Bioorganic & Medicinal Chemistry Letters 19 (2009) 1604–1609

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Morpholine containing CB2 selective agonists

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ARTICLE INFO

Article history:
Received 24 October 2008
Revised 3 February 2009
Accepted 4 February 2009
Available online 12 February 2009

Keywords:
Cannabinoid receptor
Anti-inflammatory
CB1
CB2
Agonists
Selectivity

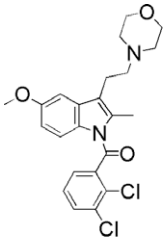
ABSTRACT

Identification and optimization of two classes of CB2 selective agonists are described. A representative from each class is profiled in a murine model of inflammation and each shows similar efficacy to prednisolone upon oral dosing.

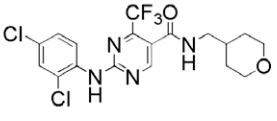
The therapeutic use and psychotropic effects of cannabis has been known for several centuries. However, it was only in the last 50 years that the active constituent has been identified as Δ^9-tetrahydrocannabinol.[1] Subsequently the receptors identified as mediating its effects were identified as the G protein-coupled receptors cannabinoid receptor 1 (CB1) and 2 (CB2).[2,3] The CB1 receptor is expressed primarily in the central nervous system and is believed to be responsible for the undesirable effects such as ataxia, hypothermia, and euphoria. The highest density of CB1 is found in the basal ganglia, cerebellum, and hippocampus.[4] CB1 is also found to a much lesser extent in peripheral tissues such as the testis, eye, uterus, ovary, lungs and heart.[5–7] The CB2 receptor is implicated in the anti-inflammatory activity of cannabis as it is expressed primarily in peripheral immune cells such as B-cells, monocytes, macrophages, and in organs such as the spleen, pancreas, thymus, lung, and tonsil.[6,8] CB2 is also found to a much lesser extent in central locations.[9]

CB2 shares 44% homology with CB1 which, coupled with differences in receptor densities in various tissues, offers the possibility to separate the immune-related effects of cannabinoids from the psychoactive effects typically associated with CB1.[10] Selective CB2 agonists should avoid unwanted psychoactive side effects attributed to CB1 while maintaining immunomodulatory and analgesic activity. CB2 selective agonists have been reported to be efficacious in both in vitro and in vivo models of inflammation[11–13] as well as inflammatory and neuropathic pain.[14–17] Some of these compounds, shown in Figure 1, are GW405833[18], GW842166[19], and PRS-211375 (structure not known)[20] with GW842166 having entered clinical trials for the treatment of inflammatory pain. Our goal is the identification of CB2 selective agonists for the modulation of inflammatory response and treatment of pain (Fig. 2).

Our starting point to identify CB2 selective agonists originated from patent application WO 2004/060882. Compound **1**, a representative example, has a binding affinity of 50 nM for CB2 when evaluated in a competitive binding assay with membranes of HEK cells expressing the human CB2 receptor against



GW405833



GW842166

Figure 1. Examples of CB2 selective agonists and modulators reported in the literature.

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doi:10.1016/j.bmcl.2009.02.033

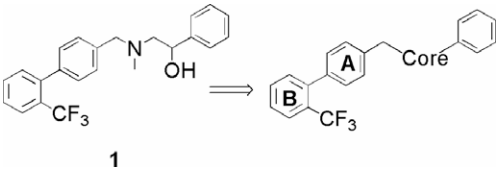
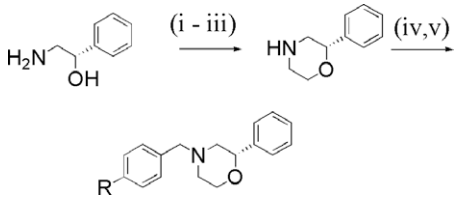


Figure 2. Starting point and modification points.



Scheme 1. Reagents and conditions: (i) bromoacetyl bromide, ethyl acetate, saturated aq $NaHCO_3$, rt, quantitative; (ii) KO^{t}Bu, ^{t}BuOH, rt, 33%; (iii) LAH, THF, 0 °C to rt, 46%; (iv) 4-bromobenzyl bromide, K_2CO_3, acetonitrile, rt, 40–80% or 4-bromobenzaldehyde, MP-BH(OAc)$_3$, THF, rt, 40–80%; (v) RB(OH)$_2$, Pd(PPh$_3$)$_4$, aq Na_2CO_3, MePh, 120 °C, 3–86%.

