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1,4-Diazepane compounds as potent and selective CB2 agonists: Optimization of metabolic stability

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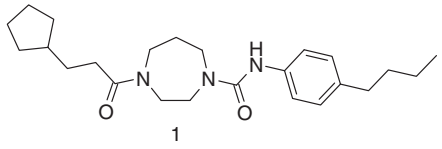
ABSTRACT

A high-throughput screening campaign has identified 1,4-diazepane compounds which are potent Cannabinoid receptor 2 agonists with excellent selectivity against the Cannabinoid receptor 1. This class of compounds suffered from low metabolic stability. Following various strategies, compounds with a good stability in liver microsomes and rat PK profile have been identified.

Cannabis has been used for its analgesic effects since the ancient dynasties of China. Cannabinoids are the pharmacologically active components of cannabis, and are known to mediate some of their actions through the cannabinoid receptors CB1 and CB2, both of which are G-protein coupled receptors. Cannabinoid CB1 receptors are located primarily at the terminals of central and peripheral neurons[1] while the CB2 receptors are expressed mainly by immune cells.[2] CB2 receptors are also present in some central and peripheral neurons,[3] and the role of neuronal CB2 receptors has still to be established. Various strategies have been developed to increase the benefit-to-risk ratio of cannabinoid therapies,[4] one of which is the development of CB2 selective agonists for circumventing the unwanted consequences of cannabinoid CB1 receptor activation.[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 as well as emesis.[9–12]

In order to identify highly selective CB2 agonists, we performed a high-throughput screen using a CB2 functional cellular assay which measures cAMP production in Forskolin stimulated CHO cells transfected with the human CB2 receptor as a primary screen.[13] The counter-screen was performed in CHO cells transfected with the human CB1 receptor, and wild-type CHO cells.[13] From this screen we identified compound **1** (Fig. 1) as an attractive hit for further evaluation. Compound **1** is a partial agonist for CB2 with an EC_{50} of 136 nM and 67% efficacy and does not activate the CB1 receptor up to 20 μM. The compound demonstrated no competitive binding for CB2 or CB1 against radiolabeled [^{3}H]-CP-55940[13] up to 5 μM suggesting that the binding site of **1** is not identical with that of CP-55940. A potentially different binding mode as



1

Figure 1. High-throughput screening hit compound **1** (EC_{50} = 136 nM (67% efficacy).

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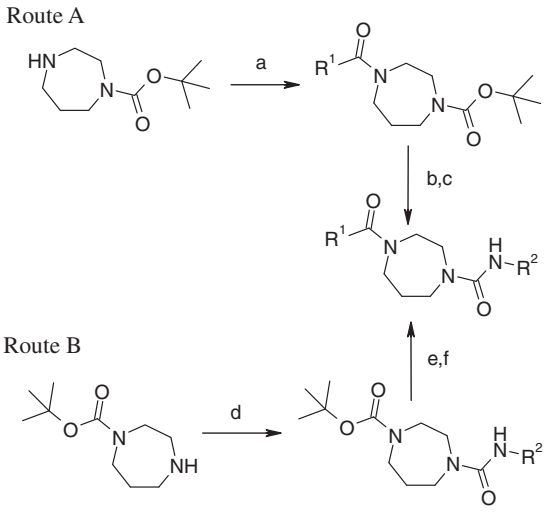
compared to classical and nonclassical cannabinoids made this compound a very interesting starting point.

When incubated with human liver microsomes (HLM),[14] **1** demonstrated a short half-life ($T_{1/2}$) of 5 min and low solubility of 3 μg/mL at pH 7.4 (as determined by a high-throughput kinetic solubility assay).[15] Our initial focus during hit evaluation was to assess whether the metabolic stability could be increased within this class of compounds while maintaining potency and selectivity.

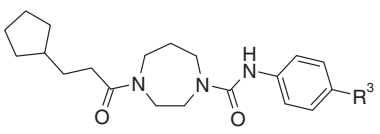
The synthesis of these compounds is outlined in Scheme 1. In route A, the commercially available *tert*-butyl homopiperazine-1-carboxylate was first coupled employing either EDC amide coupling conditions or via acid chloride, followed by Boc deprotection using HCl. The urea coupling was performed using an isocyanate as the starting material which was either commercially available or generated in situ using triphosgene. In route B, the urea was synthesized first followed by Boc deprotection and amide formation using similar conditions as in route A.

