Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 4030–4034
Bioorganic & Medicinal Chemistry Letters

2-Arylimino-5,6-dihydro-4*H*-1,3-thiazines as a new class of cannabinoid receptor agonists. Part 1: Discovery of CB_2 receptor selective compounds

Hiroyuki Kai,[a,*] Yasuhide Morioka,[b] Takami Murashi,[a] Koichi Morita,[a] Satomi Shinonome,[a] Hitoshi Nakazato,[a] Keiko Kawamoto,[a] Kohji Hanasaki,[a] Fumiyo Takahashi,[a] Shin-ichi Mihara,[a] Tohko Arai,[b] Kohji Abe,[b] Hiroshi Okabe,[b] Takahiko Baba,[b] Takayoshi Yoshikawa[a] and Hideyuki Takenaka[a]

[a]*Shionogi Research Laboratories, Shionogi & Co., Ltd., 12-4, Sagisu, 5-chome, Fukushima-ku, Osaka 553-0002, Japan*
[b]*Developmental Research Laboratories, Shionogi & Co., Ltd., 1-1, Futaba-cho 3-chome, Toyonaka, Osaka 561-0825, Japan*

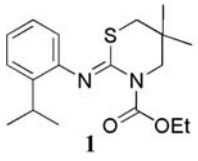
Received 13 February 2007; revised 19 April 2007; accepted 25 April 2007
Available online 30 April 2007

Abstract—2-Arylimino-5,6-dihydro-4*H*-1,3-thiazines have been identified as a novel class of cannabinoid agonists. A lead structure with moderate activity was discovered through a high throughput screening assay. Structure–activity relationships led to the discovery of potent agonists of CB_2 receptor. The most potent compound **13** displays K_i values of >5000 and 9 nM to CB_1 and CB_2 receptors, respectively.

The pharmacological activity of cannabinoids is known to be mediated by two cannabinoid receptors (CB_1 and CB_2).[1] The CB_1 receptor discovered in 1990 was mainly distributed in components of the central nervous system, such as the brain.[2] Agonists of this receptor were found to suppress the release of neurotransmitters and to cause some central actions such as euphoria or analgesia. The CB_2 receptor discovered in 1993 was mainly distributed in immune tissues, such as the spleen and tonsil.[3] It has been reported that both agonists and antagonists of this receptor have anti-inflammatory activity, and only agonists, but not antagonists, have analgesic activity. Therefore, selective agonists[4] to the CB_2 receptor were expected to be anti-inflammatory agents and analgesic agents that would not cause side effects associated with CB_1 receptors, such as euphoria or drug dependence.

To discover novel cannabinoid receptor (CBR) agonists with different pharmacophores from conventional cannabinoids,[5] we screened our compound libraries. High-throughput screening assay resulted in the discovery of compound **1**, which exhibited modest activities to CBR (binding affinity, CB_2 K_i 428 nM; CB_1 K_i >5000 nM). In attempts to improve the binding affinity of compound **1**, a series of 2-arylimino-5,6-dihydro-4*H*-1,3-thiazines have been synthesized with various substituents to find the optimal structure exhibiting the strongest binding affinity. In this paper, we describe the synthesis and the structure–activity relationships of 2-phenylimino-5,6-dihydro-4*H*-1,3-thiazines as cannabinoid receptor agonists (Fig. 1).

The 2-phenylimino-5,6-dihydro-4*H*-1,3-thiazines **5** were prepared as outlined in Scheme 1.[6] Phenylthioureas **4**



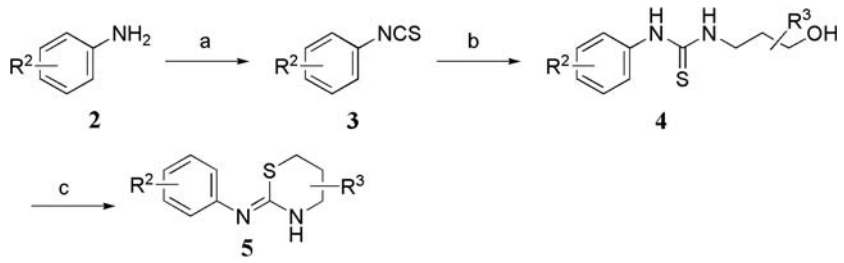
1

CB_2 K_i 428 nM
CB_1 K_i >5000 nM
cAMP (CB2, IC_{50}) : 322 nM

Figure 1. Lead structure found in a high throughput screening assay.

