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2-Amino-5-aryl-pyridines as selective CB_2 agonists: Synthesis and investigation of structure–activity relationships

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ABSTRACT

2-Amino-5-aryl-pyridines, exemplified by compound **1**, had been identified as a synthetically tractable series of CB_2 agonists from a high-throughput screen of the GlaxoSmithKline compound collection. Described herein are the results of an investigation of the structure–activity relationships (SAR) which led to the identification a number of potent and selective agonists.

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 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 CB_2 agonists with high receptor sub-type selectivity suitable for in vivo studies.

Following the analysis of results from a high-throughput screen (HTS), initial exploratory chemistry identified pyridine **1** as a low molecular weight and chemically tractable hit which showed a promising level of CB_2 potency and efficacy. Initial developability screening identified metabolic stability as a key challenge to be overcome within this series. An investigation of the SAR was initiated, with the target of improving the level of agonist activity and reducing the level of in vitro metabolism (Fig. 1).

Three initial approaches were identified with the aim of reducing the metabolic liability of the initial lead: (1) to find a replacement for the lipophilic azepine ring; (2) to introduce ring substituents which would reduce the electron density of the aminopyridine core; (3) to replace the dichlorophenyl with a less lipophilic heterocycle. After initial screening to determine potency and efficacy, analogues with a CB_2 pEC_{50} >7 would be progressed into an in vitro microsomal stability assay to determine the success of each strategy.

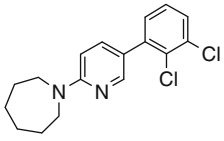

Figure 1. Pyridine analogue **1** CB_2 pEC_{50} 7.4 (efficacy 104%) (Table 1, footnote a); MW 321.

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Initial analogues were synthesized from commercially available 2,5-dibromopyridine (Scheme 1). In order to avoid problems of chemoselectivity, an aromatic S_NAr substitution was carried out with the desired amine substituent as the initial synthetic step. This reaction could be accelerated using high temperatures in a microwave reactor. The resulting 2-amino-5-bromopyridine intermediates were coupled via a Suzuki–Miyaura reaction to yield the desired analogues in overall high yield. Amide **6** and imide **7** were synthesized from 2-amino-5-bromopyridine through an initial reaction with succinic anhydride.

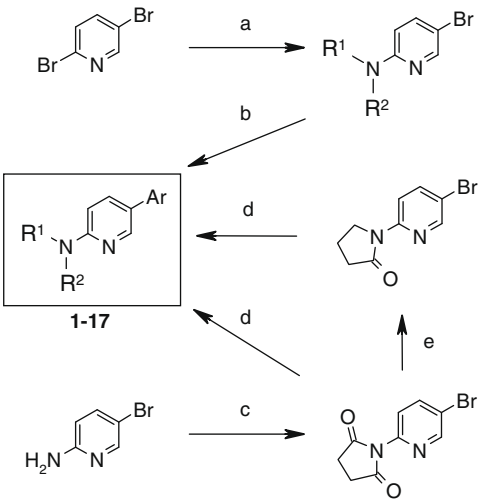
Initial screening results are summarized in Table 1. Encouragingly, CB_2 potency was retained across a range of lipohilicities and no significant CB_1 agonism was observed. Homomorpholine **4** and *cis*-2,5-dimethylpyrrolidine **8** showed equivalent potency to the lead compound. Introduction of polar and electron-withdrawing carbonyl groups (**6**, **7**) led to a decrease in potency and efficacy. This decrease in efficacy was also observed with secondary amines, as exemplified by analogue **9**. The in vitro metabolic stability of several of the analogues was assessed. The results indicated that it was possible to reduce the in vitro metabolism through modification of the amine substituent. However, the initial correlation with the calculated lipophilicity was poor and other factors, for example, increased steric hindrance (**8**) seem to have an effect.

Morpholine **2** showed a promising combination of potency and the lowest in vitro clearance, although still with a relatively high turnover. An in vivo study of rat metabolism was carried out to understand correlation with the in vitro data. This study confirmed high blood clearance with a rate around liver blood flow (**2**, Clb = 89 mL/min/kg).[14]

In order to investigate the effect of replacing the dichlorophenyl group in compound **2**, a range of substituted aryls were incorporated whilst keeping morpholine constant as the amine substituent (Table 2). The screening data from this set of analogues showed a general loss of potency and indicated that a 2,3-disubstitution pattern was important for retaining efficacy. Attempts to further reduce the lipophilicity, for example the 2,3-difluorophenyl analogue **17** also led to a loss of activity.

