Bioorganic & Medicinal Chemistry Letters 21 (2011) 2354–2358

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Imidazopyridine CB2 agonists: Optimization of CB2/CB1 selectivity and implications for in vivo analgesic efficacy

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ARTICLE INFO

Article history:
Received 10 November 2010
Revised 17 February 2011
Accepted 22 February 2011
Available online 1 March 2011

Keywords:
CB2 agonist
CB1 agonist
Selectivity
Pain
CFA model

ABSTRACT

A new series of imidazopyridine CB2 agonists is described. Structural optimization improved CB2/CB1 selectivity in this series and conferred physical properties that facilitated high in vivo exposure, both centrally and peripherally. Administration of a highly selective CB2 agonist in a rat model of analgesia was ineffective despite substantial CNS exposure, while administration of a moderately selective CB2/CB1 agonist exhibited significant analgesic effects.

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The known analgesic effects of natural cannabinoids and the identification of the CB1 and CB2 cannabinoid receptors have engendered a large and active area of research aimed at agonists of these receptors. Both endogenous and medicinal cannabinoids are known to act as agonists of CB1 and CB2.[1] Given the association of CB1 agonism with the psychotropic effects of natural cannabinoids, selective CB2 agonism has been proposed as a potential means of modulating pain-related behaviors without concomitant undesirable neurological effects.[2] Investigators in both academia and industry have pursued CB2 agonists, and a substantial body of literature has emerged detailing the design, synthesis, and in vivo evaluation of CB2-preferring compounds with good selectivity for CB2 agonism versus CB1 agonism.[3]

The vast majority of these studies involve molecules that retain some measurable CB1 agonist activity.[2] Given the existence of a large CB1 receptor reserve in the central nervous system and the demonstrated ability of weak CB1 binding to produce substantial agonism,[4] residual CB1 activity of CB2-preferring compounds may confound the dissection of CB2 and CB1 agonism in many of these studies. In this and the accompanying Letter,[5] we present the design and synthesis of two structurally distinct CB2 agonists with no measurable in vitro CB1 agonist activity. Evaluation of these highly selective compounds in vivo indicates that they do not affect rat pain responses despite high peripheral and central exposures. These results counter the perception that CB2 agonism alone can produce a significant analgesic effect.

We based our initial investigations on indole **A** (later termed GW405833, Fig. 1), which was discovered at Merck Frosst and shown to bind potently to the CB2 receptor with some selectivity over CB1.[6] Given that a number of investigators have reported variants of this [6,5] heterocyclic structure to be potent CB2 agonists,[7] we focused our own design efforts on closely related scaffolds. We report here a new variant of this theme, imidazopyridine CB2 agonists (Fig. 1). The imidazopyridines are potent CB2 agonists with highly tunable CB2/CB1 selectivity and physical properties that allow CNS penetration. Exploration of SAR in this series has produced both CB2-preferring agonists and agonists that appear to be completely selective for CB2 versus CB1. In vivo evaluation of these compounds indicates a significant impact of the degree of CB2/CB1 selectivity on analgesic effects in this series.

Synthesis of imidazopyridines relied on elaboration of appropriate aminomethyl pyridines (Scheme 1). Condensation of 2-aminomethyl pyridine with ethyl oxalyl chloride provided ester **1**.

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doi:10.1016/j.bmcl.2011.02.082

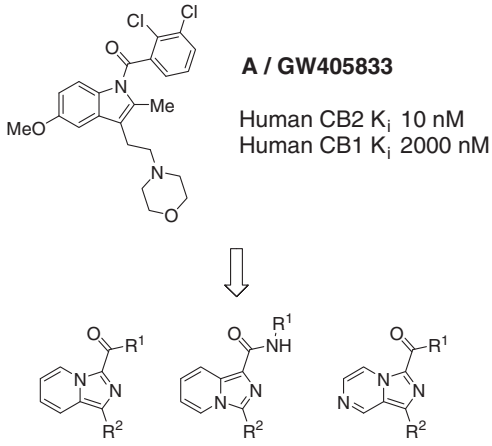
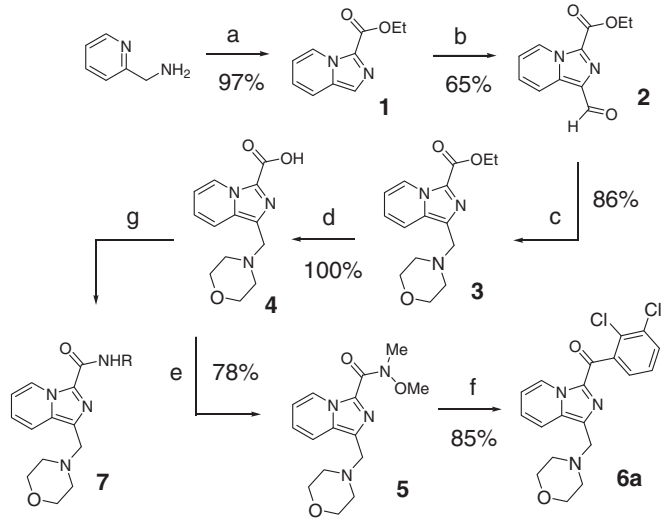


Figure 1. Imidazopyridine analogs of **A**.