[^{3}H]-CP55940 probe.[21] The ability of CB2 agonists to activate Gαi, which in turn inhibits cyclic adenosine monphosphate (cAMP) production is determined in a cellular assay employing CB2 over-expressing CHO cells stimulated with forskolin.[22,23] The CB2 cAMP EC_{50} of **1** is 2 nM with 95% efficacy and shows 120-fold selectivity over CB1 in a similar functional cAMP assay. Since interest in this program is identifying and optimizing CB2 selective agonists, the CB2 and CB1 cAMP functional assay is used to develop SAR with binding data generated on selected compounds to confirm interaction with the target.

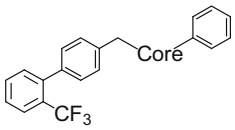
Our initial strategy was to rigidify the amino alcohol core of **1** in an effort to lock the molecule in the desired bioactive conformation which we hoped would result in improved CB2 potency and selectivity. To this end, variants of the core were investigated. Morpholine (example **2**) and piperidine (example **3**) cores show agonist activity, however piperizine, thiomorpholine and thiomorpholine dioxide lacked measurable agonist activity when tested up to 20 μM in the CB2 functional assay. Based on the overall profile, morpholine was chosen as a core for further optimization. Further results of this effort are described in this letter (Table 1).

While the morpholine containing compound **2** shows a loss of agonist activity as compared to **1**, we recognized that the enantiomers might differentiate themselves in the functional assay and therefore resolved the compound by chiral HPLC. The first eluting enantiomer **4** has a CB2 cAMP EC_{50} of 40 nM with 50% efficacy and 250 fold selectivity over CB1 while the second eluting enantiomer **5** is an inverse agonist. The 10-fold improvement in potency upon testing of the eutomer was not surprising since the racemic compound is a mixture of agonist and inverse agonist.

The synthesis of chiral analogs is depicted in Scheme 1 and can be applied to both enantiomerically pure and racemic starting materials.[24] Commercially available (S)-2-amino-1-phenylethanol is alkylated with bromoacetyl bromide and subsequently cyclized and reduced to form (S)-2-phenylmorpholine. Reaction of this intermediate under standard alkylation or reductive amination conditions followed by palladium cross coupling affords analogs **4** and **6–9**. The racemic molecules were synthesized starting from commercially available 2-phenylmorpholine (or 3-phenylpiperidine) to afford analogs **2** and **3** and those described in the text.

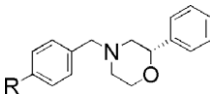
In addition to the core replacement effort, exploration of the biaryl portion of the molecule was undertaken to establish optimal spatial orientation and substitution pattern of the aromatic rings. Any racemates showing desired agonist activity were then synthesized in a chiral fashion and data is shown in Table 2.[25] Appending the B ring from the A ring 4-position was found to be optimal since appending from the 2- or 3-position results in no measurable CB2 agonist activity when tested up to 20 μM. Appending the B ring to this optimal 4-position of the A ring and exploring various chloro-substitutions demonstrated that of the 2-, 3-, and 4-chlorophenyl derivatives, only the 2-chloro-substituted phenyl (example **6**) has agonist activity. Replacing the 2-chloro substituent with electron neutral or donating moieties such as hydrogen, methyl, and methoxy lead to compounds which have no measurable agonist activity when tested up to 20 μM. Maintaining an electron withdrawing group in the 2-position while adding an additional substituent to the 3-position (example **7**) or 5-position (example **8**) not only improves potency but enhances the selectivity of the molecule as compared to the monosubstituted 2-trifluoromethyl and 2-chlorophenyl analogs (examples **4** and **6**). This data implies substitution in other positions is tolerated but requires 2-substitution for desired agonist activity. The nitrile substituted phenyl in compound

Table 1
Amino alcohol core variant



Example	Stereo-chemistry	Core	CB2 cAMP EC_{50} (nM)	Efficacy (%)	CB1 EC_{50}/CB2 EC_{50}
1	*rac*		2	95	120
2	*rac*		330	50	>60
3	*rac*		30	57	195
4	*S*		40	50	250
5	*R*		Inverse agonist		

Table 2
B ring SAR



Example	R	CB2 cAMP EC_{50} (nM)	Efficacy (%)	CB1 EC_{50}/CB2 EC_{50}
4		40	53	250
6		25	51	306
7		6	77	960
8		3	73	781
9		3	56	715

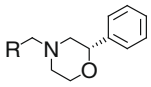
9 gave similar potency and selectivity to the disubstituted containing derivatives suggesting that in addition to the polarizing effects of the substituents on the B ring, a certain bulk imparts both the desired potency and selectivity. Thus, rigidifying the central portion of the molecule as well as manipulating the substituents of the B ring led to novel compounds showing improvement in selectivity whilst maintaining potency as compared to **1**.

