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

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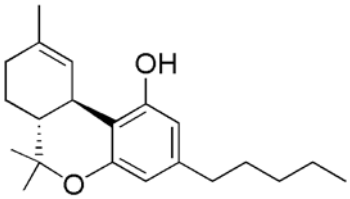
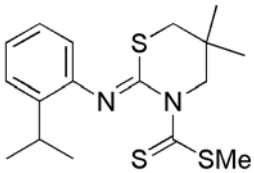
Abstract—Structure–activity relationships and efforts to optimize the pharmacokinetic profile of a class of 2-arylimino-5,6-dihydro-4*H*-1,3-thiazines as cannabinoid receptor agonists are described. Among the compounds examined, compound **14** showed potent affinity and high selectivity for CB2, and compound **23** showed potent affinities against CB1 and CB2. These compounds displayed oral bioavailability.

Cannabinoid receptor agonists, such as Δ^9-tetrahydrocannabinol (THC), have been shown to have analgesic activity in rodents. CB1 has been considered to be mainly responsible for this analgesic activity, but many recent reports have indicated that the activation of CB2 also produces analgesia.[1]



Δ^9-Tetrahydrocannabinol



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In our previous paper, we discussed our initial structure–activity relationship study of a novel series of 2-arylimino-5,6-dihydro-4*H*-1,3-thiazines as cannabinoid receptor agonists.[2] Among the derivatives examined, the most potent compound **1** displayed K_i values of >5000 and 9 nM for human CB1 (h-CB1) and human CB2 (h-CB2) receptors, respectively, and showed analgesic activity in mice when administered subcutaneously to the pain site. However, the pharmacokinetic profiles of this compound were not significant in a rat in vivo experiment. In the course of our study, compound **1** was modified structurally for further improvement of its pharmacokinetic profile. Structure–activity relationships and efforts to optimize pharmacokinetic profiles are detailed, along with a demonstration of the effectiveness of cannabinoid agonists in an animal model.

2-Arylimino-5,6-dihydro-4*H*-1,3-thiazines were prepared as outlined in Scheme 1. Phenylthioureas **III** were prepared by the reaction of anilines **I** with thiophosgene in the presence of triethylamine, followed by reaction with 3-amino-1-propanols. Thiazines **IV** were prepared by cyclization of the phenylthioureas **III** with concentrated hydrochloric acid (or CCl_4 and PPh_3

Keywords: 1,3-Thiazine; Cannabinoid receptor agonist; Oral bioavailability; CB1; CB2; Pain; Pharmacokinetic profile.
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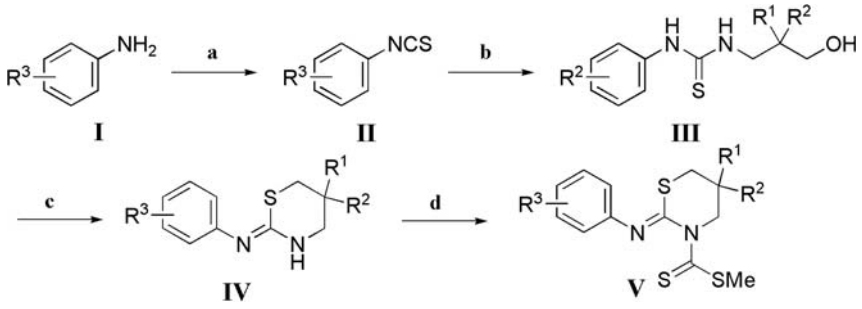
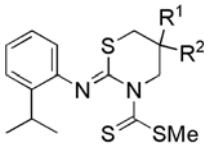


Scheme 1. Reagents and conditions: (a) $CSCl_2$, Et_3N, CH_2Cl_2, rt, 1 h; (b) 3-aminopropanol, CH_2Cl_2, rt, 2–8 h; (c) 35% HCl_{aq}, reflux 1–3 h or 1—CCl_4, Ph_3P, MeCN, rt; 2—K_2CO_3, rt; (d) 1—CS_2, NaH, DMF, 0 °C, 0.5 h; 2—MeI, 0 °C, 1 h.

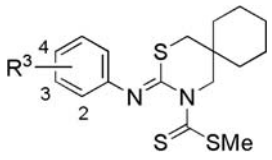
Table 1. Effects of substituents (R^1 and R^2) on the thiazine ring



Compound	R^1	R^2	h-CB2[a] K_i (nM)	h-CBl[a] K_i (nM)	CLt[b] (mL/min/kg)	BA[b] (%)
1	Me	Me	9	>5000	63.4	10
2	Et	Et	9	>5000	48.3	19
3	$(CH_2)_2$		65		85.1	14
4	$(CH_2)_4$		1	541	54.2	8.9
5	$(CH_2)_5$		0.8	122	18.6	32

CLt, total clearance; BA, oral bioavailability.
[a] See Ref. 2 for assay protocol.
[b] All compounds were administered at 0.5 mg/kg iv and 1.0 mg/kg po. These compounds were administered as a mixture of three to five compounds.

