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CB 1/2 dual agonists with 3-carbamoyl 2-pyridone derivatives as antipruritics: Reduction of CNS side effects by introducing polar functional groups

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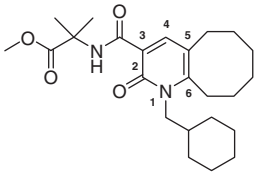
ABSTRACT

Our lead compound **1** showed high affinity for both CB1 and CB2 receptors, suggesting the possibility of inducing psychoactive side effects through the CB1 receptor in the brain. To solve this issue, polar functional groups were introduced at the 3-position of the pyridone core of compound **1** to find CB1/2 dual agonists such as **17** and **20** which did not show any CNS side effects.

Pruritus can be defined as an unpleasant cutaneous sensation associated with the immediate desire to scratch. It may be construed as part of the body's defense mechanism by which we deal with potentially dangerous organisms or stimuli. However, chronic or strong scratching leads to the development of skin lesions and the release of inflammatory mediators that induce or aggravate pruritus resulting in further scratching. Pruritus is a common symptom in dermatology and is induced by factors such as inflammation, cancer, infection, psychiatric diseases, drug application, and stress. There are many reports indicating the existence of an interactive network between the skin and not only the peripheral but also the central nervous system to regulate and respond to pruritic stimuli.[1] This means that specific sensory nerves and their receptors are involved in the pathophysiology of pruritus. Recently, among them, the cannabinoid (CB) receptor was reported to be implicated in an anti-inflammatory and anti-nociceptive action because of its abundant distribution on skin nerve fibers and mast cells.[2] Furthermore, the cannabinoid agonist (HU-210) was reported to attenuate histamine-induced itch by peripheral administration.[3] These findings suggest that the CB agonist could be useful against the various pruritic diseases such as atopic dermatitis.

CB receptors are G-protein coupled receptors (GPCRs). Two types of receptors, CB1 and CB2, have been identified in mammalian tissues and were cloned.[4,5] The CB1 receptors are widely expressed in the brain with the highest density in the cells of the basal ganglia, hippocampus and cerebellum.[6] They are also found in various peripheral tissues including the gastrointestinal tract, pancreas, liver, kidney, prostate, testis, ovary, eye, lungs and heart.[7,8] In the CNS, the CB1 receptor is responsible for cognition, memory and sensory perception, while in the periphery, it plays an important role in maintaining energy balance, metabolism, nociception and cardiovascular health.[9,10] On the other hand, the CB2 receptor is mainly located in the periphery and is associated with the cells of immune system[11,12] and, to a far lesser extent in the brain.[13] Recent data indicate that the CB2 receptor is responsible for the control of peripheral pain[14] and inflammation.[15] However, undesirable CNS side effects induced by Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive component of marijuana, are believed to be mainly mediated by the central CB1 receptor.[16] In order to avert central CB1 effects, research efforts have been focused on highly selective CB2 agonists and peripherally restricted CB1 or CB1/CB2 dual agonists.



1 Ki(hCB1): 1.7 nM
Ki(hCB2): 0.3 nM

Figure 1. Structure of lead compound **1**.

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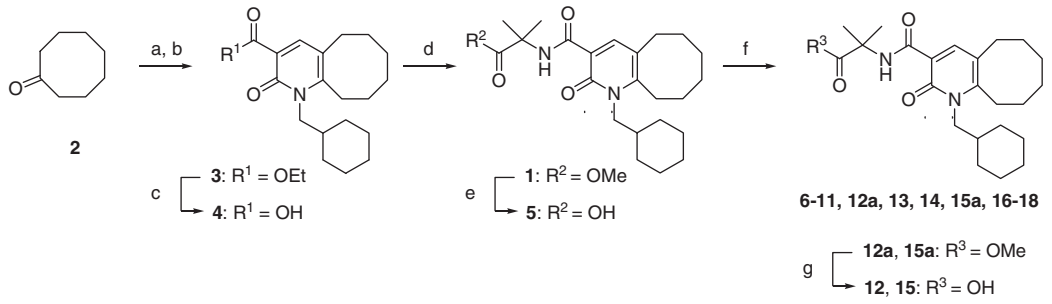
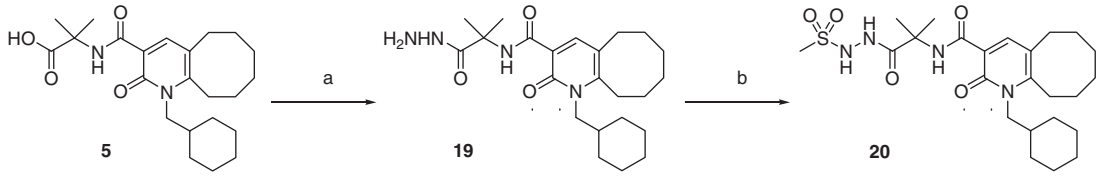


Scheme 1. Reagents and conditions: (a) cyclohexyl metylamine, toluene, reflux. (b) diethyl ethoxymethylene malonate, toluene, reflux. (c) aq. NaOH, THF. (d) Oxalyl chloride, cat. DMF, THF followed by dimethyl glycine methyl ester hydrochloride, Et_3N. (e) aq. NaOH, THF. (f) $(COCl)_2$, cat. DMF,THF followed byamines or hydrazines, Et_3N. (g) aq. NaOH, THF.