In addition to developing B ring SAR**,** we investigated incorporating a linker between the A and B rings to reduce the potential for non-specific protein binding in vivo which has been reported for biaryl containing molecules.[26] Given the differences in functional activity seen between enantiomers **4** and **5**, we chose to work with the eutomer directly in this series. Table 3 outlines our work towards this end. Replacement of the bond between the A and B rings with a nitrogen (compound **10)** or oxygen (compound **11**) shows measurable activity despite having no substituents on the B ring. This is in contrast to phenyl described above in the biaryl system which lacked agonist activity when the A and B rings are separated with a bond. Considering the wide range of activities seen within the biaryl system as described above, it was plausible to expect the potency could be improved with the appropriate substitution of the B ring. Encouraged by the activity of **10** A ring replacements were investigated which led to the identification of compound **12**. Significant improvement in the potency and selectivity is observed with this change. Unfortunately, incorporating the optimized substituted B ring phenyl moiety identified in Table 2 results in a significant loss in potency for compound **13**. This suggests introduction of a linker leads to significantly different SAR and prompted a more detailed investigation of the B ring whilst maintaining the pyridyl A ring.

Table 4 illustrates the SAR of the N-linked compounds.[27] The synthesis was similar to that shown in Scheme 1 with the exception of the last step which was a S_NAr reaction instead of the palladium cross-coupling conditions described above.[28]

Unlike the biaryl system, removing substituents from the B ring (example **12**) results in modest functional activity. Mono-substitution on the B ring (examples **14–16**), demonstrates compounds bearing substitution in the 2- or 4-position are more potent than

Table 3
Incorporation of linker between aryl rings



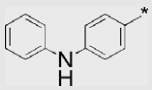
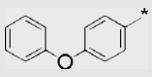
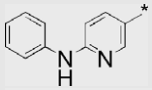
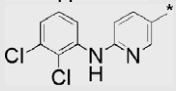
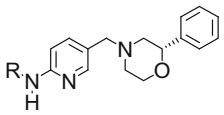
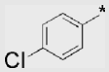
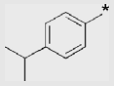
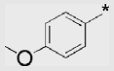
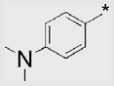
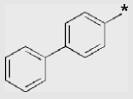
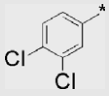
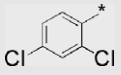
Example	R	CB2 cAMP EC_{50} (nM)	Efficacy (%)	CB1 EC_{50}/CB2 EC_{50}
10		615	60	>30
11		15,000	100	
12		140	70	>150
13		1600	70	>10

Table 4
Exploration of the B ring in the bis-aryl amine series



Example	R	CB2 cAMP EC_{50} (nM)	Efficacy (%)	CB1 EC_{50}/CB2 EC_{50}
12		140	70	>150
14		30	80	250
15		200	80	>100
16		30	80	>670
17		35	70	CB1 IA
18		100	80	>200
19		>20,000		
20		65	90	>310
21		>20,000		
22		>20,000		
23		50	80	>400
24		10	100	>2000

those which have 3-substitution. In addition to potency, 4-substitution offers improvement in selectivity over CB1. Subsequent investigation of the 4-position demonstrate that small substituents with varying electronic properties are tolerated (examples **16–18**, and **20)** but steric limitation exist as evidenced by isopropyl- (example **19)**, dimethylamine- (example **21**) and phenyl-substituents (example **22)**. Inspired by the potency and selectivity of analog **16**, di-substituted aromatics which contain a small substituent in the 4-position were investigated. Example **23** demonstrates that the 3-substituent which was detrimental to potency in example **15**, is better tolerated in combination with 4-substitution. Finally, adding the small substituent to *ortho*-position, which was well tol-

