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Arylsulfonamide CB2 receptor agonists: SAR and optimization of CB2 selectivity

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Abstract—A high-throughput screening campaign resulted in the discovery of a highly potent dual cannabinoid receptor 1 (CB1) and 2 (CB2) agonist. Following a thorough SAR exploration, a series of selective CB2 full agonists were identified.

The therapeutic usage of cannabis can be dated back to ancient dynasties of China and since then it has been administered for the treatment of various indications including lack of appetite, emesis, cramps, spasticity, pain and rheumatism.[1] The long history of cannabis therapy has prompted the development of several pharmaceutical agents, such as Marinol® and Cesamet®. Some of the physiological effects of these drugs are mediated by at least two G-protein coupled receptors, cannabinnoid receptor 1 (CB1) and 2 (CB2).[2] The CB1 receptor is expressed predominantly in the central nervous system and it regulates the release of neurotransmitters from pre-synaptic neurons.[3] CB1 is believed to mediate most of the euphoric and other CNS effects of cannabis. In contrast, the CB2 receptor is expressed almost exclusively in the periphery,[4] particularly in cells and tissues involved in immune response.[5] Selective agonists of CB2 have been shown to suppress inflammation in vivo[6] as well as inhibiting disease severity and spasticity in an animal model of multiple sclerosis.[7,8] Additionally, CB2 agonists have been shown to inhibit inflammatory and neuropathic pain and emesis.[9–12] Potent and selective inverse agonists acting on the CB2 receptor have also been reported and they are active in inflammatory models.[13]

Development of selective CB2 receptor agonists may avoid the undesirable psychoactive effects associated with the CB1 receptor and could potentially be used for the modulation of the inflammatory response as well as for the treatment of pain. Several CB2 selective agonists and modulators have been reported in the literature such as **1**[14] and GW405833 (**2**)[15] (Fig. 1) as well as others.[16–18] Recently, a novel class of pyrimidines was described[19] and the lead compound GW842166X (**3**) has entered clinical trials for inflammatory pain.

The potential utility of selective CB2 receptor agonists for the treatment of several therapeutic indications encouraged us to screen our compound collection against this target. This paper describes the identification of an initial screening hit and the subsequent optimization efforts, which resulted in a series of compounds with excellent selectivity profiles.

From our high-throughput screening programme, the commercially available arylsulfonamide **4** (Fig. 2) was

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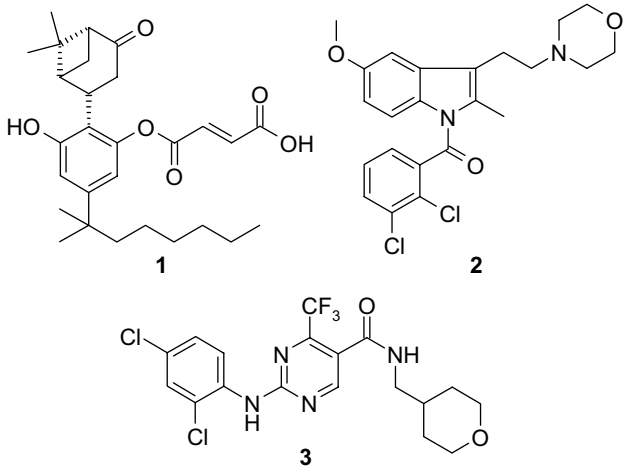


Figure 1. Structures of CB2-selective agonists.

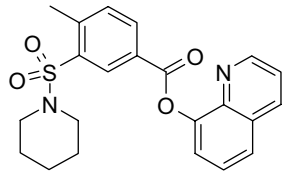


Figure 2. High-throughput screening hit **4**.

identified.[20,21] Compound **4** demonstrated high binding affinity for both the CB2 (K_i = 4 nM) and the CB1 (K_i = 3 nM) receptor, evaluated on the basis of [^{3}H]-CP-55940 binding to membranes of HEK cells expressing human CB1 or CB2.[22] The ability of **4** to inhibit cyclic adenosine monophosphate (cAMP) production was determined in a cellular assay employing CB2 over-expressing CHO cells stimulated with forskolin and it was characterized as a potent full agonist (<1 nM). In our CB1 cAMP assay, **4** was also characterized as a potent full agonist (Table 1).[23,24] As we were interested in CB2 selective agonists, we decided to focus on the functional assay, rather than binding assay, to drive the SAR.

