Bioorganic & Medicinal Chemistry Letters 19 (2009) 5931–5935

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel pyridine derivatives as potent and selective CB_2 cannabinoid receptor agonists

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ARTICLE INFO

Article history:
Received 21 May 2009
Revised 13 August 2009
Accepted 13 August 2009
Available online 21 August 2009

Keywords:
Cannabinoid receptors
Selective CB_2 agonists
Pain
Pyridine derivatives

ABSTRACT

Replacement of the phenyl ring in our previous (morpholinomethyl)aniline carboxamide cannabinoid receptor ligands with a pyridine ring led to the discovery of a novel chemical series of CB_2 ligands. Compound **3**, that is, 2,2-dimethyl-*N*-(5-methyl-4-(morpholinomethyl)pyridin-2-yl)butanamide was identified as a potent and selective CB_2 agonist exhibiting in vivo efficacy after oral administration in a rat model of neuropathic pain.

The cannabinoid receptors CB_1 and CB_2 belong to the superfamily of G-protein-coupled receptors (GPCRs).[1] Cannabinoid receptors are present at key sites involved in the relay and modulation of nociceptive information.[1] The analgesic effects of cannabinoids have been extensively studied.[2] However, administration of tetrahydrocannabinol (THC) or other non-selective synthetic cannabinoids is followed by severe psychotropic side effects mediated by activation of CB_1 receptors within the central nervous system (CNS).[2–4] The CB_1 receptors are located centrally and peripherally, whereas the CB_2 receptors are expressed primarily in immune cells and tissues.[1–3] In preclinical studies, CB_2 selective receptor agonists inhibit signs of acute nociceptive, inflammatory and neuropathic pain, and importantly they do not cause the CNS side effects typically produced by cannabinoid ligands displaying agonist activity at the CB_1 receptor.[2–7] Therefore, selective CB_2 receptor agonists are very promising candidates for the treatment of pain.

As part of a research program aimed at identifying novel selective CB_2 agonists, we identified the (morpholinomethyl)aniline carboxamide derivative **1** (Fig. 1) as the starting point for a lead optimization campaign.[8] Compound **1** bound with moderate affinity to the CB_2 receptor (K_i = 120 nM), while lacking significant affinity at the CB_1 receptor (4% inh. @ 10 μM). However, compound **1** suffered from poor in vitro metabolic stability as measured in rat and human liver microsomes (abbreviated as RLM and HLM, respectively), and the lead optimization campaign focused on improving CB_2 affinity as well as in vitro metabolic stability, while retaining good selectivity for the CB_2 receptor. As indicated in Figure 1, introduction of a methyl group at the 2-position of the benzene core of **1** (compound **2**) led to a clear improvement in the metabolic stability in rat liver microsomes. However, this subtle structural modification also led to a fivefold reduction in the CB_2 binding affinity. This result suggested that the 2-position of the benzene ring of **1** might represent one of the 'soft' spots of the molecule (position at which metabolism may occur). Based on this hypothesis, we investigated whether replacing the phenyl moiety of **1** with a pyridine core (compound **3**) would also result in an improvement in metabolic stability. As shown in Figure 1, the pyridine derivative **3** displayed increased metabolic stability in rat liver microsomes when compared to its benzene analog **1**. In addition, this modification led to a fivefold increase in the binding affinity toward the CB_2 receptor [**1**: K_i (CB_2) = 120 nM; **3**: K_i (CB_2) = 24 nM]. As a follow up to this result, we explored the structure–activity relationships (SAR) in this new series of pyridine-based CB_2 agonists (general formula **I**, Fig. 1).

The syntheses of compound **3** and its regioisomeric analogs **4**, **5** and **6** are summarized in Schemes 1 and 2. Treatment of commercially available 2-amino-4-methyl-5-bromopyridine (**17a**) with 2,2-dimethylbutanoyl chloride gave the amide **18a** (98%).

