Bioorganic & Medicinal Chemistry Letters 20 (2010) 453–457

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Conversion of 4-cyanomethyl-pyrazole-3-carboxamides into CB1 antagonists with lowered propensity to pass the blood–brain-barrier

Jean-Marie Receveur, Anthony Murray, Jean-Michel Linget, Pia K. Nørregaard, Martin Cooper, Emelie Bjurling, Peter Aadal Nielsen, Thomas Högberg *

7TM Pharma A/S, Fremtidsvej 3, DK-2970 Hørsholm, Denmark

ARTICLE INFO

Article history:
Received 24 October 2009
Revised 28 November 2009
Accepted 1 December 2009
Available online 4 December 2009

Keywords:
CB1 antagonists
CB1 inverse agonists
Obesity
DIO model
CNS
Blood–brain-barrier
Plasma/brain ratio

ABSTRACT

A series of amides, amidines and amidoximes have been made from the corresponding nitrile compounds, to provide potent antagonists and inverse agonists for the CB1 receptor with considerably lower lipophiliciy, higher polar surface area and improved plasma/brain ratios compared to the centrally acting rimonabant. Extensive investigations of ADME and in vivo pharmacological properties led to selection of the amide series and specifically the 4-(4-fluorophenyl)piperidin-4-ol derivative **D4**. A clear improvement in the peripheral profile over rimonabant was seen, although some contribution of central effect on the pronounced weight reduction in obese mice cannot be ruled out.

The endocannabinoid signaling substances are derived from arachidonic acid and act on two types of GPCR receptors, that is, cannabinoid receptors CB1 and CB2.[1,2] The CB1 receptor is widely expressed in the brain and to a lower extent in several peripheral organs such as liver, adipose tissue, gastrointestinal tract, vagal nerves, pancreas and skeletal muscle.[1–3] CB2 receptors exist mainly in the immune system, although recent publications have reported their existence also in the CNS.[1–3] The endocannabinoids play a pivotal role, through the CB1 receptor, in regulating food intake and energy expenditure in synergy with other anorexigenic and orexigenic regulatory pathways.[1,2] CB1 receptor-deficient mice are resistant to diet-induced obesity,[4] and CB1 antagonists/inverse agonists, such as rimonabant and taranabant shown in Figure 1, inhibit food intake and reduce body weight in obese animals and humans.[1,2,5] These effects are likely to be mediated by a combination of central and peripheral target organ actions.[2,6] Pharmacological blockade of the peripheral CB1 system can elicit beneficial effects on other metabolic and atherogenic risk factors that are independent of eating behavior and body weight.[2,6] Clinical trials with rimonabant also showed improvement in glycemic control and lipid profile in type 2 diabetic patients (SERENADE) and loss of visceral and hepatic fat in abdominally obese patients (ADAGIO).[6,7] These long term peripheral effects could arise from, for example, reduced hepatic lipogenesis, enhanced lipid oxidation, adipose lipolysis, and reduced insulin resistance. Recently, blockade of the CB1 receptor has also been shown in rodents to hold promise as a therapy for chronic liver diseases such as liver fibrosis.[8]

An issue associated with this drug class is the induction of central nervous system (CNS) side-effects such as anxiety and depression[2,9], effects which are evidently linked to functional CB1 receptors in brain areas such as the frontal cortex, hippocampus and amygdala.[10] A plausible working hypothesis to avoid the CNS effects seen with rimonabant and taranabant, but still achieve effects on body weight and metabolic parameters, would be to design compounds unable to pass the blood–brain-barrier (BBB).[2,11] One possibility to achieve this would be by making compounds having considerably higher polar surface area (PSA) and lower

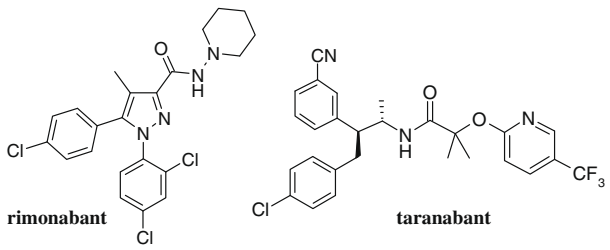


Figure 1. Two representative CB1 antagonists progressed to clinical development.

