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Synthesis and evaluation of 3-amino-6-aryl-pyridazines as selective CB_2 agonists for the treatment of inflammatory pain

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ABSTRACT

A series of 3-amino-6-aryl-pyridazines have been identified as CB_2 agonists with high efficacy and selectivity against the CB_1 receptor. Details of the investigation of structure–activity relationships (SAR) are disclosed, which led to the identification of pyridazine analogue **35**, a compound with high potency in an in vivo model of inflammatory pain.

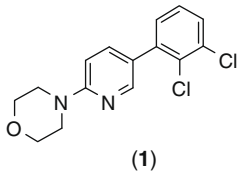
Naturally occurring cannabinoids, such as Δ^9-tetrahydrocannabinol, have been found to act as agonists at two G-protein coupled receptors CB_1 and CB_2[1] and more recently at GPR55.[2] CB_1 is found widely in the central nervous system (CNS)[3] and to a lesser extent in the periphery. CB_2 is more localized and is highly expressed in peripheral immune tissues and activated microglia.[4–6] Recent studies have also indicated the presence of CB_2 on neurons within the CNS.[7] Natural product derived and synthetic cannabinoids have shown efficacy in animal models of inflammatory and neuropathic pain.[8,9] However despite showing potential as analgesics, their use as therapeutic agents has been limited due to psychotropic effects such as euphoria/dysphoria, dry mouth and drowsiness.[9] There has therefore been great interest in the possibility of developing agonists devoid of these side effects.

One strategy to achieve an acceptable therapeutic index has been to develop selective ligands for the CB_2 receptor, therefore avoiding the behavioural effects linked to activating CB_1 within the CNS. Several classes of selective CB_2 ligands have demonstrated efficacy in pre-clinical models of inflammatory pain[10,11] and have shown a therapeutic window with regard to CNS side-effects.[12,13] We were therefore interested in developing new structural classes of CNS penetrant CB_2 agonists with high receptor sub-type selectivity suitable for in vivo studies.

A series of amino pyridines, exemplified by compound **1** (Fig. 1), had been discovered through optimization of a hit from high throughput screening.[14] The series demonstrated high potency, efficacy and selectivity and was appealing due to low molecular weight and polar surface area. Unfortunately, high in vitro metabolism was a feature of the series and limited the advancement of these compounds beyond in vitro screening.

It was decided to investigate the effect of replacing the aromatic core on metabolic stability and a series of ring isosteres were introduced (Table 1). Generally the ring substitution was well tolerated with only small variations in agonist potency. Interestingly, the phenyl analogue (**3**) had equivalent potency to the lead pyridine (**1**) suggesting the absence of any role for a ring nitrogen in binding to the receptor. Analogues **1–7** showed no significant activity at the human CB_1 receptor at concentrations up to 30 μM.

Encouragingly, several of the heterocycles showed improved metabolic stability. With the exception of triazine (**7**), the rat intrinsic clearance generally decreased with lipophilicity.



(**1**)

Figure 1. Pyridine lead from hit to lead chemistry.

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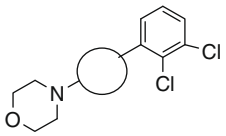
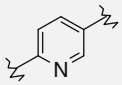
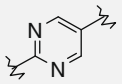
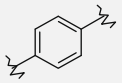
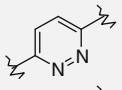
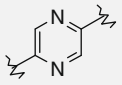
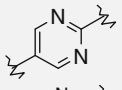
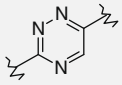
Table 1
SAR of aryl core (compounds **1–7**)