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doi:10.1016/j.bmcl.2009.08.063

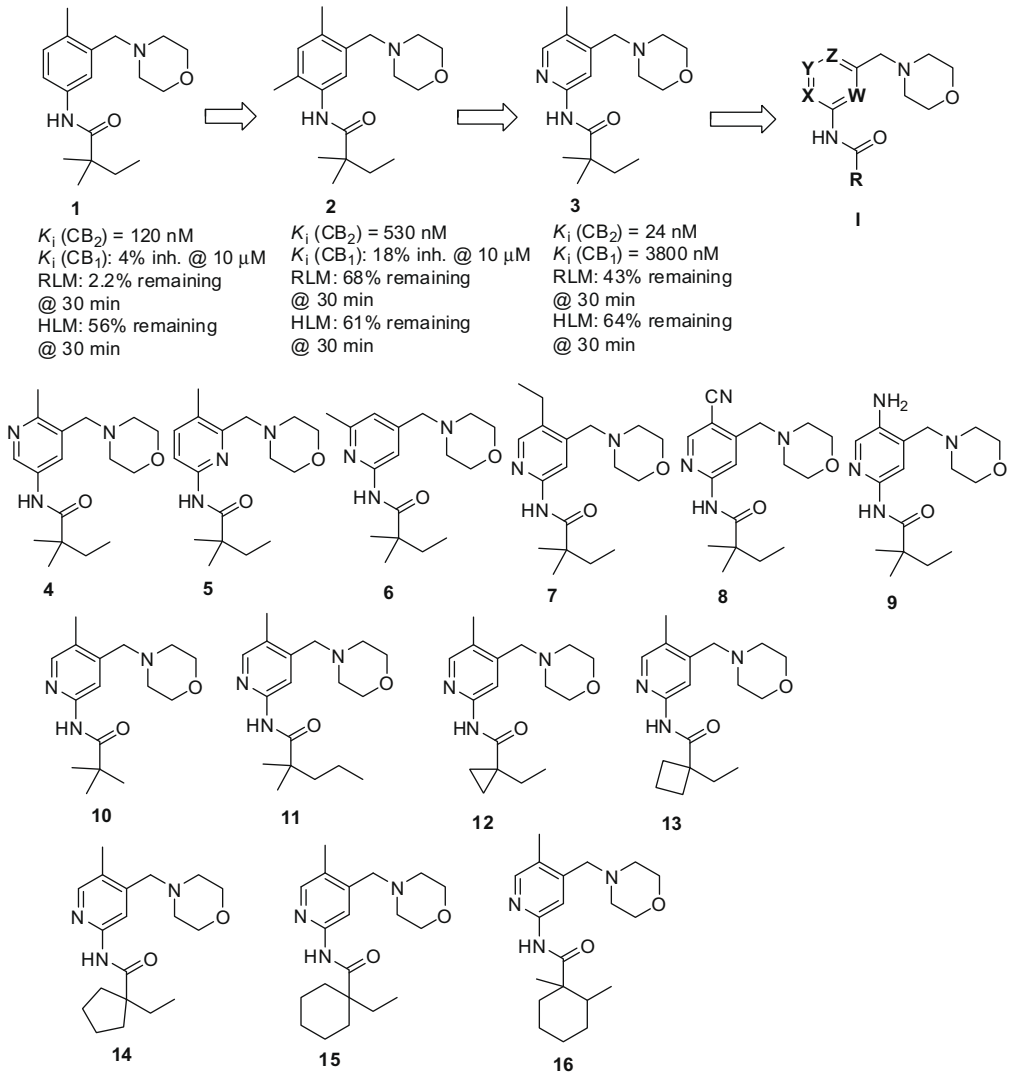
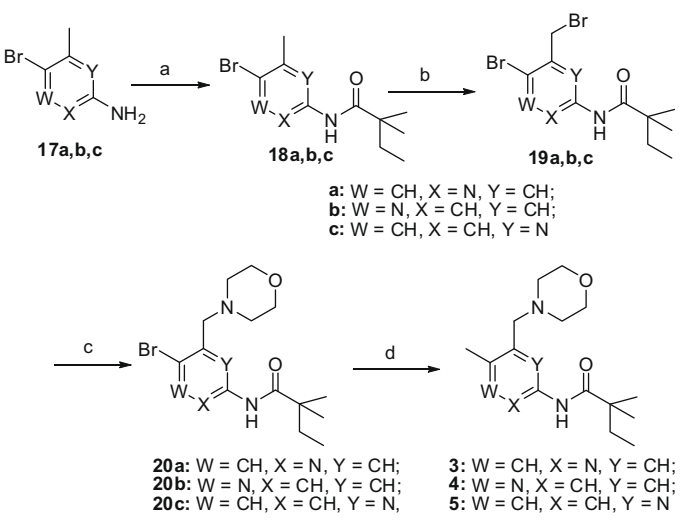