In addition to a poor CB1/2 selectivity profile, compound **4** also suffered from low chemical stability which was attributed to ease of ester cleavage. Replacement of the ester to give the amide analogue **5** improved the chemical stability and maintained excellent CB2 potency, however **5** still lacked selectivity for CB2 over CB1. In an effort to achieve selectivity, an extensive SAR study of amide substitution was undertaken. The synthesis of these compounds is outlined in Scheme 1, route A.[25] Starting from commercially available 4-methylbenzoic acid, treatment with chlorosulfonic acid provided the corresponding sulfonyl chloride. Reaction of this sulfonyl chloride with piperidine was followed by amide bond formation under standard conditions to give amides **5**–**25** (Table 2).

Table 1. Cellular potency, efficacy and selectivity over CB1 for CB2 agonists

Compound	CB2 cAMP EC_{50} [nM]	Efficacy [%]	S (CB1)[a]
2	0.65[b]	44	n/a
3	63[c]	95	>470
4	<1	99	1

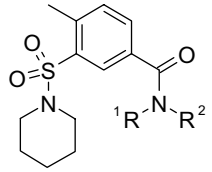
[a] S (CB1) refers to selectivity (*x*-fold) over CB1 in cAMP agonism assays.
[b] Literature data, see Ref. 15.
[c] Literature data from a reporter gene assay in yeast, see Ref. 19.

Other fused aromatic amines such as isoquinoline **6** were found to be tolerated in terms of potency on CB2, although the pattern of substitution in these analogues is important as demonstrated by a drop in potency and efficacy for compound **7**. Additionally, only modest levels of selectivity could be achieved with these compounds. Replacement of the fused system with a single unsubstituted phenyl ring resulted in a loss of potency however this could be regained by introducing substituents into the aromatic ring, preferably in the *ortho*-position as illustrated by compound **9**. It is noteworthy that substitution in the *para*-position of the aromatic ring is detrimental to activity as demonstrated by **12**. Small polar, electron-withdrawing groups in the *ortho*-position, as exemplified by compounds **15** and **16**, appear to be beneficial for CB2 potency. Importantly, compounds **15** and **16** indicated that selectivity could be improved to >50-fold.

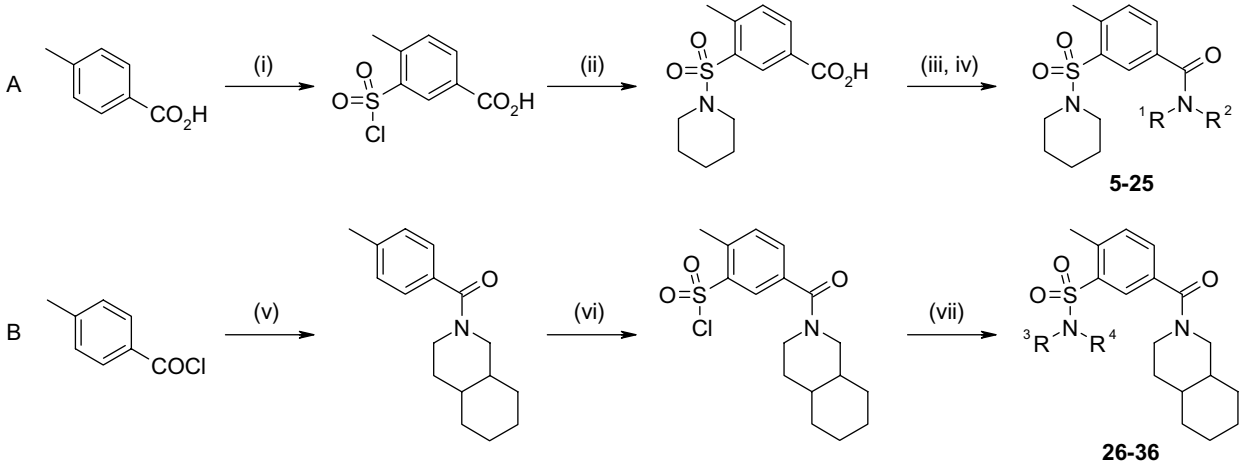
Replacing the aromatic substituent with aliphatic groups provided compounds with good CB2 potency, e.g. **17**, **18**, **20**, **21** although their selectivity over CB1 was still only modest. Disubstitution of the amide nitrogen, through introduction of a methyl group, e.g. **19** was tolerated in the aliphatic series (unlike the aromatic series (**10**)) although a drop in potency of about 10-fold was observed. Nevertheless, this prompted an investigation into other tertiary amides and piperidine amide **22** suggested that selectivities of >200-fold could be achieved. Confirmation of tertiary amides as being beneficial to the selectivity profile was illustrated by the introduction of the decahydroisoquinoline moiety as indicated by **24**. This compound has a CB2 EC_{50} of 1.5 nM with ~4000-fold selectivity over CB1. The *trans*-diastereoisomer **25** appeared to be the more potent isomer.