Keywords: 1,3-Thiazine; Cannabinoid receptor agonist; CB_2 receptor selective; CB_1; CB_2; Pain; Structure–activity relationship.
* Corresponding author. Tel.: +81 6 6458 5861; fax: +81 6 6458 0987; e-mail: hiroyuki.kai@shionogi.co.jp

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.04.093



Scheme 1. Reagents and conditions: (a) $CSCl_2$, Et_3N, CH_2Cl_2, rt 1 h; (b) 3-aminopropanol, CH_2Cl_2, rt 2–8 h; (c) 35% HClaq, reflux 1–3 h.

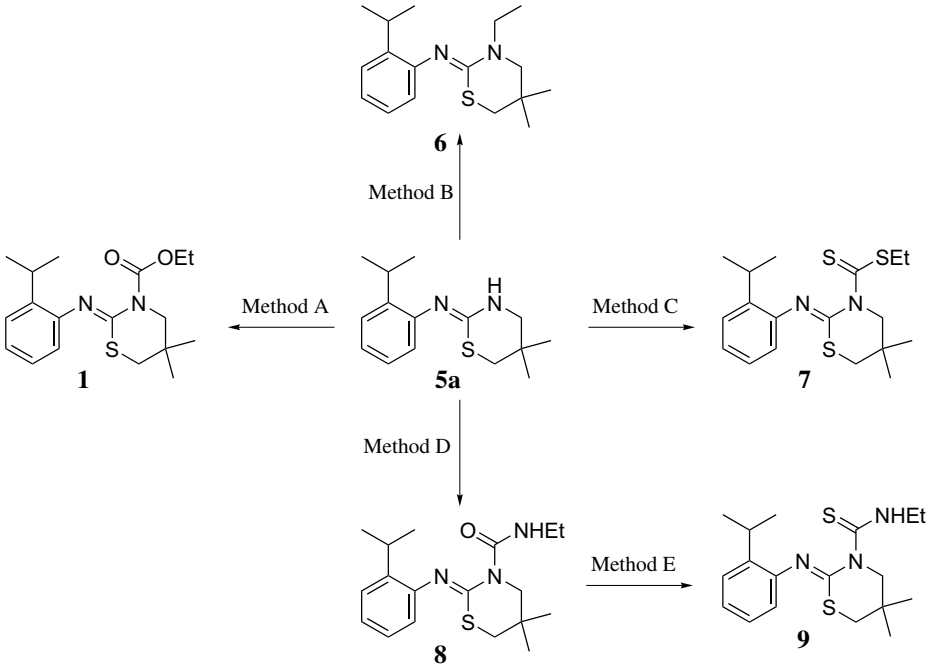
were prepared by the reaction of anilines **2** with thiophosgene in the presence of triethylamine followed by reaction with 3-amino-1-propanol. Thiazines **5** were prepared by cyclization of the phenylthioureas **4** with 35% hydrochloric acid.

The general methods for synthesis of 3-substituted 2-phenylimino-5,6-dihydro-4*H*-1,3-thiazines are shown in Scheme 2. Compound **1** was prepared by the reaction of **5a** with ethyl chloroformate in the presence of triethylamine (Method A). Compound **6** was prepared by the reaction of **5a** with iodoethane in the presence of sodium hydride (Method B). Compound **7** was prepared by the reaction of **5a** with carbon disulfide in the presence of sodium hydride followed by methylation with iodoethane (Method C). Compound **8** was prepared by the reaction of **5a** with ethyl isocyanate (Method D). Compound **9** was prepared by the reaction of **8** with Lawesson's Reagent (Method E). The thiazine analogues (**10**–**34**) were prepared using method A, B or C.

Human CB_2 receptor binding affinities[7] of 2-phenylimino-5,6-dihydro-4*H*-1,3-thiazines are shown in Tables 1–3.

