Letters

Discovery of 2-[(2,4-Dichlorophenyl)amino]-*N*-[(tetrahydro-2*H*-pyran-4-yl)methyl]-4-(trifluoromethyl)-5-pyrimidinecarboxamide, a Selective CB2 Receptor Agonist for the Treatment of Inflammatory Pain

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Abstract: Selective CB2 receptor agonists are promising potential therapeutic agents for the treatment of inflammatory and neuropathic pain. A focused screen identified a pyrimidine ester as a partial agonist at the CB2 receptor with micromolar potency. Subsequent lead optimization identified **35**, GW842166X, as the optimal compound in the series. **35** has an oral ED_{50} of 0.1 mg/kg in the rat FCA model of inflammatory pain and was selected as a clinical candidate for this indication.

The medicinal and psychoactive properties of *Cannabis sativa* have been known for hundreds of years.[1] Δ^9-Tetrahydrocannabinol, the principal active component of cannabis, has analgesic and muscle relaxant properties in addition to a range of central nervous system (CNS) mediated effects such as catalepsy, paranoia, and dependency.[2a] Consequently, although cannabis and cannabinoids merit further study in the symptomatic treatment of multiple sclerosis[2b] and for the relief of pain, it is likely that efficacy and long-term safety of cannabinoids may be compromised by undesirable CNS side effects.

Cannabinoids act at two known subtypes of the cannabinoid receptor: CB1 and CB2. The observed behavioral effects of cannabinoids are mediated centrally by the CB1 receptor.[3] The role of the CB2 receptor has, until recently, been less clear. However, a recent report from our laboratories[4] showed that a moderately selective partial CB2 receptor agonist is active in the carrageenan model of inflammatory pain and that its antihyperalgesic activity in this model is blocked by a selective CB2 antagonist[6] but not by a selective CB1 antagonist. Subsequently, this CB2 agonist was shown to be devoid of typical CB1 side effects such as sedation and catalepsy at doses that show antihyperalgesic effects.[7] Furthermore, a structurally distinct and moderately selective CB2 agonist has been reported to have activity in models of inflammatory[8] and neuropathic pain.[9]

These observations encouraged us to initiate a program to discover a CB2 agonist, with high selectivity over CB1 to avoid central side effects, for the treatment of chronic pain.

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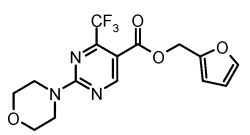
Table 1. CB2 Activity of Analogues of Screening Hit **1**[a]



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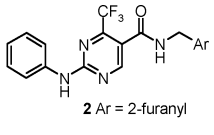

2 Ar = 2-furanyl

5 Ar = Ph

	CB2 EC_{50} (nM)	*E* (%)	*n*
1	630	55	4
2	630	62	12
5	400	45	12

[a] CB2 assay data are presented as the mean of at least two determinations. Assay reproducibility is monitored by the use of a cannabinoid control agonist HU210 ($EC_{50} = 3.0 \pm 0.5$ nM). Efficacy (*E*) at CB2 is expressed as a percentage relative to the efficacy of HU210. All analogues show no significant activity at the human CB1 receptor at concentrations up to 30 μM.

A pharmacophore model was developed on the basis of known cannabinoid ligands, and approximately 1000 samples from the corporate compound collection were selected for screening. Compounds were tested for activity at the CB1 and CB2 receptors using recombinant human receptors expressed with human G-proteins in a yeast cell line.[10]

The most promising hit, **1**, a trifluoromethyl substituted pyrimidine ester, had submicromolar potency at CB2 and an efficacy (*E*) of 55% relative to the cannabinoid analogue (6a*R*,-10a*R*)-3-(1,1-dimethylheptyl)-9-(hydroxymethyl)-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (HU210)[11] but showed no activity at CB1 at concentrations up to 30 μM, (Table 1).

The first objective was to replace the ester, and it was discovered that an amide analogue **2**, with the original 2-morpholino substituent replaced by aniline, had similar CB2 potency (Table 1). The synthesis of amide analogues is described in Figure 1. Thus, commercially available 2-chloro-4-trifluoromethyl pyrimidine ester **3** was condensed with a variety of aromatic amines. The ester product was saponified and the resultant acid **4** transformed into amides under standard conditions.

Replacement of the 2-furanylmethyl side chain with benzyl led to analogue **5** that had a similar level of CB2 potency as the 2-furanylmethyl analogue **2**. Analogue **5** had significantly better metabolic stability (rat microsomal clearance CLi < 0.5 mL min^{-1} g^{-1} liver) compared to 2-furanylmethylamide **2** (rat CLi 4.6 mL min^{-1} g^{-1}), and this template became the basis for further optimization.

