Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6299–6304
Bioorganic & Medicinal Chemistry Letters

N-Alkylidenearylcarboxamides as new potent and selective CB_2 cannabinoid receptor agonists with good oral bioavailability

Hiroshi Ohta,[a,*] Tomoko Ishizaka,[a] Makoto Tatsuzuki,[a] Mitsukane Yoshinaga,[a] Izumi Iida,[b] Yasumitsu Tomishima,[c] Yoshihisa Toda[c] and Shuji Saito[a]

[a]*Medicinal Chemistry Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403 Yoshino-cho, Kita-ku, Saitama-shi, Saitama 331-9530, Japan*
[b]*Drug Safety and Pharmacokinetics Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403 Yoshino-cho, Kita-ku, Saitama-shi, Saitama 331-9530, Japan*
[c]*Molecular Function and Pharmacology Laboratories, Taisho Pharmaceutical Co., Ltd, 1-403 Yoshino-cho, Kita-ku, Saitama-shi, Saitama 331-9530, Japan*

Received 3 July 2007; revised 22 August 2007; accepted 3 September 2007
Available online 7 September 2007

Abstract—A novel series of *N*-alkylidenearylcarboxamides **4**, a CB_2 receptor agonist, were synthesized and evaluated for activity against the human CB_2 receptor. In a previous paper, we reported that sulfonamide derivative **1** acted as a potent CB_2 receptor agonist (IC_{50} = 65 nM, EC_{50} = 19 nM, E_{max} = 90%). However, compound **1** also exhibited poor metabolic stability in human liver microsomes. During the structural modification of **1**, we found that a novel series of *N*-alkylidenearylcarboxamide, **4-1**, had a moderate affinity for the CB_2 receptor (IC_{50} = 260 nM, EC_{50} = 86 nM, E_{max} = 100%) and good metabolic stability in human liver microsomes. We explored its analogues to discover compounds with a high affinity for the CB_2 receptor and with good oral bioavailability. Among them, compounds **4-9** and **4-27** had high affinities for the human CB_2 receptor (CB_2 IC_{50} = 13 nM and 1.2 nM) and a high selectivity for CB_2 (CB_1 IC_{50}/CB_2 IC_{50} = 270 and 1600); furthermore, significant plasma levels were observed following oral administration in rats (C_{max} = 233 ng/mL and 148 ng/mL, respectively, after a dose of 10 mg/kg). Furthermore, compound **4-9** had good oral bioavailability (F = 52%, 3 mg/kg).

Marijuana (*Cannabis Sativa* L.) has been used since time immemorial as a therapeutic and recreational drug. In the 1960s, Delta ^{9}-tetrahydrocannabinol (Delta ^{9}-THC) was isolated as marijuana's major psychoactive component.[1] THC and its analogues are classified as cannabinoids and exhibit numerous biological properties, including analgesic, anti-inflammatory, anti-emetic, anti-convulsive and anti-cancer effects.[2] Despite the synthesis of analogues of Delta ^{9}-THC, difficulties in separating their beneficial and psychotropic activities have limited the therapeutic use of these compounds. The discovery of two distinct cannabinoid receptors, the CB_1 receptor[3] and the CB_2 receptor,[4] has attracted new interest in the cannabinoids. The CB_1 receptor is mainly found in the CNS but is also present in peripheral tissues. The CB_1 receptor is thought to be involved in the central effects of cannabinoids. In contrast, the CB_2 receptor is found exclusively in the periphery and is primarily associated with the cells of the immune system.[5–7] Recently, the CB_2 receptor has also been found in CNS tissue.[8] Both receptors are classified as G-protein-coupled receptors and share a 68% homology with one another at the transmembrane level and a 44% homology overall.[4] The development of highly selective CB_2 receptor ligands is important for understanding some of the physiological effects of cannabinoid, such as their anti-inflammatory, immunosuppressive, and antinociceptive activities. However, only a few compounds are known to be selective for the CB_2 receptor, such as aminopyrimidine[9] (GW842166X, CB_2 EC_{50} = 50 nM, CB_1 EC_{50} > 30,000 nM), 3-carbonylindole[10] (A-0796260, CB_2 IC_{50} = 0.77 nM, CB_1 IC_{50} = 330 nM), and amionalkylindole[11] (AM1241, CB_2 K_i = 3.4 nM, CB_1 K_i = 280 nM) (Fig. 1).