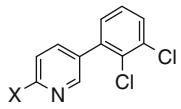
Our second strategy was to introduce electron-withdrawing substituents onto the central core. The groups selected were cyano, trifluoromethyl and carboxylic acid derivatives. Preparation of these analogues required synthesis of the 2,5-dihalopyridine intermediates from which an amine displacement and Suzuki–Miyaura coupling would lead to the desired structures. The 3-cyano pyridine **18** was formed via a pyridone ring synthesis (Scheme 2). After initial ring formation, a facile bromination led to the 3-cyano-5-bromopyridone, which could be converted to the desired intermediate using phosphorous oxychloride and phosphorous pentachloride.

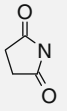
The synthesis of the 4-cyanopyridine **19** is shown in Scheme 3. Selective lithiation of 2,5-dichloropyridine was achieved at the 4-position and this was quenched by the addition of dimethylformamide. Formation of the oxime followed by dehydration led to the desired dihalo intermediate.



Scheme 1. (a) (i) R^1NHR^2, pyridine, reflux or (ii) R^1NHR^2, MeCN, NEt_3, microwave, 160 °C; 82–98%; (b) (i) $ArB(OH)_2$, $Pd(PPh_3)_4$, 2 N Na_2CO_3, DME, 80 °C or (ii) $ArB(OH)_2$, $Pd(PPh)_3Cl_2$, Cs_2CO_3, DME, EtOH, H_2O, microwave, 160 °C; 80–94%; (c) succinic anhydride, xylenes, reflux, 10%; (d) $ArB(OH)_2$, KF, Pd_2dba_3, $[^tBu_3PH]BF_4$, 1,4-dioxane, 100 °C, 18%; (e) (i) $NaBH_4$, THF, H_2O, 68% (ii) DIAD, tBu_3P, THF, 38%.

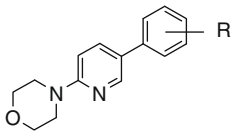
Table 1
SAR of amine substituent (compounds **1**–**9**)



	X	CB_2 pEC_{50}[a] (efficacy)	*c*Log *D* at pH 7.4[b]	Rat Cli[c] (mL/min/g)
1		7.4 (104%)	5.2	30
2		7.1 (88%)	3.2	9.5
3[d,e]		6.3 (73%)	5.7	—
4		7.2 (87%)	3.5	27
5[d]		6.8 (93%)	3.9	—
6		6.6 (59%)	2.2	—
7		5.4 (19%)	0.8	—
8		7.4 (85)	5.3	11
9		6.6 (37%)	5.8	—

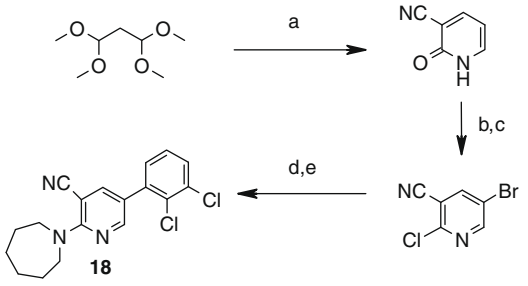
[a] Human CB_1/CB_2 assay data is the mean of at least two determinations. Assay variability is monitored by the use of a cannabinoid agonist HU210. Efficacy at CB_1/CB_2 is expressed as a percentage relative to the efficacy of HU210. See Ref. 15 for assay method. No significant CB_1 activity was observed at analogue concentrations up to 30 μM except analogue **9** CB_1 pEC_{50} 5.6 (18%).
[b] Calculated log *D* acquired using ACD v8.0 (ACDlabs).
[c] CLi = in vitro clearance, see Ref. 16 for procedure.
[d] Sample prepared as a hydrochloride salt.
[e] Compound is racemic at both stereocentres.

Table 2
SAR of aryl substituent (compounds **10**–**17**)

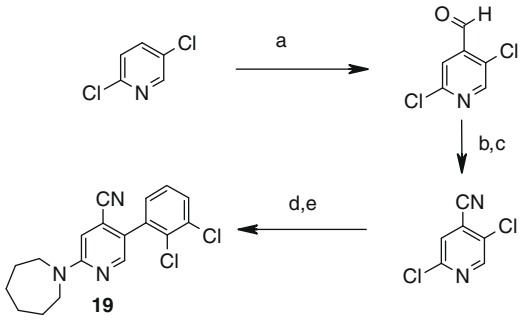


	R	CB_2 pEC_{50}[a] (efficacy)	*c*Log *D* at pH 7.4
2	2,3-diCl	7.1 (88%)	3.2
10	3,4-diCl	5.4 (11%)	3.3
11[d]	3-Cl	6.0 (20%)	2.6
12[d]	2-Cl	6.4 (43%)	2.7
13[d]	2-Me-3-Cl	6.3 (73%)	3.2
14[d]	2,5-diCl	6.2 (25%)	3.3
15[d]	2,3-diF	6.6 (58%)	2.7
16[d]	2,4-diCl	<4.5	3.4
17[d]	2,4-diF	<4.5	2.8

[a/d] See footnotes to Table 1 for details. No significant CB_1 activity was observed at analogue concentrations up to 30 μM.