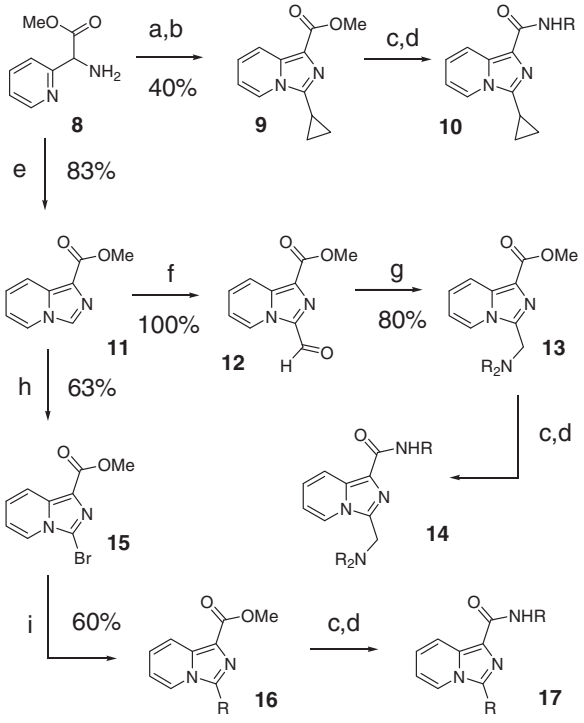
Formylation with $POCl_3$/DMF then gave imidazopyridine aldehyde **2**. Reductive amination was employed to afford **3**, which could be transformed into ketone derivatives such as **6a** via hydrolysis, Weinreb amide formation, and Grignard addition. Carboxylic acid **4** also underwent routine amide coupling reactions to provide amides of type **7**.

Isomeric imidazopyridines were prepared using aza-phenylglycine ester **8** (Scheme 2).[8] In order to prepare alkyl-substituted analogs **10**, nitrogen acylation followed by cyclization was employed to provide ester **9**. Ester hydrolysis and amide coupling afforded amides **10**. Alternatively, treatment of **8** with DMF dimethylacetal provided imidazopyridine **11**, which was a versatile intermediate for preparation of multiple 1-substituted variants. Formylation of **11** proceeded in high yield to afford **12**, which could be reductively aminated to provide esters such as **13**. Standard hydrolysis and coupling reactions provided CB2 agonists of type **14**. Bromination of **11** provided **15**, which underwent palladium-catalyzed coupling reactions with amines[9] to give **16**. The ester hydrolysis and coupling sequence then provided amides **17**.

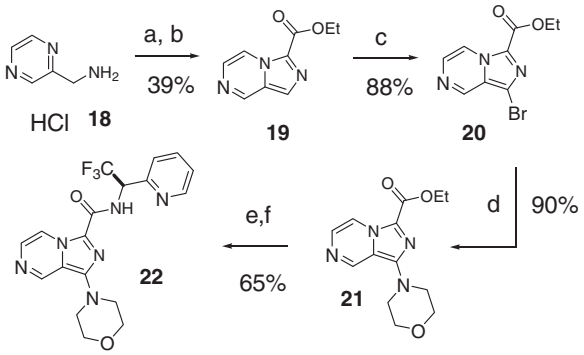
A facile synthesis of related imidazopyrazine analogs was also developed to access compounds of type **22** (Scheme 3). Application



Scheme 1. Reagents and conditions: (a) ethyl oxalyl chloride, triethylamine, THF, rt; (b) $POCl_3$, DMF, reflux; (c) morpholine, sodium triacetoxyborohydride, 1,2-dichloroethane, rt; (d) NaOH, MeOH/water, rt; (e) EDC, iPr_2NEt, DMF, MeONHMe–HCl, rt; (f) (2,3-dichlorophenyl)magnesium iodide, THF, 0 °C to rt; (g) EDC, iPr_2NEt, DMF, HOAt, RNH_2, rt.