We previously reported that **1** was a potent CB_2 agonist (IC_{50} = 65 nM, EC_{50} = 19 nM, E_{max} = 90%).[12]

Keywords: Cannabinoid; CB_2 receptor agonist; SAR; Amide.
* Corresponding author. Tel.: +81 48 663 1111; fax: +81 48 652 7254; e-mail: hiroshi.ohta@po.rd.taisho.co.jp

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.09.004

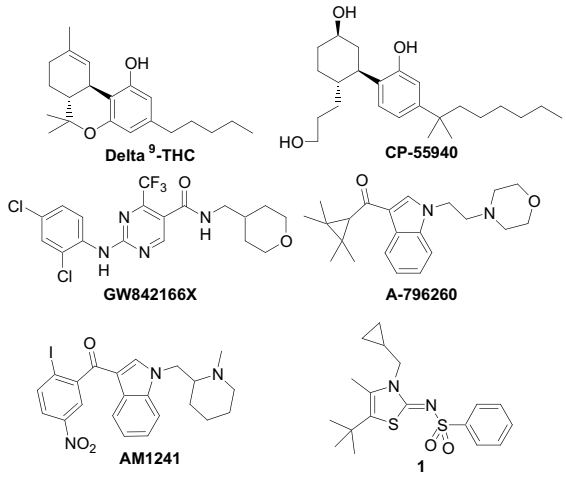
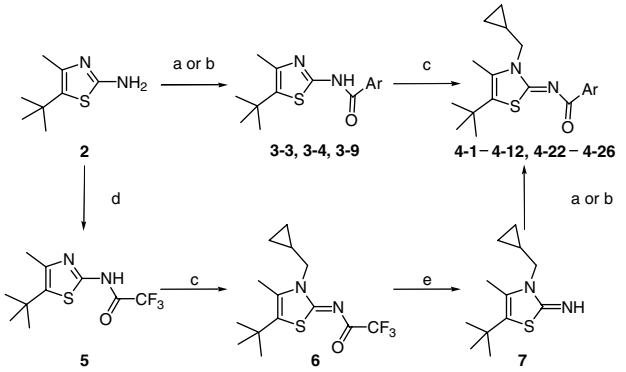


Figure 1. Structure of Delta ^{9}-THC, CP-55940, GW842166X, A-796260, AM1241 and compound **1**.

However, **1** also exhibited poor metabolic stability in human liver microsomes (metabolic stability, 35%).[13] Thus, we attempted to chemically modify **1** to improve its metabolic stability and binding affinity for the CB_2 receptor. As a result, we discovered that the conversion of a sulfonamide in **1** into an amide (**4-1**) increased its metabolic stability in human liver microsomes (**4-1**: metabolic stability, 71%).[13] We attempted to explore this compound's analogues to discover compounds with high affinities for the CB_2 receptor and with good oral bioavailability using **4-1** as the lead compound. In this paper, we discuss the synthesis and SARs of *N*-alkylidenearylcarboxamides **4**, novel CB_2 receptor agonists.

Scheme 1 shows the syntheses of *N*-Alkylidenearylcarboxamides **4-1**–**4-12** and **4-22**–**4-26**. 2-Amino-5-*tert*-butyl-4-methylthiazole (**2**), a commercially available compound (Fluorochem Ltd.), was reacted with corresponding aryl carbonyl chlorides under basic conditions or corresponding aryl carboxylic acids using coupling reagents to yield **3-3** (Ar = 3-chlorophenyl), **3-4** (Ar = 3-bromophenyl), and **3-9** (Ar = 3-trifluoromethylphenyl). Compounds **3-3**, **3-4** and **3-9** were then treated with cyclopropylmethyl bromide, NaI, and NaH to yield **4-3**, **4-4** and **4-9** in 85%, 27%, and 76% yields, respectively. Compounds **4-1**–**4-2**, **4-5**–**4-8**, **4-10**–**4-12**, and **4-22**–**4-26** were synthesized from **2** via intermediate **7**. The intermediate **7** was easily transformed into *N*-alkylidenearylcarboxamides **4** in one step. 2-Aminothiazole **(2)** was treated with trifluoroacetic anhydride and pyridine to obtain **5** with an 80% yield. The 3-position of the thiazole ring of **5** was then alkylated, followed by deprotection under basic conditions, producing the key intermediate **7**, which was in turn converted to **4-1**–**4-2**, **4-5**–**4-8**, **4-10**–**4-12**, and **4-22**–**4-26** by acylation with the corresponding aryl carbonyl chlorides under basic conditions or the corresponding aryl carboxylic acids using coupling reagents.



