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2-Arylimino-5,6-dihydro-4*H*-1,3-thiazines as a new class of cannabinoid receptor agonists. Part 3: Synthesis and activity of isosteric analogs

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ABSTRACT

Structure–activity relationships and efforts to optimize the pharmacokinetic profile of isosteric analogs of 2-arylimino-5,6-dihydro-4*H*-1,3-thiazines as cannabinoid receptor agonists are described. Among those examined, compound **25** showed potent affinity for cannabinoid receptor 1 (CB1) and receptor 2 (CB2). This compound displayed oral bioavailability and analgesic activity.

Cannabinoid receptor agonists, such as Δ^9-tetrahydrocannabinol (THC), have been shown to have analgesic activity in rodents. Cannabinoid receptor 1 (CB1) is considered to be mainly responsible for this analgesic activity, but many recent reports have indicated that the activation of cannabinoid receptor 2 (CB2) also produces analgesia.[1]

In our previous letter, we discussed 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.[2] Among the derivatives examined, compound **1** showed potent affinity for CB1 and CB2. Compounds displayed oral bioavailability and analgesic activity in mice. Recently, isosterism has represented one approach used in medicinal chemistry for the rational modification of lead compounds into safe and more clinically effective agents.[3] We chose compound **1** and presumed that the thiazine ring had the essential structure required for the activity.[2] In our continuing studies, we synthesized isosteric analogs (**2** and **3**) that possess a oxazine or pyrimidine ring instead of the thiazine ring in compound **1** to assess biological activity (Fig. 1). Structure–activity relationships and efforts to optimize

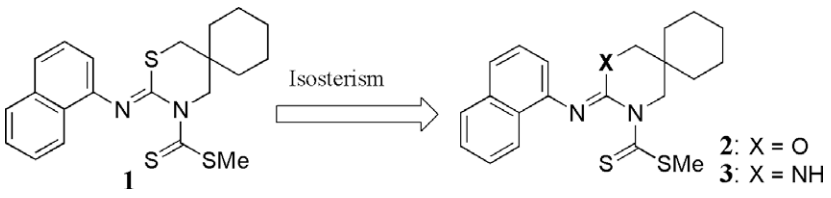

Figure 1. Compound **1** and isosteric analogs (**2** and **3**).

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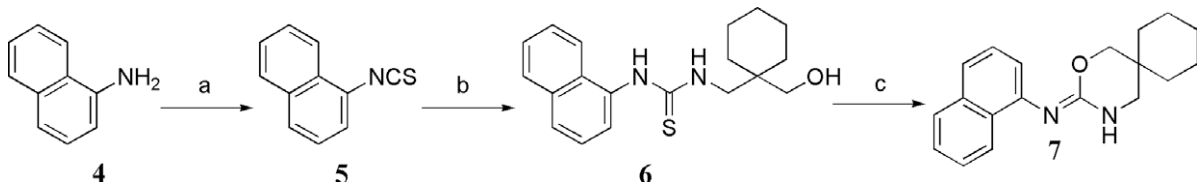
pharmacokinetic profiles are detailed, along with a demonstration of the effectiveness of cannabinoid agonists in an animal model.

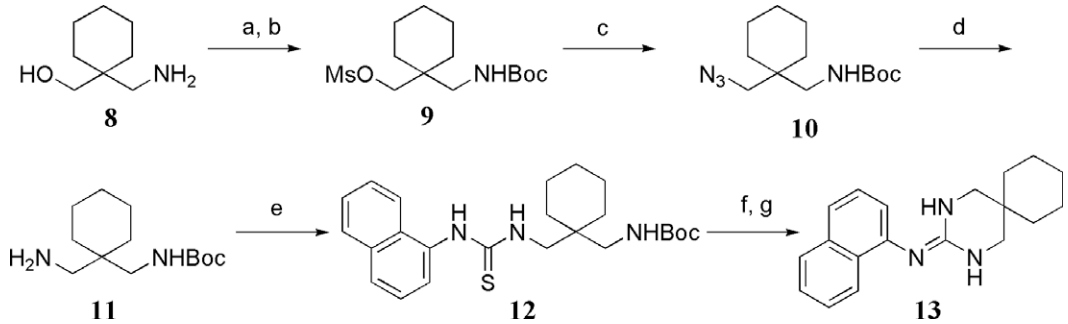
2-Imino-5,6-dihydro-4*H*-1,3-oxazine **7** was prepared as outlined in Scheme 1. Naphthylthiourea **6** was prepared by the reaction of naphthylamine **4** with thiophosgene in the presence of triethylamine, followed by reaction with 3-amino-1-propanol **8**. Oxazine ring construction was carried out by cyclization of naphthylthioura **6**, by using iodomethane followed by treatment with potassium hydroxide.[4]