Scheme 2. Reagents and conditions: (a) cyclopropylcarbonyl chloride, sodium bicarbonate, dichloromethane, rt; (b) $POCl_3$, DMF; (c) NaOH, MeOH/water, rt; (d) R-NH_2, EDC, iPr_2NEt, HOAt, DMF, rt; (e) DMF dimethyl acetal, toluene, reflux; (f) $POCl_3$, DMF, 115 °C; (g) morpholine, sodium triacetoxy-borohydride, dichloromethane, rt; (h) Br_2, acetic acid; (i) morpholine, Cs_2CO_3, XantPhos, Pd_2dba_3, 100 °C.

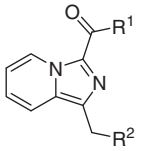


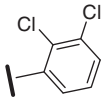
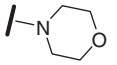
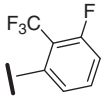
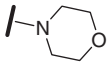
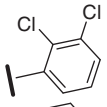
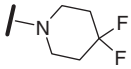
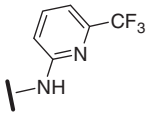
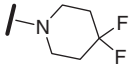
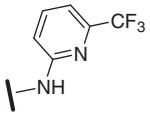
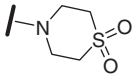
Scheme 3. Reagents and conditions: (a) ethyl oxalyl chloride, triethylamine, THF,0 °C to rt; (b) $POCl_3$, DMF, 100 °C; (c) NBS, triethylamine, acetonitrile, 0 °C to rt; (d) morpholine, Cs_2CO_3, XantPhos, Pd_2dba_3, 100 °C; (e) NaOH, MeOH/water, rt; (f) *R*-trifluoromethyl(amino)methyl-2-pyridine, EDC, iPr_2NEt, HOAt, DMF, rt.

of the acylation/cyclization sequence to aminomethylpyrazine **18** provided the imidazopyrazine scaffold **19**, which underwent NBS-mediated bromination and palladium-catalyzed amination to provide **21**. Ester hydrolysis and amide coupling provided agonists **22**.

These routes allowed facile variation of substituents on both carbon positions of the imidazo ring of the core. Optimization of compounds focused on functional CB2 agonist efficacy and CB2/CB1 selectivity.[10] We also targeted agonists with high plasma free fraction; for compounds attaining similar plasma exposures, this strategy was expected to produce compounds capable of achieving significant free concentrations in the brain.[11] As shown in Table 1, both ketone (**6**) and amide (**7**) derivatives of the 'Type I' imidazopyridine scaffold were potent CB2 agonists.

Table 1
Type I imidazopyridine CB2 agonists



Entry	R^1	R^2	hCB2 cAMP IC_{50} nM[a] (E_{max})	hCB1 cAMP IC_{50} nM[a] (E_{max})	Plasma free fraction[b]
6a			5 (94%)	2565 (66%)	24%
6b			0.7 (98%)	2229 (97%)	3%
6c			0.3 (110%)	90 (101%)	ND
7a			1.7 (98%)	347 (100%)	ND
7b			44 (80%)	12700 (54%)	11%

[a] See Ref. 10.
[b] Measured via ultrafiltration or equilibrium dialysis following incubation with rat plasma.

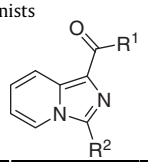
Moderate CB2/CB1 selectivity (ca. 500-fold) was generally observed. A survey of aminomethyl substituents identified moderately basic amines including morpholine and 4-difluoropiperidine that conferred good CB2 functional efficacy. Amines containing polar functionality (**6a**, **7b**) exhibited high plasma free fraction, with morpholine analogs exhibiting more potent CB2 agonism.

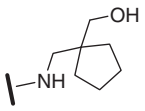
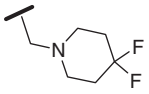
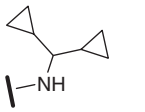
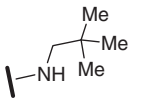
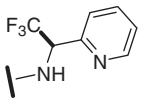
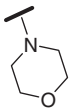
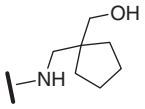
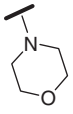
Investigation of the 'Type II' scaffold (Table 2) revealed that, in contrast to aminomethyl-substituted agonists (**14a**), analogs bearing cyclopropyl substitution (**10a**,**b**) or a directly attached morpholine substituent (**17a**,**b**) displayed improved CB2/CB1 selectivity. Variation of the amide substituent was also found to modulate efficacy of CB2 agonism as well as selectivity over CB1. Again, incorporation of polarity was pursued in order to reduce plasma protein binding. Hydroxymethyl-containing amide **17b** was a potent CB2 agonist that displayed no CB1 agonism in vitro.