Scheme 1. Reagents and conditions: (a) ArCOCl, Et_3N, $CHCl_3$, rt; (b) $ArCO_2H$, EDC, HOBt, DMF, rt; (c) cyclopropylmethyl bromide, NaI, NaH, DMF, rt; (d) $(CF_3CO)_2O$, pyridine, $CHCl_3$, 0 °C–rt; (e) K_2CO_3 aq, MeOH, rt.

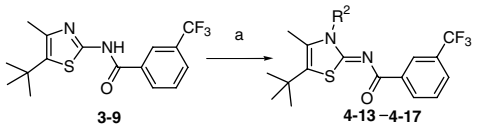
Scheme 2 shows the synthesis of **4-13**–**4-17**, with various alkyl groups at the 3-position of the thiazole ring of **4**. Compound **3-9** was treated with corresponding alkyl halides NaI and NaH to yield **4-13**–**4-17** with yields of 82%, 74%, 47%, 23% and 16%, respectively.

Scheme 3 illustrates the preparation of **4-18**–**4-20**, with various substitutes at the 5-position of the thiazole ring of **4**. The reaction of **8-18**–**8-20**[14] with 3-trifluoromethylbenzoic acid in the presence of EDC and HOBt in DMF resulted in **9-18** (R^1 = Me: 100%), **9-19** (R^1 = ^{i}Pr: 100%), and **9-20** (R^1 = CO_2Et: 100%). Compounds **9-18**–**9-20** were transformed into **4-18**–**4-20** by alkylation with cyclopropylmethyl bromide, NaI, and NaH. The regioselectivity of compound **4-18**–**4-20** was supported by NOE analysis (NOE were observed between H_a and H_b).

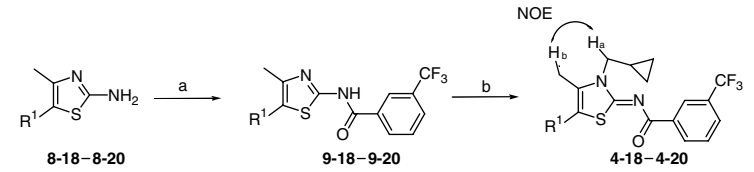
Compound **4-20** were hydrolyzed under basic condition to yield **4-29**, and the reaction of **4-29** with $HNMe_2$ produced **4-21** at a yield of 83%, as shown in Scheme 4.

The introduction of the methoxy group and the dimethylamino group onto the phenyl ring was performed via the fluorobenzene compound **4-25**. Compound **4-25** was converted to **4-27** by treatment with MeONa. The dimethylamino group was introduced by the treatment of **4-25** with dimethylamine to yield the dimethylamino compound **4-28** (Scheme 5) (The detailed procedure for synthesizing compound **4-27** is described in the note).[15]

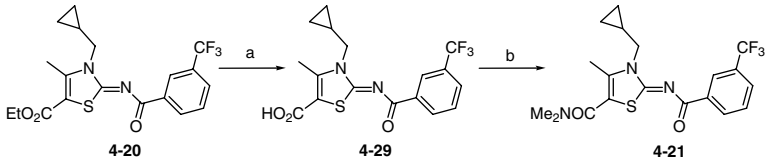
Initially, we modified the Ar group of *N*-Alkylidenearylcarboxamides to elucidate the substituent effects on the CB_2 receptor. The results of binding tests for **4-1**–**4-12** (Table 1) demonstrated significant changes in their affinities for the CB_2 receptor. The introduction of a chlorine atom or a methoxy group at the meta position of the phenyl ring in **4-1** tended to increase the binding affinity



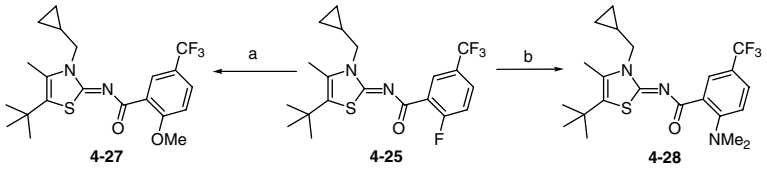
Scheme 2. Reagents and conditions: (a) R^2X, NaI, NaH, DMF, rt.