Table 5
Data of advanced compounds and GW842166

Example	CB2 cAMP EC_{50} (nM) (%)	CB1 EC_{50}/CB2 EC_{50}	CB2 Ki (nM) CP55940/Win55212
GW842166	350 (90)	>60	>5000/>10,000
7	5 (80)	960	312/104
24	10 (100)	>2000	>10,000/2650

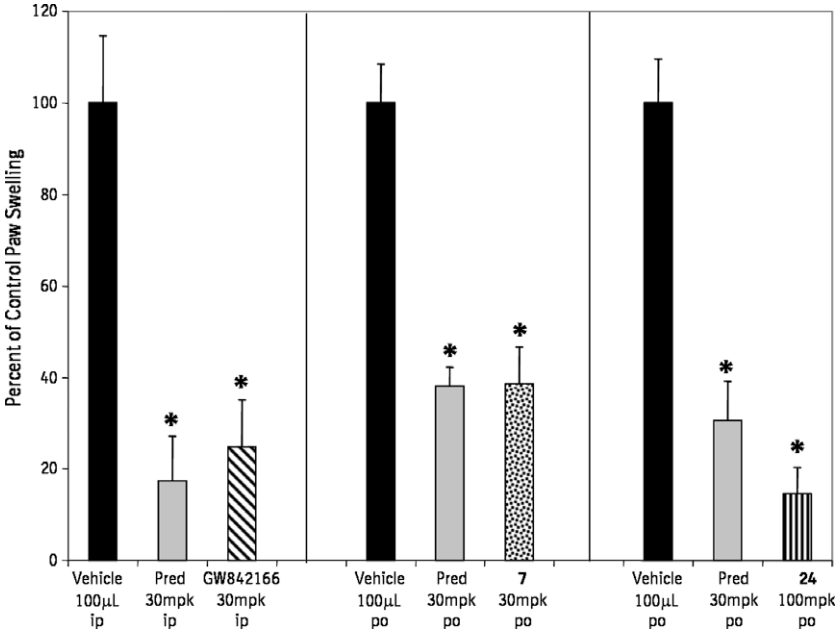


Figure 3. In vivo activity of compounds in zymosan induced paw inflammation model. Change in paw swelling of compound or prednisolone dosed mice compared to vehicle dose mice are represented. Values are mean of six mice per group ±SEM. $^{*}p < 0.001$ versus vehicle control.

erated in example **14**, leads to compound **24** which has both excellent potency and selectivity.

Based on overall profile, both **7** and **24** were chosen for further profiling as shown in Table 5. The SAR described has been developed based on the functional assays, however the binding data is also collected on selected compounds to confirm the receptor interaction. Both ^{3}H-CP55940 and ^{3}H-Win55212 are CB1/CB2 dual agonists which are used in competition assays towards this end.[21] Compound **7** shows modest competition against both radiolabeled ligands whereas **24** competes only against ^{3}H-Win55212. In addition to competing against only one of the probes, **24** shows a 25 fold loss in binding affinity as compared to **7** suggesting different binding modes of the two compounds.[29] Each of the compounds is a very potent CB2 agonist based on the functional data with very good selectivity over CB1. To examine the anti-inflammatory activity in vivo, an acute rodent inflammation model was used. Swelling is induced by zymosan and a compounds' efficacy is assessed by its ability to inhibit the swelling after an oral dose of compound.[30] The results reported in Figure 3 show compound **7** dosed at 30 mpk inhibits 54% of paw swelling as compared to vehicle control. Compound **24** dosed at 100 mpk is also efficacious with 85% inhibition of paw swelling. Both structural classes of compound show very good potency as CB2 agonists in the functional assay as well as efficacy in a murine model of inflammation.

In summary, by both rigidifying the amino-alcohol **1** to reduce the entropy of the molecule, and incorporating a linker between the aryl rings to reduce potential for unproductive protein binding, we were able to identify active molecules with improved selectivity over CB1. These compounds were dosed orally in a mouse model of inflammation and showed efficacy comparable to prednisolone. Consequently, these CB2 selective agonists should be devoid of CB1-mediated psychotropic effects with data supporting their use for the treatment of inflammatory indications.

Acknowledgements

The authors would like to thank Drs. John Regan and Matthew Netherton for their time and effort in proof reading the manuscript.