2-Imino-hexahydropyrimidine **13** was prepared as outlined in Scheme 2. Thiourea **12** was prepared in several stages from 3-amino-1-propanol **8**. Pyrimidine **13** was prepared by cyclization of naphthylthiourea **12** by using HgO in the presence of triethylamine, after deprotection of *tert*-butoxycarbonyl (Boc) group under usual conditions.[5]

The general methods for synthesis of 3-substituted 2-naphthylimino-5,6-dihydro-4*H*-1,3-thiazines and these isosteric analogs are shown in Table 1. (Methylthio)thiocarbonyl analogs (**1**–**3**) were prepared by the reaction of corresponding precursors with carbon disulfide in the presence of sodium hydride, followed by methylation with iodomethane (Method A). However, when compound **13** (X = NH) was treated with carbon disulfide in the presence of sodium hydride, the reaction did not proceed. When the sulfur atom of the thiazine ring of compound **1** was replaced by an oxygen atom (oxazine derivative **2**), the affinity towards cannabinoid receptors increased. (Methylthio)carbonyl analogs (**14**–**16**) were prepared by the reaction of corresponding precursors with methyl chlorothioformate in the presence of triethylamine (Method B) that there was a low yield of **16**, because there was a high yield of the by-product (diacylation compound). Compound **16** showed the affinity for CB1 and CB2, even though it was weak. Among the (methylthio)carbonyl derivatives (**14**–**16**), the oxazine derivative (**15**) was the most active, whereas the affinity was re-

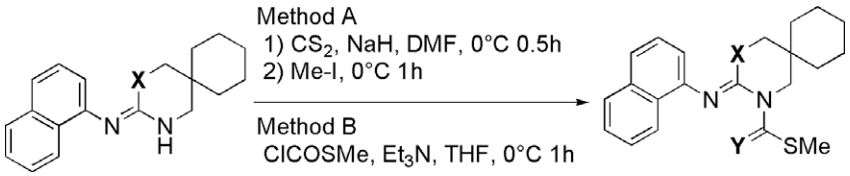


Scheme 1. Reagents and conditions: (a) $CSCl_2$, Et_3N, CH_2Cl_2, rt 1 h, 99%; (b) 3-aminopropanol (**8**), CH_2Cl_2, rt, 2 h; (c) MeI, MeOH, rt, 3 h then KOH, rt, 1 h, 86%.



Scheme 2. Reagents and conditions: (a) Boc_2O, THR, rt, 2 h, 78%; (b) MsCl, Et_3N, 0 °C, 2 h, quant; (c) NaN_3, DMF, 100 °C, 5 h, 43%; (d) H_2, 10% Pd-C, EtOH, rt, 1 h; (e) NaphNCS (**5**), CH_2Cl_2, rt, 2 h, 43% tor two steps, (f) 4 N HCl in dioxoane, quant.; (g) HgO, Et_3N, MoOH, rt, 2 h, 65%.

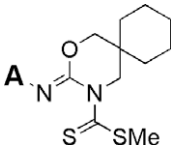
Table 1
Synthesis and biological activities of isosleric analogs.

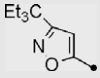


Compound	X	Y	Method	Yield (%)	h-CB2 K_i (nM)	h-CBl (nM)	CLt[a] K_i (mL/min/kg)	BA[a] (%)
1	S	S	A	66	6		17.7	53
2	O	S	A	75	0.9	9.3	35.9	24
3	NH	S	A	0[b]	—	—	—	—
14	S	O	B	65	1.5	49	43.8	75
15	O	O	B	97	5.8	31	NT	NT
16	NH	O	B	21[c]	155	624	NT	NT

CLt, total clearance; BA, oral bioavailability; NT, not tested.
[a] 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.
[b] Decomposed.
[c] By-product (diacylation compound) yielded (22%).