The methylene group between the amide and the aromatic ring of lead pyrimidine **5** was found to be optimal; extending the aniline side chain or truncating the benzylamide side chain led to analogues with no significant activity at CB2.[12] We then explored the effect of substitution of the aniline ring (Table 2). A methoxy substituent was tolerated in all three positions of the aniline ring (**6**−**8**); 2- and 3-substituted analogues appeared to provide some improvement in CB2 efficacy. *tert*-Butyl or amide substituents led to inactive **9**−**11**. A chloro substituent was well tolerated (analogues **12**−**14**), and encouragingly, the 3-chloro substituted analogue **13** had high CB2 efficacy. Cyano

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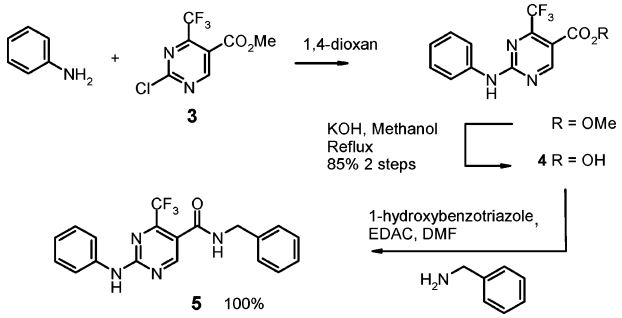
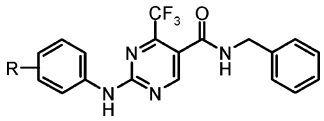


Figure 1. Synthesis of pyrimidine CB2 agonists.

Table 2. Substitution of the Aniline[a]



compd	R	CB2 EC_{50} (nM)	*E* (%)	*n*
5	H	400	45	12
6	2-OMe	158	77	12
7	3-OMe	465	76	2
8	4-OMe	200	32	12
9	4-^{t}Bu	>25000		2
10	3-NHAc	>25000		2
11	4-NHAc	>25000		2
12	2-Cl	250	48	12
13	3-Cl	245	82	3
14	4-Cl	126	49	12
15	3-CN	455	76	2
16	4-CN	400	67	8
17	3-F	310	88	2
18	3-Br	230	81	2

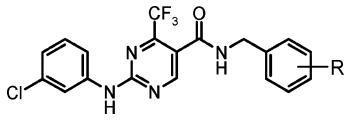
[a] CB2 assay data are presented as the mean of at least two determinations. Assay reproducibility is monitored by the use of a cannabinoid control agonist HU210 (EC_{50} = 3.0 ± 0.5 nM). Efficacy (*E*) at CB2 is expressed as a percentage relative to the efficacy of HU210. All analogues show no significant activity at the human CB1 receptor at concentrations up to 30 μM.

substituted analogues **15** and **16** were equipotent with unsubstituted analogue **5**. The 3-fluoro- and 3-bromo analogues **17** and **18** had high CB2 efficacy. The 3-chloro analogue **13** was selected as the basis for further analogue synthesis. None of these analogues show significant agonist effects at the human CB1 receptor at concentrations up to 30 μM.

The effect of substitution of the aromatic ring of the benzylamide was subsequently investigated using the preferred 3-chloroaniline template (Table 3). Substitution of this phenyl ring is less well tolerated than the aniline. For example, both electron-withdrawing and electron-donating substituents in the 2- and 3-positions (**19**−**22**) have reduced CB2 activity. However, substitution of the 4-position is more promising. The 4-methoxy **24** and 4-cyano **25** analogues retain good CB2 potency, and the 4-fluoro analogue **26** has much higher CB2 potency and efficacy. However, because of an unfavorable cytochrome P450 (CYP450) enzyme inhibition profile (Table 3), indicating a potential for clinical drug−drug interactions, analogue **26** was not evaluated further. The tight SAR around the phenyl ring of the benzylamide encouraged us to evaluate replacements for the phenyl ring.

A range of amide analogues containing heteroaromatic rings gave low-potency CB2 agonists (data not shown) with the exception of a 4-pyridylmethylamide analogue that had high CB2 potency but an unacceptable CYP450 profile.[13] Further analogue synthesis resulted in the identification of a series of analogues with non-aromatic rings, some of which have

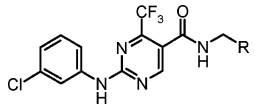
Table 3. Amide Aromatic Substituent[a]



compd	R	CB2 EC_{50} (nM)	*E* (%)	*n*
13	H	245	82	3
19	2-Cl	10000	21	12
20	3-Cl	1580	18	12
21	2-OMe	>25000		11
22	3-OMe	25000	20	8
23	4-Cl	400	65	12
24	4-OMe	214	66	2
25	4-CN	220	69	2
26[b]	4-F	63	78	12

[a] CB2 assay data are presented as the mean of at least two determinations. Assay reproducibility is monitored by the use of a cannabinoid control agonist HU210 (EC_{50} = 3.0 ± 0.5 nM). Efficacy (*E*) at CB2 is expressed as a percentage relative to the efficacy of HU210. All analogues show no significant activity at the human CB1 receptor at concentrations up to 30 μM. [b] CYP450 profile (IC_{50} μM) of **26**: 1A2, 8; 2C9, 8; 2C19, 12; 2D6, 1.5; 3A4, 5.