Scheme 3. Reagents and conditions: (a) 3-trifluoromethylbenzoic acid, EDC, HOBt, DMF, rt; (b) cyclopropylmethyl bromide, NaI, NaH, DMF, rt.



Scheme 4. Reagents and conditions: (a) NaOHaq., THF, EtOH, rt; (b) $HNMe_2$, EDC, HOBt, DMF, rt.



Scheme 5. Reagents and conditions: (a) NaOMe, THF, EtOH, 95 °C; (b) $HNMe_2$, K_2CO_3, DMSO, H_2O, 100 °C.

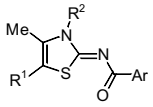
by about 10-fold (**4-3** and **4-7** vs. **4-1**). Next, various alternative 3-substituents were evaluated. 3-Bromo (**4-4**), 3-dimethylamino (**4-5**), 3-methyl (**4-6**), 3-trifluoromethoxy (**4-8**), and 3-trifluoromethyl (**4-9**) compounds exhibited high CB_2 receptor affinities (IC_{50} = 1.2, 17, 22, 5.7 and 13 nM, respectively). Among these, bulky functional groups like bromo (**4-4**), trifluoromethoxy, (**4-8**) and trifluoromethyl (**4-9**) exhibited much higher CB_2 affinities. In contrast, small groups like fluoro (**4-2**) exhibited a lower CB_2 affinity (44% inh [100 nM]). Although hydrophilic substituents like dimethylamino (**4-5**) yielded a high CB_2 affinity, the metabolic stability of **4-5** was low (metabolic stability, 44%).[13] These findings suggest the following: (1) the electron density on the benzene ring might not affect the binding affinity for the CB_2 receptor, and (2) the CB_2 binding affinity increased as the size of the functional group at the meta position of the benzene ring increased. Chemical modification of the meta position of the phenyl ring suggested that the most suitable substituent for selectivity was a trifluoromethyl group (**4-9**). Compound **4-9** exhibited good selectivity (CB_1 IC_{50}/CB_2 IC_{50} = 270), and had a good metabolic stability in human liver microsomes (**4-9**: metabolic stability, 93%).[13] Next, we examined the influence of the position of a trifluoromethyl group in the benzene ring on CB_2 binding affinity. Compound **4-11**, containing a para-trifluoromethyl group, exhibited a lower affinity than that of **4-9**. In contrast, an ortho-trifluoromethyl compound, **4-10**, exhibited a much higher CB_2 binding affinity (**4-10**: IC_{50} = 0.50 nM). However, **4-10** also exhibited a high affinity for the CB_1 receptor (CB_1IC_{50} = 140 nM). The 3,5-bis(trifluoromethyl) compound **4-12** exhibited a lower affinity for the CB_2 receptor than that of the mono-trifluoromethyl compound **4-9**.

Next, the $\mathbf{R}^2$ in the 3-position of the thiazole ring was investigated. Exchanging the cyclopropylmethyl group in **4-9** with a straight-chain alkyl group yielded a lower CB_2 affinity (**4-9** vs. **4-13**, **4-14**, and **4-15**). The introduction of an alkoxyalkyl group significantly increased CB_2 affinity (**4-9** vs. **4-16** and **4-17**). However, **4-16** and **4-17** were partial agonists (**4-16**: E_{max} = 59%, **4-17**: E_{max} = 45%).