Scheme 2. Reagents and conditions: (a) NH_2NH_2 H_2O, WSCD, HOBt, DMF. (b) $MeSO_2Cl$, Et_3N, THF.

Our lead compound **1** from our compound library showed high affinity for both CB1 and CB2 receptors with K_i values of 1.7 and 0.3 nM, respectively (Fig. 1). To evaluate the CNS side effects of our compounds, the psychoactivity scoring test[17] was performed, and compound **1** exhibited strong CNS side effects such as catalepsy. Considering the CNS side effects by CB1 activation, exploring CB2 selective agonists would be one option. Alternatively, the CB1/2 dual agonist with poor brain penetration could eliminate the CNS side effect, especially when the compounds are administered topically. In this study, we adopted the latter approach by introducing a polar functional group at the 3-position of the pyridone core which restricts BBB permeability. Topological polar surface area (tPSA)[18] was utilized as the index to analyze the polarity of our compounds. This knowledge led to the finding of the clinical candidate of S-444823 and S-777469. The SAR of these compounds will be described in subsequent reports.[19]

The compounds used in this study were prepared as shown in Schemes 1 and 2. Cyclooctanone (**2)** was treated with cyclohexyl methylamine in refluxing toluene to give imine in situ, then diethyl ethoxymethylene malonate was reacted with refluxing to give **3** with a 3-carbamoyl 2-pyridone skeleton.[20] Hydrolysis of compound **3** afforded the core compound **4**. The carboxylic acid **4** was converted to acid chloride then condensed with dimethyl glycine methyl ester to give lead compound **1**. The key intermediate **5** was obtained by alkaline hydrolysis of **1** (Scheme 1). Compound **5** was converted to acid chloride as described above, then coupled with several amines to give the amide derivatives **6**–**11**, **12a**, **13**, **14**, **15a**, **16**–**18** (46–99%). Hydrolysis of esters (**12a** and **15a)** afforded the corresponding carboxylic acids **12** and **15** in 99% and 85%, respectively.

The syntheses of **19** and **20** are outlined in Scheme 2. Compound **19** was obtained by coupling compound **5** with hydrazine (81%). Treatment of **19** with methanesulfonyl chloride provided **20** (80%).

The affinities for CB1/CB2 of the compounds **1–20** were evaluated and the K_i values are shown in Table 1. Carboxylic acid **5** showed very weak affinities for both hCB1 and hCB2 receptors compared with the corresponding methyl ester **1**, suggesting that carboxylic functionality is not tolerated to keep the affinities. This trend was also found for other carboxylic acids, such as **12** and **15**, so we next focused on the amide derivative with a polar functional group at the chain terminal. As a clue of incorporation of the polar group, compound **6**, replacing by the partial structure of anandamide,[21] possessed affinities as high as **1**. In addition, other amide derivatives **7–9** with a terminal hydroxyl group also showed strong affinities for CB1 receptors, while compound **10** with a terminal amino group had decreased affinities. When the terminal carboxylate or amino functional group was converted to the corresponding $-CONH_2$ or acetamide, the affinities were comparable with the terminal –OH analogues (**11** and **13**). Piperidine analogues (**14**, **15** and **17**) with a functional group at the 4-position of the piperidine ring also showed comparable affinity as found with open chain analogues.

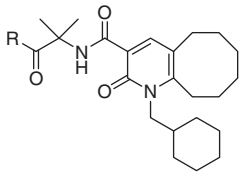
With some potent analogues in hand, we next examined the CNS side effects responsible for CB1 affinity. To qualitatively estimate the level of CB1-induced CNS side effects, we conducted the psychoactivity scoring (PS) test using mice.[17] The apparent behaviors of mice ($n = 3$) at 15 min after intravenous injection were evaluated with the score based on Table 2. PS ($0 \leqslant PS \leqslant 10$) represents the total of the average score at two doses (1.0 mg/kg and 0.1 mg/kg). tPSA values were also calculated as physicochemical parameters to estimate the polarities of each molecule. The relationships between PS and tPSA are shown in Table 3.