Figure 1. Design of novel pyridine derivatives as CB_2 cannabinoid receptor agonists.

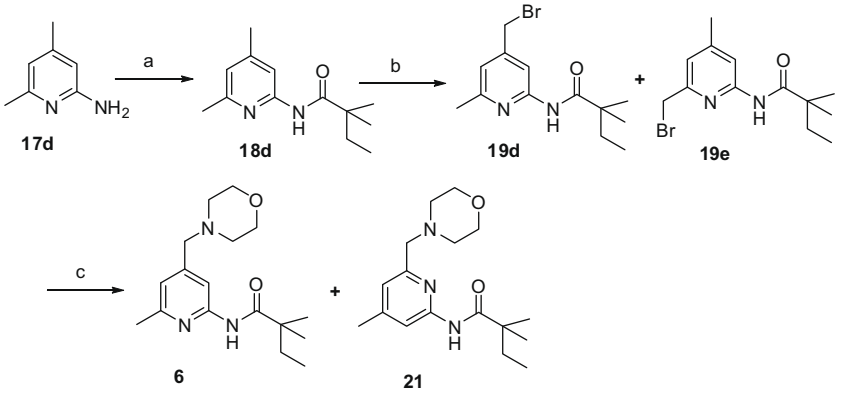


Scheme 1. Synthesis of compound **3**–**5**. Reagents: (a) 2,2-dimethylbutanoyl chloride, TEA, DCM, 93–96%; (b) NBS, AIBN, CCl_4; (c) morpholine, THF, $NaHCO_3$, 10–65% (two steps); (d) MeZnCl, $Pd(PPh_3)_4$, THF, 76–88%.

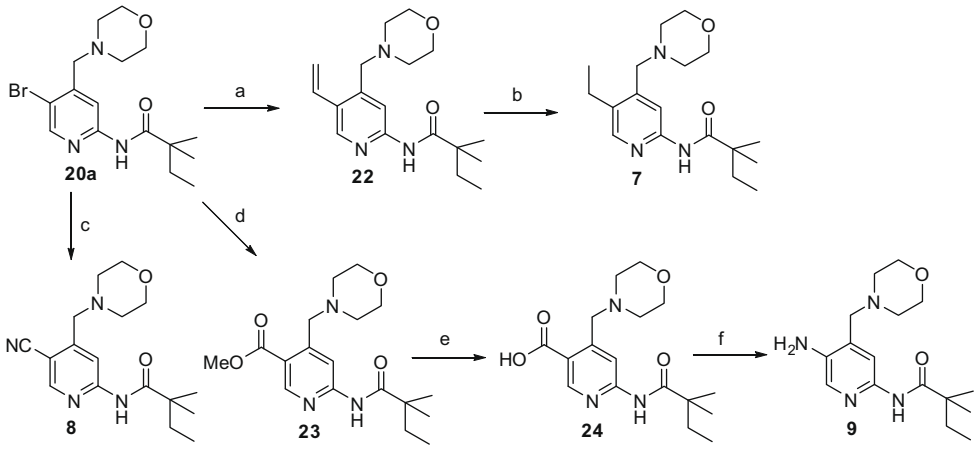
Treatment of amide **18a** with NBS in the presence of AIBN provided the bromide **19a** which reacted with morpholine to yield **20a** (40% for two steps). Compound **20a** was subjected to a Negishi coupling reaction by treatment with methylzinc chloride to afford the desired target compound **3** (84%). Compounds **4** and **5** were synthesized following the same route described for the preparation of **3** (Scheme 1).