Imidazopyrazines incorporating directly attached amino substituents were also found to display generally high CB2/CB1 selectivity (Table 3). As observed for the des–aza scaffolds, CB2 efficacy could be tuned by variation of both the amide and amino functionality, and plasma free fraction was impacted by incorporation of polar atoms (**22a**,**b** vs **22c**,**d**). The impact of nitrogen incorporation in the core is illustrated by comparison of **22c** and **17a** (Table 2); while CB2 efficacy is reduced somewhat, both selectivity and free fraction are significantly improved upon migration to the imidazopyrazine core.

These efforts provided us with a collection of imidazopyridine CB2 agonists with a range of selectivity profiles and physicochemical attributes. Compounds **6a** and **17b** (Fig. 2) were identified as

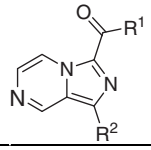
Table 2
Type II imidazopyridine CB2 agonists

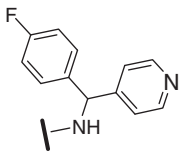
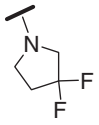
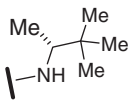
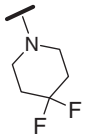
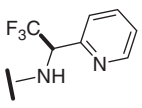
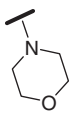
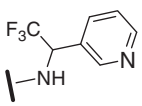
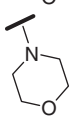


Entry	R^1	R^2	hCB2 cAMP IC_{50} nM (E_{max})[a]	hCB1 cAMP IC_{50} nM (E_{max})[a]	Plasma free fraction[b]
14a			3 (88%)	68 (99%)	ND
10a			7 (99%)	>17000	3%
10b			21 (97%)	>17000	5%
17a			21 (97%)	705 (55%)	10%
17b			33 (99%)	>17000	16%

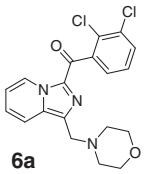
[a] See Ref. 10.
[b] Measured via ultrafiltration or equilibrium dialysis following incubation with rat plasma.

Table 3
Imidazopyrazine CB2 agonists



Entry	R^1	R^2	hCB2 cAMP IC_{50} nM[a] (E_{max})	hCB1 cAMP IC_{50} nM[a] (E_{max})	Plasma free fraction[b]
22a			92 (96%)	>17000	3%
22b			36 (99%)	>17000	6%
22c			139 (94%)	>17000	18%
22d			69 (98%)	>17000	25%

[a] See Ref. 10.
[b] Measured via ultrafiltration or equilibrium dialysis following incubation with rat plasma.

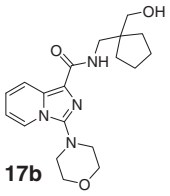


6a

hCB2 cAMP IC_{50} 5 nM (94% activity)
hCB1 cAMP IC_{50} 2565 nM (66% activity)

Rat CB2 cAMP IC_{50} 11 nM (87% activity)
Rat CB1 cAMP IC_{50} 1068 nM (104% activity)

Rat Plasma Protein Binding 76%



17b

hCB2 cAMP IC_{50} 33 nM (99% activity)
hCB1 cAMP IC_{50} >17000 nM

Rat CB2 cAMP IC_{50} 58 nM (87% activity)
Rat CB1 cAMP IC_{50} >17000 nM

Rat Plasma Protein Binding 84%

Figure 2. CB2 agonists for in vivo dosing.

appropriate tools to elucidate the impact of CB2/CB1 selectivity on in vivo analgesic effects. Both compounds exhibited potent CB2 agonism in vitro as well as high rat plasma free fraction. Thus it was anticipated that both compounds would effect CB2-mediated pharmacology both in the CNS and the periphery when dosed to rats. The key difference between the two compounds concerned CB2/CB1 selectivity. **6a** retained moderate but significant CB1 agonism in vitro, while **17b** exhibited no in vitro agonism against either the human or rat CB1 receptor (Fig. 2).

Analgesic effects of the two compounds were evaluated in a rat CFA hyperalgesia model.[12] Both **6a** and **17b** were dosed either orally or subcutaneously to rats in order to maximize peripheral and CNS exposure. Plasma, brain, and CSF[11] levels were measured for each compound in order to verify that adequate exposure for CB2 activity was achieved. Moderately selective agonist **6a** exhibited dose-dependent effects in the CFA model, giving naproxen-like reversals in paw withdrawal threshold at 100 mpk (Fig. 3). Plasma, brain, and CSF levels at the 100 mpk dose (60 min timepoint) were 4500, 5400, and 110 nM, respectively.

