Bioorganic & Medicinal Chemistry Letters 22 (2012) 2803–2806

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of S-777469: An orally available CB2 agonist as an antipruritic agent

Masahide Odan *, Natsuki Ishizuka, Yoshiharu Hiramatsu, Masanao Inagaki, Hiroshi Hashizume, Yasuhiko Fujii, Susumu Mitsumori, Yasuhide Morioka, Masahiko Soga, Masashi Deguchi, Kiyoshi Yasui, Akinori Arimura

Shionogi Pharmaceutical Research Center, Shionogi & Co., Ltd 1-1, Futaba-cho 3-chome, Toyonaka, Osaka 561-0825, Japan

ARTICLE INFO

Article history:
Received 23 January 2012
Revised 16 February 2012
Accepted 23 February 2012
Available online 3 March 2012

Keywords:
Cannabinoid
CB receptor agonist
GPCR
Antipruritics

ABSTRACT

The discovery of novel CB2 ligands based on the 3-carbamoyl-2-pyridone derivatives by adjusting the size of side chain at 1-, 5- and 6-position is reported. The structure–activity relationship around this template lead to the identification of **S-777469** as a selective CB2 receptor agonist, which exhibited the significant inhibition of scratching induced by Compound 48/80 at 1.0 mg/kg po and 10 mg/kg po (55% and 61%, respectively).

CB1 is mainly expressed in neurons in the brain[1,2] and is responsible for most the psychoactive properties of cannabinoids. CB2 is abundant in splenocytes and leukocytes[3] and is responsible for many of the immunomodulatory and anti-inflammatory effects of cannabinoids.[4] Because of the predominant peripheral distribution of the CB2 receptor, CB2 selective agonists are very attractive therapeutic agents without CB1 mediated psychoactive effects.

Recently, various chemical scaffolds having high affinity and selectivity for CB2 receptor have been reported. Preclinical data support the effects of these cannabinoids in a wide range of pain models, including neuropathic pain and chronic pain. In spite of the high CB2 selectivity, most of these compounds exhibit CB1 mediated side effects at higher doses. For example, CB2 selective ligand A-836339 (Abbott) possesses high hCB2 affinity (0.64 nM) with 425-fold selectivity against hCB1[5] and does not display significant affinity at other GPCRs and ion channels. However, at high doses, A-836339 displayed brain CB1 mediated decrease of spontaneous motor activity.[6]

Cannabinoid ligands often have highly lipophilic structures that facilitate passage through the blood–brain barrier (BBB). One way to limit CNS exposure is to add polar functional groups such as carboxylic acid. Ajulemic acid, CB1/CB2 dual agonist, exhibits analgesic and anti-inflammatory activity.[7–9] However, some CNS side effects were displayed. This compound may pass through the BBB and activate the central CB1 receptor.[10] These findings indicate the need to discover a safer CB agonist having high CB2 selectivity and CNS exposure restriction.

Recently, we reported on CB receptor agonists having carboxylic acid at the 3-position in the 3-carbamoyl-2-pyridone system (Fig. 1). Among these compounds, compound **1** exhibited CB2 selectivity (K_i (hCB1): 793 nM, K_i (hCB2): 6.2 nM). However, in spite of good selectivity for CB2 (>130) and the presence of carboxylic acid which could limit BBB penetration, compound 1 displayed CNS side effects in our psychoactive test (PS = 0.7).[11] It seemed that the high lipophilicity of compound **1** facilitated passage through the BBB and could induce CNS side effects by activating CB1 receptors. Therefore, we directed our attention to reducing the lipophilicity by using *C*log*P* as an index to design an analogue of compound **1** which would not show any CNS side effects.

The syntheses and structures of the compounds in this Letter were presented in Scheme 1 and Table 1. As a general synthetic scheme, amines were reacted with various ketones in refluxing toluene to give the corresponding imines in situ, then diethyl ethoxymethylene malonate was reacted with refluxing to give 3-carbamoyl-2-pyridones **1a**–**16a** (24–98%).[12] Hydrolysis of compound **1a**–**16a** afforded the core compound **1b**–**16b** (47–98%). Carboxylic acid **1b**–**16b** were converted to acid chloride then condensed with the appropriate amino acid methyl esters to give compound **1c**–**16c** (66–97%). Compound **1**–**16** were obtained by alkaline hydrolysis of the corresponding esters (75–98%).

First, we investigated various Z groups to reduce *C*log*P*. We utilized various α-amino acids in the pyridone ring-constructing step to introduce various Z groups (**1**–**6**). The binding affinities of these compounds with different Z groups are summarized in Table 2.

