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Benzimidazole CB2 agonists: Design, synthesis and SAR

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ABSTRACT

A new series of CB2-selective agonists containing a benzimidazole core is reported. Design, synthesis, SAR and pharmacokinetic data for selected compounds are described.

Cannabis, originating from central Asia, is probably the oldest psychotropic drug known to humanity and has been used for recreational as well as medicinal (analgesic, anticonvulsant, antiemetic, appetite stimulant, etc.) purposes.[1] Cannabinoids are pharmacologically active components of cannabis (*Cannabis sativa*) and partially act on two known subtypes of cannabinoid receptors, CB1[2] and CB2,[3] which are members of the G-protein coupled receptor (GPCR) family. The CB1 receptor is abundantly expressed in the central nervous system (CNS)[4] as well as in the periphery[5,6] and is believed to be responsible for the psychotropic effects of cannabinoids.[7] Conversely, CB2 receptors are expressed predominantly, but not exclusively, outside of the CNS, where they are found mainly in the cells of the immune system, though they may be up-regulated in the CNS under pathological conditions (inflammation, pain).[8,9]

Activation of CB2 receptors triggers the signal transduction pathway through Gi proteins resulting in inhibition of adenylate cyclase activity.[10] In contrast to CB1 receptors, CB2 receptors do not couple to calcium-Q or inward-rectifying potassium channels,[11] whereas agonism of CB1 receptors suppresses calcium and activates inward-rectifying potassium conductance, associated with depression and neuronal excitability. Additionally, the first moderately selective CB2-agonist, HU-308 showed antiinflammatory and antihyperalgesic properties in mice which were reversed by a CB2 antagonist, but not by a CB1 antagonist.[12] Moreover, HU-308 showed no activity in mice in the tetrad[13] of behaviorial tests. It has thus been hypothesized that CB2-selective agonists could be therapeutically useful to treat pain without the undesirable psychotropic side effects. Based on this hypothesis, significant efforts have been dedicated to the pursuit of CB2-selective agonists, both in academia and in industry.[14,15]

Large numbers of publications have detailed CB2-agonists with varying degrees of selectivity over CB1. In our own efforts to find CB2-selective agonists, we have previously reported imidazopyridines[16] and decahydroquinolines[17] as potent and selective CB2 agonists. In these studies, we observed[16] that highly selective CB2 agonists (e.g., **2**, Fig. 1) did not show efficacy[18] in a rat CFA hyperalgesia model[19] despite high exposure in vivo, both peripherally and centrally, while moderately selective CB2/CB1 agonists (e.g., **1**, Fig. 1) show a significant analgesic effect.[18] In light of the above observations, the question remains—how does residual CB1 agonism affect the outcome of agonist dosing in the in vivo pain models? Continuing to identify novel structural classes of CB2-selective agonists may enable additional experiments towards answering this question.

In the course of our imidazopyridine series efforts, we identified hetero-aryl alcohol substituents as viable analogs of the morpholinomethylene substituent of imidazopyridine **1** (imidazopyridine **3**,[20] Fig. 2). Examination of **3** suggested that a similar display of

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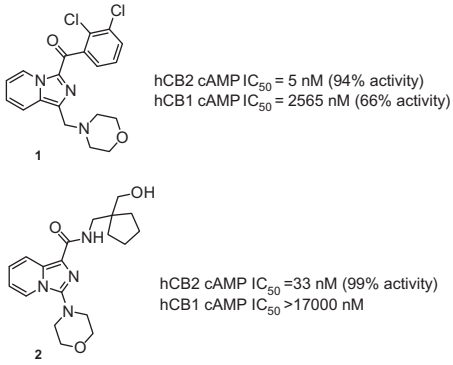


Figure 1. Imidazopyridine CB2 agonists.