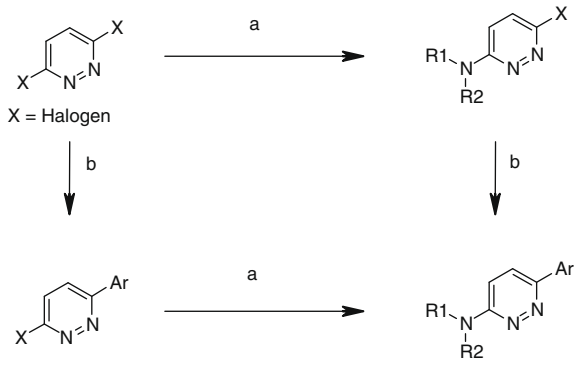
	Aryl	CB_2 pEC_{50} (Efficacy)[a]	Rat CLi (mL/min/g)[b]	Aqueous Solubility (μg/mL)	*c* log D @pH7.4[c]
1		7.1 (88%)	9.5	—	3.2
2		6.7 (67%)	—	—	3.4
3		7.4 (86%)	20	—	4.0
4		7.1 (76%)	2.8	6	2.1
5		7.4 (89%)	—	1	3.0
6		7.3 (92%)	2.8	<1	2.3
7		7.0 (78%)	4.7	—	0.6

[a] Human CB_1/CB_2 assay data is the mean of at least two determinations. Assay variability is monitored by the use of a cannabinoid agonist HU210. Efficacy at CB_1/CB_2 is expressed as a percentage relative to the efficacy of HU210. See Ref. 15 for assay method.
[b] CLi = microsomal clearance, see Ref. 16 for procedure.
[c] Calculated log *D* acquired using ACD v8.0 (ACDlabs).

Pyridazine (**4**) and pyrimidine (**6**) demonstrated particularly encouraging profiles. Of concern was the low solubility, however pyridazine (**4**) showed measurable aqueous solubility and it was found this could be improved through salt formation (HCl salt, aqueous solubility = 120 μg/mL). This promising data led us to initiate further SAR studies around the pyridazine template. The aim of this investigation was to increase the potency of the lead pyridazine (**4**), whilst maintaining or reducing the level of in vitro metabolic turnover. Improvements in solubility would also be beneficial in achieving oral bioavailability. It was hoped that this investigation would lead to analogues with suitable properties to profile in vivo.

Pyridazine analogues were synthesized from the commercially available 3,6-dichloro and 3,6-dibromo pyridazines according to Scheme 1. Synthesis proceeded via a palladium catalysed cross coupling combined with an aromatic halide displacement. In cases where the yield from the Suzuki–Miyaura coupling was low, 3-chloro-6-iodopyridazine could be prepared via an aromatic Finkelstein reaction[17] from 3,6-dichloropyridazine. Halide displacement was carried out at high temperature in a microwave reactor. Reaction times were significantly longer for sterically hindered amines. The order of the two reactions was dependant upon the desired point of variation, however carrying out the amine displacement first avoided the separation of mono and bis-arylated pyridazines.

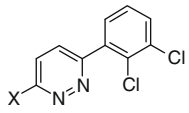
Initial work concentrated on the amine substituent and a range of tertiary and secondary amines were introduced (Table 2). Increasing the lipophilicity, for example, piperidine (**8**), led to an increase in CB_2 activity. This however was accompanied by a decrease in stability which was not affected by the introduction of fluorine substituents (**9**). Pyrrolidine was also a successful replacement for potency and this could be increased further through the introduction of *cis*-2,5-methyl substituents (**12**, **13**). These analogues showed very high CB_2 agonism and a small amount of CB_1 partial agonism although a significant selectivity window remained. Secondary amines, as exemplified by compounds **14** and **15**, maintained potency only with the most lipophilic group.

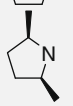
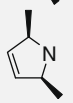


Scheme 1. Reagents and conditions: (a) R^1NHR^2, MeCN, NEt_3, microwave, 160 °C, 30 min–5 h, 2–89%; (b) (i) $ArB(OH)_2$, $Pd(PPh_3)_4$, 2 N Na_2CO_3, DME, 90 °C or (ii) $ArB(OH)_2$, KF, Pd_2dba_3, $[tBu_3PH]BF_4$, 1,4-dioxane, 100 °C, 10–68%.

