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Identification of a highly potent and selective CB2 agonist, RQ-00202730, for the treatment of irritable bowel syndrome

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ABSTRACT

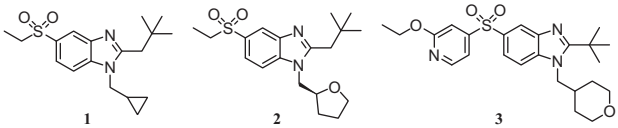
Herein we report the identification of a highly potent and selective CB2 agonist, RQ-00202730 (**40**), obtained by lead optimization of the benzimidazole scaffold. Compound **40** showed strong agonistic activity with an EC_{50} of 19 nM and excellent selectivity (>1300-fold) over the CB1 receptor. Compound **40** displayed a dose dependent analgesic effect on TNBS-induced visceral hypersensitivity in rats by oral administration (ED_{50} 0.66 mg/kg at 2.5 h after oral administration). In addition, **40** did not show a significant effect on body temperature in rats after oral administration at 300 mg/kg. These findings suggest that highly selective CB2 agonists will be effective agents for IBS therapy.

The endocannabinoid system plays an important role in complex biological processes.[1] These processes are regulated by two subtypes of cannabinoid receptors, the CB1 and CB2 receptors. While the CB2 receptor is suggested to regulate nociception and gut motility, the CB1 receptor, which is activated by cannabinoids, is believed to be related to CNS side effects, such as hypothermia, catalepsy, and hypolocomotion. Such CNS side effects should be avoided upon clinical use of cannabinoid agonists.

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain and abnormal bowel habits. IBS is classified into three categories based on the bowel habits and stool forms: IBS with diarrhea (IBS-D), IBS with constipation (IBS-C) and mixed state thereof (IBS-M).[2]

The CB2 receptor is known to be highly expressed in the myenteric and submucosal plexus in the gut.[3] CB2 agonists exhibit an analgesic effect in the trinitrobenzenesulfonic acid (TNBS)-induced visceral hypersensitivity model,[4] a well-known animal model of IBS.[5] Therefore, selective CB2 agonists are of particular interest as an emerging therapy for IBS.

Previous studies by Watson et al.[6] and Gijsen et al.[7] have demonstrated that the benzimidazole ring system would be a promising scaffold for selective CB2 agonists (Fig. 1). While these compounds exhibited excellent in vitro agonistic activity on CB2 and high selectivity over the CB1 receptor, there remains room for improvement in terms of the in vivo pharmacokinetics profile.



1

hCB2 EC_{50}: 2.7 nM
hCB1 EC_{50}: >10.0 μM

Rat PK (1mg/kg i.v.)
Cl: 20 mL/min/kg
Vd: 3.2 L/kg
$T_{1/2}$: 1.9 h

2

hCB2 EC_{50}: 11 nM
hCB1 EC_{50}: 15 μM
HLM Clint: 12 μL/min/mg
RLM Clint: 17 μL/min/mg

Rat PK (1mg/kg i.v)
Cl: 27 mL/min/kg
Vd: 1.0 L/kg
$T_{1/2}$: 0.4 h

3

hCB2 EC_{50}: 0.3 nM
hCB1 EC_{50}: 1280 nM
metabolism (HLM, 15 min): 48%

Figure 1. Structures of CB2 agonists of benzimidazole scaffold.

In particular, in vivo clearance of compounds **1** and **2** in rats was still high for oral use in persistent symptoms associated with IBS, while the rapid metabolism and low aqueous solubility of compound **3** should limit its use in animal and human dosing.