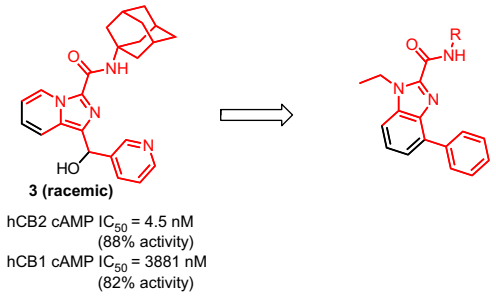


Figure 2. Design of benzimidazole core from imidazopyridine scaffold.

substituents could be achieved using a completely different benzimidazole scaffold (Figs. 2 and 3).

To test this hypothesis, synthesis of benzimidazole containing compounds was investigated. The initial route employed 3-chloro-1,2-diaminobenzene **4** as starting material (Scheme 1). Reaction[21] of **4** with methyl-2,2,2-trichloroacetimidate in acetic acid provided 2-trichloromethyl benzimidazole **5**. Unfortunately, ethanolysis of **5** to give **6** also provided 50% decarboxylated byproduct **7**.

Although the reason behind the formation of decarbonylated byproduct **7** during ethanolysis of **5** was not well understood, an alternate route was devised where the 7-chloro group of **5** was substituted with a phenyl group prior to ethanolysis of the 2-trichloromethyl group. In this route (Scheme 2), phenyl boronic acid was coupled to 3-chloro-2-nitroaniline to give **10**. The nitro group of **10** was then reduced with tin(II)chloride to give **11**, which in turn was transformed to 2-trichloromethyl benzimidazole **12** via

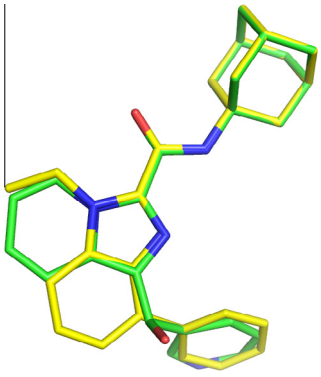
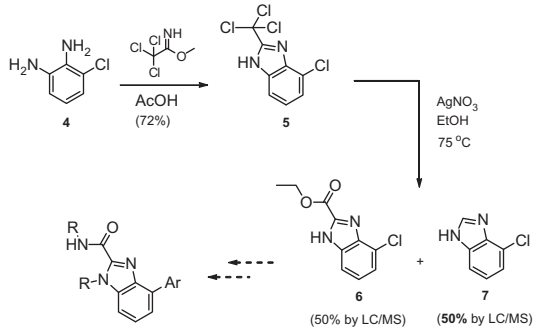
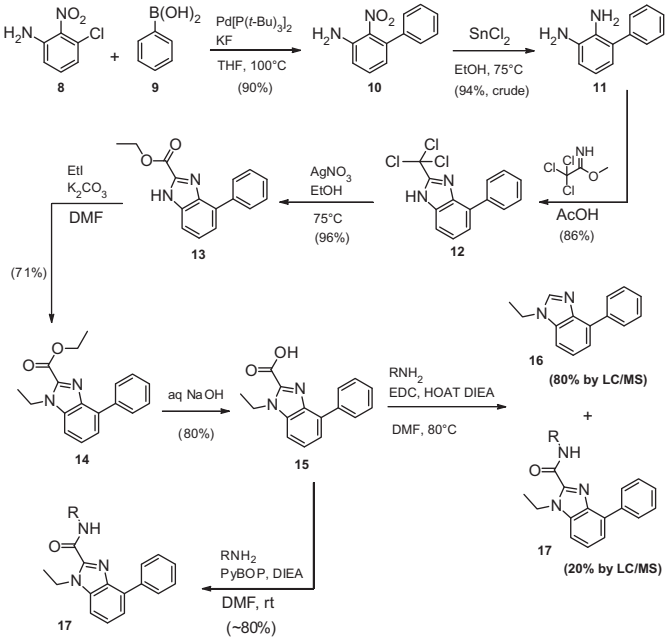


Figure 3. Overlay of gas phase conformations (within 3 kcal/mol of global minimum) of imidazopyridine **3** and benzimidazole **12**.



Scheme 1.

