/EtOAc 1:1 as eluent yielded **9b** as white crystals (165 mg, 38%). ^{1}H and ^{13}C NMR ($CDCl_3$); ESI-MS: m/z = 439 $(M + H)^+$. Anal. ($C_{24}H_{26}N_2O_6$) C, H, N.

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid Benzylamide (9c). Prepared as **9b** from **6** (150 mg, 0.49 mmol) and benzylamine (0.081 mL, 0.74 mmol). Flash chromatography with petroleum ether/EtOAc 2:1 as eluent yielded **9c** as a light solid product (129 mg, 67%). ^{1}H and ^{13}C NMR (DMSO); ESI-MS: m/z = 395 $(M + H)^+$. Anal. ($C_{23}H_{26}N_2O_4$) C, H, N.

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid Phenethylamide (9d). Prepared as **9b** from **6** (200 mg, 0.66 mmol) and phenethylamine (0.13 mL, 1.04 mmol). Recrystallizing from methanol yielded **9d** as white crystals (150 mg, 56%). ^{1}H and ^{13}C NMR ($CDCl_3$); ESI-MS: m/z = 409 $(M + H)^+$. Anal. ($C_{24}H_{28}N_2O_4$) C, H, N.

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid (3-phenylpropyl)amide (9e). Prepared as **9b** from **6** (150 mg, 0.49 mmol) and 3-phenylpropylamine (0.105 mL, 0.74 mmol). Flash chromatography with petroleum ether/EtOAc 2:1 as eluent yielded **9e** as a white solid product (134 mg, 65%). ^{1}H and ^{13}C NMR (DMSO); ESI-MS: m/z = 423 $(M + H)^+$. Anal. ($C_{25}H_{30}N_2O_4$) C, H, N.

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid [2-(4-nitrophenyl)ethyl]amide (9f). Prepared as **9b** from **6** (300 mg, 0.98 mmol) and 2-(4-nitrophenyl)ethylamine hydrochloride (298 mg, 1.47 mmol). Flash chromatography using

petroleum ether/EtOAc 1:2 as eluent yielded **9f** as a yellow solid product (140 mg, 32%). ^{1}H and ^{13}C NMR ($CDCl_3$); ESI-MS: m/z = 454 (M + H) $^+$. Anal. ($C_{24}H_{27}N_3O_6$·0.18 hexane·0.16EtOAc) C, H, N.

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid [2-(4-chlorophenyl)-ethyl]-amide (9g). Prepared as **9b** from **6** (150 mg, 0.49 mmol) and 2-(4-chlorophenyl)ethylamine (0.103 mL, 0.74 mmol). Purification by flash chromatography using petroleum ether/EtOAc 1:1 as eluent yielded **9g** as a whitish solid product (95 mg, 44%). ^{1}H and ^{13}C NMR (DMSO); ESI-MS: m/z = 443 (M + H) $^+$. Anal. ($C_{24}H_{27}N_2O_4Cl$) C, H, N.

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid [2-(4-hydroxyphenyl)ethyl]amide (9h). Prepared as **9b** from **6** (150 mg, 0.49 mmol) and tyramine hydrochloride (127 mg, 0.73 mmol). Flash chromatography with petroleum ether/EtOAc 2:3 as eluent yielded **9h** as a whitish solid product (112 mg, 54%). ^{1}H and ^{13}C NMR ($CDCl_3$); ESI-MS: m/z = 425 (M + H)$^+$. Anal. ($C_{24}H_{28}N_2O_5$·0.12H_2O) C, H, N.

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid [2-(4-aminophenyl)ethyl]amide (9i). Concentrated HCl (0.1 mL) and Fe-powder (0.65 g, 11.6 mmol) were added to the solution **9f** (110 mg, 0.24 mmol) in EtOH/AcOH/water 2:2:1 (20 mL). The reaction mixture was refluxed for 15 min and stirred at room temperature for 1 h. The mixture was filtered, and the filtrate was extracted with EtOAc (3 × 20 mL). The organic layer was washed with saturated $NaHCO_3$ (3 × 15 mL) and saturated NaCl (20 mL), dried over Na_2SO_4, and then purified by flash chromatography using petroleum ether/EtOAc 1:2 as eluent yielding **9i** (59 mg, 58%).^{1}H and ^{13}C NMR ($CDCl_3$); ESI-MS: m/z = 424 (M + H) $^+$. Anal. ($C_{24}H_{27}N_3O_6$·0.75H_2O) C, H, N.

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid [2-(3,4-dihydroxyphenyl)ethyl]amide (9j). Prepared as **9b** from **6** (200 mg, 0.66 mmol) and dopamine (150 mg, 0.98 mmol). Flash chromatography eluting with 2% 2-propanol in DCM yielded **9j** as a white solid product (62 mg, 21%). ^{1}H and ^{13}C NMR (DMSO); ESI-MS: m/z = 441 (M + H) $^+$. Anal. ($C_{24}H_{28}N_2O_6$) C, H, N.