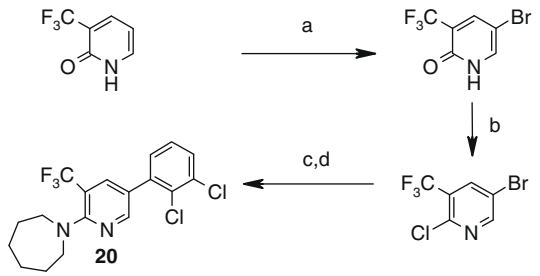
Scheme 2. (a) (i) HCl, (ii) cyanoacetamide, NEt_3, 25%; (b) Br_2, AcOH, 63%; (c) PCl_5, $POCl_3$, reflux, 82%; (d) homopiperidine, THF, 94%; (e) $Pd(PPh_3)_4$, 2 N Na_2CO_3, DME, 80 °C, 37%.



Scheme 3. (a) (i) LDA, THF, (ii) DMF, 16%; (b) $NH_2OH{\cdot}HCl$; (c) HCl, *i*PrOH, 94%; (c) $POCl_3$, MeCN, 90%; (d) homopiperidine, THF, 93%; (e) $ArB(OH)_2$, KF, Pd_2dba_3, $[tBu_3PH]BF_4$, 1,4-dioxane, 100 °C, 20%.

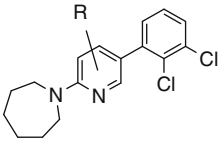
The synthesis of 3- and 4-trifluoromethylpyridines (**20, 21**) was achieved through bromination of the corresponding trifluoromethylpyridones (exemplified for 3-CF_3 in Scheme 4). Finally, acid derivatives (**22–24**) were synthesized from the commercially available 5-bromo-2-chloronicotinic acid.

The results of the biological screening of these analogues are given in Table 3. Generally ring substituents were not well tolerated with the exception of 4-cyano **19**. 3-Substitution seems detrimental to efficacy, although interestingly the relatively large morpholino amide **24** still shows some activity. Some CB_1 partial agonism was observed with analogues **19** and **24**, although greater than 100-fold separation remained between CB_1 and CB_2 activity. Unfortunately those analogues tested show no reduction in the level of in vitro metabolism.



Scheme 4. (a) Br_2, MeOH; (b) $PhPOCl_2$,75%; (c) homopiperidine, THF, 94%; (d) $Pd(PPh_3)_4$, 2 N Na_2CO_3, DME, 80 °C, 70%.

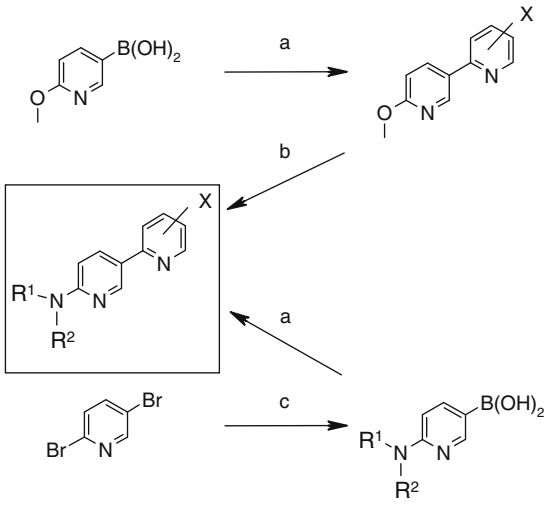
Table 3
SAR of pyridine ring substituents (compounds **18**–**24**)



	R	CB_2 pEC_{50}[a] (efficacy)	CB_1 pEC_{50}[a] (efficacy)	Rat Cli[c] (mL/min/g)
1	—	7.4 (104%)	5.0 (4%)	
18	3-CN	5.4 (41%)	<4.5	—
19	4-CN	7.0 (86%)	5.0 (13%)	>50
20	3-CF_3	5.2 (12%)	<4.5	—
21	4-CF_3	5.6 (79%)	<4.5	—
22[d]	3-CO_2H	5.8 (41%)	<4.5	—
23[d]	3-$CONMe_2$	6.1 (37%)	<4.5	—
24[d]	3-CO-morpholine	7.1 (67%)	5.0 (19%)	>50

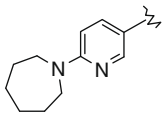
[a,c,d] See footnotes to Table 1 for details.