* Corresponding author.
E-mail address: masahide.oodan@shionogi.co.jp (M. Odan).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2012.02.072

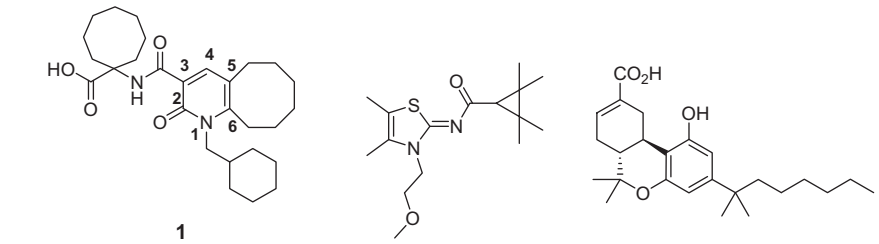
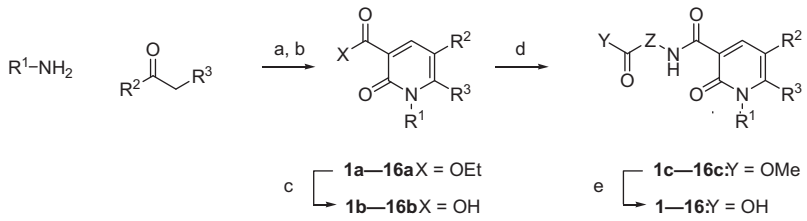


Figure 1. Structures of the lead compound **1**.



Scheme 1. Reagents and conditions : (a) toluene, reflux. (b) diethyl ethoxymethylene malonate, toluene, reflux. (c) aq NaOH, THF. (d) oxalyl chloride, cat. DMF, THF followed by amino acid methyl ester, Et_3N. (e) aq NaOH, THF.

Unsubstituted methylene derivative **2** showed decreased affinities for CB receptors, especially for the CB2 receptor, compared with compound **1**. Substitution with small alkyl groups such as the *gem*-dimethyl group did not improve the affinities. Among the *spiro*-derivatives, cyclohexylidene analogue **5** had high affinities as compound **1**. Cyclohexyl substituted congener **6** resulted in reduction of affinities. These results suggest that a *spiro*-framework and an appropriate ring size are crucial for the affinities for CB receptors. In our psychoactive test, compound **5** did not show CNS side effects (PS = 0).[11] Judging from the *C*log*P* value, compound **5** had still high lipophilicity (*C*log*P* = 6.20), so we next explored the possibility of further reducing the lipophilicity by modification of other moieties.

To reduce the lipophilicity, less lipophilic R1 groups were introduced. The binding affinities of the compounds having several side chains at the 1-position of pyridone skeleton are summarized in Table 3.

The compound substituted with *n*-butyl: **7** exhibited moderate affinity and strong IC_{50} value for CB2 receptor. Interestingly, the affinity for CB1 receptor was drastically reduced in comparison with **5**. However, the introduction of methoxyethyl moiety: **8** led to marked reduction in affinities for CB2 receptor compared with **7**. As another approach to reduce the lipophilicity, introduction of a polar group such as tetrahydrofuranyl methyl: **9** and *N*-ethyl pyrrolidinyl methyl: **10** led to marked reduction in affinities for CB receptors. In the case of a compound having a benzyl group with slightly reduced *C*log*P*, introduction of *p*-fluorobenzyl: **11** led to moderate affinities for CB receptors compared with **5**. In contrast, *p*-cyano derivative **12** led to low affinities for CB receptors. From these data, the 1-position of the pyridone skeleton was restricted at the lipophilic site and did not tolerate the polar groups. However, these results suggest that reduction of lipophilicity at the 1-position contributes to improve the selectivity for CB2 against CB1.

To optimize the 5- and 6-substituents of the pyridone skeleton, we directed our attention toward opening the 8-membered ring to reduce lipophilicity. The CB receptors affinities of those compounds (**13**–**16**) are shown in Table 4.