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21. Human CB2 receptor binding assay: CB2 membranes were isolated from HEK cells stably transfected with the CB2 receptor. The membrane preparation was bound to scintillation beads (Ysi-Poly-L-lysince SPA beads, GE Healthcare) in assay buffer containing 50 mM Tris, pH 7.5, 2.5 mM EDTA, 5 mM $MgCl_2$, 0.8% fatty acid in free Bovine Serum Albumin. Unbound membrane was removed by washing in assay buffer. Membrane-bead mixture was added to the 96-well assay plates in the amounts of 2 mg membrane per well (CB2) and 1 mg SPA bead per well. Compounds were added to the membrane-bead mixture in dose-response concentrations ranging from 1×10^{-5} M to 1×10^{-10} M. The competition reaction was initiated with the addition of [^{3}H]-CP55940 or [^{3}H]-Win55212 as noted (Perkin–Elmer Life and Analytical Sciences) at a final concentration of 4 nM. IC50 values for each compound were calculated as the

concentration of compound that inhibits the specific binding of the radioactively labeled ligand to the receptor by 50% using the XLFit 4.1 four-parameter logistic model. IC_{50} values were converted to inhibition constant (Ki) values using the Cheng–Prusoff equation.
22. CB2 and CB1 cAMP assays: CHO cells expressing human CB2 or CB1 (Euroscreen) were plated at a density of 5000 cells per well in 384-well plates and incubated overnight at 37 °C. After removing the media, the cells were treated with test compounds diluted in stimulation buffer containing 1 mM IBMX, 0.25% BSA and 10 mM forskolin. The assay was incubated for 30 min at 37 °C. Cells were lysed and the cAMP concentration was measured using DiscoveRx XS+ cAMP kit, following the manufacturer's protocol. The maximal amount of cAMP produced by forskolin compared to the level of cAMP inhibited by 200 nM CP-55940 is defined as 100%. The EC50 value of each test compound was determined as the concentration at which 50% of the forskolin-stimulated cAMP synthesis was inhibited using a four-parameter logistic model.
23. Reproducibility of the cAMP assays is assessed using the control compound CP-55940 which is tested twice on every assay plate to rule out any plate artifacts. Efficacy at CB1 and CB2 is expressed as a percentage relative to the efficacy of CP-55940. Each compound is tested in triplicate at least three times with individual dilutions from the stock. The results reported in the table are the mean values of the measurements and individual values for the compounds do not differ by more than a factor of three from the mean.
24. All compounds described gave satisfactory ^{1}H NMR and LC–MS analytical data.
25. Boehringer Ingelheim Corp. WO 2007/070760.
26. Hajduk, P. J.; Bures, M.; Praestgaard, J.; Fesik, S. W. *J. Med. Chem.* **2000**, *43*, 3443.
27. Boehringer Ingelheim Corp. WO2008/048914.
28. Synthetic scheme varies from Scheme 1 in that (iv) employs 6-chloro-pyridine-3-carbaldehyde as the aldehyde to afford (S)-4-(6-chloro-pyridin-3-yl)-2-phenyl-morpholine. (v) RNH2, NaHMDS, DMSO, 60 °C, 23–60%.
29. Literature suggests there is more than one plausible binding site and orientation for CB ligands. Xie, X.; Chen, J. Z.; Billings, E. M. *Proteins: Structure, Function, and Genetics* **2003**, *53*, 307; Raitio, K. H.; Salo, O. M. H.; Nevalainen, T.; Poso, A.; Jarvinen, T. *Curr. Med. Chem.* **2005**, *12*, 1217.
30. In vivo zymosan-induced paw edema assay: Male Balb/c mice (Jackson Laboratories, Bar Harbor, ME), between 6–10 weeks of age were used. Mice were fed and watered *ad libitum*, and housed in compliance with IACUC guidelines. On the day of study, mice were administered the test compounds, and appropriate vehicles (95%PEG400/5%Tween 80 for po or 80% Cremophor EL for ip) approximately 60 min prior to a challenge of a dorsal metatarsal injection of 0.5% zymosan (Sigma Z-4250) in 0.9% saline in each hind paw. Volume injected was 0.025 microliters. Paw volumes were ascertained before challenge and 3 h after challenge by a water-based plethysmometer (Ugo Basile) by the following method: The hind paws of mice are extended and placed in the water chamber to the level of the lateral malleous, and the volume displacement is electronically recorded. Six animals (12 paws) were used in each group.