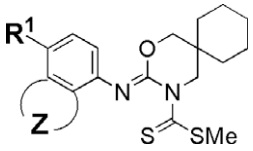
Table 2
Effects of substituents (A) on the imino moiety.

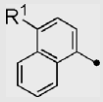
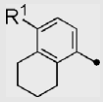


Compound	A	h-CB2 K_i (nM)	h-CB1 K_i (nM)	CLt[a] (mL/min/kg)	BA[a] (%)
2		0.9	9.3	35.9	24
17		11	515	NT	NT
18		0.9	59	70.5	4.3
19		6.8	152	NT	NT
20		1.0	1.0	223	19
21		1.0	0.9	74.6	8.9
22		1.0	1.0	50.7	NC
23		4.7	17	NT	NT

NC, not calculated. See footnote a of Table 1.

Table 3
Effects of bicyclic moiety.



Compound		R1	h-CB2 K_i (nM)	h-CB 1 K_i (nM)	CLt[a] (mL/min/kg)	BA[a] (%)
2		H	0.9	9.3	35.9	24
24		F	0.8	4.9	24.4	37
25		Cl	0.9	3.4	12.2	39
26		CN	1.8	9.2	377	13
27		NO_2	2.4	14	NT	NT
28		NMe_2	6.6	4.9	NT	NT
29		H	1.0	14	25.5	19
30		Cl	5.8	76	NT	NT
31		CN	1.9	101	NT	NT

See footnote a of Table 1.

duced more than that of the methylthiocarbonyl type (**2**). As these compounds showed affinity for CB1 and CB2, the oxazine and pyrimidine rings were assumed to correspond to the isosterism of the thiazine ring.

Since the oxazine type was found to be favorable for high affinity and good pharmacokinetic profiles, the naphthyllimino moiety of compound **2** was modified structurally.

Table 2 shows the effects of substituents (A) on the imino position of the oxazine ring on binding affinity and pharmacokinetic profiles. The naphthyl derivative **2** showed high affinity for CB1 and CB2, with a good pharmacokinetic profile. On the other hand, the 5-membered heterocyclic derivatives (**20**–**23**) showed a greater increase in affinity than compound **2**, however, their pharmacokinetic profiles were not particularly good. One possible explanation for this is that a favorable steric conformation of the naphthalene ring is required for a good pharmacokinetic profile.

Table 3 shows the effects on the binding affinity and pharmacokinetic profiles of various substituents (R^1) on the naphthyl moiety of 3-(methylthio)thiocarbonyl-2-naphthylimino-5,5-pentamethylene-5,6-dihydro-4*H*-1,3-oxazines. Substitution with a fluoro (**24**) or chloro (**25**) of the napthyl ring resulted in the most favorable affinity for CB1, whereas strong electron-withdrawing groups (CN; **26**, NO_2; **27**) reduced the affinity for CB1. On the other hand, the tetrahydronaphthyl derivative **29** showed high affinity for CB1 and CB2, with a moderate pharmacokinetic profile. However, introduction of substituents (R^1) to the tetrahydronaphthyl ring of compounds **30** and **31** did not improve their binding affinity.

From the results described above, we selected compound **25** and compared the in vitro and in vivo activities to those of compound **1**. Compound **25** showed higher binding activity for human CB1, CB2, and mouse CB1 compared to compound **1**, with higher inhibitory activity for cAMP production in human-CB1-expressing CHO cells. The bioavailability of compound **25** was lower, but the total clearance was slower than that of compound **1**. The analgesic activity and side effect of compounds were evaluated by using the formalin test[6] and the ring-catalepsy test in mice.[7,8] The analgesic activity of compound **25** was slightly stronger, and the ratio of the analgesic activity to side effects was superior to that of compound **1** (Table 4). The isosterism was used for the rational modification of lead compounds into safer and more clinically effective agents. As we expected, replacement of the oxazine ring of the thiazine ring part of the lead compound did not affect biological activity and reduced the side effects. These results might be explained by the restricted brain penetration of compound **25** compared to that of compound **1** (data not shown). Compound **25** may act more potently on peripheral CB receptors than compound **1**.[9] The isosteric modification of the lead compound was effective for the improvement of pharmacokinetic profiles without any loss of activity, which demonstrated a rational approach in drug design.