Structure–activity relationship efforts began by truncating the alkyl groups on the urea substituent of the molecule (Table 1). Removal of the *n*-butyl substituent on the phenyl ring as in **2** or truncating it to a methyl group (compound **3**) led to a loss in CB2 agonism while compounds with larger R^3 groups such as ethyl, *iso*-propyl or *tert*-butyl (compounds **4**–**6**) had similar potency as compared to **1**. Compound **6** had a high selectivity ratio of ~200 fold, however showed some activation of the CB1 receptor as compared to the other analogs. Compounds **2**–**6** suffered from low microsomal stability (HLM $T_{1/2} \leqslant 7$ min) and it was demonstrated in a metabolite ID study of **5** using human and mouse liver microsomes that the benzylic position was the single site of oxidative metabolism. Although analog **6** suffered from low HLM stability as well indicating that metabolism can also occur at other sits, it lacked benzylic hydrogens prone to oxidative metabolism and offered a good balance of CB2 potency and selectivity. Thus, **6** was selected for an initial evaluation of the structure–activity relationship at R^1 (Table 2). This effort was focused on truncating the cyclopentylethyl group to potentially improve metabolic stability.



Scheme 1. Reagents and conditions: Route A: (a) R^1COOH, EDC, HOBt, DIEA, catalytic DMAP, DMF; or R^1COCl, TEA, THF; (b) 4 N HCl in dioxane, DCM; (c) R^2NCO, TEA, THF or R^2NH_2, triphosgene, DIEA, DCM; Route B: (d) R^2NH_2, triphosgene, DIEA, DCM; (e) 4 N HCl in dioxanes, DCM; (f) R^1COOH, EDC, HOBt, DIEA, catalytic DMAP, DMF; or R^1COCl, TEA, THF.

Table 1
SAR: evaluation of the phenyl substituents



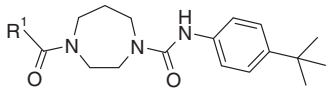
Example	R^3	CB2 EC_{50} (nM) efficacy (%)	CB1 EC_{50} (nM) efficacy (%)	HLM $t_{1/2}$ (min)
1	*n*-Bu	136 (67%)	>20,000	5
2	H	>20,000	>20,000	3
3	Me	>20,000	>20,000	4
4	Et	226 (69%)	>20,000	5
5	*iso*-Pr	123 (81%)	>20,000	7
6	*tert*-Bu	85 (95%)	19,300 (65%)	3

Cyclopentylmethyl (**7**) or cyclopentyl (**8**) as R^1 demonstrated comparable or slightly lower CB2 potency while cylohexyl (**9**) led to an improvement in CB2 potency with an EC_{50} of 20 nM. Retaining the ethyl spacer of **6**, but replacing cyclopentyl with *iso*-propyl (compound **10**) had no significant effect on potency and selectivity. Since these changes did not affect the metabolic stability, as demonstrated by a HLM half-life of less than 5 min for compounds **7–10**, aromatic substituents at R^1 were next investigated. Compared with the excellent potencies of 4-substituted phenyl analogs **11** and **14** other substitution patterns such as dichloro analogs **12** and **13** had attenuated potencies as well as efficacies. Electron donating 4-substituents such as NMe_2 as in **15** were less tolerated for potency and efficacy than the electron withdrawing Cl- and CF_3-substituent in **11** and **14**.