Next, we performed modifications focusing on $\mathbf{R}^1$ in the 5-position of the thiazole ring. Replacement of the *tert*-butyl group using a methyl group (**4-18**), an isopropyl (**4-19**) group, and hydrophilic groups like ester (**4-20**) and amide (**4-21**) groups decreased the affinity for the CB_2 receptor. In particular, amide compound **4-21** did not exhibit a significant affinity for the CB_2 receptor. These findings suggested that there was a requirement for a lipophilic substituent at 5-position for CB_2 activity.

The final modifications focused on the introduction of various substituents at the Ar group of **4-9**. First, a chlorine atom was introduced at the Ar group of compound **4-9** (**4-22**, **4-23**, and **4-24**). One of the resulting compounds—**4-24**, containing a 2-chloro-5-trifluoromethyl group—exhibited a high affinity for the CB_2 receptor and a good selectivity (IC_{50} = 2.1 nM, CB_1 IC_{50}/CB_2 IC_{50} = 400). For this reason, we examined 2-substituted-5-trifluoromethyl compounds (**4-25**, **4-26**, **4-27**, and **4-28**). These compounds had much higher affinities for the CB_2 receptor, with good selectivity (**4-25**–**4-28**: IC_{50} = 3.4, 2.1, 1.2, and 9.4 nM, respectively; CB_1 IC_{50}/CB_2 IC_{50} = 440, 570, 1600, and 1000, respectively). In particular, the 2-methoxy-5-trifluoromethyl compound **4-27** exhibited an excellent affinity and selectivity for the CB_2 receptor. Furthermore, compound **4-27** had

Table 1. Pharmacological profile of *N*-alkylidenearylcarboxamide



Compound	Ar	R^1	R^2	Binding affinity IC_{50}[a] (nM)		Agonist activity	
				CB_2	CB_1	CB_2 EC_{50}[b] (nM)	E_{max}[c] (%)
4-1	Ph	*t*-Bu	CH_2-(cyclopropyl)	260	—	86	100
4-2	3-F-Ph	*t*-Bu	CH_2-(cyclopropyl)	44% inh (100 nM)	—	—	—
4-3	3-Cl-Ph	*t*-Bu	CH_2-(cyclopropyl)	20	—	—	—
4-4	3-Br-Ph	*t*-Bu	CH_2-(cyclopropyl)	1.2	53	0.3	96
4-5	3-NMe_2-Ph	*t*-Bu	CH_2-(cyclopropyl)	17	—	—	—
4-6	3-Me-Ph	*t*-Bu	CH_2-(cyclopropyl)	22	—	—	—
4-7	3-OMe-Ph	*t*-Bu	CH_2-(cyclopropyl)	24	—	—	—
4-8	3-OCF_3-Ph	*t*-Bu	CH_2-(cyclopropyl)	5.7	1100	7.6	72
4-9	3-CF_3-Ph	*t*-Bu	CH_2-(cyclopropyl)	13	3500	10	91
4-10	2-CF_3-Ph	*t*-Bu	CH_2-(cyclopropyl)	0.50	140	—	—
4-11	4-CF_3-Ph	*t*-Bu	CH_2-(cyclopropyl)	24% inh (100 nM)	—	—	—
4-12	3,5-2CF_3-Ph	*t*-Bu	CH_2-(cyclopropyl)	10% inh (100 nM)	—	—	—
4-13	3-CF_3-Ph	*t*-Bu	Me	220	—	—	—
4-14	3-CF_3-Ph	*t*-Bu	Et	74	—	—	—
4-15	3-CF_3-Ph	*t*-Bu	*n*-Pr	46	—	—	—
4-16	3-CF_3-Ph	*t*-Bu	C_2H_4-OMe	10	4100	5.6	59
4-17	3-CF_3-Ph	*t*-Bu	C_2H_4-OEt	2.3	690	2	45
4-18	3-CF_3-Ph	Me	CH_2-(cyclopropyl)	25	—	—	—
4-19	3-CF_3-Ph	*i*-Pr	CH_2-(cyclopropyl)	29	—	—	—
4-20	3-CF_3-Ph	CO_2Et	CH_2-(cyclopropyl)	110	—	—	—
4-21	3-CF_3-Ph	$CONMe_2$	CH_2-(cyclopropyl)	1000	—	—	—
4-22	2-Cl-3-CF_3-Ph	*t*-Bu	CH_2-(cyclopropyl)	0.41	19	—	—
4-23	4-Cl-3-CF_3-Ph	*t*-Bu	CH_2-(cyclopropyl)	230	—	—	—
4-24	2-Cl-5-CF_3-Ph	*t*-Bu	CH_2-(cyclopropyl)	2.1	830	4.0	95
4-25	2-F-5-CF_3-Ph	*t*-Bu	CH_2-(cyclopropyl)	3.4	1500	1.5	85
4-26	2-Me-5-CF_3-Ph	*t*-Bu	CH_2-(cyclopropyl)	2.1	1200	2.7	99
4-27	2-OMe-5-CF_3-Ph	*t*-Bu	CH_2-(cyclopropyl)	1.2	1900	0.62	100
4-28	2-NMe_2-5-CF_3-Ph	*t*-Bu	CH_2-(cyclopropyl)	9.4	>10,000	7.3	89
CP-55,940[16]				0.82	2.1	0.86	100

