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The SAR studies of novel CB2 selective agonists, benzimidazolone derivatives

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Abstract—Benzimidazolone derivatives were discovered as novel CB2 selective agonists. Structure Activity Relationship (SAR) studies around them were examined to improve metabolic stability. Compound **39** exhibited excellent metabolic stability in human liver microsomes (HLM) and significant attenuation of the chronic colonic allodynia in the TNBS-treated rats by po administration.

A human cannabinoid receptor 2 (hCB2)[1,2] was cloned in 1993 and has been known to express mainly in the cells of immune systems. In contrast, CB1 receptor[3] is expressed principally in the central nervous system (CNS) and responsible for the overt physiological effect of cannabinoids. CB2 selective agonists[4] represented by AM1241 (**2**) [5] and HU-308[6] were reported to mediate peripheral antinociception without CNS related side-effects.[5,7] Therefore, CB2 receptor has a potential to be a promising drug target. In this paper, we report the SAR studies of novel CB2 selective agonists, 1,3-dihydro-2*H*-benzimidazol-2-one (benzimidazolone) derivatives, for the treatment of chronic colonic allodynia.[8]

The representative CB2 agonists (**1**–**4**),[5,9–11] are listed in Figure 1. These compounds have a couple of common structural features such as an amine (e.g., piperidine or morpholine) at 1-position and a lipophilic moiety at 3-position on the indole or indazole cores. In order to find novel CB2 agonists, we tried to replace the indole and indazole cores with benzimidazolone and 3,4-dihydro-2(1*H*)-quinazolinone. For initial studies, we selected naphthalene as the lipophilic moiety and morpholine as the amine.

The benzimidazolone derivatives (**8** and **9**) were synthesized in four steps starting from 2-fluoronitrobenzene (**5**) (Scheme 1). Morpholinoethylamine was introduced by S_NAr reaction followed by reduction to give diaminobenzene **6**. The subsequent carbonylation with 1,1′-carbonyldiimidazole (CDI) gave benzimidazolone **7**. The external urea formation was carried out via carbonate intermediate using *p*-nitrophenyl chloroformate followed by addition of 1-naphthylamine or its non-aniline[12] analog, (*R*)-1,2,3,4-tetrahydro-1-naphthylamine. This procedure is useful for the syntheses of various

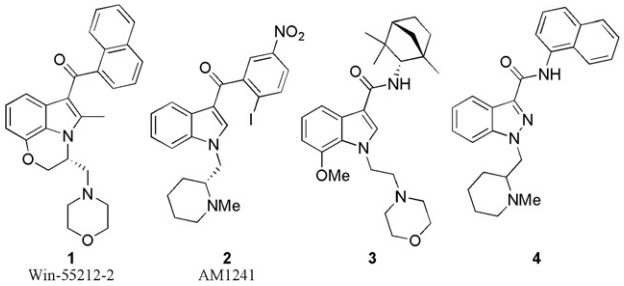

Figure 1. Structures of representative CB2 agonists.

Keywords: Cannabinoid; CB2 agonist; Benzimidazolone; Metabolic stability.
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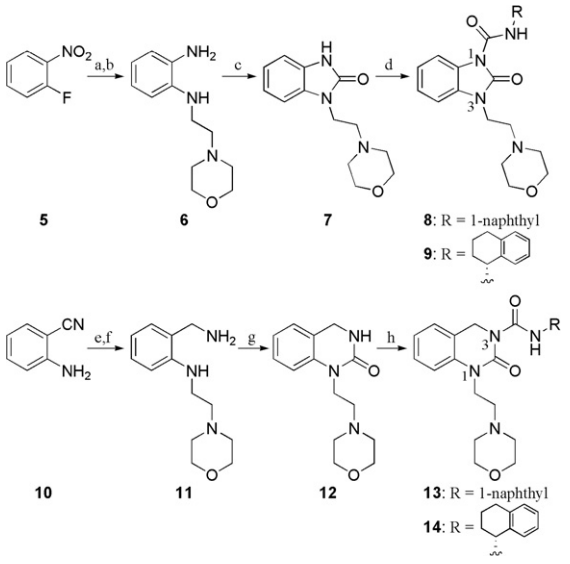


Scheme 1. Reagents and conditions: (a) 4-(2-aminoethyl)morpholine, K_2CO_3, THF, rt, 20 h, 97%; (b) H_2 (4 atm), Pd/C, THF, rt, 5 h; (c) CDI, THF, rt, 20 h, 83% (2 steps); (d) 4-nitrophenyl chloroformate, triethylamine, dichloromethane, rt, 3 h; then 1-naphthylamine or (*R*)-1,2,3,4-tetrahydro-1-naphthylamine, 81% or 67%, respectively (2 steps); (e) *N*-(2-chloroethyl)morpholine hydrochloride, sodium hydride, DMF, 80 °C, 2 h, 51%; (f) lithium aluminum hydride, THF, rt, 4 h; (g) CDI, THF, rt, 4 h, 49% (2 steps); (h) 4-nitrophenyl chloroformate, triethylamine, dichloromethane, rt, 3 h; then 1-naphthylamine or (*R*)-1,2,3,4-tetrahydro-1-naphthylamine, 15% or 55%, respectively (2 steps).

benzimidazolone derivatives for optimization at 1- and 3-positions.[13]

The quinazolinone derivatives (**13** and **14**) were also synthesized in four steps starting from 2-aminobenzonitrile (**10**) (Scheme 1). Alkylation of **10** followed by reduction gave diamine **11**. The subsequent carbonylation and urea formation process were the same process as benzimidazolone derivatives **8** and **9**.