Hence, we embarked on a structure activity relationship (SAR) study using the benzimidazole scaffold, aiming for an improvement in the pharmacokinetic profile, particularly microsomal stability, while maintaining in vitro potency and selectivity. Our design strategy was to introduce a cyclic amine onto the sulfonyl group at the 5-position of the benzimidazole ring system, thereby adjusting the pharmacological and pharmacokinetic profile, since it had been suggested by the previous work that the position would tolerate various substituents while maintaining in vitro

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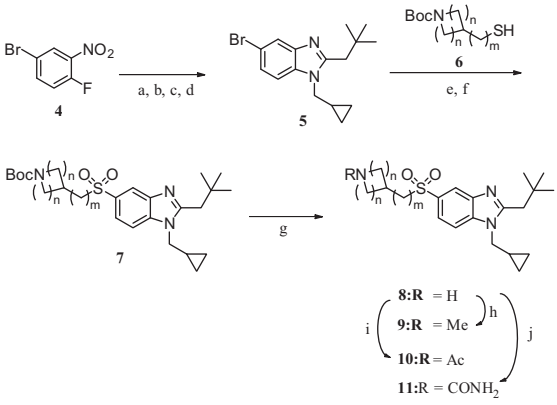


Scheme 1. Reagents and conditions: (a) cyclopropylmethylamine (1.2 equiv), Et_3N (1.2 equiv), EtOH, reflux; (b) Fe (8 equiv), 2 N HCl (5 equiv), reflux; (c) 3,3-dimethylbutanoyl chloride (1 equiv), Et_3N (1.5 equiv), CH_2Cl_2, 0 °C; (d) 5 N HCl (3 equiv), MeCN, reflux, 69% from **4**; (e) **6** (1.3 equiv), Pd_2dba_3 (0.025 equiv), xantphos (0.05 equiv), Et_3N (2 equiv), 1,4-dioxane, microwave irradiation, 170 °C, 1 h, 66–99%; (f) 30% H_2O_2, Na_2WO_4, MeOH, 0 °C–rt; (g) trimethylsilyl chloride (4 equiv), MeOH, reflux, 72%-quant; (h) $(CH_2O)_n$ (3 equiv), $NaBH(OAc)_3$ (12 equiv), AcOH (6 equiv), MeOH, rt; (i) AcCl (1.3 equiv), Et_3N (3.6 equiv), CH_2Cl_2, rt; (j) trimethylsilyl isocyanate (2 equiv), CH_2Cl_2, rt.

potency. Azetidine and piperidine were selected as the cyclic amine moieties.

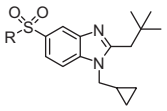
The syntheses of target compounds were conducted as outlined in Scheme 1.[8] Benzimidazole intermediate **5** was prepared according to a conventional reaction sequence via addition of cyclopropylmethylamine to the fluoronitrobenzene **4**, reduction of the nitro group, acylation, then cyclization. Cross coupling reaction using a palladium catalyst was performed under microwave irradiation, followed by oxidation with sodium tungstate to give intermediate **7**. Removal of the Boc protecting group and subsequent functionalization of the nitrogen atom using standard procedures delivered the various analogues shown in Table 1.

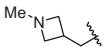
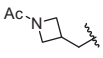
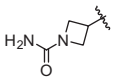
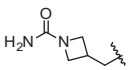
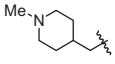
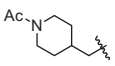
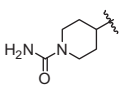
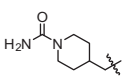
In the first cycle of the SAR study, the substituents at the 1- and 2-positions of benzimidazole were held constant to cyclopropylmethyl and neopentyl, respectively (Table 1). Interestingly, slight changes in the ring size and the length of a linker had a significant impact on CB2 agonistic activity (compounds **12** vs **20**, **13** vs **21**, **14** vs **22**, **15** vs **23**, **12** vs **16** and **13** vs **17**). Azetidine was found to be the more favored cyclic amine at this position for in vitro potency (compounds **12**–**15**). And in the piperidine subseries, keeping the piperidine basic resulted in lower potency compared to compounds where the nitrogen was part of an amide or urea moiety (compounds **16**, **17**, **24**, **25** vs **18**, **19**, **26**, **27**).