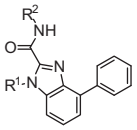


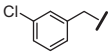
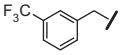
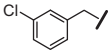
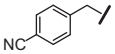
Scheme 2.

treatment with methyl-2,2,2-trichloroacetimidate. Ethanolysis of **12** cleanly gave benzimidazole-2-ethyl ester in 96% yield. Alkylation of **13** produced exclusively one product whose regiochemistry was determined by NMR spectrometry on the basis of ROESY correlations.[22] The alkylated product, **14** was then hydrolyzed to **15**. When standard peptide coupling conditions (EDC, HOAT, DIEA in DMF) were employed to couple amines with **15**, reactions did not proceed at rt. At higher temperature (80 °C), predominantly decarboxylated byproduct **16** was observed. This problem was alleviated by using a more active coupling agent, PyBOP, which yielded the desired amides in good yields.

This synthetic route allowed us to make benzimidazole analogs for which the amide group as well as the *N*-alkyl group could be varied. Evaluation of the initial compounds revealed that this chemotype did afford potent CB2 agonists with a high degree of CB2/CB1 selectivity.[23] Table 1 lists selected compounds containing arylmethyl amides at the benzimidazole 2-position. As evident from Table 1, *N*-ethyl benzimidazoles show more potent CB2 agonism (lower EC_{50} value and higher E_{max}) when compared to N-unsubstituted benzimidazoles. The most potent compound in this series is 2-chlorobenzyl amide **7**, which shows 92-fold selectivity over CB1. 3-Chlorobenzyl amide **8**, although it exhibits 4-fold weaker potency vs CB2 (compared with **7**), shows >425-fold selec-

Table 1
SAR: arylmethyl amides



Entry	R^1	R^2	hCB2 cAmp EC_{50} nM[a] (E_{max})[b]	hCB1 cAmp EC_{50} nM[a] (E_{max})[b]
1	H		165 (67%)	>17,000
2	H		256 (49%)	>17,000
3	H		256 (69%)	>17,000
4	H		443 (64%)	>17,000
5	H		991 (64%)	>17,000
6	H		1437 (39%)	>17,000
7	Et		9.9 (101%)	913 (87%)
8	Et		40 (87%)	>17,000
9	Et		45 (98%)	>17,000
10	Et		84 (99%)	>17,000
11	Et		1104 (89%)	>17,000

[a] See Ref. 23.
[b] See Ref. 24.

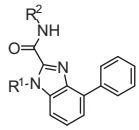
tivity over CB1. SAR also reveals that heteroatoms in the aromatic ring are not favored (**6** and **11**).

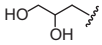
Table 2 lists benzimidazoles where the 2-position is substituted with aliphatic amides and the 1-position is either unsubstituted or substituted with alkyl groups. A bulky, lipophilic adamantyl amide gives a very potent CB2 agonist (**12**), but its selectivity over CB1 is poor (65-fold). When the adamantyl group is replaced with a *t*-butylmethyl group (**14**), a potent CB2 agonist is obtained which shows >1156-fold selectivity over CB1. In an effort to reduce lipophilicity, aliphatic amides bearing hydroxy groups were prepared. As evident from Table 2, these molecules were potent and selective CB2 agonists. The most potent and selective compound from this category is **13**. As noted for the arylmethyl amides (Table 1), aliphatic amides bearing *N*-ethyl substitution of the benzimidazole were more potent than their unsubstituted counterparts (compare **14** and **18**; **15** and **19**).

As evident from Table 2 (**20**, **21**, **22**), introduction of an electron withdrawing trifluoroethyl group makes these compounds much less active as CB2 agonists.

Evaluation of analgesic effects in animal models of pain requires pharmacokinetic profiles that provide adequate exposures for CB2 agonism. Following our PK screening paradigm for this series, representative compounds were selected for initial pharmacokinetic profiling in dog i.v. cassette PK experiments. Figure 4 shows data for compounds **9**, **14** and **15**. 2-Fluorobenzyl amide **9** shows moderate clearance (21 mL/min/kg) whereas two aliphatic amide containing compounds (**14** and **15**) show moderately higher clearance. This data suggests that, although further optimization is needed, compounds with suitable pharmacokinetic profile for in vivo dosing could be obtained from this chemical series.