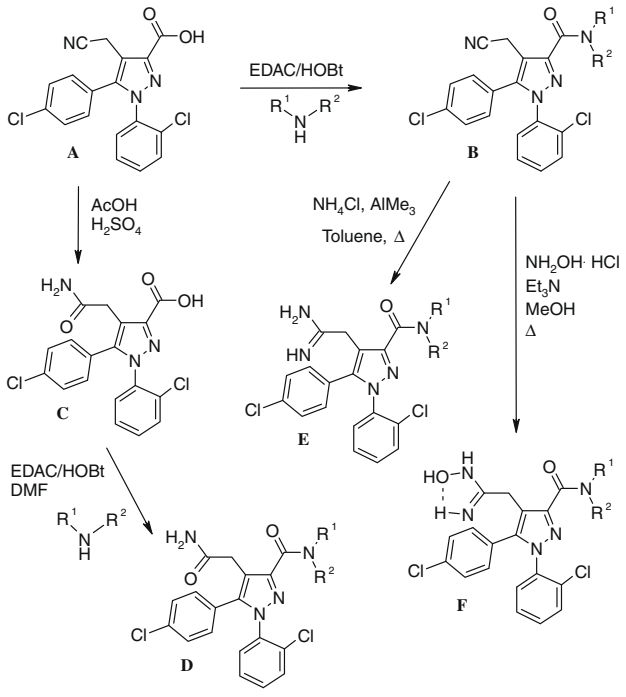
* Corresponding author. Tel.: +45 3925 7760; fax: +45 3925 7776.
E-mail address: th@7tm.com (T. Högberg).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.12.003

log *D* than the centrally acting drugs. We have previously described the synthesis and SAR features of a diverse library of 4-cyanomethyl-1,5-diphenyl-1*H*-pyrazole-3-carboxamides **B** (Scheme 1).[12] In this report we present the synthesis and characterization, as CB1 antagonists, of subsequent compound series in which the nitrile has been converted into more polar neutral (amides **D** and amidoximes **F**) or positively charged (amidines **E**) functional groups.

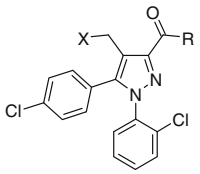
For the conversion of nitriles to amides, the standard procedure was hydrolysis of the intermediate acid **A** to amide acid **C** by treatment with a mixture of acetic acid/concentrated sulphuric acid (2:1) at 100 °C for 30 min, followed by coupling with the amine using standard conditions (Scheme 1). When salt forms of amines were used the coupling was conducted in the presence of diisopropylethylamine. The nitriles **B** were used directly in the conversion to amidines **E** and amidoximes **F**. The former were obtained by firstly reacting ammonium chloride with trimethylaluminium in dry toluene, followed by addition of nitrile **B** and raising the temperature to 160 °C in a microwave oven. The amidoximes **F** were conveniently obtained by reaction of **B** with hydroxylamine in methanol at 40 °C.

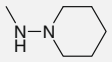
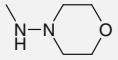
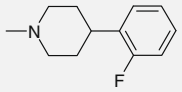
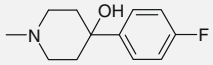
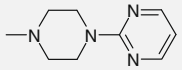
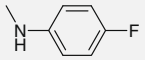
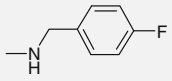
The compounds were tested against the human CB1 receptor using a GTPγS antagonist assay with CP55940 as agonist and membranes produced from CHO-K1 cells stably transfected with recombinant human CB1 receptor.[13] Several compounds were also investigated for inverse agonism, by running the same assay without agonist, and for selectivity versus the CB2 subtype receptor in a GTPγS antagonist assay using CP55940 as agonist. Table 1 displays a comparison of a small set of amides **D**, amidines **E** and amidoximes **F** in comparison with the corresponding nitriles **B,** described earlier. Some general trends can be mentioned about the impact of lowering chromatographically measured log *D* values[14] and raising polar surface areas of the new western chemotypes **D**, **E** and **F**. For example, compared to the nitrile **B1** (log *D* 3.6/ PSA 74 Å^2) the following differences are observed for the corresponding amide **D1** (−1.0/+19), amidine **E1** (−1.7/+26) and amidoxime **F1** (−0.7/+35). The somewhat surprisingly small effect on log *D* resulting from the conversion of nitriles to amidoximes **F** might be attributable to the intra-molecular hydrogen bonding indicated in Scheme 1 – a possibility that would make this series less attractive as it would facilitate BBB penetration. We also observed an inappropriate brain to plasma ratio for the amidoximes and for some amides (see below).