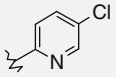
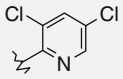
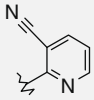
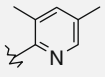
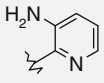
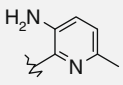
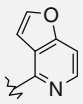
Another approach was the introduction of a second pyridyl ring to lower the overall lipophilicity of the analogues. A bi-pyridyl template also had the potential to improve aqueous solubility. It was decided to introduce the second pyridine with the bi-aryl bond ortho to the ring nitrogen. This pyridine isomer would enable us to maintain a chloro substituent ortho to the bi-aryl bond without introducing a chemically reactive site. The synthesis of these analogues is given in Scheme 5. Compounds could be synthesized from the commercially available [6-(methyloxy)-3-pyridinyl]boronic



Scheme 5. (a) ArX, $Pd(PPh_3)_4$, 2 N Na_2CO_3, DMF, 80 °C, 35–70%; (b) (i) $POCl_3$, DMF, 53–85%, (ii) R^1R^2NH, NMP, microwave, 250 °C, 7–77%; (c) (i) R^1NHR^2, pyridine, reflux, 96%, (ii) *n*BuLi, $B(O^iPr)_3$, Et_2O, 48–61%.

Table 4
SAR of heteroaryl substituents (compounds **33–38**)



	Aryl	CB_2 pEC_{50}[a] (efficacy)	*c*Log *D* at pH 7.4	Rat Cli (mL/min/g)
25		7.0 (101)	3.9	13
26		6.5 (100%)	4.8	—
27		5.0 (29%)	3.1	—
28		6.3 (103%)	3.9	—
29[d]		6.4 (75%)	2.8	—
30[d]		6.1 (86%)	3.4	—
31[d]		5.9 (102%)	3.8	—
32[d]		6.0 (91%)	2.2	—
33		6.9 (86%)	2.9	—
34		7.2 (80%)	3.5	>50

[a/d] See footnote to Table 1 for details. No significant CB_1 activity was observed at analogue concentrations up to 30 μM.

acid via a Suzuki–Miyaura coupling then reaction with $POCl_3$ to replace the methoxy with a chloro substituent. An alternative strategy was also developed to enable the aryl to be introduced as the final synthetic step. This involved introducing the amine substituent first, followed by boronic acid formation via lithiation and Suzuki–Miyaura coupling with the desired 2-halopyridine.

The screening results (Table 4) revealed some interesting SAR. The introduction of a second pyridine seemed to be well tolerated, although substituents are required for activity (**27**). The 5-chloro pyridine (**25**) shows improved potency over the 3-chloropyridine (**28**), however a combination of these substituents shows no improvement (**26**). Polar substituents are tolerated at the 3-position, for example, **33**. Whilst the 3,4-dichloro analogue was not synthesised due to concerns over reactivity, the bicyclic analogue **34** shows encouraging potency and could be an interesting lead for further chemistry.

Analogue **25** had yielded a decrease in metabolic turnover when compared to the lead. It was decided to combine the morpholine substituent, found in compound **2**, with the bi-pyridyl template, however all analogues synthesized had no significant CB_2 activity.

In conclusion, investigation of SAR around the lead 2-amino pyridine (**1**) revealed a range of potent CB_2 agonists with high CB_1 receptor selectivity. Several strategies were used to address the observed metabolic liability. An investigation of the SAR with the aim of lowering the overall lipophilicity was carried out. The observed correlation of metabolic stability with calculated lipophilicity was poor, however this work identified some analogues with improved in vitro stability. This however did not translate to an acceptable value in vivo. Introduction of an electron-withdrawing group to the central ring generally led to a loss of activity and showed no benefit in terms of stability. Finally introduction of a second heteroaryl was well tolerated by the receptor, and led to some reduction in the in vitro metabolic turnover, however low in vivo metabolism remained elusive.

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14. Intravenous pharmacokinetics were assessed using the hydrochloride salt dissolved in 0.9% (w/v) saline containing 2% (v/v) DMSO, 10% (w/v) Kleptose. Following a 1 h intravenous infusion (target dose 1 mg/kg), **2** showed a blood clearance of 89 mL/min/kg, with a steady-state volume of distribution (Vss) of 7.5 L/kg and a terminal half-life of 1.7 h.
15. Details of the yeast CB-2 reporter assay used to assess potency are given in: Eartherton, A. J.; Giblin, G. M. P.; Mitchell, W. L.; Naylor, A.; Rawlings, D. A. World patent WO 2005 074939.
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