Table 4. Nonaromatic Amide Side Chains[a]



					CYP450 IC_{50} (μM)				
compd	R	CB2 EC_{50} (nM)	*E* (%)	*n*	1A2	2C9	2C19	2D6	3A4
27	cyclohexyl	80	82	12	>100	9	19	>100	82
28	4-piperidinyl	5000	46	2	NT	NT	NT	NT	NT
29	4-tetrahydro-pyranyl	50	89	8	>100	18	56	51	51
30	cyclopentyl	80	83	12	32	6	14	>100	65
31	cyclobutyl	40	82	12	8	3	8	22	75
32	1-hydroxy-1-cyclohexyl	125	83	12	11	10	17	13	10
33	cyclopropyl	80	79	12	NT	NT	NT	NT	NT

[a] CB2 assay data are presented as the mean of at least two determinations. Assay reproducibility is monitored by the use of a cannabinoid control agonist HU210 (EC_{50} = 3.0 ± 0.5 nM). Efficacy (*E*) at CB2 is expressed as a percentage relative to the efficacy of HU210. All analogues show no significant activity at the human CB1 receptor at concentrations up to 30 μM. NT = not tested.

improved CB2 potency and high efficacy (Table 4). Carbocyclic analogues **27**, **30**, **31**, and **33** had high CB2 potency and efficacy. However, three of these analogues had IC_{50} of less than 10 μM at one or more human CYP450 isozymes. Analogue **29** with a 4-tetrahydropyranylmethylamide had good CB2 potency and the highest efficacy. Encouragingly, this analogue showed a reduced inhibitory profile at the CYP450 enzymes.

On the basis of the optimal 4-tetrahydropyranylmethylamide, the SAR of the aniline was reinvestigated. We discovered that most dihalo substituted analogues were high efficacy CB2 agonists but potency varied (Table 5). The 2,4-dichloroanilino analogue **35** was notable for its high CB2 efficacy and very low inhibition of the CYP450 isozymes and so was selected for further evaluation.

Compound **35** shows similar potency and efficacy for rat and human recombinant CB2 receptors (for rat, CB2 EC_{50} = 91 nM, *E* = 100%, *n* = 6; for human, CB2 EC_{50} = 63nM, *E* = 95%, *n* = 20). It has no significant agonist activity at concentrations up to 30 μM in human and rat CB1 recombinant assays. When dosed orally in the rat, **35** has an oral bioavail-

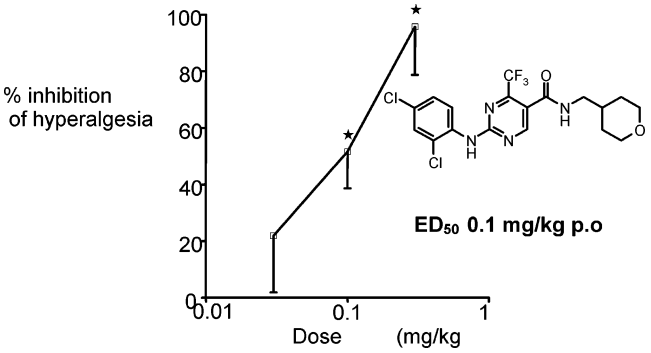
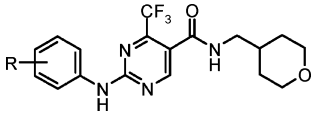


Figure 2. FCA model activity of **35**.

Table 5. Dihaloanilino Analogues[a]



					CYP450 IC_{50} (μM)				
compd	R	CB2 EC_{50} (nM)	*E* (%)	*n*	1A2	2C9	2C19	2D6	3A4
34	2,3-dichloro	80	90	8	NT	NT	NT	NT	NT
35	2,4-dichloro	63	95	20	>100	>100	>100	>100	>100
36	2,5-dichloro	100	85	12	NT	NT	NT	NT	NT
37	2,6-dichloro	630	86	12	NT	NT	NT	NT	NT
38	3,4-dichloro	49	83	12	8	>25	>25	>25	>25
39	3,5-dichloro	33	85	12	>100	13	14	27	38
40	3-chloro-2-fluoro	125	90	12	99	75	>100	>100	>100
41	5-chloro-2-fluoro	125	86	12	26	37	>100	>100	68
42	2-chloro-4-fluoro	400	85	12	NT	NT	NT	NT	NT
43	4-chloro-2-fluoro	200	80	12	>100	>100	>100	>100	>100
44	2,3-difluoro	400	90	12	NT	NT	NT	NT	NT
45	3,5-difluoro	125	91	12	24	>100	91	45	>100
46	2,4-difluoro	630	81	12	NT	NT	NT	NT	NT