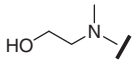
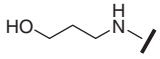
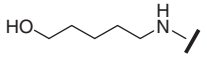
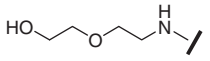
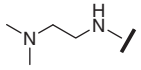
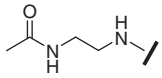
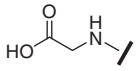
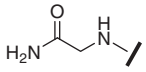
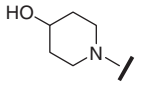
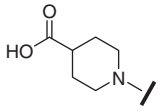
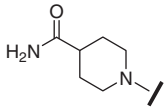
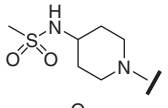
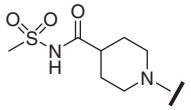
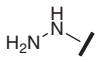
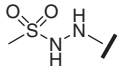
The lead compound **1**, which has a tPSA value of 75.71 Å^2, induced spontaneous catalepsy by intravenous injection, exhibited very strong CNS activity (PS = 9.5) as expected. Terminal alcohol derivatives, such as **6** and **14**, which have the same tPSA value (89.95 Å^2) improved the PS score, causing mild catalepsy (PS = 6.7 and 3.6, respectively). The compounds with much higher tPSA value (**7–9)** also reduced the CNS side effects ($PS \leqslant 3.6$) in spite of high affinities for CB1 receptors as the lead compound **1**.

Compound **11**, **13** and **16**, with carboxamide derivatives at the terminal position, have tPSA values over 100 Å^2 and displayed a mild CNS side effect. From these results, there seems to be a good inverse relationship between PS and tPSA (Fig. 2) suggesting that the introduction of a polar functional group may successfully reduce the CNS effects caused by CB1 receptor affinity.

Encouraged by the above findings, we next introduced a sulfonyl moiety as a more polar functional group. Sulfonamide **17** had slightly weak affinity for CB1 receptor compared with **16**.

Table 1
Affinities for CB1 and CB2 receptor



Compd	R	K_i (hCB1) (nM)[a]	K_i (hCB2) (nM)[a]
1	MeO—	1.7	0.3
5	HO—	1351	349
6		2.9	0.6
7		6.3	1.0
8		5.8	1.2
9		3.4	0.6
10		242	81
11		4.7	2.5
12		>5000	2823
13		11	6.0
14		8.0	1.1
15		2970	377
16		4.9	3.0
17		39	4.4
18		218	128
19		30	2.8
20		32	7.3

[a] See Ref. 22 for assay protocol.

Compound **18**, with acyl sulfonamide introduced as the more polar functional group, showed loss of affinities. In the case of hydrazides, **19** and **20** showed moderate affinities without improving the affinity for CB1 receptors. Compound **17** with a high tPSA value (115.89 Å^2) showed no CNS side effect on injection to mice (PS = 0). While hydrazide **19** represented medium PS (PS = 3.0), its methanesulfonylated compound **20** increased tPSA (tPSA = 124.68)

Table 2
The score for the level of CB1-induced side effects

Status	Score
Spasm, rigor	5
Catalepsy	4
Stretching the body without moving	3
Walking with lying on the belly	2
Moving sluggishly	1
Moving actively	0

Table 3
Psychoactive score (PS) and tPSA

Compd	PS [a]	tPSA (Å^2)[b]
1	9.5	75.71
6	6.7	89.95
7	3.6	98.74
8	2.5	98.74
9	1.9	107.97
11	2.2	107.61
13	0.2	121.6
14	3.6	89.95
16	0.2	112.81
17	0	115.89
19	3.0	104.53
20	0	124.68

[a] See Ref. 17 for assay protocol.
[b] Calculated by ChemDraw Ultra 9.0.7.

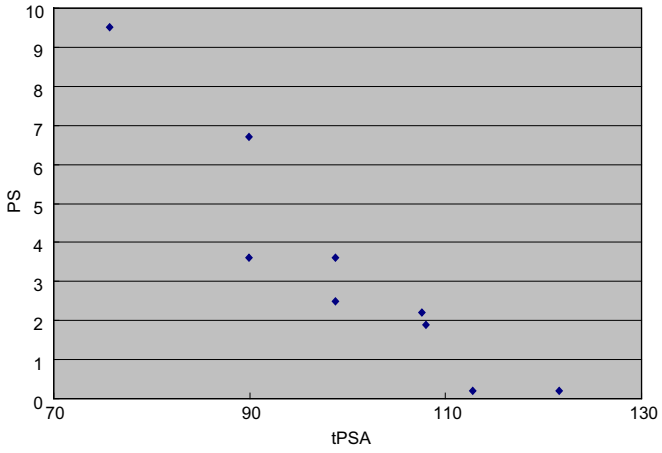


Figure 2. Relationship between psychoactive score (PS) and tPSA.

and showed no side effects (PS = 0). The representative compounds (**1**, **6** and **20**) were evaluated for their ability to penetrate the CNS in rats. Compound **1** that showed strong catalepsy exhibited good brain penetrability with brain/plasma ratio of 3.35. Compound **6**, by converting the ester moiety to a more polar functional group exhibited a lower brain/plasma ratio (0.28). Compound **20** without CNS side effects had a very low brain/plasma ratio (0.02). These results supported the relationship between PS and brain/plasma ratio.