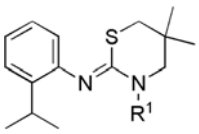
First, the ethoxycarbonyl moiety of the thiazine ring of compound **1** was modified structurally to attain more potent affinity. Table 1 shows the effects of substituent (R^1) on 3-position of the thiazine ring on binding affinity. The reaction precursor (**5a**) of **1** was inactive. When the oxygen atom of the ethoxycarbonyl group of **1** was replaced by a sulfur atom, the affinity increased (**7**, **11**, and **12**). Alkyl (ethyl, **6**; propyl, **16**; benzyl, **17**) and propionyl (**10**) derivatives exhibited extremely weak affinity. Ethylcarbamoyl (**8**) and ethylthiocarbamoyl (**9**) derivatives were inactive. Among the (alkylthio)thiocarbonyl derivatives (**7**, **13**–**15**), the (methylthio)thiocarbonyl derivative (**13**) was the most active, whereas substituents larger than the methyl group (ethyl, **7**; propyl, **14**; isopropyl, **15**) reduced the affinity. These results suggested that the physicochemical characteristics or hydrophobic influence of the sulfur atom into a substituent at 3-position might lead to enhancement of the affinity, but no definitive explanation for this observation is yet available.

Table 2 shows the effect of substituents on the phenyl-moiety of 5,5-dimethyl-3-(methylthio)thiocarbonyl-2-



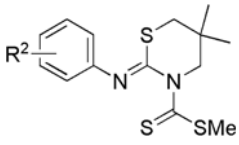
Scheme 2. Reagents and conditions: (method A) EtOCOCl, Et_3N, THF, rt 2 h, 68%; (method B) EtI, NaH, DMF, rt 2 h, 71%; (mehtod C) CS_2, NaH, DMF, 0°C 0.5 h, then EtI, 0°C 1 h, 70%; (method D) EtNCO, THF, rt 1 h, 87%; (method E) Lawessons Reagent, pyridine, 80°C 2 h, 65%.

Table 1. Lead optimization study of R^1 part



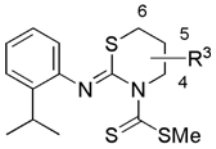
Compound	R^1	Method	h-CB_2 K_i (nM)
1	COOEt	A	428
10	COEt	A	2090
11	COSEt	A	48
12	CSOEt	A	230
7	CSSEt	C	35
13	CSSMe	C	9
14	CSSPr	C	119
15	CSS-*i*-Pr	C	275
8	CONHEt	D	>5000
9	CSNHEt	E	>5000
5a	H	–	>5000
6	Et	B	4880
16	Pr	B	915
17	CH_2Ph	B	1360

Table 2. Lead optimization study of R^2 part



Compound	R^2	CB_2 K_i (nM)
7	2-*i*-Pr	9
18	3-*i*-Pr	33
19	4-*i*-Pr	22
20	H	316
21	2-Me	202
22	2-Et	54
23	2-Pr	57
24	2-*t*-Bu	313
25	2-F	252
26	2-Cl	722
27	2-Br	371
28	2-MeO	18
29	2-EtO	84
30	2-CF_3	141
31	2-Ph	989
32	2-PhO	2920
33	2,6-Me_2	>5000

Table 3. Lead optimization study of R^3 part



Compound	R^3	CB_2 K_i (nM)
7	5,5-Me_2	9
34	H	1581
35	4-Me	>5000
36	5-Me	98
37	6-Me	>5000

phenylimino-5,6-dihydro-4*H*-1,3-thiazines. When the isopropyl group at 2-position of compound **13** was changed to 3- or 4-position removed, the affinity was reduced (**18**–**20**). Among the 2-substituted derivatives (**13**, **18**–**34**), the isopropyl derivative (**13**) was the most active, whereas bulky groups (*t*-Bu; **24**, Ph; **31**, PhO; **32**) or electron-withdrawing groups (F; **25**, Cl; **26**, Br; **26**, CF_3; **30**) reduced the affinity. Furthermore, the 2,6-dimethyl derivative (**33**) was inactive. These findings suggested that the presence of a hydrophobic group as well as a steric effect of the substituent at 2-position on the phenyl moiety were responsible for increasing affinity.