Aromatic substituents at R^1 however had also no effect on the metabolic stability (HLM $T_{1/2} \leqslant 6$ min). A metabolite ID study was performed for a compound with R^1 = 4-Cl-phenyl and R^2 = 4-CF_3-phenyl (compound not shown), which demonstrated that oxidation occured on R^2 as well as the 1,4-diazepane core. This data in conjunction with an evaluation of all microsomal stability data suggested that the strategy to block sites of metabolism or reduce the number of metabolically labile groups had a low likelihood of success. Therefore we adjusted our optimization strategy and aimed to decrease the lipophilicity of compounds, thus making them less susceptible to microsomal metabolism.[16] The tetrahydropyran analog **16** ($c \log P = 1.4$) showed a 5.5-fold loss in CB2 potency as compared to the cyclohexyl analog **9** ($c \log P = 3.8$), however it was the first compound within this series that demonstrated an improved HLM half-life of 11 min. Having identified a more polar R^1 substituent, we returned our attention to the right hand side of the molecule (R^2 in Table 3) in an attempt to further decrease lipophilicity by replacing the phenyl group with a heterocycle. Isoxazole isomers **17** and **18** demonstrated a 2- and 4-fold increase in CB2 potency, respectively, compared to the phenyl analog **16**, while maintaining a high selectivity. The in vitro half-life was significantly improved to >120 min. In the case of the 3-amino-5-*tert*-butyl isoxazole analogue **17**, we further investigated the role of the heterocyclic substitution and found that a bulky substituent was required for CB2 potency, as demonstrated by compounds **19** and **20**. Further, a *t*-butyl substituent was preferred over a phenyl substituent as in **21**. We also identified a series of 2-aminothiazoles **22**–**25**, which were generally less potent than the isoxazoles and had a preference for substitution at the 5-position (**22**–**23**) over the 4-position (**24**–**25**) of the thiazole heterocycle, with both a *tert*-butyl and a phenyl group being tolerated at position 5 and only a phenyl group being allowed at position 4. R^2 being *tert*-butyl pyrazole group as in **26** and **27** was not well tolerated for CB2 potency.

With the discovery of *tert*-butyl-isoxazoles as R^2 offering good CB2 potency, selectivity against CB1 and most importantly acceptable metabolic stability, we decided to further expand the structure–activity relationship at R^1 (Table 4). First we evaluated

Table 2
SAR: aliphatic and aromatic amide groups



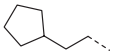
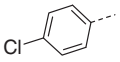
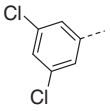
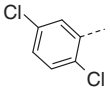
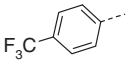
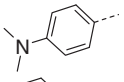
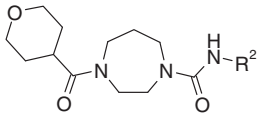
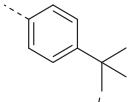
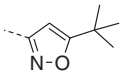
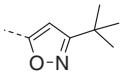
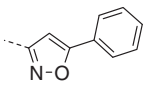
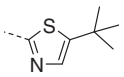
Example	R^1	CB2 EC_{50} (nM) efficacy (%)	CB1 EC_{50} (nM) efficacy (%)	HLM $T_{1/2}$ (min)
6		85 (95%)	19,300 (65%)	3
7		60 (95%)	>20,000	3
8		168 (99%)	>20,000	3
9		20 (101%)	>20,000	4
10		96 (94%)	19,800 (100%)	3
11		9.8 (96%)	14,700 (100%)	3
12		215 (75%)	>20,000	nt
13		87 (72%)	>20,000	6
14		2.7 (99%)	~20,000 (100%)	4
15		49 (78%)	>20,000	4
16		111 (104%)	>20,000	11

Table 3
SAR: heteroaromatic urea substituents R^2



Example	R^2	CB2 EC_{50} (nM) efficacy (%)	CB1 EC_{50} (nM) efficacy (%)	*c* log *P*	HLM $T_{1/2}$ (min)
16		111 (104%)	>20,000	1.4	11
17		67 (104%)	>20,000	0.14	>120
18		26 (99%)	>20,000	0.14	>120
19		>20,000	>20,000	−1.1	nt
20		>20,000	>20,000	−1.5	nt
21		1940 (97%)	>20,000	0.64	nt
22		150 (106%)	>20,000	0.77	27

(*continued on next page*)

Table 3 (*continued*)