Compound **6** was prepared from commercially available 2-amino-4,6-dimethylpyridine (**17d**) according to Scheme 2. Bromination of amide **18d** with NBS yielded a mixture of two regioisomeric bromides **19d** and **19e**, which was reacted with morpholine to furnish the desired compound **6** as the minor product (~10%), and the regioisomeric analog of **6**, that is, compound **21**, as the major product (45%).

Scheme 3 outlines the synthesis of analogs **7**–**9**. Suzuki–Miyaura cross-coupling of the bromide **20a** with 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane in the presence of $Pd(PPh_3)_4$ under microwave conditions gave the vinyl compound **22** (60%), which was hydrogenated to furnish the 5-ethyl analog **7** (70%). Substitution reaction of **20a** with copper(I) cyanide in refluxing DMF afforded the 5-cyano substituted analog **8** (21%). Palladium-catalyzed carbon monoxide insertion reaction of the bromide **20a** under

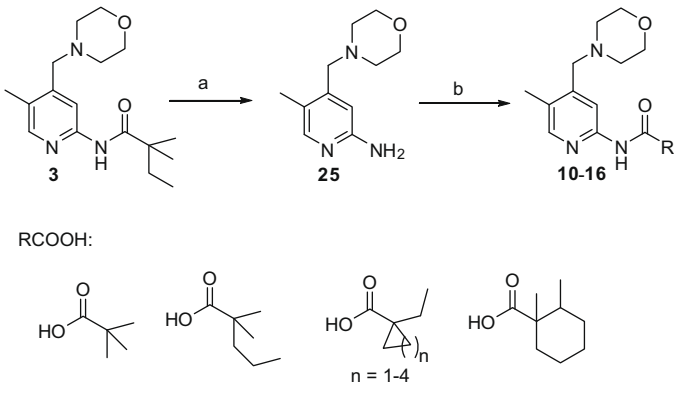


Scheme 2. Synthesis of compound **6**. Reagents: (a) 2,2-dimethylbutanoyl chloride, TEA, DCM, 95%; (b) NBS, AIBN, CCl_4; (c) morpholine, THF, $NaHCO_3$, 10% for **6**, 45% for **21** (two steps).



Scheme 3. Synthesis of compounds **7**–**9**. Reagents: (a) 4,4,5,5-tertamethyl-2-vinyl-1,3,2-dioxaborlane, $Pd(PPh_3)_4$, K_2CO_3, dioxane–H_2O, microwave, 120 °C, 60%; (b) H_2, Pd/C, MeOH, 70%; (c) CuCN, DMF, reflux, 21%; (d) MeOH, CO, dppp, $Pd(OAc)_2$, TEA, DMF, 54%; (e) LiOH, THF–MeOH–H_2O, 98%; (f) (i) DPPA, TEA, dioxane, *t*-BuOH; (ii) HCl, MeOH, Et_2O, 26%.

standard conditions gave the methyl ester **23** (54%) which was hydrolyzed to the corresponding carboxylic acid **24** (98%) by treatment with lithium hydroxide. Curtius rearrangement of **24** by reaction with diphenylphosphoryl azide (DPPA) in the presence of *t*-BuOH[9] followed by treatment of the resulting *tert*-butyl carbamate with HCl yielded the 5-amino substituted analog **9** (26%).



Scheme 4. Synthesis of compounds **10**–**16**. Reagents: (a) 6 N HCl, reflux, 82%; (b) (i) RCOOH, $SOCl_2$; (ii) **25**, TEA, DCM or RCOOH, bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl), DIEA, DCM, 7–55%.