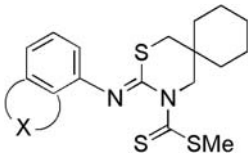
Table 2. Effects of substituents (R^3) on the benzene ring

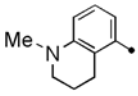


Compound	R^3	h-CB2[a] K_i (nM)	h-CBl[a] K_i (nM)	CLt[b] (mL/min/kg)	BA[b] (%)
5	2-*i*-Pr	0.8	122	18.6	32
6	3-*i*-Pr	2.5	100		
7	4-*i*-Pr	23	1260		
8	2-MeO	1.1	13	75.9	0.4
9	3-MeO	1	108	45.1	13
10	4-MeO	3.8	671		
11	4-Et	1.2	95		
12	4-Pr	7	514		
13	4-CF_3	18	1730		
14	4-CF_3O	6	1500	7.4	24
15	4-CN	2.3	173		
16	2-EtO	6.3	358		
17	2-CF_3	1.1	418		
18	2-CF_3O	12	711		
19	2,3-Me_2	1	114		
20	2,3-$(CH_2)_3$	5	243		
21	2,3-$(CH_2)_4$	0.2	15	19.1	44
22	3,4-$(CH_2)_4$	24	396		

See footnotes a and b of Table 1.

Table 3. Effects of bicyclic moiety



Compound		h-CB2[a] K_i (nM)	h-CBl[a] K_i (nM)	CLt[b] (mL/min/kg)	BA[b] (%)
21		0.2	15	19.1	44
23		6	30	17.7	53
24		11	763	120.7	42
25		1.3	44	60.4	NC
26		0.9	246		
27		1.1	85		

NC: not calculate.
See footnotes a and b of Table 1.

then K_2CO_3). Products **V** were prepared by the reaction of **IV** with carbon disulfide in the presence of sodium hydride, followed by methylation with iodomethane.

We evaluated the metabolic stability of compound **1** in vitro to identify the cause of its poor pharmacokinetic profile. We found that cleavage of the (methylthio)thiocarbonyl moiety at the 3-position of the thiazine ring appeared to be the main metabolized part. In our continuing studies, we synthesized 2-arylimino-5,6-dihydro-4*H*-1,3-thiazines possessing a (substituted thio)thiocarbonyl or heteroaryl group instead of the (methylthio)thiocarbonyl moiety in compound **1**, to assess their in vitro activities and pharmacokinetic profiles. The compound satisfying the target value could not be obtained by conversion of this part, although some improvements were seen in the pyridyl compounds (data not shown). This led us to hypothesize that cleavage of the (methylthio)thiocarbonyl moiety was prevented by the steric effect of 5-position of the thiazine ring. Table 1 shows the effects of substituents (R^1 and R^2) on 5-position of the thiazine ring on binding affinity and pharmacokinetic profiles.

The introduction of large substituents (diethyl, **2**; spirohexyl, **5**) at 5-position decreased the total clearance compared to that with dimethyl groups (**1**), whereas, the introduction of small substituents (spiropropyl, **3**) increased the total clearance to more than that with dimethyl groups. These results suggest that the presence of a bulky group at 5-position may enhance the metabolic stability. Furthermore, the spirohexyl type (**5**) improved bioavailability and increased the binding affinity against h-CB1.

Since the spirohexyl type was found to be favorable for high affinity and good pharmacokinetic profiles, the phenylimino moiety of compound **5** was modified structurally.

Table 2 shows the effects on the binding affinity and pharmacokinetic profiles of various substituents (R^3) on the phenyl moiety of 3-(methylthio)thiocarbonyl-2-phenylimino-5,5-pentamethylene-5,6-dihydro-4*H*-1,3-thiazines. Substitution with an isopropyl or a methoxy group at 2-position (**5** and **8**) or 3-position (**6** and **9**) of the phenyl ring resulted in the most favorable affinity for h-CB1, while substitution at 4-position (**7** and **10**) led

