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Selective CB2 receptor agonists. Part 3: The optimization of a piperidine-based series that demonstrated efficacy in an in vivo neuropathic pain model

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ABSTRACT

A novel class of potent cannabinoid receptor 2 (CB2) agonists based on a (*S*)-piperidine scaffold was identified using ligand-based pharmacophore models. Optimization of solubility and metabolic stability led to the identification of several potent CB2 agonists (e.g., **30**) that displayed selectivity over cannabinoid receptor 1 (CB1) and acceptable drug like properties. In rats, compound **30** demonstrated a favorable pharmacokinetic profile and efficacy in a Streptozotocin-induced diabetic neuropathy model, with full reversal of mechanical hyperalgesia.

Cannabinoids, the active ingredients of cannabis, are a class of compounds of known analgesic and anti-inflammatory properties.[1] Their effects are due to the interaction with several receptors, mostly members of the G-protein-coupled receptor (GPCR) superfamily such as the well profiled cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2). The CB1 receptor is highly expressed in the central and peripheral nervous system[2,3] and is responsible for the psychotropic effects of cannabinoids. This vastly limits the pharmaceutical application of this target. The CB2 receptor on the other hand, was initially discovered on immune cells[3,4] which suggests it would be an interesting target for inflammatory settings. A number of more recent reports have indicated that CB2 may in fact be also expressed in the CNS,[5,6] with its expression level depending on the activation state of the cell.[7] This makes it an attractive target for pain treatment. The search for selective CB2 agonists throughout the pharmaceutical industry in the last decade has identified multiple compounds that demonstrated efficacy in several animal models.[8] So far, only a few have entered clinical trials.[9,10]

In the context of investigating the therapeutic potential of CB2 receptor modulation, we started a program with the goal of identifying potent and selective CB2 agonists. This Letter is the third part in a series of publications documenting the work that was performed to find suitable replacements for the 1,4-diazepane core[11] previously reported by our group (Fig. 1).

Ligand-based pharmacophore models identified the azetidines, proline and piperidine scaffolds as promising starting points that were able to reproduce what we believe to be the bioactive conformation of the diazepane core.[12] The positive results that were obtained from our optimization of the proline-based scaffold[13] encouraged us to also explore the SAR of piperidine-based templates.

Compound **1**, the six-membered ring analogue of proline **2** and the first synthesized compound in the piperidine class, demonstrated potency and full efficacy[14] as a CB2 agonist in an assay measuring the inhibition of cyclic adenosine monophosphate (cAMP) production in forskolin stimulated recombinant CHO cells expressing CB2[15] (Table 1).

As **1** suffered from low solubility,[16] less than desirable metabolic stability in human liver microsomes (HLM)[17] (HLM $t_{1/2}$ >120 min is considered ideal) and an insufficient CB2 versus CB1

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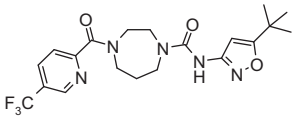
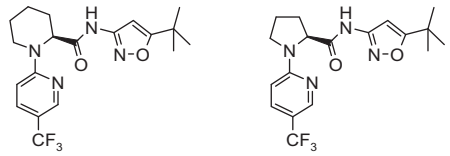


Figure 1. Representative compound from diazepane series.

Table 1
Direct comparison of **1** and **2**



1 **2**

Example	hCB2 EC_{50} (nM)	hCB1 EC_{50} (nM)	Solubility @ pH 6.8 (μg/mL)	HLM $t_{1/2}$ (min)
1	0.093	250	<0.1	74
2	0.070	53	0.3	120

selectivity window, we pursued SAR exploration with the goal of identifying potent CB2 agonists (targeting hCB2 EC_{50} <50 nM) while improving on the identified liabilities. We aimed to identify compounds with hCB1 EC_{50} >20 μM, wary of potential side effects caused by interaction with the CB1 receptor. Aqueous solubility and metabolic stability became two key SAR optimization parameters, with the goal to identify compounds with solubility >50 μg/mL and HLM $t_{1/2}$ >120 min.

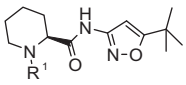
We first set out to investigate the effects on compound properties of the *N*-substituents on the piperidine ring; SAR was explored by replacing the 5-trifluoromethyl-2-pyridyl group in **1** while maintaining the 3-amino-5-*tert*-butyl isoxazole as the amide substituent (Table 2). Aware of the chiral preferences demonstrated by the CB2 receptor towards the (*S*)-versus the (*R*)-enantiomers in the proline series, we first confirmed that the (*S*)-piperidine isomer was preferred over the (*R*)-isomer (data not shown) and then conducted further SAR exploration exclusively on the *S*-enantiomer. Compounds in Table 2 were synthesized according to Scheme 1.