The selected compounds **17** and **20** without CNS side effects were tested with our pruritic model induced by Compound 48/80 (Fig. 3).[23] Painting at 1.0% of these compounds strongly inhibited scratching with percent inhibition levels of 97% and 87%, respectively.

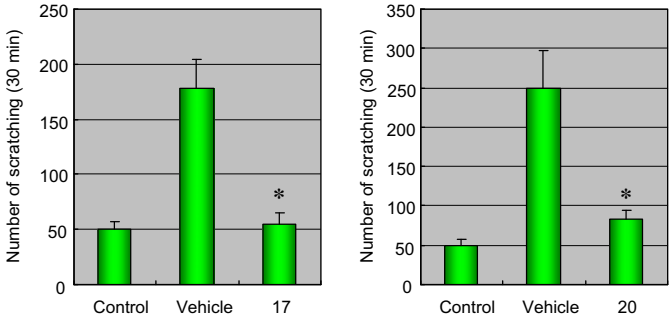


Figure 3. Inhibition scratching of compound **17** and **20** in the pruritic model induced by Compound **48**/**80** in mice.

In summary, hypothesizing that addition of polar functional groups would limit CNS exposure, we successfully decreased the side effects by incorporating polar functional groups on the terminal side chain of the 3-position on the lead compound **1**. Compounds **13**, **16**, **17** and **20** with higher tPSA values (tPSA >110) displayed little psychoactive side effect. Compound **17** and **20**, in particular, showed no CNS side effects via intravenous administration in mice. Both strongly inhibited scratching induced by Compound 48/80 in the pruritic model. This study showed that it is possible to reduce the CB1-induced CNS side effects of CB1/2 dual agonists to treat atopic disease.

Acknowledgments

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17. Psychoactivity scoring test (Psychoactive score: PS): In order to qualitatively estimate the level of CB1-induced CNS side effects, test compounds were dissolved in MDAA and PEG solution and intravenously injected into the tail of the ICR mice (0.1 mg/kg and 1.0 mg/kg, $n = 3$ each). The apparent behavior of each mouse was observed at 15 min after injection and the score (5: Tonic convulsion; 4: Catalepsy; 3: Prone position, Sedation; 2: Crawling; 1: Decrease in locomotor activity; 0: Normal) was determined. The higher the score, the more potent were the CNS side effects.
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19. (a) Odan, M.; Ishizuka, N.; Hiramatsu, Y.; Inagaki, N.;Hashizume, H.; Fujii, Y.; Mitsumori, S.; Morioka, Y.; Soga, M.; Deguchi, M.; Yasui, K.; Arimura, A. Bioorg. Med. Chem. Lett. accepted for publication, doi:10.1016/j.bmcl.2012.02.050; (b) Odan, M.; Ishizuka, N.; Hiramatsu, Y.; Inagaki, N.;Hashizume, H.; Fujii, Y.; Mitsumori, S.; Morioka, Y.; Soga, M.; Deguchi, M.; Yasui, K.; Arimura, A. Bioorg. Med. Chem. Lett., doi:10.1016/j.bmcl.2012.02.072.
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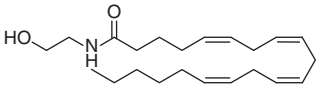
21. Structure of 2-arachidonoylethanolamine (anandamide)



22. Binding assay: CB receptor binding assay was carried out using the membrane; recombinant human CB1 (hCB1), CB2 (hCB2), radioligand [3H]-CP55940. Membrane fractions, used for the measurement of binding activity, were prepared as reported elsewhere and stored in a deep freezer (−80 °C). In brief, confluent cultures of the hCB1 and hCB2 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, 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.
23. In vivo assay (pruritic model): Crj:CD-1 (ICR) (Japan Charles River Lab.) mice were used for scratching tests to investigate the antipruritic effect. Test compounds were dissolved in acetone (Sigma). Compound **48**/**80** as a pruritogen was dissolved at 60 μg/ml in isotonic saline (Otsuka Pharma.). Test compounds were painted on the shaved back of mice. After fifteen minutes, 50 μl of pruritogen was injected intradermally into the back of the mice. Thereafter, their behavior was videotaped to count the scratching behavior for 30 min. The value of% inhibition was calculated by fitting the equation:% inhibition = (B–A) – (C–A)/(B–A/100); scratching of the control ($n = 29$) as A, scratching of vehicle ($n = 6$) as B, scratching of test mice ($n = 6$) as C.