The synthesis of compounds **10**–**16** is described in Scheme 4. Treatment of **3** with 6 N HCl at reflux provided the aminopyridine **25** (82%), a common intermediate used for the preparation of **10**–**16**. Compounds **10**–**16** were prepared in 7–55% yield by coupling the aminopyridine derivative **25** with carboxylic acids under standard peptide coupling conditions using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOPCl) as coupling reagent or by coupling **25** with the corresponding acyl chloride derivatives.

The novel pyridine derivatives **3**–**16** were evaluated for their affinity toward the CB_2 and the CB_1 receptors as measured by their ability to displace [^{3}H]-CP-55960 from its specific binding sites in membranes expressing CB_1 and CB_2 receptors.[10] The agonist potencies of selected compounds (CB_2: K_i <100 nM) were assessed by their abilities to stimulate guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding to membranes containing CB_2 cannabinoid receptors.[10] Compounds displaying good affinity at CB_2 receptors (K_i <50 nM) were also evaluated for in vitro metabolic stability in rat and human liver microsomes.[11] As indicated in Table 1, compounds **4** and **5**, the regioisomeric analogs of **3**, displayed weak affinity at the CB_2 receptor. Changing the methyl moiety of **3** from the 5-position to the 6-position relative to the pyridine ring (compound **6**)[12] also led to a threefold reduction in the CB_2 binding affinity, indicating that the substitution pattern of the pyridine ring has a significant impact on the optimal CB_2 binding affinity. The 5-ethyl analog of **3** (compound **7**) displayed potent affinity at the CB_2

Table 1
Cannabinoid receptor binding and in vitro metabolic stability of compounds **1–16**

Compd	K_i CB_1[a]	K_i CB_2[a]	Ratio (CB_1/CB_2)	$EC_{50}CB_2$[a] (nM)	Microsomal stability[b]	
	(nM or % inh @ 10 μM)				RLM	HLM
1	4%	120	nd	nd[c]	2.2	56
2	18%	530	nd	nd	68	61
3	3800	24	160	41	43	64
4	4%	46%	nd	nd	nd	nd
5	28%	1800	nd	nd	nd	nd
6	0%	67	nd	260	nd	nd
7	4300	7.9	550	9.6	29	34
8	11%	100	nd	200	nd	nd
9	10%	870	nd	nd	nd	nd
10	37%	81	nd	70	nd	nd
11	1800	5.3	330	4.1	nd	15
12	25%	63	nd	62	nd	nd
13	13%	45	nd	140	48	51
14	1100	13	85	13	nd	12
15	36%	7.0	nd	8.8	0	0
16	2500	1.9	1300	2.8	0	1

[a] For assay description see Ref. 10.
[b] % remaining of parent compound after 30 min, see Ref. 11.
[c] nd: not determined.

receptor. However, this modification also led to a decrease in the in vitro metabolic stability (see Table 1). Replacement of the 5-methyl group of **3** with a polar substituent such as CN (compound **8**) or NH_2 (compound **9**) caused a significant decrease in CB_2 binding affinity. We investigated further the SAR at the 2-position of **3**, concentrating on carboxamide derivatives. Substitution of the 2,2-dimethylbutanoyl moiety of **3** with the less lipophilic pivaloyl group (compound **10**) led to a threefold reduction in CB_2 binding affinity. In contrast, extending the acyl substituent from dimethylbutanoyl to 2,2-dimethylpentanoyl, as in **11**, was beneficial to improve the binding affinity at the CB_2 receptor. Unfortunately, compound **11** displayed poor in vitro metabolic stability. Among the constrained analogs of **3** that were prepared, the two cyclohexyl derivatives **15** and **16** were identified as potent and selective CB_2 agonists. However, as was the case for **11**, compounds **15** and **16** could not be studied further due to their poor in vitro metabolic stability.