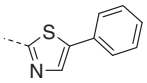
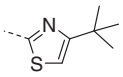
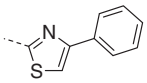
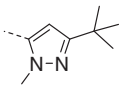
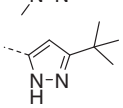
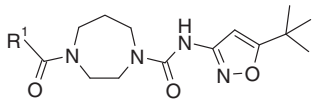
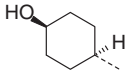
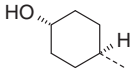
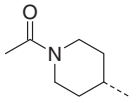
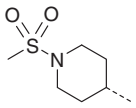
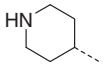
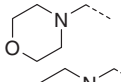
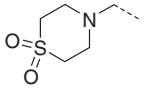
Example	R^2	CB2 EC_{50} (nM) efficacy (%)	CB1 EC_{50} (nM) efficacy (%)	*c* log *P*	HLM $T_{1/2}$ (min)
23		135 (88%)	>20,000	1.1	23
24		1700 (105%)	>20,000	0.77	nt
25		450 (114%)	>20,000	1.1	nt
26		14,850 (100%)	>20,000	−0.18	106
27		13,150 (100%)	>20,000	0.24	nt

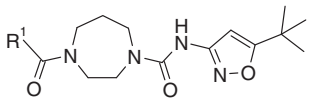
Table 4
SAR: amide groups R1

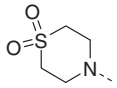
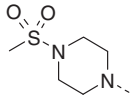
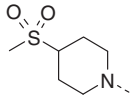
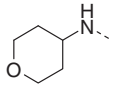


Example	R^1	CB2 EC_{50} (nM) efficacy (%)	CB1 EC_{50} (nM) efficacy (%)	*c* log *P*	HLM $T_{1/2}$ (min)
17		67 (104%)	>20,000	0.14	>120
28		2045 (96%)	>20,000	1.37	nt
29		750 (100%)	>20,000	1.85	nt
30		4030 (102%)	>20,000	1.42	nt
31		2300 (100%)	>20,000	0.95	nt
32		8.4 (95%)	>20,000	0.46	>120
33		11,600 (102%)	>20,000	0.46	nt
34		1350 (100%)	>20,000	−0.83	nt
35		1690 (91%)	>20,000	−0.23	nt
36		>20,000	>20,000	0.12	nt
37		157 (89%)	>20,000	1.3	35
38		107 (100%)	>20,000	0.35	38

tetrahydropyran isomers **28** and **29** and tetrahydrofurans **30** and **31**, all of which demonstrated a loss in CB2 potency suggesting that there is a strict requirement for the positioning of the oxygen. Moving the oxygen outside the ring, thus replacing the

Table 5
SAR: ureas



Example	R^1	CB2 EC_{50} (nM) efficacy (%)	CB1 EC_{50} (nM) efficacy (%)	*c* log *P*	HLM $T_{1/2}$ (min)
39		177 (101%)	>20,000	1.9	58
40		135 (100%)	>20,000	0.91	>120
41		2865 (95%)	>20,000	1.87	nt
42		426 (95%)	>20,000	0.02	nt
43		814 (94%)	>20,000	0.82	nt

hydrogen-bond acceptor with a hydrogen bond donor, as in *cis*-hydroxycyclohexyl analog **32** resulted in excellent potency with acceptable HLM stability, while the *trans*-isomer **33** showed attenuated CB2 potency. Similarly, a loss of potency was observed for piperidine analogs **34**–**36**. Morpholinomethyl and dioxothiomorpholinomethyl analogs **37** and **38** demonstrated CB2 potencies of 157 and 107 nM, a lack of CB1 activity and acceptable HLM $T_{1/2}$ of 35 and 38 min, respectively. The SAR exploration at R^1 and R^2 overall demonstrated a dependance of metabolic stability on lipophilicity (*c* log *P*).

Based on the acceptable profile of compounds **37** and **38**, a small series of urea analogues at R^1 was investigated (Table 5). From this set, the morpholino and thiomorpholino urea analogs **39** and **40** were the most potent analogues with comparable potency and selectivity profiles to amides **37**–**38** and improved HLM $T_{1/2}$ of 58 and >120 min, respectively. The tetrahydropyrano urea **43** demonstrated a loss in CB2 potency.