As a result, benzimidazolone **8** was found to have CB2 agonist activity (Table 1). Its non-aniline derivative, benzimidazolone **9**, also showed potent (0.7 nM) agonist activity and 250-fold selectivity for CB2 receptor over CB1. In contrast, quinazolinone **13** was a partial agonist and its non-aniline derivative, quinazolinone **14** was an inverse agonist. Therefore, **9** was selected as a lead compound.

A half-life of compound **9** in human liver microsomes (HLM) was not calculated due to its rapid metabolism (Table 1). The major metabolite of **9** in HLM was identified as a product oxidized on the tetrahydronaphthalene ring. Therefore our effort was focused on the optimization at tetrahydronaphthalene moiety at 1-position on the benzimidazolone ring to address the metabolic liability.

In order to improve metabolic stability, two general strategies were applied; (1) structural changes around metabolic sites to block metabolism, (2) reduction of molecular lipophilicity.[14] Table 2 shows the results of metabolic stabilities of representative compounds prepared using various amines according to Scheme 1.

Removal of benzene ring remained metabolically unstable with decreased CB2 binding activity (**15**). On the other hand, secondary amine-derived compounds represented by **16** did not have CB2 binding activity. This result suggested that the fixed conformation at 1-position on the benzimidazolone ring of **15** presumably controlled by hydrogen bonding between hydrogen on urea and oxygen of benzimidazolone was necessary for CB2 binding activity.[15] Therefore, further optimization was carried out by using primary amine-derived compounds. Reducing alkyl portion still remained metabolically unstable as exemplified by **17** and **18**.

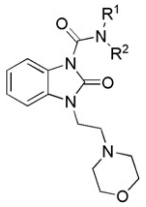
Among compounds possessing hydroxyl groups, only **20** had improved metabolic stability (HLM $T_{1/2}$ = 40 min) though its potency was weak. Introduction of amino acids-derived amides was effective to improve metabolic stability (**23** and **24**). Especially **24** was the best compound in terms of both CB2 binding activity and metabolic stability. Through intensive studies of various alkyl groups instead of *tert*-butyl group as exemplified by **25**, **26**, and **27**, *tert*-butyl moiety was found to be the best to show potent CB2 binding activity. Replacement of the amide moiety of **24** with nitrile, tetrazole, and oxadiazole decreased half-lives in HLM, although they showed

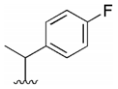
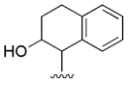
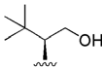
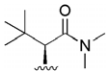
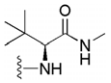
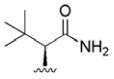
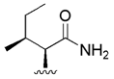
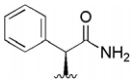
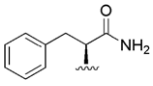
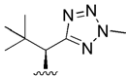
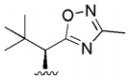
Table 1. Benzimidazolone and quinazolinone derivatives

Compound	hCB2 K_i [a] (nM)	hCB2 EC_{50} [b] (nM)	hCB2 E_{max} [b] (%)	hCB1 K_i [c] (nM)	HLM $T_{1/2}$ (min)
8	8	8	95	NT[d]	11
9	7	0.7	107	1730	NC[e]
13	41	22	28	NT[d]	4
14	9	ND[f]	−98[g]	1510	NC[e]

[a] Measured as the K_i value for the displacement of tritiated CP55940 from membrane of human CB2 transfectant cell.
[b] The inhibition of forskolin-stimulated cAMP production by CB2 agonists was measured. E_{max} values represent the relative efficacy that is defined as the ratio of the maximum response of test compound to the maximum response of 2-arachidonoylglycerol.
[c] Measured as the K_i value for the displacement of tritiated SR141716A from membrane of human CB1 transfectant cell.
[d] Not tested.
[e] Not calculated because the compound was not detected at 10 min after incubation in HLM.
[f] Not determined.
[g] % inhibition at 30 nM.