After exploring the SAR in this new chemical series of pyridine-based CB_2 agonists, 2,2-dimethyl-*N*-(5-methyl-4(morpholinomethyl)pyridin-2-yl)butanamide (**3**) was the best compound identified in terms of CB_2 affinity, selectivity versus CB_1 and metabolic stability. We then determined the pharmacokinetic profile of **3** in rat and dog after IV and PO administration. As indicated in Table 2, **3** is a high clearance compound in rat. The elimination of **3** was high with a half-life of 0.5 h after a single IV dose of 1 mg/kg. After a 10 mg/kg PO dose, maximum plasma concentrations were reached at around 2 h post-dose. The oral bioavailability of **3** in rat was 57%. Table 2 summarizes the pharmacokinetics of **3** in male Beagle dogs after single IV and PO doses of **1** and 3 mg/kg, respectively. In contrast to the high clearance observed in rat, the systemic plasma clearance of **3** in dog was low (0.9 L/h/ kg). The half-life of **3** in dog was 1.4 h after a 1 mg/kg IV dose. After a single 3 mg/kg oral dose of **3**, maximum plasma concentrations averaging 535 ng/mL were reached 30 min post-dose. The oral bioavailability of **36** in dog was 29%. Additional studies demonstrated that **3** exhibited antiallodynic activity in the L5 spinal nerve ligation (L5 SNL) rat model[13] of neuropathic pain at a dose of 100 mg/kg PO (Fig. 2). The weak in vivo activity of the compound **3** is not optimized and possibly caused by its rapid clearance. Further work is underway to enhance in vivo activity of this class of CB_2 agonists.

Table 2
Pharmacokinetics of **3** in male Sprague-Dawley rats and male Beagle dogs after IV and PO administration

Route	IV		PO	
Species	Rat	Dog	Rat	Dog
Dose (mg/kg)	1.0	1.0	10	3.0
CLs (L/h/kg)	5.8 ± 0.8	0.9 ± 0.0	—	—
Vdss (L/kg)	3.5 ± 0.8	0.6 ± 0.1	—	—
[a]$t_{1/2}$ (h)	0.5 ± 0.2	1.4 ± 0.0	2.1 ± 2.4	1.1 ± 0.1
$AUC_{0-\infty}$ (ng h/mL)	158 ± 23	1127 ± 30	893 ± 487	977 ± 227
[b]T_{max} (h)	—	—	2.0 (0.25–2.0)	0.5 (0.25–2.0)
C_{max} (ng/mL)	—	—	185 ± 133	535 ± 225
F (%)	—	—	57 ± 31	29 ± 7

Values represent the mean ± standard deviation of three animals.
[a] Expressed as harmonic mean.
[b] Expressed as median and range.

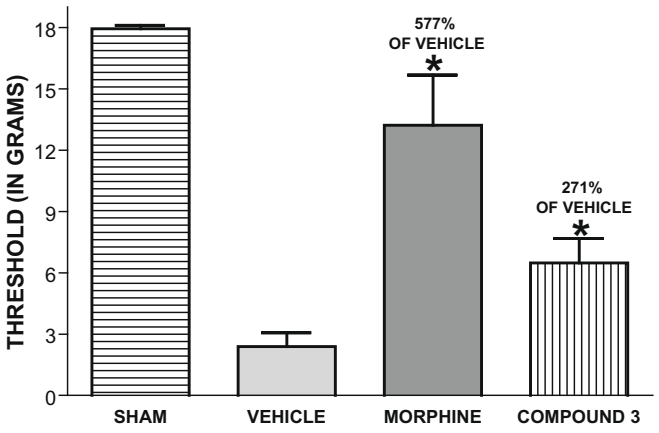


Figure 2. Antiallodynic activity of compound **3** (100 mg/kg PO) in the L5 SNL model (9 days after surgery) (morphine: 3 mg/kg s.c.). *p <0.05 compared to vehicle-treated rats. $n = 8$ (SNL Groups), $n = 4$ (Sham-operated animals); Vehicle threshold = 2.4 grams.