We next explored the SAR at the 1-position of benzimidazole with the 5-position held constant to an azetidinosulfonyl group. The in vitro agonistic activities on the CB2 and CB1 receptors and HLM clearance of these analogues are shown in Table 2. While lipophilic groups such as cyclopropylmethyl or trifluoromethoxyethyl afforded highly potent compounds **13**, **15**, **28** and **33**, HLM stability of these analogues was decreased. On the other hand, introduction of a polar group was effective for improving HLM stability as demonstrated by compounds **29**–**32** and **34**–**37**. The dimethylaminoethyl group exhibited the best balance between potency, selectivity and metabolic stability (**35**).

With these findings, we proceeded to further optimize the substituent on the nitrogen atom of the azetidine ring (Table 3). Although methyl (**30**) as an R group demonstrated a good profile with respect to agonistic activity on CB2 receptor, HLM stability and membrane permeability as measured in parallel artificial membrane permeability assay (PAMPA), the compound resulted in some activation of the CB1 receptor. R groups such as ethyl (**38**), trifluroethyl (**39**) and methanesulfonyl (**42**) led to decreased

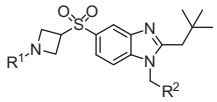
Table 1
In vitro agonistic activity of compounds **12**–**27** toward human CB2 receptor



Compound	R	hCB2 EC_{50}[a] (nM)	Compound	R	hCB2 EC_{50}[a] (nM)
12		8.9	**20**		104
13		2.0	**21**		32
14		9.6	**22**		73
15		1.2	**23**		13
16		62	**24**		278
17		37	**25**		209
18		6.2	**26**		7.7
19		6.3	**27**		0.99

[a] EC_{50} values based on inhibition of forskolin-stimulated cAMP production in CHO cells expressing CB2 receptor.[4]

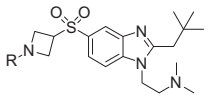
Table 2
In vitro agonistic activities for human CB1 and CB2 receptors, and HLM stability of compounds **13**, **15** and **28**–**37**



Compound	R^1	R^2	hCB2 EC_{50} (nM)	hCB1[a] EC_{50} (nM)	HLM Clint (mL/min/kg)
13	Me		2.0	1600	57
28	Me		2.6	Not Tested	86
29	Me		40	>25,000	13
30	Me		12	16,000	<7
31	Me		7.9	>25,000	12
32	Me		56	>25,000	<7
15			1.2	2800	47
33			5.9	>25,000	62
34			14	>25,000	11
35			4.8	>25,000	<7
36			21	10,000	<7
37			40	>25,000	13

[a] EC_{50} values based on inhibition of forskolin-stimulated cAMP production in CHO cells expressing CB1 receptor.[4]

Table 3
In vitro agonistic activities for human CB1 and CB2 receptors, HLM clearance, and PAMPA permeability coefficient (Pe) of compounds **30**, **35** and **38**–**42**



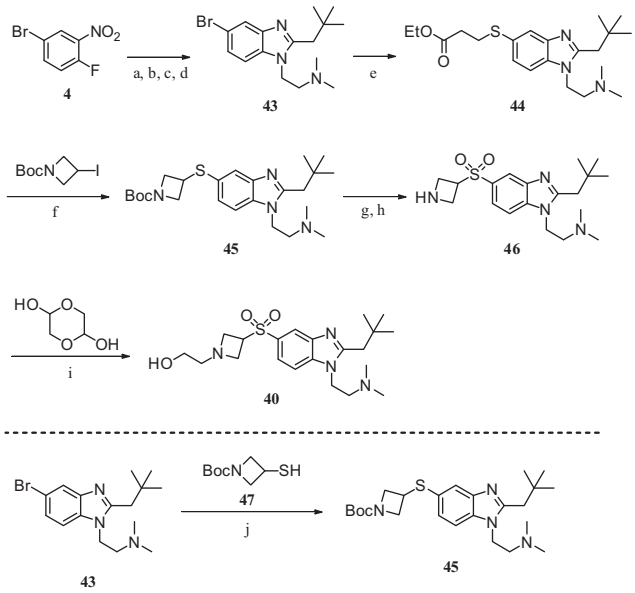
Compound	R	hCB2 EC_{50} (nM)	hCB1 EC_{50} (nM)	HLM Clint (mL/min/kg)	PAMPA Pe (10^{-6} cm/s)
30	Me	12	1600	<7	7.8
38	Et	16	>25,000	16	10
39	CF_3CH_2	14	>25,000	58	18
40 (RQ-00202730)	$HOCH_2CH_2$	19	>25,000	9.2	1.5
41	$MeOCH_2CH_2$	47	>25,000	Not tested	Not tested
42	$MeSO_2$	14	>25,000	19	5.9
35	H_2NCO	4.8	>25,000	<7	0.6