[a] Evaluated by using [^{3}H]-CP-55,940 binding to the membranes of Chinese hamster ovary (CHO) cells expressing the human CB_2 or CB_1 receptor.
[b] Evaluated by using a functional assay based on [^{35}S]-GTPγS binding to the membranes of CHO cells expressing the human CB_2 receptor.
[c] Compared to the maximal response to CP-55,940, a full agonist of human CB_2 receptor.

a good metabolic stability in human liver microsomes (**4-27**: metabolic stability, 85%).[13]

Finally, we evaluated the plasma levels of **4-9** and **4-27**. These compounds showed high affinities for the CB_2 receptor and good selectivity. Table 2 shows the plasma levels and pharmacokinetic parameters for **4-9** and **4-27** following oral administration in rats.

Maximal plasma **4-9** concentrations (C_{max}) of 43.1, 233, and 498 ng/mL were reached 1.67–2.67 h after the oral administration of **4-9** at doses of 3, 10, or 30 mg/kg. The C_{max} values and the corresponding exposure, as measured using AUC_{0-24h}, increased less than dose, proportionality. The plasma **4-27** level following oral administration was also evaluated at a dose of 10 mg/kg in rats. The C_{max} level of **4-27** was 148 ng/mL, slightly lower than that of **4-9**, and the corresponding AUC_{0-24h} value was 1120 ng h/mL.

Table 2. Pharmacokinetic paremeters of **4-9** and **4-27** following oral administration to rats

Compound	**4-9**			**4-27**
Dose (mg/kg)	3	10	30	10
T_{max} (h)	1.67 ± 0.58	2.00 ± 0.00	2.67 ± 1.15	1.00 ± 0.00
C_{max} (ng/ml)	43.1 ± 11.7	223 ± 18.1	498 ± 72.6	148 ± 10.4
$t_{1/2}$ (h)	4.80 ± 0.25	4.96± 0.08	4.70 ± 0.16	4.07 ± 0.31
AUC_{0-24h} (ng h/mL)	326 ± 30.4	2050 ± 243	4240 ± 254	1120 ± 118

Each value represents the means ± SD of three animals.

The oral administration of **4-9** and **4-27** at dose of 10 mg/kg (po) in rats resulted in significant plasma concentrations. Furthermore, **4-9** had good oral bioavailability in rats (F = 52%) when administrated at a dose of 3 mg/kg.

In this paper, we report the synthesis and SAR of **4**, a novel CB_2 receptor ligand. The SAR study showed that the functional groups at 3- and 5-position in the thiazole ring and the Ar group in the amide greatly affect the affinity and selectivity for the CB_2 receptor. Furthermore, it was found that (1) introduction of the cyclopropylmethyl group at 3-position of the thiazole ring was suitable to obtain a high affinity for the CB_2 receptor,

(2) presence of a bulky lipophilic group like *tert*-butyl at 5-position of the thiazole ring was important for obtaining a high affinity for the CB_2 receptor, and (3) a 2-substituted-5-trifluorometylphenyl amide yielded high affinity and selectivity for the CB_2 receptor. These studies led to compounds **4-9** and **4-27**, which showed a combination of good potency for the CB_2 receptor and excellent pharmacokinetics in rats.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.004.