Scheme 1.

Table 1
Antagonism and inverse agonism (shown in brackets) of hCB1 by representative 4-substituted pyrazole-3-carboxamides



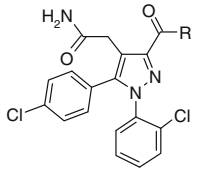
R		IC_{50} GTPγS[a] (nM)			
		B[b]	D	E	F
	**1**	7.5 [1.8]	1.4 [0.82]	0.93 [0.40]	1.8 [1.3]
	**2**	71	7.0	26	51
	**3**	nd	0.51	nd	0.54
	**4**	3.0	0.19	1.9	nd
	**5**	3.8	0.71	9.7	21
	**6**	11	nd	1.5 [0.59]	1.1
	**7**	2.1	nd	1.2 [0.79]	nd

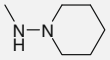
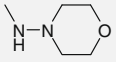
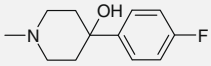
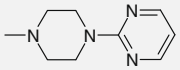
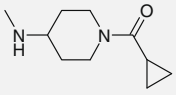
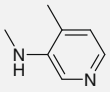
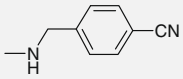
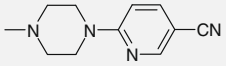
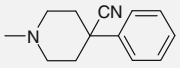
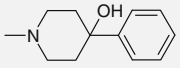
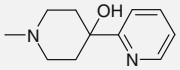
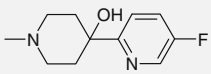
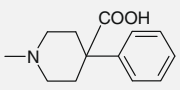
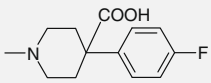
[a] Antagonist (using CP55940 as agonist) and inverse agonist activity in GTPγS assay with membranes produced from CHO-K1 cells stably transfected with hCB1 receptor. All compounds displayed efficacy above 100% and all values are single or mean of double determinations.[13]
[b] Nitriles described in earlier publication.[12]

When comparing the CB1 potency for the same set of compounds (**B1-F1**), an encouraging trend of increased potency compared to the nitriles, was observed for the three more polar derivatives. The corresponding morpholine derivatives (**2**) showed a reduced potency, consistent with previous findings.[15,16] The related polar members of phenyl piperidines (**3** and **4**) and piperazine (**5**) displayed variable potencies, however the amides **D** were consistently very potent, which might indicate some cooperative binding interactions. The homologous amidines **E6** and **E7** also show pronounced antagonistic and inverse agonistic activity. Even if the comparisons should not be stretched too far, this small set of compounds demonstrated that lowering log *D* and increasing PSA had no negative effect on potency, which supported our current strategy to modulate polarity by modifications in this part of the molecules.

Although, the amidines **E** had very good microsomal stability they showed insufficient in vivo ADME properties. For example, **E6** remained unaffected after 30 min in rat or mouse microsomes, but it showed a modest bioavailability of 19% in rat even after intraperitoneal administration (no detectable levels were found after po administration). However, the plasma/brain ratio of 3.2 of **E6** was an improvement compared to rimonabant having a ratio of 0.22 in rat (Table 3). In mouse, rimonabant displays plasma/brain ratios in the range of 0.3 to 0.5 throughout the period of 2 and 13 h after oral administration. As mentioned the amidoximes, exemplified by **F1,** showed a poor plasma/brain ratio of 1.2 after

Table 2
Antagonism, inverse agonism and receptor binding of hCB1 by representative 4-carbamoylmethyl-pyrazole-3-carboxamides.