Table 1
Structure of compounds in this manuscript

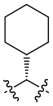
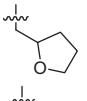
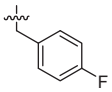
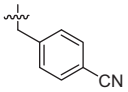
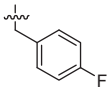
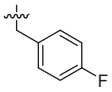
Compd	Z	R^1	R^2	R^3
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				

Table 1 (*continued*)

Compd	Z	R^1	R^2	R^3
11				
12				
13			Me	Et
14			Br	*n*-Pr
15			Me	Et
16			Et	*n*-Pr

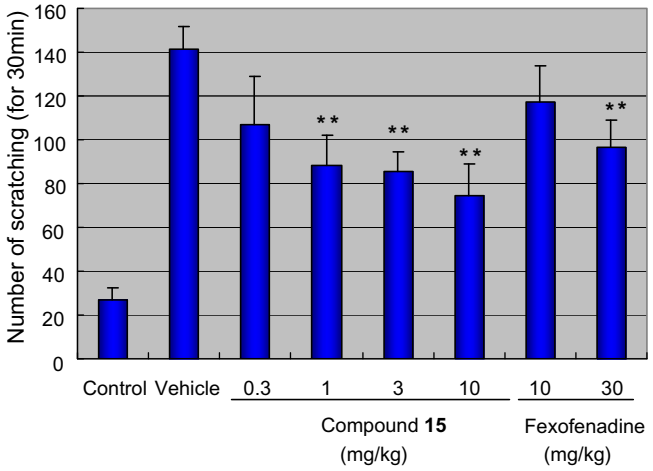


Figure 2. Evalution of **15** with the pruritic model of the ICR mice. Compound **15** was dosed after injection of Compound 48/80 as a pruritogen. $n = 9–11$ per group. Data are expressed as a mean percentage of vehicle ± SEM and the results of two experiments. $^*P$ <0.05 versus vehicle by Welch test.

Table 2
*C*log*P* and CB receptor affinities of compound **1–6**

Compd	*C*log*P* [a]	K_i[b] (hCB1) (nM)	K_i[b] (hCB2) (nM)	IC_{50}[c] (nM) CB1 (cAMP)	IC_{50} (nM)[c] CB2 (cAMP)
1	7.32	793	6.2	468	1.0
2	4.27	1056	177	—	—
3	4.89	1354	349	—	—
4	5.64	258	56	113	14
5	6.20	181	6.7	76	7.0
6	6.70	3062	111	>2000	51

[a] Calculated by ChemDraw Ultra 9.0.7.
[b] See Ref. 13 for assay protocol.
[c] See Ref. 14 for assay protocol.

Table 3
*C*log*P* and CB receptor affinities of compound **7–12**

Compd	*C*log*P*[a]	K_i[b] (hCB1) (nM)	K_i[b] (hCB2) (nM)	IC_{50}[c] (nM)CB1 (cAMP)	IC_{50}[c] (nM) CB2 (cAMP)
5	6.20	181	6.7	76	7.0
7	5.14	>5000	56	>2000	7.0
8	3.77	>5000	294	—	—
9	3.99	>5000	247	—	—
10	2.61	—	2114	—	—
11	5.46	266	16	514	9.0
12	4.75	3952	144	—	—

[a] Calculated by ChemDraw Ultra 9.0.7.
[b] See Ref. 13 for assay protocol.
[c] See Ref. 14 for assay protocol.

Table 4
*C*log*P* and CB receptor affinities of compound **13–16**

Compd	*C*log*P*[a]	K_i[b] (hCB1) (nM)	K_i[b] (hCB2) (nM)	IC_{50}[c] (nM) CB1 (cAMP)	IC_{50}[c] (nM) CB2 (cAMP)
13	3.92	—	348	—	—
14	4.98	>5000	382	—	—
15	4.25	4607	36	>2000	24
16	5.304	>5000	263	—	—

[a] Calculated by ChemDraw Ultra 9.0.7.
[b] See Ref. 13 for assay protocol.
[c] See Ref. 14 for assay protocol.

The *n*-butyl (**13** and **14**) or *p*-fluorobenzyl (**15** and **16**) group was selected as the R^1 substituent due to their favorable CB2 affinity and the selectivity against CB1 receptor for optimization. For analogues with the *n*-butyl group at the 1-position, ring opening resulted in loss of affinities for CB receptors (**13** and **14**). However, when the *p*-fluorobenzyl group was used as R^1, compound **15** exhibited moderate potency for CB2 receptor and excellent selectivity (>120). In addition, *C*log*P* of compound **15** was dramatically reduced to 4.25. The reduction of lipophilicity might avert the undesired CNS side effects (PS = 0). Introducing the longer alkyl chain **16** led to decreased affinities for CB receptors.

The selected compound **15** was further evaluated with the in vivo animal model.[15] The inhibition of scratching induced by Compound 48/80 is shown in Figure 2. In this test, compound **15** was compared with Fexophenadine, which is a type of antihistamine. Fexophenadine was mild inhibition of scratching at 30 mg/kg (39%). On the other hand, compound **15** revealed significant inhibitions when dosed at 1.0 mg/kg (46%), 3.0 mg/kg (48%) and 10 mg/kg (59%).