In summary, the discovery of a new class of cannabinoid receptor agonists in which the oxazine ring has been incorporated as an isosteric replacement for the thiazine ring of original compound **1** was reported. Among those examined, compound **25**[10] showed potent affinity for CB1 and CB2. This compound displayed oral bioavailability and analgesic activity. Furthermore, compound **25** showed a tendency to cause fewer side effects than compound **1**. This novel series of cannabinoid agonists is expected to be useful for characterizing the functions of cannabinoid receptors and evaluating their potential as a new class of analgesic drugs.

Table 4
Biological tests of selected compounds.

Compound	In vitro assay (nM)				Rat pharmacokinetics (iv 2 mg/kg; po 3 mg/kg)				
	h-CB2 (K_i)	h-CBI (K_i)	m-CRI (K_i)	cAMP (CB1 IC_{50})	$T_{1/2}$ (h)	CLt (mL/min/kg)	C_{max} (po) (μg/ml)	AUC (po) (μg h/mL)	BA (%)
25	0.9	3.4	0.7	8.0	2.2	14.7	0.28	1.21	35
1	6.0	30	6.8	10	1.5	30.1	0.16	1.39	80

Compound	Mouse formalin test ED_{50} (mg/kg, po)		Mouse ring-catalepsy test ED_{50} (mg/kg, po)	Ratio (side efect[b]/analesic action[a])
	Early	Late		
25	1.9	0.64	25.6	40
1	1.5	1.0	19.2	19.2

[a] Mouse formalin test late phase.
[b] Mouse ring-catalepsy test.