Table 2
SAR: aliphatic amides



Entry	R^1	R^2	hCB2 cAmp EC_{50} nM[a] (E_{max})[b]	hCB1 cAmp EC_{50} nM[a] (E_{max})[b]
12	H		1.9 (99%)	123 (100%)
13	H		6.3 (75%)	>17,000
14	H		14.7 (95%)	>17,000
15	H		24.6 (93%)	>17,000
16	H		43 (92%)	>17,000
17	H		236 (82%)	>17,000
18	Et		490 (67%)	913 (87%)
19	Et		1255 (68%)	>17,000
20	CH_2CF_3		1973 (45%)	>17,000
21	CH_2CF_3		>17,000	>17,000
22	CH_2CF_3		>17,000	>17,000

[a] See Ref. 23.
[b] See Ref. 24.

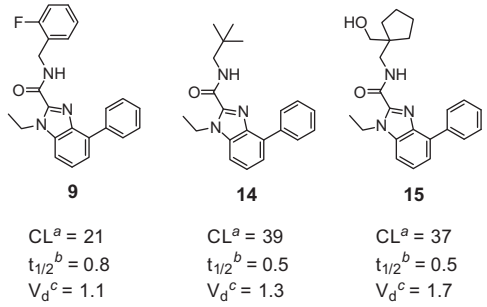


Figure 4. PK (dog cassette) data. [a]mL/min/kg; [b]h; [c]L/kg.

In summary, a new structural class of CB2 agonists has been identified. Exploratory efforts have provided CB2-selective compounds with moderate pharmacokinetic profiles. Further optimization could provide new tools for understanding the viability of selective CB2 agonists for the treatment of pain.

Acknowledgment

We thank Deping Wang for generation of the overlay of gas phase conformations of imidazopyridine and benzimidazole containing structures.

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18. *Compound* ***1***: rat CB2 cAMP EC_{50} = 11 nM (87% activity); rat CB1 cAMP EC_{50} = 1068 nM (104% activity); rat plasma protein binding 76%. Compound **1** exhibited naproxen-like reversal in paw withdrawal threshold in rat CFA model at 100 mpk (PO dosing) at 60 min post dose. Plasma, brain and CSF levels at 100 mpk dose (60 min timepoint) were 4500, 5400 and 110 nM, respectively.
Compound ***2***: rat CB2 cAMP EC_{50} = 58 nM (87% activity); rat CB1 cAMP EC_{50} > 17,000 nM; rat plasma protein binding 84%. Compound **1** exhibited no change in paw withdrawal threshold in rat CFA model at 100 mpk (PO dosing) at 120 min post dose. Plasma, brain and CSF levels at 100 mpk dose (120 min timepoint) were 4300, 1300 and 300 nM, respectively.
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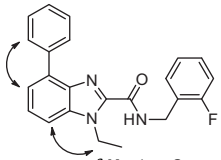
22. Diagnostic NOE correlations (indicated by the arrows) to assign regiochemistry of the alkylated product:



23. Assay results represent the average of *N* = 1 to 3 results. Inter-assay variability was ±20%.
24. Compounds were added to a Greiner black 384 well low volume assay plate. 1000 CHO-K1 cells (expressing human or rat CB1 or CB2) were added to each well of the assay plate containing compound, then incubated at room temperature for 15 min. Then, forskolin at EC_{70} was added and incubated at room temperature for an additional 30 min. Detection of cAMP was performed using Cisbio's HRTF cAMP dynamic 2 kit following the manufacturer's protocol for cAMP detection. After adding d2-cAMP and anti-cAMP-cryptate to all the wells, there was a final incubation at room temperature for 1 hour. Plates were then read on an EnVision plate reader (Perkin Elmer). CV values calculated for a positive control compound (>250 replicates) were 59% and 70% respectively, for CB1 and CB2 IP values.