3,4-Dihydroxybenzylamine. BBr_3 (0.40 mL, 4.23 mmol) was added to a solution of 3,4-dimethoxybenzylamine in anhydrous DCM (15 mL) at −78 °C. The reaction mixture was stirred at room temperature under argon for 24 h, water (10 mL) was added dropwise at 0 °C, and the aqueous layer was washed with DCM (3 × 20 mL). The aqueous layer was evaporated, and the brown solid crude product was dried under vacuum. The reaction was assumed to have proceeded quantitatively, and the product (670 mg) was not separated from the mainly inorganic impurities.

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid 3,4-dihydroxybenzylamide (9k). Prepared as **9b** from **6** (200 mg, 0.66 mmol) and 3,4-dihydroxybenzylamine (138 mg, 0.99 mmol). Purification by flash chromatography eluting with 4% 2-propanol in DCM yielded **9k** as a yellow solid product (33 mg, 12%). ^{1}H and ^{13}C NMR ($CDCl_3$); ESI-MS: m/z = 427 (M + H) $^+$. Anal. ($C_{23}H_{26}N_2O_6$·0.12H_2O) C, H, N.

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid (2-aminophenyl)amide (9l). Prepared as **9b** from **6** (300 mg, 0.98 mmol) and 1,2-phenylenediamine dihydrochloride (266 mg, 1.47 mmol). Purification by flash chromatography eluting with 2% methanol in DCM yielded **9l** (189 mg, 49%) as a yellow solid product. ^{1}H and ^{13}C NMR ($CDCl_3$); ESI-MS: m/z = 396 (M + H) $^+$. Anal. ($C_{22}H_{25}N_3O_4$·0.08EtOAc, based on ^{1}H NMR) C, H, N.

7-Methoxy-2-oxo-8-pentyloxy-1,2-dihydroquinoline-3-carboxylic Acid [2-(1*H*-indol-3-yl)ethyl]amide (9m). Prepared as **9b** from **6** (300 mg, 0.98 mmol) and tryptamine (236 mg, 1.47 mmol). Flash chromatography with petroleum ether/EtOAc 1:2 as eluent yielded **9m** as a white solid product (270 mg, 60%). ^{1}H and ^{13}C NMR (DMSO); ESI-MS: m/z = 448 (M + H) $^+$. Anal. ($C_{26}H_{29}N_3O_4$) C, H, N.

Preparation of CHO Cell and Rat Cerebellar Membranes. Stably transfected CHO-hCB2 cells (B_{max} 20.4 ± 1.7 pmol/mg) were generated as previously described.[17] These and control CHO cells were cultured as monolayers with 100 μg/mL G-418 (Euroclone) in Ham's F-12 nutrient mixture (Euroclone), containing 10% fetal calf serum (Euroclone), 100 U/mL penicillin, and 100 μg/mL streptomycin (Euroclone) at 37 °C in a humidified atmosphere of 5% CO_2/95% air. CHO cell membranes were prepared as previously described.[17] Rat cerebellar membranes were prepared as previously described.[20]All animal experiments were conducted according to the Declaration of Helsinki and were approved by the local ethics committee.

[^{35}S]GTPγS-Binding Assays. Incubations with CHO cell membranes were carried out as previously described.[11] Briefly, the final incubation contained 5 μg of membrane protein, 55 mM Tris-HCl pH 7.4, 1.1 mM EDTA, 100 mM NaCl, 5 mM $MgCl_2$, 0.5% (w/v) BSA, 11 μM GDP, ∼150 pM [^{35}S]GTPγS with the studied ligands in ethanol (final concentration 1%, v/v) or in DMSO (final concentration 0.5%, v/v). Incubations for measuring CB1 receptor activities with rat cerebellar membranes were conducted under optimized conditions, essentially as previously described.[21]

Data Analysis. The experimental data from at least three independent experiments performed in duplicate have been expressed as means and variability as SEM. Values for IC_{50}, maximal effects (I_{max}) and for the SEM have been calculated by nonlinear regression analysis using the equation for a sigmoidal concentration−response curve (GraphPad Prism 4). Statistical differences between groups have been compared using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test with P value < 0.05 considered to be significant.

Acknowledgment. The authors are grateful to Ms. Miia Reponen, Ms. Minna Glad, Ms. Katja Tiainen, Ms. Helly Rissanen, and Ms. Tiina Koivunen for their outstanding technical assistance. We also thank Outi Salo, M.Sc (Pharm.) for her consulting regarding SARs. This study was financially supported by the National Technology Agency of Finland, the Emil Aaltonen Foundation and the Academy of Finland (grant # 107300).

Supporting Information Available: Elemental analysis data and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM050879Z