With the optimized decahydroisoquinoline amide, the SAR around the sulfonamide bond (Table 3) was explored to determine its effects on potency and selectivity. To enable the rapid evaluation of this region of the molecule, the order of the synthetic steps was altered as outlined in Scheme 1, route B.[25] This facilitated the introduction of the sulfonamide substituent in the last step. Replacement of the piperidine with primary amines, compounds **26**–**28**, generally resulted in a reduction in potency, which was most significant for aromatic amine **26**. In contrast, a range of secondary cyclic amines are well tolerated in this position. Expansion or reduction of the ring size (compounds **29**–**31**) quickly established that 4–7 membered systems provided compounds with excellent CB2 potency and selectivity

Table 2. Amide SAR



Compound	R^1	R^2	CB2 cAMP EC_{50} [nM]	Efficacy [%]	S (CB1)
5	8-Quinolinyl	H	4	100	7
6	1-Isoquinolinyl	H	1	98	25
7	2-Quinolinyl	H	195	45	n/a
8	Phenyl	H	139	74	6
9	2-Chlorophenyl	H	5	89	13
10	2-Chlorophenyl	Methyl	>20000	—	—
11	3-Chlorophenyl	H	27	80	31
12	4-Chlorophenyl	H	14100	117	—
13	2-Methoxyphenyl	H	21	91	9
14	2-Methylphenyl	H	154	76	1.5
15	2-Trifluoromethylphenyl	H	2	92	96
16	2-Methylsulfonylphenyl	H	6	96	66
17	1-Methylcyclohexane	H	18	58	n/a
18	Cyclohexyl	H	14	86	24
19	Cyclohexyl	Methyl	119	86	38
20	(*S*)-1-Cyclohexylethyl	H	5	73	2
21	(*R*)-1-Cyclohexylethyl	H	18	85	45
22	Piperidine		82	85	>240
23	Decahydroquinoline		6	82	94
24	Decahydroisoquinoline		1.5	95	4326
25	*trans*-Decahydroisoquinoline		1	93	6894

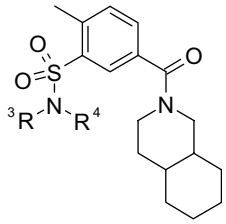


Scheme 1. Reagents and conditions: (i) chlorosulfonic acid, 65 °C, 88%; (ii) piperidine, MeCN, 0 °C to rt, 52%; (iii) $SOCl_2$, DMF (cat.); (iv) R^1R^2NH, DIPEA, CH_2Cl_2, ~75% (two steps); (v) decahydroisoquinoline, MeCN, rt, 90%; (vi) chlorosulfonic acid, dichloroethane, 80 °C, 90%; (vii) R^3R^4NH, DIPEA, MeCN, rt, ~70%.

profiles. Introduction of a heteroatom into the ring system, such as in morpholine **32** or thiomorpholine **33** was also well tolerated (potency and selectivity), however addition of a hydroxyl group as in compound **35** reduced the potency. The basic centre in compound **36** was detrimental to activity.