Table 2. Representative SAR data at 1-position



Compound	R^1	R^2	hCB2 K_i (nM)	hCB1 K_i (nM)	HLM $T_{1/2}$ (min)
15		H	60	>10,000[a]	4
16		Me	>1000[a]	NT[b]	NT[b]
17		H	232	>10,000[a]	2
18		H	74	2100	2
19		H	51	>10,000[a]	2
20		H	484	>10,000[a]	40
21		H	15	>10,000[a]	5
22		H	25	>10,000[a]	3
23		H	20	2720	20
24		H	5	>10,000[a]	35
25		H	67	>4040	17
26		H	>2500[a]	NT[b]	NC[c]
27		H	>2500[a]	NT[b]	NC[c]
28		H	5	>10,000[a]	3
29		H	5	1480	4
30		H	15	>10,000[a]	3

[a] IC_{50}.
[b] Not tested.
[c] Not calculated because the compound was not detected at 10 min after incubation in HLM.

potent CB2 binding activity working as bioisosteres of the amide. Therefore, R^1 was fixed with *tert*-leucinamide for further modifications.

Although the half-life of **24** was improved compared to lead compound **9**, it was still insufficient. One of major metabolic parts of **24** in HLM was identified as morpholine moiety by a metabolite identification study. Therefore, the optimization at morpholine group was examined in order to improve metabolic stability (Table 3).

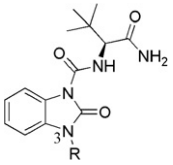
Removals of oxygen and/or nitrogen of the morpholine group were attempted as structural changes around the metabolic site to block metabolism. Eliminating amine moiety (**31** and **32**) did not improve metabolic stability while retaining potent CB2 binding activity. On the other hand, **33** and **34** without ether moiety showed HLM half-lives of >120 min suggesting that ether moiety in morpholine of **24** was a major metabolic site, although their CB2 binding activities were weak.[16] Removal of both ether and amine moieties (**35**) resulted in poor metabolic stability while showing the most potent CB2 binding activity.

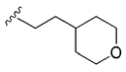
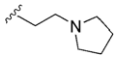
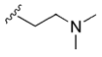
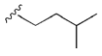
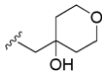
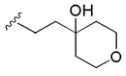
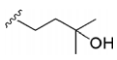
To reduce molecular lipophilicity, introductions of hydroxyl group into the potent compounds **31** and **35** were carried out (**36**–**39**). As a result, **39** showed significant improvement of metabolic stability (>120 min) while preserving potent CB2 binding activity (8 nM).

The profile of compound **39**[17] is summarized in Table 4. It showed potent CB2 agonist activity and approximately 180-fold selectivity for CB2 over CB1. Compound **39** exhibited a good PK profile (33% of bioavailability in rat) and significantly attenuated the chronic colonic allodynia in the TNBS-treated rats[18] (3 mg/kg, po).

In summary, benzimidazolone derivatives were identified as novel CB2 agonists based on structural analogy of existent CB2 agonists. The SAR study of benzimidazolone derivative was focused on the improvement

Table 3. Representative SAR data at 3-position



Compound	R	hCB2 K_i (nM)	hCB1 K_i (nM)	HLM $T_{1/2}$ (min)	log D[a]
31		4	1470	15	2.4
32		6	1320	37	2.1
33		242	NT[b]	>120	1.5
34		338	NT[b]	>120	1.5
35		1	69	10	3.4
36		92	3010	>120	1.6
37		144	>10,000[c]	84	1.9
38		196	>10,000[c]	>120	2.2
39		8	1459	>120	2.2

[a] At pH 7.4.
[b] Not tested.
[c] IC_{50}.

Table 4. The pharmacological profile of compound **39**

hCB2 K_i	8 nM
hCB2 EC_{50}	2 nM
hCB2 E_{max}	118%
hCB1 K_i	1459 nM
Selectivity CB1/CB2	182
HLM $T_{1/2}$	>120 min
Bioavailability in rat	33%
log D[a]	2.2

[a] At pH 7.4.

of metabolic stability by structural changes around metabolic sites and/or the reduction of molecular lipophilicity. The SAR study starting from **9** resulted in the discovery of **39** as the best compound. **39** showed 180-fold CB2 selectivity over CB1, excellent metabolic stability in HLM, and oral activity in rat visceral allodynia.

Acknowledgments

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11. Compound **4**: Makriyannis, A.; Liu, Q. WO03/035005.
12. Toxicity of aniline was reported. see: (a) Famulok, M.; Boche, G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 468; (b) Marques, M. M.; Mourato, L. L. G.; Amorim, M. T.; Santos, M. A.; Melchior, W. B., Jr.; Beland, F. A. *Chem. Res. Toxicol.* **1997**, *10*, 1266.
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15. Similar trends were reported by other research groups. see: Refs. 2e,10b.
16. The introduction of a methyl group at 4-position on the benzimidazolone ring of compound **34** enhanced its CB2 binding activity up to 5 nM from 338 nM. Similar study of substituent effect was reported. see: Ref. 10a.
17. Spectrum data of compound **39** were described in Ref. 13.
18. See Ref. 13 for the assay protocol.