From this initial investigation the observed SAR seemed to correlate well with the pyridine template previously investigated.[14]

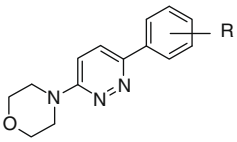
Table 2
SAR of amine substituent (compounds **4**, **8–15**)



	X	CB_2 pEC_{50} (Efficacy)[a]	CB_1 pEC_{50} (Efficacy)[a]	Rat CLi (mL/min/g)[b]
4		7.1 (76%)	<4.5	2.8
8		8.2 (86%)	<4.5	35
9		7.8 (83%)	<4.5	29
10		6.2 (89%)	<4.5	—
11		7.6 (56%)	<4.5	—
12		8.9 (83%)	5.6 (57%)	9.7
13		9.2 (93%)	5.2 (21%)	8.7
14		6.7 (69%)	<4.5	—
15		<4.5	<4.5	—

[a/b] See footnotes to Table 1 for details.

Table 3
SAR of aryl substituent (compounds **16**–**30**)



	R	CB_2 pEC_{50} (Efficacy)[a]	CB_1 pEC_{50} (Efficacy)[a]
4	2,3-Di-Cl	7.1 (76%)	<4.5
16	2-Cl	5.8 (77%)	<4.5
17	2-Me	5.1 (38%)	<4.5
18	2-CF_3	5.9 (78%)	<4.5
19	2-OMe	4.9 (37%)	<4.5
20	2-OCF_3	5.9 (55%)	<4.5
21	3-CF_3	6.0 (15%)	<4.5
22	2,3-Di-Me	5.7 (71%)	<4.5
23	2,3-Di-CF_3	6.8 (75%)	<4.5
24	2,5-Di-Cl	6.4 (39%)	<4.5
25	2,3,5-Tri-Cl	7.3 (78%)	<4.5
26	2,4-Di-Cl	<4.5	<4.5
27	2-Cl-4-Me	<4.5	<4.5
28	2-Cl-4-OMe	<4.5	<4.5
29	2-OBn	6.2 (45%)	5.4 (11%)
30	3-OBn	<4.5	<4.5

[a] See footnotes to Table 1 for details.

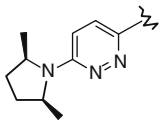
Although high levels of potency had been obtained, this set of pyridazine analogues indicated that metabolic stability remained a key challenge and it looked difficult to replace the morpholine group and retain a low turnover in vitro. It was decided to look at whether the potency could be improved through modification of the aryl substituent and a number of analogues were prepared with the morpholine kept constant (Table 3).

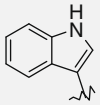
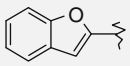
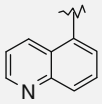
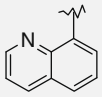
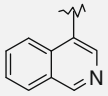
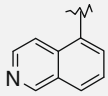
Generally changes to the aryl substitution pattern were not well tolerated. A comparison of the mono substituted analogues, for example, **18** (2-CF_3) and **21** (3-CF_3), indicated that 2-substitution was important for maintaining efficacy. As observed in the pyridine template, 2,4-disubstituted analogues (**26–28**) lost all potency, possibly indicating restricted space within the binding site. Overall, only 2,3-di-trifluoromethyl (**23**) and 2,4,5-trichloro (**25**) maintain equivalent agonism to the original 2,3-dichloro. As 2,3-substitution seemed optimal we decided to look at bi-aryl substituents. For heterocyclic bi-aryls, it was decided to synthesise analogues using the potent *cis*-2,5-dimethlpyrrolidine group, therefore balancing the reduced aryl lipophilicity and hopefully obtaining high potency (Table 4).

Encouragingly, CB_2 agonism was observed across a range of heterocycles in particular quinolines and iso-quinolines (**33**–**36**).