Table 4. Biological tests of selected compounds

Compound	In vitro assay						Rat pharmacokinetics (iv, 2 mg/kg; po, 30 mg/kg)				Mouse formalin test (ED_{50})			
	h-CB2	h-CBl	m-CB2	m-CBl	cAMP	cAMP	$T_{1/2}$	CLt	AUC (po)	BA	iv		po	
	(K_i, nM)	(K_i, nM)	(K_i, nM)	(K_i, nM)	(CB2 IC_{50})	(CB1 IC_{50})	(h)	(mL/min/kg)	(μg·h/mL)	(%)	Early	Late	Early	Late
5	0.8	122	1.2	80	8	69	2.5	38.2	3.2	24	1.2	1.1	17.5	17.5
14	6	1500	7	1210	9	>5000	2.4	25.9	3.9	20	>20	>20	>100	>100
21	0.2	15	0.7	6	0.2	3.2	4.1	23.3	1.5	7	0.3	0.2	2.5	4.0
23	6	30	4	6.8	1.7	10	1.5	30.1	6.1	36	0.4	0.4	1.5	1.0
THC	3.3	17	9.2	40	6.6	8.5	NT				0.4	0.3	NT	
Morphine											1.1	1.1	27.3	9.8

NT, not tested.

to weak affinity. A 2-methoxy derivative (**8**) showed high affinity, but its pharmacokinetic profile was not particularly good.

While all compounds showed high affinity for h-CB2, no influence of substitution groups (electron-withdrawing or hydrophobic effect) was noted. Among the 4-substituted phenyl derivatives (**7**, **10**–**15**), the 4-trifluoromethoxy derivative (**14**) showed high selectivity against h-CB2, with good pharmacokinetic profiles. Among the 2- or 3-substituted phenyl derivatives (**7**, **10**–**15**), the 2,3-tetramethylene derivative (**21**) showed affinity against h-CB1 and good pharmacokinetic profiles, but the 3,4-tetramethylene derivative (**22**) showed reduced affinity. One possible explanation for this is that a favorable conformation of the benzene ring for the steric influence of substituents, or at the *ortho* or *meta* position, is required for affinity to h-CB1.

These results revealed that the 2- or 3-substituted phenyl derivatives were favorable for high affinity, and led us to examine the activity of the bicyclic type (Table 3). The naphthyl derivative **23** showed high affinity against h-CB1, with a good pharmacokinetic profile. However, introduction of a heteroatom to the bicyclic ring of compounds **24**–**27** did not improve the binding affinity and pharmacokinetic profile.

From the results described above, compounds **5**, **14**, **21**, and **23** were selected for further evaluation (Table 4). Compounds **5**, **21**, and **23** showed analgesic activity (formalin test[3]) when administered intravenously and orally. The analgesic potency of compounds **21** and **23** (CB1/CB2 dual agonist) was equal to that of THC and superior to that of morphine, while the analgesic potency of compound **14** (CB2 selective agonist) was low. In our previous study, compounds **1**, CB2 selective agonists, showed an analgesic effect against formalin-induced pain by local administration.[2] On the other hand, compound **14** which has sufficient efficacy against CB2 did not show any analgesic effect by systemic administration. This might account for the insufficient tissue concentration of this compound.

Figure 1 shows the analgesic activity of compound **23**. Orally administered compound **23** caused significant dose-dependent inhibition of both early (acute pain) and late (inflammatory pain) phases of formalin-induced licking[3]; ED_{50} was 1.5 and 1.0 mg/kg, respectively. These effects were reversed by a CB1 antagonist, but not by a CB2 antagonist. On the other hand, the ED50 of CB1-mediated motor impairment was more than 10 times higher than that of the analgesic effects (data not shown).

In summary, this paper has described the structure–activity relationships of a class of 2-arylimino-5,6-dihydro-4*H*-1,3-thiazines as cannabinoid receptor agonists, and our efforts to optimize their pharmacokinetic profiles. Among the compounds examined, compound **14** showed potent affinity and high selectivity for CB2, and compound **23** showed potent affinity for CB1 and CB2.[4] These compounds displayed oral bioavailability. This novel series of cannabinoid agonists is expected

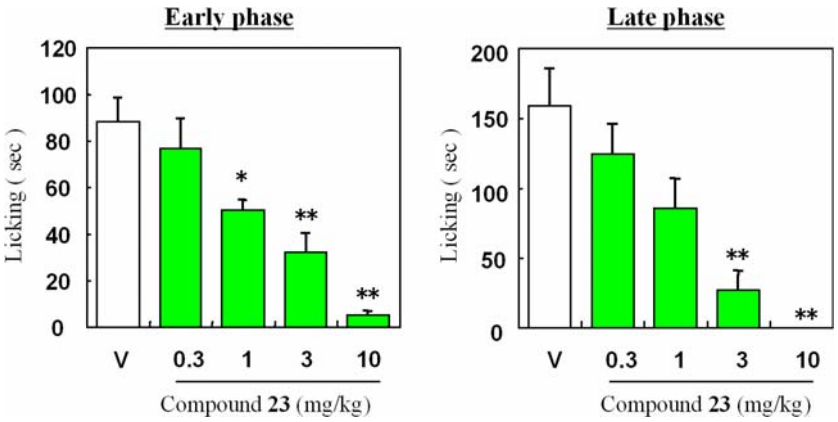


Figure 1. Analgesic effects of compound **23** on formalin-induced pain compound **23** was orally administered 2 h prior to formalin injection. $^{**}P < 0.01$; V, vehicle; $n = 8$ (mice).