In summary, replacement of the benzene template in our previous (morpholinomethyl)aniline carboxamide cannabinoid receptor ligands with a pyridine core led to the discovery of a novel chemical series of pyridine-based potent and selective CB_2 agonists. Compound **3**, that is, 2,2-dimethyl-*N*-(5-methyl-4-(morpholinomethyl)pyridin-2-yl)butanamide, the best compound in this series, displayed good affinity at the CB_2 receptor (K_i = 24 nM), 160-fold selectivity versus CB_1 and moderate metabolic stability in rat and human liver microsomes. Importantly, compound **3** displayed antiallodynic activity after oral administration in a rat model of neuropathic pain.

References and notes

1. Pan, H.-L.; Wu, Z.-Z.; Zhou, H.-Y.; Chen, S.-R.; Zhang, H.-M.; Li, D.-P. *Pharmacol. Ther.* **2008**, *117*, 141.
2. Malan, T. P., Jr.; Ibrahim, M. M.; Lai, J.; Vanderah, T. W.; Makriyannis, A.; Porreca, F. *Curr. Opin. Pharmacol.* **2003**, *3*, 62.
3. Ermann, M.; Riether, D.; Walker, E. R.; Mushi, I. F.; Jenkins, J. E.; Noya-Marino, B.; Brewer, M. L.; Taylor, M. G.; Kahrs, A. F.; Ebneth, A.; Lobbe, S.; O'Shea, K.; Shih, D.-T.; Thomson, D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1725.
4. Giblin, G. M. P.; O'Shaughnessy, C. T.; Naylor, A.; Mitchell, W. L.; Eatherton, A. J.; Slingsby, B. P.; Rawlings, D. A.; Goldsmith, P.; Brown, A. J.; Haslam, C. P.; Clayton, N. M.; Wilson, A. W.; Chessell, I. P.; Wittington, A. R.; Green, R. *J. Med. Chem.* **2007**, *50*, 2597.
5. Hohmann, A. G.; Farthing, J. N.; Zvonok, A. M.; Makriyannis, A. *J. Pharmacol. Exp. Ther.* **2004**, *308*, 446.
6. Ibrahim, M. M.; Porreca, F.; Lai, J.; Albrecht, P. J.; Rice, F. L.; Khodorova, A.; Davar, G.; Makriyannis, A.; Vanderah, T. W.; Mata, H. P.; Malan, T. P., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 3093.
7. Worm, K.; Zhou, Q. J.; Saeui, C. T.; Green, R. C.; Cassel, J. A.; Stabley, G. J.; DeHaven, R. N.; Conway-James, N.; LaBuda, C. J.; Koblish, M.; Little, P. J.; Dolle, R. E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2830.

8. Worm, K.; Weaver, D. G.; Green, R. C.; Saeui, C. T.; Dulay, D.-M. S.; Barker, W. M.; Cassel, J. A.; Stabley, G. J.; DeHaven, R. N.; LaBuda, C. J.; Koblish, M.; Brogdan, B. L.; Smith, S. A.; Dolle, R. E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5004.
9. Ninomiya, K.; Shioiri, T.; Yamada, S. *Tetrahedron* **1974**, *30*, 2151.
10. Binding assays were performed by modification of the method of Pinto, J. C.; Potie, F.; Rice, K. C.; Boring, D.; Johnson, M. R.; Evans, D. M.; Wilken, G. H.; Cantrell, C. H.; Howlett, A. *Mol. Pharmacol.* **1994**, 46, 516–522: (b) The [^{35}S]GTPγS binding method is a major modification of the method by Selley, D. E.; Stark, S.; Sim, L. J.; Childers, S. R. *Life Sci.* **1996**, 59, 659–668. Additional details are given in Ref. 7; (c) All the compounds listed in Table 1 are full CB_2 receptor agonists as determined in a [^{35}S]GTPγS functional binding assay.
11. Obach, S. R. *Drug Metab. Dispos.* **1999**, *27*, 1350.
12. Compound **21**, the regioisomeric analog of **6**, exhibited much weaker CB_2 binding affinity as compared to **6** and **3** with K_i (CB_2) = 470 nM.
13. LaBuda, C. J.; Little, P. J. *J. Neurosci. Methods* **2005**, *144*, 175.