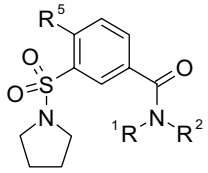
In parallel to the extensive amide and sulfonamide SAR study, we also investigated the role of the substituent in the 4-position (R^5) of the phenyl core (Table 4). The synthesis of compounds **37**–**45** proceeded by adaptation of the routes illustrated in Scheme 1 (not shown);[25] R^3 and R^4 were fixed as pyrrolidine for this study. Substitution in the 4-position was found to be essential for activity as exemplified by the unsubstituted analogues **38** and **44** which are considerably less potent (EC_{50} > 20000 nM) in the cAMP cellular assay. Replacement of the methyl in **37** or **29** with an isopropyl group as shown with **39** and **41** gave compounds with improved potency and similar selectivity profiles, however, further increasing

Table 3. Sulfonamide SAR



Compound	R^3	R^4	CB2 cAMP EC_{50} [nM]	Efficacy [%]	S (CB1)
24	Piperidine		1.5	95	4326
26	Phenyl	H	>20000	—	—
27	Isopropyl	H	2284	101	0.1
28	Isobutyl	H	14	96	939
29	Pyrrolidine		4	94	2238
30	Azetidine		13	94	>1500
31	Homopiperidine		2	100	3276
32	Morpholine		5	104	2638
33	Thiomorpholine		1	90	3247
34	Dioxothiomorpholine		2	99	7744
35	4-Hydroxypiperidine		215	86	37
36	*N*-Isopropylpiperazine		588	45	n/a

Table 4. SAR of phenyl substitution



Compound	R^5	R^1	R^2	CB2 cAMP EC_{50} [nM]	Efficacy [%]	S (CB1)
37	Methyl	Cyclohexyl	H	21	81	181
38	H	Cyclohexyl	H	>20000	—	—
39	Isopropyl	Cyclohexyl	H	4	86	60
40	Chloro	Cyclohexyl	H	3	74	85
29	Methyl	Decahydroisoquinoline		4	94	2238
41	Isopropyl	Decahydroisoquinoline		0.4	101	2864
42	Cyclohexyl	Decahydroisoquinoline		>20000	—	—
43	Chloro	Decahydroisoquinoline		4	93	520
44	H	*trans*-Decahydroisoquinoline		>20000	—	—
45	Chloro	*trans*-Decahydroisoquinoline		3	89	3768

the size and lipophilicity of the substituents as in compound **42** resulted in a significant loss of potency. A reduction in potency was also observed when an electron-donating substituent was introduced, such as a methoxy group (data not shown). The effect of electron-withdrawing substituents were demonstrated by the chloro-substituted analogues **40**, **43** and **45** and generally resulted in compounds with good potency and selectivity profiles.

Compound **29** was tested further for its affinity to the human CB2 receptor in a competitive radiolabeled ligand binding assay using either [^{3}H]-CP-55940 or [^{3}H]-WIN-55212-2 as probes. Both probes were used to evaluate whether this class of compounds adapt a CP- or WIN-like binding mode as two partially overlapping binding sites can be proposed based on the mutagenesis studies.[26,27] Compound **29** demonstrated K_i's of 130 nM against [^{3}H]-CP-55940 and 20 nM against [^{3}H]-WIN-55212-2. This suggests a better overlap between **29** and WIN-55212-2 within the binding pocket. In a CB1 binding assay using [^{3}H]-CP-55940 as a probe, **29** did not demonstrate any competition up to 5000 nM.

In conclusion, an SAR exploration of a non-selective, chemically unstable high-throughput screening hit resulted in the discovery of new series of highly selective CB2 receptor agonists. Several compounds with selectivities, in functional agonism assays, of >2000-fold over CB1 were identified. Further optimization and in vivo evaluation of compounds from this chemical series will be reported in due course.

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22. Human CB2 and CB1 receptor binding assays: CB2 and CB1 membranes were isolated from HEK cells stably transfected with the respective receptors. The membrane preparation was bound to scintillation beads (Ysi-Poly-L-lysine 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 free Bovine Serum Albumin. Unbound membrane was removed by washing in assay buffer. Membrane–bead mixture was added to 96-well assay plates in the amounts of 2 μg membrane per well (CB2) or 2.5 μg per well (CB1) 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]-CP-55940 (Perkin-Elmer Life and Analytical Sciences) at a final concentration of 4 nM (CB2) or 2.5 nM (CB1), or [^{3}H]-WIN-55212-2 at 1.6 nM. IC_{50} 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 (K_i) values using the Cheng-Prusoff equation.
23. 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 μM 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 EC_{50} 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.
24. 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 artefacts. 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 three from the mean.
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