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

References and notes

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3. Formalin test: 20 μl formalin solution (2% in saline) was injected subcutaneously into the dorsal surface of the right hindpaw of mice (ICR). The total time the mouse spent licking in the early phase (acute pain, 0–5 min) or the late phase (inflammatory pain, 10–30 min) was measured.
4. Experimental procedure for the preparation of compound **23**. To a solution of 1-naphthylamine (35.8 g, 250 mmol) and triethylamine (55.7 g, 550 mmol) in dichloromethane (450 ml), thiophosgene (31.6 g, 275 mmol) was added dropwise under ice-cooling conditions, over a 30-min period. The mixture was stirred at room temperature for 1 h. The mixture was poured into ice-cold water (1000 ml) and extracted with ethyl ether (1000 ml). The organic layer was washed with brine (1000 ml), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to give 1-naphthyl isothiocyanate (46.0 g, yield: 99%) as brown oil.
To a solution of 1-naphthyl isothiocyanate (1.85 g, 10 mmol) in dichloromethane (5 ml), 3-amino-2,2-pentamethylenepropanol (1.50 g, 10.5 mmol) in dichloromethane (5 ml) was added. The mixture was stirred at room temperature for 2 h and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/hexane) to give *N*-(1-naphthyl)-*N*′-(1-hydroxy-2,2-pentamethylene)propylthiourea (2.70 g, yield: 82%) as a white powder.
A mixture of *N*-(1-naphthyl)-*N*′-(1-hydroxy-2,2-pentamethylene)propylthiourea (2.63 g, 8 mmol) and concentrated hydrochloric acid (8 ml) was heated under reflux with stirring for 3 h. The reaction solution was cooled to room temperature and poured into an aqueous solution of 10% sodium hydroxide (50 ml). The mixture was poured into water (50 ml) and extracted with chloroform (100 ml). The organic layer was washed with brine (50 ml), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The crude product was recrystallized from ethyl acetate and hexane to give 2-(1-naphthyl)imino-5,5-pentamethylene-5,6-dihydro-4*H*-1,3-thiazine (1.06 g, yield: 43%) as white crystals (mp 178–179 °C). ^{1}H NMR (δ ppm TMS/$CDCl_3$ 270 MHz) 1.40–1.64 (10H, m), 2.71 (2H, s), 3.17 (2H, s), 5.73 (1H, br s), 7.01 (1H, d, $J = 6.9$ Hz), 7.35–7.47 (3H, m), 7.55 (1H, d, $J = 8.3$ Hz), 7.81 (1H, m), 8.01 (1H, m).
To a mixture of 2-(1-naphthyl)imino-5,5-pentamethylene-5,6-dihydro-4*H*-1,3-thiazine (0.25 g, 0.8 mmol), carbon dioxide (0.06 ml, 1.04 mmol) and *N*,*N*-dimethylformamide (2.4 ml), 60% sodium hydride (0.04 g, 1.04 mmol), was added under ice-cooling conditions. The mixture was stirred for 1 h, then methyliodide (0.06 ml, 1.04 mmol) was added. This mixture was stirred at 0 °C for 1 h. Water (80 ml) was added to the solution, which was then extracted with diethyl ether (80 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 **23** (0.21 g, yield: 66%). The product was recrystallized from ethyl acetate and hexane to give yellow crystals, mp 120–121 °C. Anal. Found: C, 63.06; H, 5.98; N, 7.10; S, 23.95, Calcd for $C_{21}H_{24}N_2S_3$: C, 62.96; H, 6.04; N, 6.99; S, 24.01%. ^{1}H NMR (δ ppm TMS/$CDCl_3$ 270 MHz) 1.30–1.85 (10H, m), 2.66 (2H, s), 2.70 (3H, s), 4.65 (2H, s), 7.09 (1 H, d, $J = 7.3$ Hz), 7.42–7.53 (3H, m), 7.67 (1H, d, $J = 8.2$ Hz), 7.85 (1H, m), 8.07 (1H, m). Compound **14** was synthesized by the same procedure described above: yellow crystals, mp 98–99 °C. Anal. Found: C, 49.68; H, 4.78; F, 13.34; N, 6.38; S, 22.11, Calcd for $C_{18}H_{21}F_3N_2S_3$: C, 49.75; H, 4.87; F, 13.12; N, 6.45; S, 22.14%. ^{1}H NMR (δ ppm TMS/$CDCl_3$ 270 MHz) 1.36–1.61 (10H, m), 2.58 (3H, s), 2.93 (2H, s), 3.61 (2H, s), 7.25–7.29 (2H, m), 7.47–7.52 (2H, m).