The data indicated that CB2 selective agonists had the antipruritic effect. The pharmacokinetic properties of **15** were evaluated in several species (Table 5). Compound **15** had moderate clearance and absorption across species.

In summary, we discovered novel CB2 receptor agonists containing a 3-carbamoyl-2-pyridone system with carboxylic acid at the 3-position. Among them, we selected compound **15** (**S-777469**) as a CB2 selective agonist for development. This compound is considered to be a promising candidate for an orally active antipruritic.

Table 5
Pharmacokinetic Properties of **15**

Species	Clearance (mL/min/kg)[a]	$t_{1/2}$[b] (h)	V_{dss}[c] (L/Kg)	BA[d] (%)
Rat	11.3	6.20	1.90	37.9
Dog[c]	6.00	12.8	1.23	18.7
Monkey[d]	13.6	7.43	0.87	9.01

[a] iv dose at 3.0 mg/kg.
[b] Oral dose at 10 mg/kg.
[c] Beagle dog.
[d] Cynomolgus monkey.

Acknowledgments

The authors thank Drs. Hiroki Sato and Yutaka Yoshida for many helpful suggestions.

References and notes

1. Herkenham, M.; Lynn, A. B.; Little, M. D.; Johnson, M. R.; Melvin, L. S.; de Costa, B. R.; Rice, K. C. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 1932.
2. Mailleux, P.; Vanderhaeghen, J. J. *Neuroscience* **1992**, *48*, 655–668.
3. Munro, S.; Thomas, K. L.; Abu-Shaar, M. *Nature* **1993**, *365*, 61.
4. Ashton, J. C. *Curr. Opin. Investig. Drugs* **2007**, *8*, 373.
5. McGaraughty, S.; Chu, K. L.; Dart, M. J.; Yao, B. B.; Meyer, M. D. *Neuroscience* **2009**, *158*, 1652.
6. Yao, B. B.; Hsieh, G.; Daza, A. V.; Fan, Y.; Grayson, G. K.; Garrison, T. R.; Kouhen, O. E.; Hooker, B. A.; Pai, M.; Wensink, E. J.; Salyers, A. K.; Chandran, P.; Zhu, C. Z.; Zhong, C.; Ryther, K.; Gallagher, M. E.; Chin, C.; Tovcimak, A. E.; Hradil, V. P.; Fox, G. B.; Dart, M. J.; Honore, P.; Meyer, M. D. *J. Pharmacol. Exp. Ther.* **2009**, *328*, 141.
7. Burstein, S. *AAPS J.* **2005**, *7*, E143.
8. Burstein, S. H.; Karst, M.; Schneider, U.; Zurier, R. B. *Life Sci.* **2004**, *75*, 1513.
9. Burstein, S. H.; Zurier, R. B. *AAPS J.* **2009**, *11*, 109.
10. Vann, R. E.; Cook, C. D.; Martin, B. R.; Wiley, J. L. *J. Pharmacol. Exp. Ther.* **2007**, *320*, 678.
11. Estimation of in vivo psychoactivity: 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 are the CNS side effects.
12. Bondavalli, F.; Bruno, O.; Presti, E. L.; Menozzi, G.; Mosti, L. *Synthesis* **1999**, *7*, 1169–1174.
13. **Binding assay**: CB receptor binding assay was carried out using the membrane; recombinant human CB1 (hCB1), CB2 (hCB2), radioligand [^{3}H]-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 100,000g 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.
14. *Cyclic AMP assay method*: The CHO cells expressing hCB1 or hCB2 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, 0.1% BSA). The cells were stimulated with 4 μM forskolin at 37 °C for 25 min (hCB1 cells) or 45 min (hCB2 cells). The cAMP concentrations in the cells were measured using cAMP kits (CIS Bio International).
15. *In vivo assay (pruritic model)*: Crj:CD-1 (ICR) (Japan Charles River Lab.) mice were used for the scratching test to investigate the antipruritic effect. Test compounds were dissolved in acetone (Sigma). Compound 48/80 as a pruritogen dissolved at 75 μg/ml in isotonic saline (Otsuka Pharma.). Test compounds were administered as forced oral dosages to mice. After 1 h, 40 μl of pruritogen was injected intradermally into the back of mice. Thereafter, their behavior was videotaped to count the scratching behavior for 30 min. The % inhibition value was calculated with the equation: % inhibition = (B–A) – (C–A)/(B–A/100); scratching of control ($n = 29$) as A, scratching of vehicle ($n = 6$) as B, scratching of test mice ($n = 6$) as C.