In contrast, the more selective agonist **17b** elicited no change in paw withdrawal threshold when dosed at 100 mpk (Fig. 4). Plasma, brain, and CSF levels (120 min timepoint) were measured at 4300, 1300, and 300 nM, respectively, all well above the in vitro CB2 IC_{50} values.

Our results indicate that two compounds with a high degree of structural similarity, differing mainly with respect to their CB2/CB1 selectivity profiles, exhibit markedly different efficacy profiles in the rat CFA model. By obtaining plasma, brain, and CSF exposure data, we have demonstrated that both compounds are present in both the systemic circulation and the CNS in levels exceeding their CB2 IC_{50} values. The analgesic effect observed for **6a** combined with the lack of effect seen with the more selective agonist **17b** suggests that CB1 agonism may play a role in the efficacy of **6a**. In fact, almost all selective CB2 agonists that have been evaluated in rodent pain models do exhibit measurable CB1 agonism.[13,14] The in vitro profile of **6a** is similar to many agonists reported in the literature. It is selective against CB1 (rat CB2/CB1 ~ 100-fold) but nonetheless exhibits significant efficacy as a CB1 agonist.[15]

Relevant to these observations is the demonstration by Gifford et al. that a large CB1 receptor reserve exists in the CNS. These investigators demonstrated that major downstream effects of CB1 agonism can be observed at <1% occupancy and that 95% inhibition of these effects occurs at <8% occupancy of the CB1 receptor. Thus even a compound with a relatively high EC_{50} for CB1 agonism may significantly affect CB1 signaling.[4]

An important caveat to this work (and to published CB2 agonist studies to date) is the absence of a direct demonstration of CB2 and CB1 agonism in vivo by our compounds. However, we have demonstrated that the compounds achieve high exposure in vivo, both peripherally and centrally, with a significant fraction of unbound compound available. In the accompanying paper, study of a structurally dissimilar series of CB2 agonists provides further evidence that CB2 agonism alone may be insufficient for analgesic activity.[5]

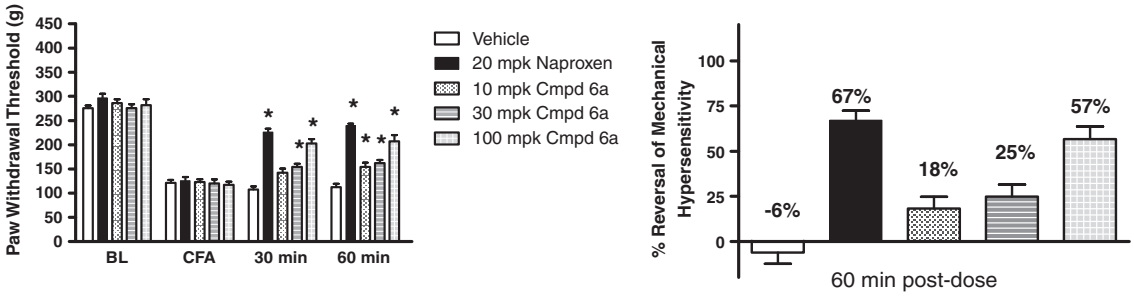


Figure 3. Agonist **6a** dosed in the rat CFA model.

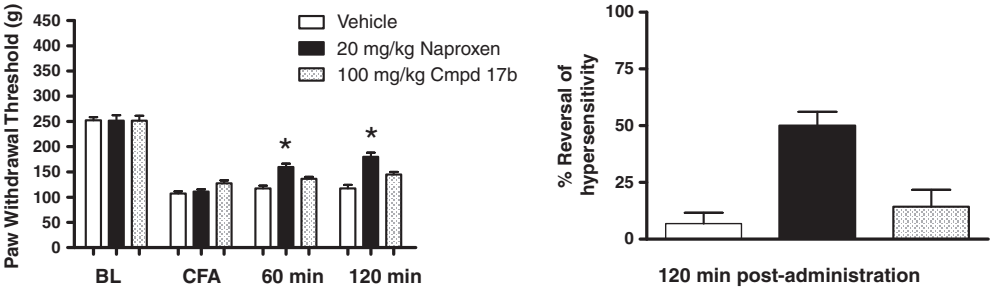


Figure 4. Agonist **17b** dosed in the rat CFA model.

Acknowledgments

We would like to thank Joan S. Murphy and Dr. Charles W. Ross III for HRMS analysis. We would also like to thank Janine N. Brouillette and Dr. Steven M. Pitzenberger for NMR support.

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10. 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 h. 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.
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