The optimization of the 1,4-diazepane scaffold has generated molecules which have improved in vitro clearance as compared to the high-throughput screening hit **1** and are attractive starting points for further optimizations. Because of their superior profile, we examined compounds **17**, **18**, **38** and **40** in various in vitro assays (Table 6). The solubility of amide compounds **17**, **18** and **38** was more favorable than the one of urea **40**. Compounds **17**, **38** and **40** have a clean profile in the CYP inhibition assay[17] against various isoforms and also demonstrated no hERG inhibition[18] when assessed at 1 μM concentrations in a Patch Express assay. The pharmacokinetic profile of **17** was evaluated in Wistar rat demonstrating a low volume of distribution and medium clearance which correlates to the in vitro clearance in rat liver microsomes. The bioavailability was 28% (Table 7).

Table 6
In vitro profile of 1,4-diazepane compounds **17**, **18**, **38** and **40**

Compound	**17**	**18**	**38**	**40**
Sol pH 7.4 (μg/mL)	>37	>37	>96	18
CYP IC_{50} (μM) 2C9, 2D6, 3A4	>30	nt	>30	>30
hERG (% inhibition @ 1 μM)	1	nt	0	1
HLM $\%Q_h$	<24	<24	50	<24
RLM $\%Q_h$	35	<23	27	42

Table 7
Wistar rat pharmacokinetic profile of **17** dosed at 1 μmol/kg i.v. and 10 μmol/kg p.o. (suspension) ($n = 3$ animals, mean ± SD)

Route	Compound	**17**
i.v.	V_{SS} (L/kg)	0.87 ± 0.21
	CL (mL/min/kg)	34 ± 3.7
	$T_{1/2}$ (h)	1.4 ± 0.76
p.o.	F (%)	28 ± 9.8
	AUC_{0-inf} (nmol h/L)	1380 ± 485
	C_{max} (ng/mL)	1760 ± 1120

In summary, we have discovered a novel series of CB2 receptor agonists that are potent, selective, and orally bioavailable in rats. The focus of the optimization effort was to preserve the CB2 potency and selectivity against CB1 while increasing the stability in human liver microsomes which was achieved by decreasing lipophilicicty (*c* log *P*). The work culminated in compounds with overall attractive in vitro and rat pharmacokinetic profiles. These compounds offer attractive starting points for further optimization and represent pharmacological tools to evaluate the therapeutical effects of CB2 agonists in various disease settings. Further they could be used to investigate whether CB2 agonists with a different binding mode from CP-55, 940 exhibit different receptor biology given the recent findings on functional selectivity of cannabinoid signaling.[19]

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14. Test compounds were incubated in Duplicate Matrix MultiScreen mintubes (Matrix Technologies, Hudson, NH) with liver microsomes (Xenotech, Lenexa, KS). Each assay is performed in 50 mM potassium phosphate buffer, pH 7.4, and 2.5 mM NADPH. Compounds were tested at a final assay concentration of 1.0 μM. The protein concentration in the reaction mix was 1 mg/mL. Compounds were preincubated for 5 min at 37 °C and the metabolic reactions were initiated by the addition of NADPH. Aliquots of 80 μL were removed from the incubation mix at 0, 5 and 30 min after the start of the reaction for screening data. Each aliquot was added to 160 μL acetonitrile for extraction by protein precipitation. These samples were mixed for 1 min by vortexing, and a volume of the mixture was filtered through wells in 0.25 mm glass fiber filter plates by centrirfugation at 3000 rpm for5 min. Sample extracts were analyzed by LC–MS–MS to determine parent compound levels. Percent loss of parent compound was calculated from the peak area at each time point to determine the half-life for test compounds ($T_{1/2}$, min) and clearance ($T_{1/2}$ expressed as percent hepatic blood flow, $\%Q_h$).
15. 6 μL of a 10 mM DMSO stock solution is spiked into pH 4.5 and 7.4 buffers targetting 50–200 μM final concentrations in buffers in duplicate deep well plates. The DMSO content is 0.5%. The samples are incubated for 16–18 h, filtered and analyzed using spectrophotometer. The UV spectra of samples and reference are scanned from 230 to 500 nm. Solubility is measured taking the ratio of area under the curve of reference to sample.
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18. hERG assessment used the automated PatchXpress 7000A patch clamp robot (Molecular Devices).
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