References and notes

1. Gaoni, Y.; Mechoulam, R. *J. Am. Chem. Soc.* **1964**, *86*, 1646.
2. Watson, S. J.; Benson, J. A., Jr.; Joy, J. E. *Arch. Gen. Psychiatry* **2000**, *57*, 547.
3. Devane, W. A.; Dysarz, F. A.; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. *Mol. Pharmacol.* **1988**, *34*, 605.
4. Munro, S.; Thomas, K. L.; Abu-Shaar, M. *Nature* **1993**, *365*, 61.
5. Howlett, A. C.; Barth, F.; Bonner, T. I.; Cabral, G.; Casellas, W. A.; Devane, W. A.; Felder, C. C.; Herekenham, M.; Mackie, K.; Martin, B. R.; Mechoulam, R.; Pertwee, R. G. *Pharmacol. Rev.* **2002**, *54*, 161.
6. Klein, T. W.; Friedman, H.; Specter, S. *J. Neuroimmunol.* **1998**, *83*, 102.
7. Buckley, N. E.; McCoy, K. L.; Mezey, E.; Bonner, T.; Zimmer, A.; Felder, C. C.; Glass, M.; Zimmer, A. *Eur. J. Pharmacol.* **2000**, *396*, 141.
8. Fernandez-Ruiz, J.; Romero, J.; Velasco, G.; Tolon, R. M.; Ramos, J. A.; Guzman, M. *Trends Pharmacol. Sci.* **2007**, *28*, 39.
9. Giblin, G.; O'Shaughnessy, C.; Naylor, A.; Mitchell, W. L.; Eatherton, A.; Jandu, K.; Slingsby, B.; Sweeting, J.; Wall, I.; Goldsmith, P.; Brown, A. J.; Haslam, C.; Wilson, A.; Whittington, N. C.; Green, R. *Abstracts of Papers*, 229th National Meeting of the American Chemical Society, Sandiego, CA, March 13–17, 2005; American Chemical Society: Washington, DC, 2005; MEDI 5; Eatherton, A. J.; Giblin, G. M. P.; Green, R. H.; Mitchell, W. L.; Naylor, A.; Rawlings, D. A.; Slingsby, B. P.; Whittington, A. R. WO2004/018433; 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. *J. Med. Chem.* **2007**, *50*, 2597.
10. Meyer, M.; Hsieh, G.; Pai, M.; Zhu, C.; Chandran, P.; Honore, P.; Dart, M.; Frost, J.; Daza, T.; Grayson, G.; Mukherjee, S.; Fan, Y.; Yao, B. *Abstracts of Papers*, 16th Annual Symposium on the Cannabinoids, Tihany, Hungary, Jun 24–28, 2006; International Cannabinoid Research Society, Burington,VT, 2006; p 147; Pace, J. M.; Tietje, Ka.; Dart, M. J.; Meyer, M. D. WO2006/069196.
11. Ibrahim, M. M.; Deng, H.; Zvonok, A.; Cockayne, D. A.; Kwan, J.; Mata, H. P.; Vanderah, T. W.; Lai, J.; Porreca, F.; Makriyannis, A.; Malan, T. P., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 10529.
12. Ohta, H.; Ishizaka, T.; Yoshinaga, M.; Morita, A.; Tomishima, Y.; Toda, Y.; Saito, S. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5133.
13. In vitro metabolic stability expressed as a residual percentage of parent compound (5 μM) after 15 min. *Procedure*: Each compound was incubated with 1.0 mg protein/mL of the liver microsomal fractions from Xenotech in 250 mM phosphate buffer containing 69 mM KCl (pH 7.4) at a final drug concentration of 5 μM in the presence of an NADPH-generating system (2.4 mM $MgCl_2$, 1.4 mM glucose-6-phosphate, 0.18 U glucose-6-phosphate dehydrogenase) at 37 °C for 15 min. The stability of the sample was checked in boiled liver microsomes fractions. All experiments were performed in triplicate. After incubation, twofold volume of DMSO was added to incubation medium, the tube was vortexed and centrifuged at 2000*g* (4 °C) for 10 min. The resulting supernatant was analyzed by LC–MS/MS system.
14. **8-18** and **8-20** are commercially available compounds (Aldrich Chemical Company, Inc.). **8-19** was prepared by Ido's procedure. Ueda, S.; Terauchi, H.; Kawashima, M.; Yano, A.; Ido, M. *Chem. Pharm. Bull.* **2004**, *52*, 634.
15. Typical procedure: synthesis of *N*-[5-*tert*-butyl-3-(cyclopropylmethyl)-4-methyl-1,3-thiazol-2(3*H*)-ylidene]-2-methoxy-5-(trifluoromethyl)benzamide (**4-27**). To a mixture of 2-amino-5-*tert*-butyl-4-methylthiazole **2** (0.10 g, 0.59 mmol), pyridine (0.051 g, 0.65 mmol) and $CHCl_3$ (1.0 ml) was added trifluoroacetic anhydride (0.14 g, 0.65 mmol) with ice-cooling and the mixture was stirred for 1 h. To the reaction mixture was added water and the mixture was extracted with $CHCl_3$. The organic layer was dried over sodium sulfate, evaporated in vacuo, and purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 2/1) to give 0.13 g (yield; 80%) of **5** as colorless powder. To a mixture of **5** (0.12 g, 0.45 mmol), sodium iodide (0.010 g 0.068 mmol) and DMF (1.5 ml) was added sodium hydride (0.011 g, 0.45 mmol), and the mixture was stirred at room temperature for 15 min. To the reaction mixture was added cyclopropylmethyl bromide (0.073 g, 0.54 mmol), and the reaction mixture was stirred overnight. To the reaction mixture was added water, and the mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, evaporated in vacuo, and purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 1/1) to give 0.090 g (yield; 62%) of **6** as colorless powder. To a mixture of **6** (0.080 g, 0.25 mmol), MeOH (2.0 ml) and H_2O (0.50 ml) was added K_2CO_3 (0.069 g, 0.50 mmol) and the mixture was stirred at room temperature for 2 h. The mixture was extracted with ethyl acetate. The organic layer was washed with brine, dried over sodium sulfate, evaporated in vacuo, and purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 3/1) to give 0.047 g (yield; 84%) of **7** as colorless oil. To a mixture of 2-fluoro-5-(trifluoromethyl)benzoic acid (0.56 g, 2.7 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.51 g, 2.7 mmol), 1-hydroxybenzotriazole hydrate (0.41 g, 2.7 mmol) and DMF (7.0 ml) was added **7** (0.50 g, 2.2 mmol), and the mixture was stirred at room temperature overnight. To the reaction mixture was added water and the mixture was extracted with ethyl acetate. The organic layer was dried over sodium sulfate, evaporated in vacuo, and purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 3/1) to give 0.91 g (yield; 99%) of **4-25** as colorless powder. To a mixture of sodium hydride (0.087 g 2.2 mmol) and DMF (9.0 ml) was added MeOH (0.084 g, 2.6 mmol), and the mixture was stirred at room temperature for