HLM stability. The analogue bearing a carbamoyl group on this position (**35**) exhibited strong potency and excellent selectivity over CB1, and proved to be metabolically stable. On the other hand, however, membrane permeability of the compound was significantly decreased, rending it much less attractive for oral use. Looking at the chemical structure, it is possible that the poor permeability may be attributed to excessive polarity and hydrogen bonding ability of the urea group.

Compound **40**, which bears a hydroxyethyl group as a side chain on the azetidine ring, demonstrated the best overall profile in terms of potency, selectivity, HLM stability and membrane permeability. Given its encouraging in vitro dataset, compound **40** was prioritized for further evaluation in animal studies.

The synthesis of compound **40** is shown in Scheme 2. Starting from fluoronitrobenzene (**4**), the benzimidazole core was prepared in a similar sequence to Scheme 1. Selective hydrogenation of the nitro group was accomplished by use of a platinum catalyst. Attempts at cross coupling between bromobenzimidazole (**43**) and azetidinethiol (**47**) without microwave irradiation were not successful, providing compound **45** in low yield (step j). Instead, the introduction of azetidinylthio group was enabled by cross coupling reaction with ethyl mercaptopropanoate, followed by a one pot sequence of β-elimination and S-alkylation, giving compound **45** in quantitative yield for two steps. After removal of the Boc protecting group, the hydroxyethyl group was finally introduced onto the azetidine ring via reductive amination with 1,4-dioxane-2, 5-diol to give compound **40**.

The in vivo pharmacokinetics of compound **40** was evaluated in rats and dogs (Table 4). Single oral administration of **40** to rats at 3 mg/kg ($n = 2$) provided a C_{max} of 38 ng/mL, AUC_{0-inf} of



Scheme 2. Reagents and conditions: (a) *N*,*N*-dimethylethylenediamine (1.02 equiv), K_2CO_3 (0.5 equiv), THF, 60 °C; (b) H_2 (3–4 atm), Pt–C type 128 (Johnson Matthey), THF, rt; (c) 3,3-dimethylbutanoyl chloride (1.05 equiv), Et_3N (1.1 equiv), THF, 0 °C; (d) 12 N HCl (2 equiv), MeCN, 80 °C, 53% for four steps; (e) ethyl 4-mercaptopropanoate (1.05 equiv), $Pd_2(OAc)_2$ (0.005 equiv), xantphos (0.01 equiv), *i*Pr_2Net (1.1 equiv), DMA, 150 °C, 1.5 h; (f) iodide (1.05 equiv), *t*-BuOK (1.4 equiv), THF, 0 °C, quant for two steps; (g) 30% H_2O_2 (2.1 equiv), Na_2WO_4 (0.02 equiv), MeOH, 0 °C–rt, 86%; (h) 2 N HCl (2.7 equiv), 0 °C–rt, 93%; (i) 1,4-dioxane-2,5-diol (0.51 equiv), $NaBH(OAc)_3$ (1.5 equiv), CH_2Cl_2, rt, 95%; (j) **47**, Pd_2dba_3 (0.1 equiv), xantphos (0.2 equiv), Et_3N (2 equiv), 1,4-dioxane, reflux.