D	R	log D[c]	Solubility (μM)	IC_{50} GTPγS[a] Antagonism (nM)	IC_{50} GTPγS[a] Inverse agonism (nM)	IC_{50} Bind[b] (nM)
	rimonabant	4.7	10	4.5	2.9	5.1
1		2.6	4.5	1.4	0.82	1.8
2		1.8	nd	7.0	1.7	7.9
4		2.8	75	0.19	0.10	2.6
5		2.6	1.1	0.71	0.30	1.4
8		2.3	80	nd	1.2	3.7
9		2.4	200	3.0	1.7	0.55
10		2.9	16	0.66	0.49	0.34
11		2.8	nd	1.2	1.4	4.6
12		3.4	9.0	0.23	0.11	0.78
13		2.7	105	0.26	0.11	2.9
14		2.3	400	3.6	2.0	1.9
15		2.5	290	nd	2.1	5.5
16		1.5	425	16	10	23
17		1.6	500	30	nd	nd

[a] As in Table 1.
[b] Receptor binding in COS7 (rimonabant, 10, 13, 15, 17) or CHO-K1 (other) cells using [^{3}H]-CP55940 as tracer.[13]
[c] Chromatographically determined log D values.[14]

Table 3
Plasma/brain ratios in lean rodents of selected compounds

Compd	Species	Admin. route	Dose (mg/kg)	Time (h)	Plasma/brain[a]
Rimonabant	Mouse	po	10	6	0.42
Rimonabant	Rat	po	5	6	0.22
E6	Rat	ip	10	6	3.2
F1	Mouse	po	10	3	1.2
D4	Mouse	po	10	6	10.2
D8	Mouse	po	10	6	6.1
D9	Rat	po	5	6	1.2
D14	Mouse	po	10	6	10.2

[a] Plasma/brain ratios were calculated after subtraction of 1% of the plasma concentration from the brain levels to account for contaminations in homogenized brain.[18]

oral administration in mouse and was accordingly not pursued further. A compilation of plasma/brain ratios in lean rodents is shown in Table 3.

With the limitations seen with the amidines and amidoximes, we focused on the amide series **D** (Table 2), to capitalize on the SAR information we gained earlier, with the intention to arrive at compounds with log *D* values below 2.5 and good solubilities. One of the first amides **D1** showed very poor oral bioavailability in rat (2%) which was attributed to a very low solubility, since both PAMPA permeability and microsomal stability in rat were good (72% remain after 30 min). Introduction of a cyclopropyl-carboxamide (**D8**) improved solubility over **D1** while retaining pronounced inverse agonism and receptor binding. The pyridyl derivative **D9** displayed good solubility, log *D* and CB1 potency. This derivative **D9,** with a microsomal stability of 63% comparable to **D1**, had on the other hand a reasonable oral bioavailability of 39% in rat, but penetrated into the brain over time (ratio ~1.0 at 6 h). As the piperidinylamide **D8** showed a plasma/brain ratio of 6.1, we decided to investigate larger compounds to determine if they would have improved separation. The cyano substituted benzylamine **D10**, which was the most polar and yet potent analogue of this substance class identified earlier among the nitrile derivatives **B**,[12] did not show the anticipated improvement in log *D* and solubility, so this series was not progressed further.

Disappointingly, the very potent piperazines **D5** and **D11** and the 4-cyanopiperidine **D12** did not have sufficiently good physicochemical properties to warrant further studies. However, the 4-hydroxy-4-phenylpiperidines **D4** and **D13** and the pyridyl analogues **D14** and **D15** combined good potency with appropriate physicochemical properties. The pyridyl compounds improvement in log *D* and solubility are only at the slight expense of inverse agonistic potency. Further improvement of these physicochemical parameters was achieved by the introduction of a 4-carboxyl moiety, however, in this case with greater loss of CB1 potency (**D16** and **D17**). The acid **D17** also showed a very poor oral bioavailability of 4% in mouse and hence was not explored.