References and notes

1. Pertwee, R. G. *Curr. Med. Chem.* **1999**, *6*, 129.
2. (a) Kai, H.; Morioka, Y.; Tomida, M.; Takahashi, T.; Hattori, M.; Hanasaki, K.; Koike, K.; Chiba, H.; Shinohara, S.; Kanemasa, T.; Iwamoto, Y.; Takahashi, K.; Yamaguchi, Y.; Baba, T.; Yoshikawa, T.; Takenaka, H. *Bioorg. Med. Chem. Lett.* **2007**, *11*, 3925; (b) Kai, H.; Morioka, Y.; Murashi, T.; Morita, K.; Shinonome, S.; Nakazato, H.; Kawamoto, K.; Hanasaki, K.; Takahashi, F.; Mihara, S.; Arai, T.; Abe, K.; Okabe, H.; Baba, T.; Yoshikawa, T.; Takenaka, H. *Bioorg. Med. Chem. Lett.* **2007**, *11*, 4030.
3. Patani, G. A.; LaVoie, E. J. *Chem. Rev.* **1996**, *96*, 3147.
4. Csomós, P.; Bernátha, G.; Sohárc, P.; Csámpaic, A.; Kimped, N. D.; Fülöp, F. *Tetrahedron* **2001**, *57*, 3175.
5. Jochen, J.; Bearice, R. *Liebigs Ann. Chem.* **1994**, 805.
6. *Formalin test:* Twenty microliters 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) were measured.
7. *Ring-catalepsy test:* Male ddY mice were obtained from Japan SLC (Shizuoka, Japan) and housed in a temperature-controlled (22–26 °C) environment, with a 12/12-h light/dark cycle. Food and water were available ad libitum in their home cages. The ring-catalepsy test was performed according to the method of Pertwee.[8] Briefly, the mouse was positioned with front and rear paws on a horizontal wire ring (5.5 cm diameter and 16 cm high). The immobility index was calculated as a percentage of time that the animal spent motionless during the 5-min test session on the ring. If an animal fell or jumped off from the ring, it was immediately placed on the ring again. Immobility index = (time motionless × 100/duration of the test session).
8. Pertwee, R. G. *Br. J. Pharmacol.* **1972**, *46*, 753–763.
9. (a) Richardson, J. D.; Kilo, S.; Hargreaves, K. M. *Pain* **1998**, *75*, 111; Pertwee, R. G. *Life Sci.* **1999**, *65*, 597; (b) Dyson, A.; Peacock, M.; Chen, B.; Courade, J.-P.; Yaqoob, M.; Groarke, A.; Brain, C.; Loong, Y.; Fox, A. *Pain* **2005**, *116*, 129.
10. *Experimental procedure for the preparation of compound **25**:* To a solution of 4-chloro-1-naphthylamine (7.99 g, 45 mmol) and triethylamine (10.02 g, 99 mmol) in dichloromethane (90 ml), thiophosgene (5.69 g, 49.5 mmol) was added dropwise under ice-cooling conditions, over a 20-min period. The mixture was stirred at room temperature for 1 h. The mixture was poured into ice-cold water (500 ml), and extracted with dichloromethane (300 ml). The organic layer was washed with brine (500 ml), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure to give crude 4-chloro-1-naphthyl isothiocyanate. To a solution of crude 4-chloro-1-naphthyl isothiocyanate in dichloromethane (60 ml), 3-amino-2,2-pentamethylenepropanol (8.38 g, 58.5 mmol) in dichloromethane (30 ml) was added. The mixture was stirred at room temperature for 3 h. The mixture was poured into water (400 ml), and extracted with dichloromethane (250 ml). The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (ethyl acetate/hexane) to give *N*-(4-chloro-1-naphthy)-*N′*-(1-hydroxy-2,2-pentamethylene)propylthiourea (13.63 g, yield: 83%) as a white powder.
A mixture of *N*-(4-chloro-1-naphthy)-*N′*-(1-hydroxy-2,2-pentamethylene)-propylthiourea (1.09 g, 3 mmol), methyliodide (0.93 ml, 15 mmol) and methanol (9 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure. To a solution of the residue in methanol (6 ml), potassium hydroxide (1.68 g, 30 mmol) in methanol (9 ml) was added dropwise under ice-cooling conditions, over a 5-min period. The mixture was stirred at room temperature for 2 h. The mixture was poured into ice-cold water (200 ml), and extracted with dichloromethane (2× 100 ml). The organic layer was dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The crude product was recrystallized from ethyl acetate and hexane to give 2-(4-chloro-1-naphthy)imino-5,5-pentamethylene-5,6-dihydro-*4H*-1,3-oxazine (0.73 g, yield: 73%) as a white crystals (mp 177–178 °C). ^{1}H NMR (δ ppm TMS/$CDCl_3$ 270 MHz) 1.40–1.64 (10H, m), 3.07 (2H, s), 3.94 (2H, s), 7.06 (1H, m), 7.45–7.59 (3H, m), 8.14 (1H, d, *J* = 8.2 Hz), 8.21 (1H, d, *J* = 8.6 Hz).
To a mixture of 2-(4-chloro-1-naphthy)imino-5,5-pentamethylene-5,6-dihydro-*4H*-1,3-oxazine (0.66 g, 2 mmol), carbon dioxide (0.18 ml, 3 mmol) and *N*,*N*-dimethylformamide (2.4 ml), 60% sodium hydride (0.10 g, 2.4 mmol) was added under ice-cooling conditions. The mixture was stirred for 20 min, then methyliodide (0.19 ml, 3 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 diethylether (100 ml), dried over anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (toluene/hexane/triethylamine, 70:30:1) to give compound **25** (0.52 g, yield: 62%). The product was recrystallized from ethyl acetate and hexane to give yellow crystals, mp 118–119 °C. Anal. Found: C, 60.15; H, 5.42; N, 6.57; Cl, 8.25; S, 15.41, Calcd. for $C_{21}H_{23}ClN_2OS_2$: C, 60.20; H, 5.53; N, 6.69; Cl, 8.46; S, 15.31%. ^{1}H NMR (δ ppm TMS/$CDCl_3$ 270 Mz) 1.30–1.70 (10H, m), 2.62 (3H, s), 3.90 (2H, s), 4.34 (2H, s), 7.10 (1H, d, *J* = 8.2 Hz), 7.50–7.62 (3H, m), 8.21–8.26 (2H, m).