Table 4
In vivo pharmacokinetics parameters of compound **40** in rats and dogs

PK parameters	Rat (IGS) 3 mg/kg p.o. (n = 2)[a]	Dog (Beagle, Marshall) 1 mg/kg p.o. (n = 2)[a]
C_{max} (ng/mL)	38	102
AUC_{inf} (ng h/mL)	300	1000
$T_{1/2}$ (h)	3.3	8.6
Bioavailability (%)	20	72
Cl (mL/h/kg)	2605	725
Vdss (mL/kg)	5696	5694

[a] Vehicle: 0.5% methyl cellulose.

Table 5
In vitro ADME profile of compound **40**

PK parameters	Rat	Dog	Human
Clint (mL/min/kg) (liver microsome)	<10	<8.3	9.2
CYP IC_{50} (μM) (1A2, 2C9, 2D6, 3A4)	—	—	>3
Plasma protein binding (free fraction %)	78	48	50

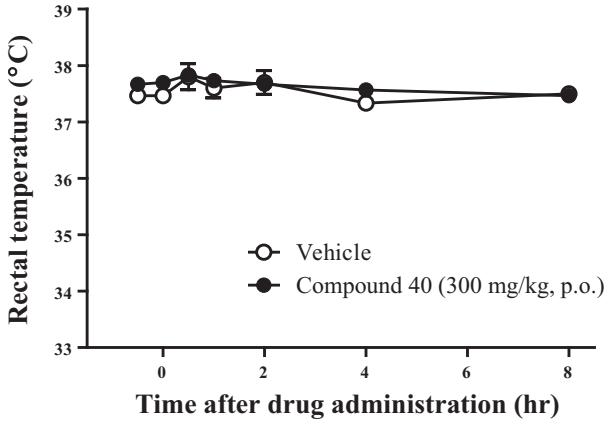


Figure 3. Effect of compound **40** on body temperature in rats. Results are shown as mean ± SD (n = 3).

298 ng h/mL and $T_{1/2}$ of 3.3 h. Bioavailability in rats was calculated to be 20% based on the AUC_{0-inf} of intravenous administration of the compound at 1 mg/kg (391 ng h/mL). In dogs, more encouraging results were obtained. At 1 mg/kg oral administration (n = 2), compound **40** demonstrated high and sustained exposure (C_{max} 102 ng/mL; AUC_{0-inf} 999 ng/mL h; $T_{1/2}$ 8.6 h). Its bioavailability was calculated to be 72%. In vitro ADME evaluation was also conducted, demonstrating favorable, drug-like, characteristics (Table 5).

Compound **40** showed a dose-dependent analgesic effect with an ED_{50} of 0.66 mg/kg on TNBS-induced visceral hypersensitivity in rats after oral administration (Fig. 2). A similar analgesic activity was observed after 1 h and 2.5 h at low dose, thereby demonstrating a sustained effect of the compound, which should be a positive attribute for the treatment of IBS, since patients often suffer recurrent abdominal pain after the first symptoms throughout the day.

Next, we evaluated the effect of compound **40** on body temperature in rats because hypothermia is a pharmacological biomarker of activation of CB1 receptor. Notably, even after administration of 300 mg/kg p.o. of compound **40**, where C_{max} reached 1800 ng/mL, no significant hypothermia was observed compared with control group (Fig. 3).

Taken together, these results suggest that compound **40** has a potent CB2-related analgesic effect, which is separated from CB1-related side effects with a wide margin of safety.