Encouragingly the 4-hydroxypiperidine **D4** and the pyridyl analogue **D14** showed 10-fold higher concentrations in plasma than brain, 6 h after oral administration to mouse (Table 3). Both compounds showed excellent CB1 selectivity, with greater than 1000-fold lower potency on CB2. As the phenylpiperidine **D4** also showed better ADME properties than the pyridyl compounds **D14** and **D15,** with high oral bioavailability in mouse (~100%) and rat (70%), we continued in vivo investigations with **D4** as tool compound. The amide **D4** was initially assessed for its central pharmacodynamic effect in the hypothermia model, one of the four tests that constitute the 'tetrad'. CB1 agonists induce a reduction in body temperature by acting on CB1 receptors in the hypothalamus,[17] an effect that can be reversed by an antagonist. Notably, **D4** gives a partial reduction only in the highest dose of 10 mg/kg, whereas rimonabant already at 1 mg/kg gives a full blockade (Fig. 2).

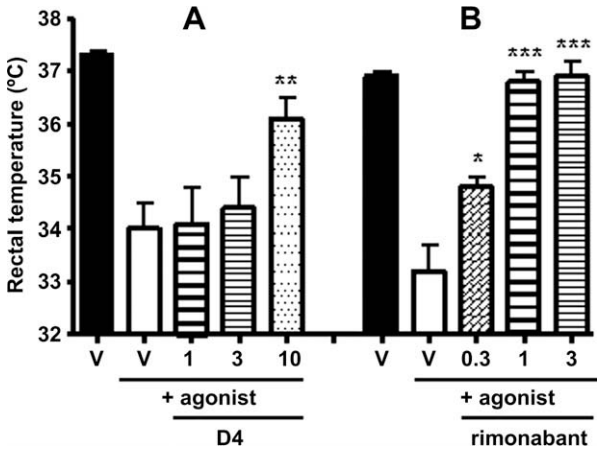


Figure 2. Blockade of CB1 agonist (WIN-55,212, 2.5 mg/kg, sc) induced hypothermia in mouse of amide **D4** (A:, 1, 3, 10 mg/kg, po) and rimonabant (B: 0.3, 1, 3 mg/kg, po). Temperature measured 1 h after agonist dosing. The antagonists were administered 45 min prior to the agonist. V is denoting vehicle treatment. Student's *t* test: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared with agonist alone.

The effect of **D4** on food intake (data not shown) and body weight was investigated in diet-induced obese (DIO) mice over seven days (Fig. 3). The plasma/brain ratio of **D4** was found to be 13 in these obese animals after 6 h. A clear dose-dependant

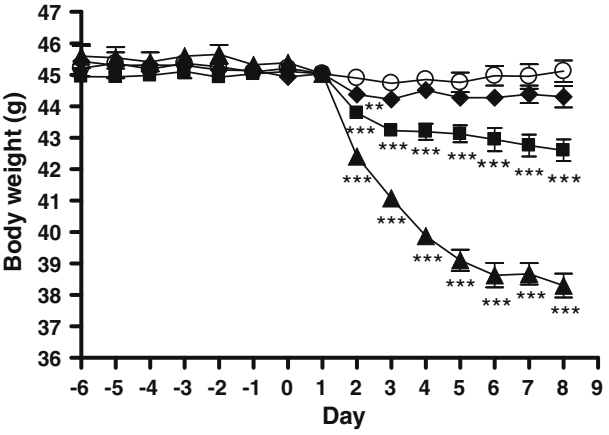


Figure 3. Effect on body weight after subchronic oral administration of **D4** (▲10, ■ 3 and ♦1 mg/kg) to DIO mice versus vehicle control animals (○). Data analyzed by ANCOVA with body weight on day 1 as covariate followed by Williams' test: $^{**}p < 0.01$, $^{***}p < 0.001$.

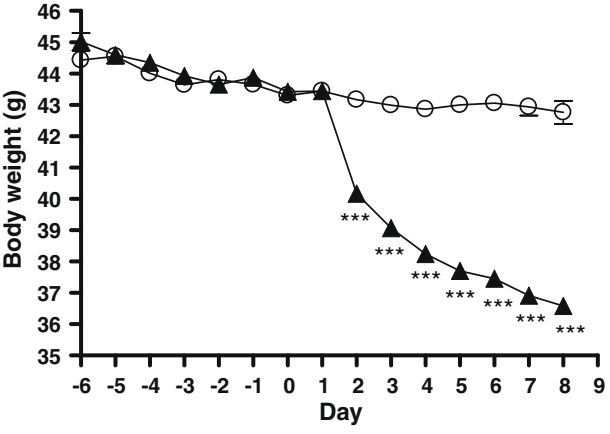


Figure 4. Effect on body weight after subchronic oral administration of rimonabant (▲10 mg/kg) to DIO mice versus vehicle control animals (○). Statistics as in Figure 3.

reduction of body weight was observed reaching 5.6% and 15.1% at 3 and 10 mg/kg, respectively. Rimonabant at 10 mg/kg gives comparable reductions in body weight (14.5%) as **D4** (Fig. 4), but exhibits more pronounced central effects already at 1/10 of the dose (cf. Fig. 2).