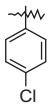
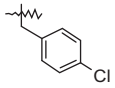
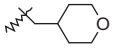
Aromatic and benzyl *N*-substitutions (**3**–**5**) afforded potent CB2 agonists that were however still affected by low solubility and decreased metabolic stability compared to **1**. Similarly, aliphatic (**6**–**9**) and heteroaliphatic (**10** and **11**) substitutions led to potent CB2 agonists, with the most potent compounds being the ones carrying a methylene linker alpha to the ring nitrogen (i.e., **7**, **8** and **11**). Overall aqueous solubility was improved by the replacement of aromatic groups with aliphatic and heteroaliphatic ones, however further improvement in solubility was still necessary. Metabolic stability remained an issue that needed to be addressed.

With the goal of improving solubility and microsomal stability of the compounds, we focused the SAR investigation on improving the physiochemical properties of the molecules by increasing polarity. With compounds **1** and **3** having *c*Log*P*/TPSA (topological polar surface area) values of 4.3/71 Å^2 and 5.1/58 Å^2, respectively, we aimed to reduce *c*Log*P* and increase TPSA targeting values of *c*Log*P* <3.0 while keeping TPSA <120 Å^2.

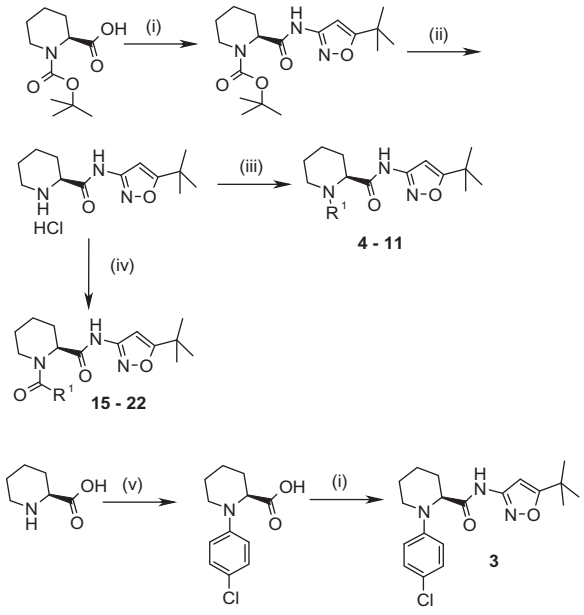
The introduction of a carbonyl group on the proline core[12] led to the identification of oxo-proline compounds that displayed an appropriate balance of potency, selectivity, pharmacokinetic and physicochemical properties (**12**–**13**). A similar approach was therefore pursued in the piperidine series with the goal to identify compounds where all properties would be balanced. Compound **14** was synthesized according to Scheme 2 and is representative of the SAR exploration that was conducted. Contrary to what was

Table 2
SAR: evaluation of the *N*-substituent R^1



Example	R^1	CB2 EC_{50} (nM)	CB1 EC_{50} (nM)	Solubility @ pH 6.8 (μg/mL)	HLM $t_{1/2}$ (min)
3		0.8	1170	<0.1	14
4		2.0	>20,000	<0.1	10
5		3.0	217	<0.1	9
6		31	>20,000	38	11
7		0.9	2940	nd	9
8		1.5	1965	32	6
9		14	13,000	40	5
10		33	>20,000	74	19
11		0.2	600	72	11

nd = not determined.



Scheme 1. Reagents and conditions: (i) 3-amino-5-*tert*-butyl isoxazole (1.1 equiv), pyridine, $POCl_3$ (1.1 equiv) addition at 0 °C then rt for 12 h; (ii) 4 N HCl in dioxane (3.8 equiv), DCM, rt, 12 h; (iii) benzyl or alkyl aldehyde (2.0 equiv), DMF, AcOH (2.0 equiv), Na_2SO_4 (10 equiv), 30 min, then $NaBH_3CN$ (1.0 equiv), rt, 12 h or (iii) for compound **6**: [(1-ethoxycyclopropyl)oxy]trimethylsilane (5.8 equiv), AcOH (10 equiv), $NaBH_3CN$ (4.5 equiv), MeOH, reflux, 5 h; (iv) carboxylic acid (1.1 equiv), DMF, HOBt hydrate (1.1. equiv), EDC hydrochloride (1.1 equiv), rt, 15 min then amine, triethylamine (1.0 equiv), DMAP (0.05 equiv), rt, 18 h; (v) 1-bromo-4-chlorobenzene (1.0 equiv), K_2CO_3 (1.5 equiv), CuI (0.1 equiv), DMA, 100 °C, 3 days.