Since 5,5-dimethyl-3-(methylthio)thiocarbonyl-2-phenylimino-5,6-dihydro-4*H*-1,3-thiazines were found to have favorable affinity for the human CB_2 receptor, the dimethyl moiety of the thiazine ring of compound **13** was modified structurally. Table 3 shows the effects of substituent (R^3) on 4-, 5- or 6-position of the thiazine ring on binding affinity. Among them, the 5,5-dimethyl derivative (**13**) showed the greatest affinity, followed by the 5-methyl derivative (**36**). On the other hand, hydrogen (**34**), 4-methyl (**35**) and 6-methyl (**37**) derivatives were weak or had no activity. These results revealed that the methyl groups at 5-position of the thiazine ring of 3-(methylthio)thiocarbonyl-2-phenylimino-5,6-dihydro-4*H*-1,3-thiazines seem to play a significant role in raising affinity.

Based on the findings described above, compound **13** was selected for further evaluation (Table 4). The affinity of compound **13** for CB_2 receptors was high, and its potency (K_i value) did not differ between the two animal species. Also, compound **13** exhibited extremely weak affinities against CB_1 receptor, and higher CB_2 selectivity than WIN55212-2. Both CB_1 and CB_2 are coupled with Gi proteins and known to reduce the cyclic AMP concentration in cells upon receptor stimulation.[8] Compound **13** showed the inhibitory activity of cyclic AMP production in the assay by using CHO cells expressing human CB_2.[9] The compound showed a fast clearance rate of 83.3 mL/min/kg, a short half life ($t_{1/2}$ = 0.6 h), and low oral bioavailability of 18%. Unfortunately, the pharmacokinetics profile of this compound was not suitable in a rat in vivo examination.

Compound **13** showed analgesic activity[10] when administered subcutaneously to the pain site, and caused significant inhibition of both early and late phases of formalin-induced licking when co-injected with formalin. These effects were reversed by SR144528 (CB_2 antagonist), but not by SR141716A (CB_1 antagonist) (Fig. 2). In addition, SR144528 by itself did not affect analgesic activity (data not shown). These findings suggest that compound **13** has analgesic activity due to activation of CB_2.

In conclusion, the structure–activity relationships of a lead structure found in high throughput screening led to the discovery of new cannabinoid CB_2 selective agonists with approximately 500-fold selectivity in potency. Compound **13** showed an analgesic effect.[11] This novel series of cannabinoid agonists should be useful for

Table 4. In vitro data and pharmacokinetic profiles in rat of selected compounds

Compound	In vitro assay (nM)						Rat PK (iv: 2, po: 30 mg/kg)			
	h-CB_2 (K_i)	h-CB_1 (K_i)	m-CB_2 (K_i)	m-CB_1 (K_i)	cAMP (CB_2, IC_{50})	cAMP (CB_1, IC_{50})	$T_{1/2}$ (h)	CLt (mL/min/kg)	AUC (po) (μg-h/mL)	BA (%)
1	428	>5000	187	>5000	322	>5000			NT	
13	9	>5000	9	2020	6.1	>5000	0.6	83.3	1.1	18
WIN55212-2	2.2	14	6	7	1.1	17			NT	