In conclusion, we have developed potent antagonists and inverse agonists of the CB1 receptor, incorporating either an amide, amidine or amidoxime group which gives rise to considerably lower lipophilicity, higher polar surface area and improved plasma/brain ratios compared to the centrally acting rimonabant. Extensive investigation of ADME and in vivo pharmacological properties led to selection of the amide series and specifically the 4-(4-fluorophenyl)piperidin-4-ol derivative **D4**. Although the exposure of **D4** in brain is considerably lower than that of rimonabant, it nevertheless displays comparable efficacy in terms of weight reduction. This observation supports the concept that efficacious drugs for the treatment of obesity and associated cardiometabolic risk factors are feasible without CNS liabilities, In our search for peripherally acting CB1 antagonists, we will report further studies, with negatively charged compounds in due course.

Acknowledgements

The authors thank Rokhsana Andersen, Niklas Sköld, Kirsten Nymann Petersen, Peter Andersen, Joan Gredal, Ann Christensen and Helle Iversen, for excellent technical assistance.

References and notes

1. (a) Di Marzo, V. *Pharmacol. Res.* **2009**, *60*, 77; (b) De Petrocellis, L.; Di Marzo, V. *Best Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 1; (c) Di Marzo, V.; Ligresti, A.; Cristino, L. *Int. J. Obesity* **2009**, *33*, S18.
2. (a) Janero, D. R.; Makriyannis, A. *Expert Opin. Emerging Drugs* **2009**, *14*, 43; (b) Di Marzo, V. *Drug Discov. Today* **2008**, *13*, 1026; (c) Akbas, F.; Gasteyger, C.; Sjödin, A.; Astrup, A.; Larsen, T. M. *Obes. Rev.* **2009**, *10*, 58.
3. (a) DiMarzo, V. *Diabetologia* **2008**, *51*, 1356; (b) Mackie, K. *Neuroendocrinology* **2008**, *20*, 10.
4. Ravinet Trillou, C.; Delgorge, C.; Menet, C.; Arnone, M.; Soubrie, P. *Int. J. Obes.* **2004**, *28*, 640.
5. Medicinal chemistry reviews (a) Lee, H.-K.; Choi, E. B.; Pak, C. S. *Curr. Topics Med. Chem.* **2009**, *9*, 482; (b) Jagerovic, N.; Fernandez-Fernandez, C.; Goya, P. *Curr. Topics Med. Chem.* **2008**, *8*, 205.
6. (a) Scheen, A. J.; Paquot, N. *Best Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 103; (b) Samaha, F. F.; Chou, C. M. *Obesity* **2008**, *17*, 220.
7. (a) Rosenstock, J.; Hollander, P.; Chevalier, S.; Iranmanesh, A. *Diabetes Care* **2008**, *31*, 2169; (b) Després, J. P. *Arterioscler. Thromb. Vasc. Biol.* **2009**, *29*, 416; (c) Després, J. P. *Curr. Pharm. Des.* **2009**, *15*, 553.
8. Caraceni, P. *Best Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 65.
9. (a) Moreira, F. A.; Grieb, M.; Lutz, B. *Best Pract. Res. Clin. Endocrinol. Metab.* **2009**, *23*, 133; (b) Viveros, M. P.; de Fonseca, F. R.; Bermudez-Silva, F. J.; McPartland, J. M. *Endocrinol. Metab. Immune. Disord. Drug Targets* **2008**, *8*, 220.
10. Viveros, M. P.; Marco, E. M.; Llorente, R.; López-Gallardo, M. *Neural Plast.* **2007**, *2007*, 52908.
11. Kunos, G.; Osei-Hyiaman, D.; Bátkai, S.; Sharkey, K. A.; Makriyannis, A. *Trends Pharmacol. Sci.* **2009**, *30*, 1.