15 min. To the reaction mixture was added **4-25** (0.90 g, 2.2 mmol), and the reaction mixture was stirred at 95 °C for 2 h. To the reaction mixture was added 2 M HCl, and the mixture was extracted with ethyl acetate. The organic layer was dried over sodium sulfate, evaporated in vacuo, and purified by silica gel column chromatography (eluent: hexane/ethyl acetate = 3/1) to give 0.60 g (yield; 65%) of **4-27** as colorless powder: mp109–110 °C; ^{1}H NMR (200 MHz, chloroform-*d*) δ ppm 0.48–0.66 (m, 4H) 1.07–1.34 (m, 1 H) 1.43 (s, 9H) 2.44 (s, 3 H) 3.95 (s, 3H) 4.18 (d, J = 7.0 Hz, 2H) 7.02 (d, J = 9.2 Hz, 1H) 7.60 (d, J = 9.2 Hz, 1H) 8.27 (s, 1H); MS (ESI) *m/z* 427 (M+H); Anal. Calcd for $C_{21}H_{25}F_3N_2O_2S$: C, 59.14; H, 5.91; N, 6.57. Found: C, 59.11; H, 5.89; N, 6.51.

16. Johnson, M. R.; Melvin L. S., Jr. US Patent 4,371,720, 1981.