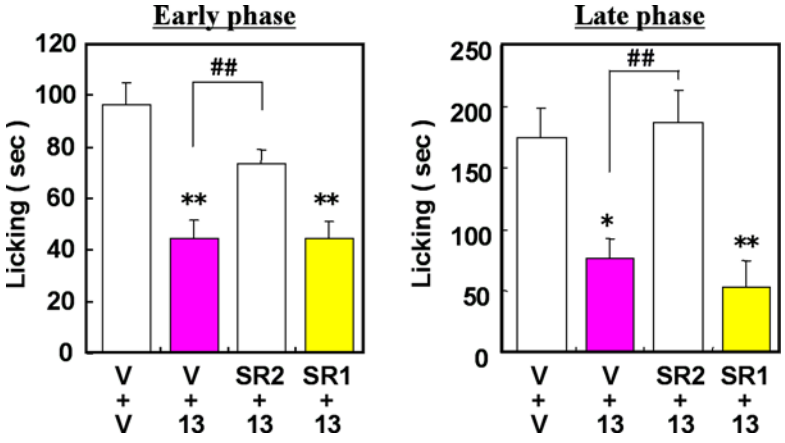


Figure 2. Analgesic effects of compound **13** reversed by SRI44528 The CB_1 selective antagonist SRI41716A (SRI, 0.3 mg/kg) or CB_2 selective antagonist SR144528 (SR2, 0.3 mg/kg) was administered iv 1 min prior to compound **13** administration, compound **13** (500 μg/site) was dissolved in 20 μL of formalin solution (2% in saline), and sc-injected into the dorsal surface of the right hindpaw of mice. $^{*}p < 0.05$, $^{**}p < 0.01$ (vs vehicle + vehicle), $^{\#\#}p < 0.01$ (vehicle + compound **13**), V: vehicle, **13**: compound **13**, $n = 6$–8.

characterizing the functions of cannabinoid receptors and evaluating their potential as a new class of analgesic drugs.

References and notes

1. Pertwee, R. G. *Curr. Med. Chem.* **1999**, *6*, 129.
2. Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. *Nature* **1990**, *346*, 561.
3. Munro, S.; Thomas, K. L.; Abu-Shaar, M. *Nature* **1993**, *365*, 61.
4. (a) Hanus, L.; Breuer, A.; Tchilibon, S.; Shiloah, S.; Goldenberg, D.; Horowitz, M.; Pertwee, R. G.; Ross, R. A.; Mechoulam, R.; Fride, E. *Proc. Nat. Acad. Sci. U.S.A* **1990**, *96*, 14228; (b) Whiteside, G. T.; Gottshall, S. L.; Boulet, J. M.; Chaffer, S. M.; Harrison, J. E.; Pearson, M. S.; Turchin, P. I.; Mark, L.; Garrison, A. E.; Valenzano, K. J. *Eur. J. Pharmacol.* **2005**, *528*, 65.
5. Pertwee, R. G. *Prog. Neurobiology* **2001**, *63*, 569.
6. (a) Ichinari, M.; Sato, T.; Hayase, Y. *Heterocycles* **1988**, *27*, 227; (b) Tisler, M. *Tetrahedron Lett.* **1959**, *1*, 12.
7. Receptor binding assay method: Membrane; recombinant human CB_1 (h-CB_1), CB_2 (h-CB_2), mouse spleen (m-CB_2), brain (m-CB_1); radioligand [^{3}H]-CP55940. Membrane fractions, used for the measurement of binding activity, were prepared as in the other study and stored in a deep freezer (−80 °C). In brief, confluent cultures of the h-CB_1, h-CB_2, m-CB_1, and m-CB_2 cells were harvested. The harvested cells were sonicated in a buffer for membrane suspensions (membrane buffer: 20 mM Tris–HCl, pH 7.4, 2 mM EDTA, and 0.25 M sucrose containing protease inhibitor) on ice, and centrifuged at 3000 rpm for 10 min at 4 °C. The supernatants were centrifuged at 100,000*g* for 60 min at 4 °C. The pelleted membrane fractions were homogenized in the membrane buffer and stored in a deep freezer (−80 °C). The K_d values of [^{3}H]-CP55940 for each membrane fraction were determined by Scatchard plot analysis.
8. Felder, C. C.; Joyce, K. E.; Briley, E. M.; Mansouri, J.; Mackie, K.; Blond, O.; Lai, Y.; Ma, A. L.; Mitchell, R. L. *Mol. Pharmacol.* **1995**, *48*, 9. 443.
9. Cyclic AMP assay method: The CHO cells expressing h-CB_1 or h-CB_2 were seeded into 24-well plates. The cells were incubated at 37 °C for 20 min with compounds in the cAMP assay buffer (Hanks' solution with 20 mM Hepes, 0.1 mM IBMX, 0.2 mM Ro20-1724, and 0.1% BSA). The cells were stimulated with 4 μM forskolin at 37 °C for 25 min (h-CB_1 cells) or 45 min (h-CB_2 cells). The cAMP concentrations in the cells were measured using cAMP kits (CIS Bio International).
10. Formalin test: Twenty microliter of formalin solution (2% in saline) was injected subcutaneously into the dorsal surface of the right hindpaw of mice (ICR). The total amount time the mouse spent licking in the early phase (acute pain, 0–5 min) and the late phase (inflammatory pain, 10–30 min) was measured.
11. Experimental procedure for preparation of compound **13**: To a solution of 2-isopropylaniline (29.4 g, 217.5 mmol) and triethylamine (44.0 g, 434.8 mmol) in dichloromethane (200 ml) was added dropwise under ice-cooling over 20 min thiophosgene (25.0 g, 217.5 mmol). The mixture was stirred at room temperature for 1 h. The mixture was poured into ice-cold water (500 ml) and then extracted with ethyl ether (500 ml). The organic layer was washed with brine (500 ml), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to give