Compound **40** was further characterized in order to demonstrate its potential as a drug candidate. The compound did not show genotoxicity in bacterial reverse mutation and in vitro micronucleus assays, and exhibited excellent selectivity in broad ligand profiling on 64 target molecules including a variety of

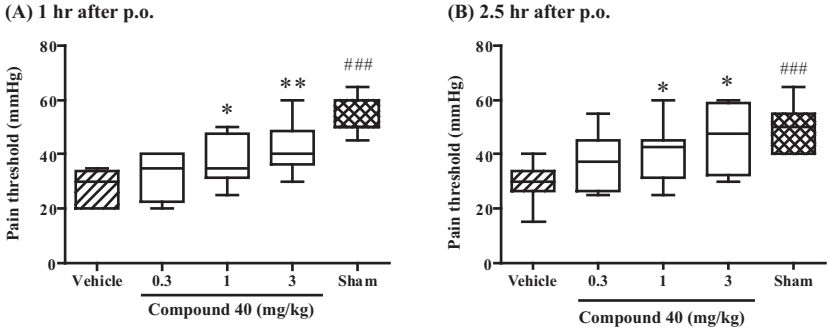


Figure 2. Effect of compound **40** on TNBS-induced visceral hypersensitivity in rats. Results were expressed as median pressure threshold (mmHg). Boxes were representing the 25th and 75th percentiles, and vertical bars represent ranges (n = 7–8/group). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{###}p < 0.001$, significant difference compared to vehicle-treated rats by Mann–Whitney U test.

receptors, ion channels and transporters. Considering its excellent pharmacological profile, the compound was selected as a preclinical candidate, with code name RQ-00202730.

In summary, we have identified a highly potent and selective CB2 agonist, RQ-00202730 (compound **40**), starting from a well known benzimidazole scaffold. Tailored adjustment of in vitro potency, selectivity and pharmacokinetic parameters led to the discovery of compound **40** which exhibited a dose dependent analgesic effect on TNBS-induced visceral hypersensitivity in rats by oral administration. In addition, **40** did not show significant effect on body temperature in rats, a typical side effect caused by activation of CB1, after oral administration of 300 mg/kg. These findings suggest that highly selective CB2 agonists will be an attractive target for IBS therapy. Further studies of compound **40** are underway, the results of which will be reported separately in the future.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.11.062.

References and notes

1. (a) Goutopoulos, A.; Makriyannis, A. *Pharmacol. Ther.* **2002**, *95*, 103; (b) Pertwee, R. G. *Br. J. Pharmacol.* **2008**, *153*, 263.
2. Longstreth, G. F.; Thompson, W. G.; Chey, W. D.; Houghton, L. A.; Mearin, F.; Spiller, R. C. *Gastroenterology* **2006**, *130*, 1480.
3. Wright, K. L.; Duncan, M.; Sharkey, K. A. *Br. J. Pharmacol.* **2008**, *153*, 263.
4. Kikuchi, A.; Ohashi, K.; Sugie, Y.; Sugimoto, H.; Omura, H. *J. Pharmacol. Sci.* **2008**, *106*, 219.
5. (a) Ohashi, K.; Sato, Y.; Iwata, H.; Kawai, M.; Kurebayashi, Y. *J. Vet. Med. Sci.* **2007**, *69*, 1223; (b) Rijnierse, A.; Nijkamp, F. P.; Kraneveld, A. D. *Pharmacol. Ther.* **2007**, *116*, 207.
6. Watson, C.; Owen, D.; Harding, D.; Kon-I, K.; Lewis, M. L.; Mason, H. J.; Matsumizu, M.; Mukaiyama, T.; Rodriguez-Lens, M.; Shima, A.; Takeuchi, M.; Tran, I.; Young, T. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4284.
7. (a) Verbist, B. M. P.; De Cleyn, M. A. J.; Surkyn, M.; Fraiponts, E.; Aerssens, J.; Nijsen, M. J. M. A.; Gijsen, H. J. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2574; (b) Gijsen, H. J. M.; De Cleyn, M. A. J.; Surkyn, M.; Van Lommen, G. R. E.; Verbist, B. M. P.; Nijsen, M. J. M. A.; Meert, T.; Van Wauwe, J.; Aerssens, J. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 547.
8. Refer to patent WO 2,010,084,767 for detailed procedures.