[a] CB2 assay data are presented as the mean of at least two determinations. Assay reproducibility is monitored by the use of a cannabinoid control agonist HU210 (EC_{50} = 3.0 ± 0.5 nM). Efficacy (*E*) at CB2 is expressed as a percentage relative to the efficacy of HU210. Analogues show no significant activity at the human CB1 receptor at concentrations up to 30 μM except **38**, which had weak but significant activity at the CB1 receptor (approximately 10% efficacy at 2 μM). NT = not tested.

ability of 58% and a half-life of 3 h. In the FCA[a] model of inflammatory pain[14] **35** has extremely high potency with an oral ED_{50} of 0.1 mg/kg and shows full reversal of hyperalgesia at 0.3 mg/kg as determined by a weight bearing protocol (Figure 2). The blood concentrations of **35** in this experiment were 30 nM (0.03 mg/kg), 130 nM (0.1 mg/kg), and 370 nM (0.3 mg/kg) 1 h after dosing. Thus, efficacy in this model is observed when blood concentrations of drug are in excess of the EC_{50} of **35** (91 nM) at rat CB2 receptors. Furthermore, this activity was reversed by administration of the selective CB2 antagonist {6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl}[4-(methyloxy)phenyl]methanone (AM630).[15] These data support the conclusion that the antihyperalgesia properties of **35** in the FCA model are mediated by the CB2 receptor.

The potential for **35** to show tolerance in vivo by desensitizing the CB2 receptor was also assessed. After dosing for 4 days in the FCA model, no statistical difference in antihyperalgesic response was observed on day 4 relative to day 1,[16] indicating that tolerance did not occur. Finally, although **35** readily partitions into the CNS (rat brain/blood ratio, 0.8:1), no evidence of CB1 mediated side effects (catalepsy or hypothermia) was observed in rats at doses 100-fold higher than the effective dose in FCA model.[17]

In conclusion, starting from a low molecular weight screening hit with micromolar potency at the CB2 receptor and good selectivity against CB1, **35 (**GW842166X),[18] a potent and highly selective full agonist at the CB2 receptor, was discovered. This compound is a very potent analgesic in the FCA model of inflammatory pain and has a high therapeutic index and a promising pharmacokinetic profile in the rat. Compound **35** was selected as a clinical candidate for pain associated with osteoarthritis and rheumatoid arthritis. Further details of the pharmacology of **35** will be reported elsewhere.

Supporting Information Available: Details on the assay methods, synthetic procedures, and compound characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) 2-Benzylamino-*N*-benzyl-4-(trifluoromethyl)-5-pyrimidinecarboxamide and 2-phenylamino-*N*-phenyl-4-(trifluoromethyl)-5-pyrimidinecarboxamide had no significant activity in the CB2 assay at 10 μM.
(13) 2-[(3-Chlorophenyl)amino]-*N*-(4-pyridinylmethyl)-4-(trifluoromethyl)-5-pyrimidinecarboxamide. CB2: EC_{50} = 60 nM, *E* = 94%. CYP450 (IC_{50} μM): 1A2, 0.4; 2C9, 0.9; 2C19, 1.9; 2D6, <0.1; 3A4, <0.1.

[a] Abbreviations: FCA, Freund's complete ajuvant; EDAC, N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride.

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(16) Dosing of **35** started 23 h after 100 μL of intraplantar FCA and continued at 1 mg/kg po three times per day for 4 days. The effect of **35** on the FCA-induced decrease in weight bearing was determined each day 1 h after the morning dose. **35** reversed the FCA-induced hyperalgesia 1 h after the first dose, and this effect was maintained throughout the study. There was no evidence of tolerance in this study.

(17) For methods, see the following: Fox, A.; Kesingland, A.; Gentry, C.; McNair, K.; Patel, S.; Urban, L.; James, I. The roleof central and peripheral cannabinoid 1 receptors in the anti-hyperalgesic activity of cannabinoids in a model of neuropathic pain. *Pain* **2001**, *92*, 91−100. In our experiments, a 10 mg/kg po dose of **35** showed no significant effect in the catalepsy or hypothermia behavioural modalities; HU210 was used in both experiments as a positive control. Blood concentrations were not assayed in this experiment.

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