(2-isopropylphenyl)isothiocyanate (38.2 g, yield: 99%) as a brown oil.

To a solution of (2-isopropylphenyl)isothiocyanate (20.0 g, 112.8 mmol) in diethylether (30 ml) was added 3-amino-2,2-dimethylpropanol (11.63 g, 112.8 mmol). The mixture was stirred at room temperature for 1 hour and concentrated under reduced pressure to give *N*-(2-isopropylphenyl)-*N*′-(1-hydroxy-2,2-dimethyl)propylthiourea (31.6 g, yield: 99%) as a yellow oil. ^{1}H NMR (δ ppm TMS/$CDCl_3$ 270 MHz) 0.82(6 H, s), 1.25(6H, d, J = 6.7 Hz), 3.11(1H, sept, J = 6.7 Hz), 3.25(2H, s), 3.55(2H, d, J = 6.3 Hz), 6.05(1H, m), 7.17–7.40 (4H, m).

A mixture of *N*-(2-isopropylphenyl)-*N*′-(1-hydroxy-2,2-dimethyl)propylthiourea (31.6 g, 112.7 mmol) and concentrated hydrochloric acid (30 ml) was heated under reflux with stirring for 3 hours. The reaction solution was cooled to room temperature and poured into an aqueous solution of 10% sodium hydroxide (100 ml). The mixture was poured into water (400 ml) and extracted with chloroform (100 ml × 2). The organic layer was washed with brine (100 ml), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude product was recrystallized from ethyl acetate and hexane to give 2-(2-isopropylphenyl)imino-5,5-dimethyl-5,6-dihydro-4*H*-1,3-thiazine (22.5 g, yield: 76%) as a white crystals (mp 155–157 °C). ^{1}H NMR (δ ppm TMS/$CDCl_3$ 270 MHz) 1.15 (6H, s), 1.20 (6H, d, J = 6.7 Hz), 2.67 (2H, s), 3.09 (2H, s), 3.15 (1H, sept, J = 6.7 Hz), 6.88 (1H, m), 7.05–7.11 (2H, m), 7.20 (1H, m).

To a mixture of 2-(2-isopropylphenyl)imino-5,5-dimethyl-5,6-dihydro-4*H*-1,3-thiazine (3.15 g, 12.0 mmol), carbon dioxide (1.08 ml, 18.0 mmol), and *N*,*N*-dimethylformamide (36 ml), 60% sodium hydride (0.72 g, 18 mmol) was added under ice-cooling. The mixture was stirred for 1 h. Methyliodide (0.17 g) was added to it. The mixture was stirred at 0 °C for 1 h. To the solution was added water (150 ml), extracted with diethyl ether (150 ml), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/hexane) to give compound **13** (2.53 g, yield: 72%). The product was recrystallized from ethyl acetate and hexane to give yellow crystals, mp 86–87 °C. Anal. Found: C, 57.85; H, 6.80; N, 8.13; S, 27.28, Calcd for $C_{17}H_{24}N_2S_3$: C, 57.91; H, 6.86; N, 7.95; S, 27.28%. ^{1}H NMR (δ ppm TMS/$CDCl_3$ 270 Mz) 1.20 (6H, d, J = 6.9 Hz), 1.23 (6H, s), 2.65 (3H, s), 2.68 (2H, s), 3.11 (1H, sept, J = 6.9 Hz), 4.51 (2H, s), 6.87 (1H, m), 7.11–7.18 (2H, m), 7.32 (1H, m).