12. Cooper, M.; Receveur, J.-M.; Bjurling, E.; Nørregaard, P. K.; Aadal Nielsen, P.; Sköld.; Högberg, T. *Bioorg. Med. Chem. Lett.* **2009**. doi:10.1016/j.bmcl.2009.11.047.
13. CB1 antagonism was assessed by measuring inhibition of CP55940-induced [^{35}S]GTPγS binding to membranes prepared from CHO-K1 cells expressing the human CB1 receptor (Harrison and Traynor, *Life* Sci. **2003,** *74*, 489). Assessment of inverse agonism was conducted analogously without addition of the agonist CP55940. CB1 receptor binding (Bylund and Toews, *Am J Physiol*. **1993**, *265*, L421-9.) was analyzed by measuring displacement of [^{3}H]-CP55940 binding to COS7 or CHO-K1 cells expressing the human CB1 receptor. CP55940 is a well known non-selective CB1 and CB2 receptor agonist (e.g., Felder et al., *Mol. Pharmacol.* **1995**, *48*, 443). **Experimentals**: The [^{35}S]GTPγS SPA (Scintillation Proximity Assay; cf. Glickman et al., *Assay Drug Dev. Technol.* **2008,** *6*, 433) binding assay was performed by incubating 5 μg hCB1-membranes with 1 nM [^{35}S]GTPγS in the presence of 3 nM of CP55940 (for antagonist assessment) and various concentrations of the test compounds at room temperature for 1 h 0.4 mg/well SPA beads (PVT-WGA; RPNQ0001 Amersham Pharmacia Biotech) were then added and the incubation continued for further 30 min on an orbital shaker. The assay buffer contained 50 mM HEPES (pH 7.5), 50 mM NaCl, 2.5 mM $MgCl_2$, 0.1% BSA, 1 μM GDP and 100 μg/ml Saponin. Microtiter plates were centrifuged at 1500 rpm for 5 min and radioactivity was read immediately using a Topcounter (PerkinElmer Life Sciences). The CB1 receptor binding assay was performed by incubating 30,000 cells with 0.15 nM [^{3}H]-CP55940 in the presence of various concentrations of the test compounds at 4 °C for 3 h. The assay buffer contained 50 mM Tris (pH 7.5), 3 mM $MgCl_2$, 1 mM EDTA and 0.5% BSA. The cells were washed twice and then lysed for 30 min at room temperature with 0.1N NaOH. After overnight incubation with scintillation fluid (Microscint 20) radioactivity was read using a Topcounter. Data from assays were analyzed and IC_{50} values determined by non-linear regression using the PRISM software (GRAPHPAD Software, San Diego).
14. Valko, K.; Du, C. M.; Bevan, C.; Reynolds, D. P.; Abraham, M. H. *Curr. Med. Chem.* **2001**, *8*, 1137.
15. Lan, R.; Liu, O.; Fan, P.; Lin, S.; Fernando, S. R.; McCallion, D.; Pertwee, R.; Makriyannis, A. *J. Med. Chem.* **1999**, *42*, 769.
16. Lange, J. H. M.; van Stuivenberg, H. H.; Coolen, H. K. A. C.; Adolfs, T. J. P.; McCreary, A. C.; Keizer, H. G.; Wals, H. C.; Veerman, W.; Borst, A. J. M.; de Looff, W.; Verveer, P. C.; Kruse, C. G. *J. Med. Chem.* **2005**, *48*, 1823.
17. (a) Martin, B. R.; Compton, D. R.; Thomas, B. F.; Prescott, W. R.; Little, P. J.; Razdan, R. K.; Johnson, M. R.; Melvin, L. S.; Mechoulam, R.; Ward, S. J. *Pharmacol. Biochem. Behav.* **1991**, *40*, 471; (b) Rawls, S. M.; Cabassa, J.; Adler, M. W.; Geller, E. B. *J. Pharmacol. Exp. Ther.* **2002**, *301*, 963.
18. Pardridge, W. M.; Kang, Y. S.; Buciak, J. L. *Pharm. Res.* **1994**, *11*, 738.