The 4-substituted isoquinoline (**35**) showed a promising combination of agonist activity, in vitro metabolic stability and high aqueous solubility (di-hydrochloride salt, >1 mg/mL). The compound was therefore progressed into a rat in vivo DMPK study

Table 4
SAR of aryl substituent (compounds **31**–**36**)



	Aryl	CB_2 pEC_{50} (Efficacy)[a]	CB_1 pEC_{50} (Efficacy)[a]	Rat CLi (mL/min/g)[b]
31		6.6 (61%)	<4.5	–
32		6.8 (70%)	<4.5	–
33		6.7 (75%)	<4.5	–
34		7.1 (82%)	<5.0	6.0
35		8.0 (85%)	5.1 (71%)	3.7
36		7.4 (93%)	4.7 (52%)	5.1

[a/b] See footnotes to Table 1 for details.

(as a di-hydrochloride salt) in order to see how the in vitro data translated in vivo (Table 5).

The in vivo DMPK profile was characterized by a moderate-high blood clearance, a volume of distribution which indicated a good potential to distribute into the tissues and oral bioavailability of ca. 26%. This data enabled us to select **35** as a tool for further investigation of the in vivo profile of this class of compounds. The compound was initially evaluated in the rat Freund's Complete Adjuvant (FCA) model of inflammatory pain using a weight bearing protocol,[18] and produced a significant reversal of the FCA induced hypersensitivity at all doses tested (0.1–1 mg/kg po) (Fig. 2). Tissue samples from this study at 1 mg/kg (1 h post dose) showed a mean blood concentration of 0.3 μM and a brain concentration of 0.5 μM indicating a brain:blood ratio of approximately 1.7:1.

Table 5
Rat DMPK parameters for analogue **35**

Dose route	Parameter	Mean (n = 3)
Intravenous[a]	Clb (mL/min/kg)	57 ± 5
	V_{SS} (L/kg)	1.7 ± 0.3
	$T_{½}$ (h)	0.3 ± 0.1
Oral[b]	C_{max} (μM)	0.340 ± 0.181
	T_{max} (h)	0.9 (0.5–1.0)
	F (%)	26 ± 13

[a] Compound dissolved in 0.9% (w/v) aqueous saline. Rats received an infusion of **35** at a target dose of 1 mg free base/kg.
[b] Compound formulated in 1% (w/v) aqueous methylcellulose. Single oral dose via gastric gavage to achieve a target dose level of 3 mg/kg of free base.

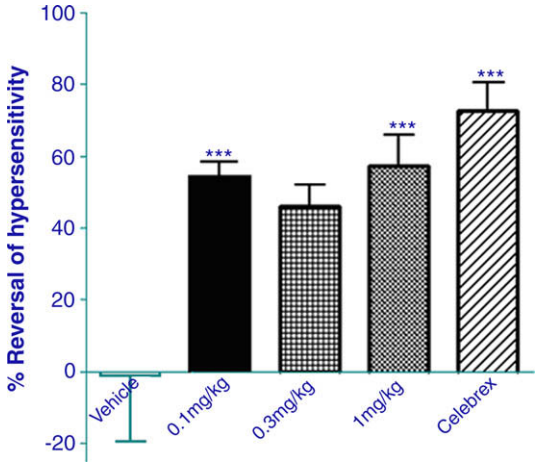


Figure 2. Dose–response effect of compound **35** and Celebrex (10 mg/kg po) in the FCA model of inflammatory pain.

Compound **35** had shown the ability to penetrate the CNS and had measurable CB_1 agonism, therefore an in vivo study was undertaken to look into the effect of **35** on rat core temperature after dosing, an indicator for CB_1 mediated side effects.[19] Compound **35** showed no significant hypothermia at doses up to and including 300 mg/kg po (C_{max} blood 6.6 μM and brain ca 11 μM) and therefore demonstrated the potential to achieve a high therapeutic index with this class of compound.

In summary, replacement of the pyridine core of the initial lead (**1**) with a pyridazine led to the discovery of a number of analogues with increased in vitro metabolic stability. Introduction of the *cis*-2,5-dimethylpyrrolidine moiety was found to greatly increase the CB_2 activity. Combining this group with a number of bicyclic heterocycles led to the identification of compound **35**, which showed high potency in a model of inflammatory pain.

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