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Rapid assessment of a novel series of selective CB_2 agonists using parallel synthesis protocols: A Lipophilic Efficiency (LipE) analysis

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ABSTRACT

A series of libraries were designed using the 1-(cyclopropylmethyl)-2-alkyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridin-5-ium templates **2a**–**b**, and Sulfonamide derivatives **11a**–**n** proved to be potent agonists of the CB_2 receptor. Analysis of the Lipophilic Efficiency (LipE) of potent compounds provided new insight for the design of potent, metabolically stable CB2 agonists.

The cannabinoid system has emerged as an important modulator of several key processes,[1] such as mood, appetite, metabolic regulation, immunity, cell proliferation and pain.[2] While nociception is modulated by both CB_1 and CB_2 receptors, the CB_2 subtype has emerged as a more attractive target since the CNS-expressed CB_1 receptor is involved in the psychotropic side-effects of cannabinoids.[3] There is, therefore, a growing interest[4] in the development of selective CB_2 agonists for the treatment of pain.[5]

Compound **1**[6] is a selective, potent (0.2 nM) full agonist of the CB_2 receptor. Ongoing interest in this family led us to seek new lead matter from a structurally similar series. We decided to rapidly evaluate new series based on saturation of the benzene ring in **1** by preparing compounds **2a** and **2b**. Introduction of a nitrogen 'handle' in the saturated ring allows rapid diversification through substitution in that position (Fig. 1).

We now report our use of parallel chemistry to prepare and assess this new class of compounds in terms of in vitro potency and metabolic stability. The design criteria (in terms of physicochemical properties) of target compounds will also be discussed.

Target templates were prepared from 4-chloro-3-nitro pyridine **3** according to Scheme 1. Addition[7] of cyclopropyl methyl amine to the 4-chloro-3-nitro-pyridine (**caution**: pyridine **3** and adduct **4** both display a sharp exotherm around 230 °C) proceed smoothly

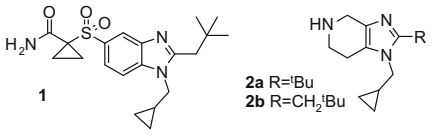
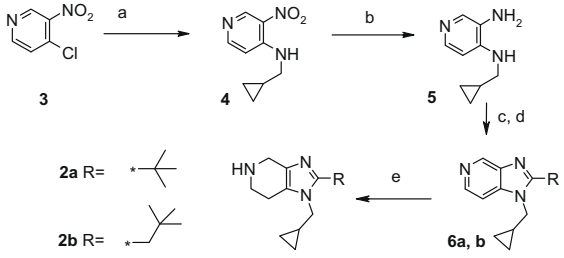


Figure 1. Structures of starting lead **1** and templates **2a** and **2b**.



Scheme 1. Synthesis of templates **3** and **4**. Reagents and conditions: (a) cyclopropylmethyl amine, TEA, Toluene reflux 16 h 70%; (b) Pd/C 20%, EtOH, H_2 50 psi, rt 60 h 88%; (c) Amide formation: acid chloride, NMM, DCM rt 16 h 67–83%; (d) Cyclisation AcOH, sealed tube, 200 °C 10 h **8** 42% **9** 40%; (e) PtO_2, AcOH, H_2 50 psi, 50 °C 72 h. Crude compounds were converted to BOC derivatives, purified and deprotected with TFA at 0 °C affording **2a** 35% and **2b** 50%.

at 110 °C in 70% yield. Reduction of the nitro group of **4** using Pd/C followed by the two-step cyclisation gave the azabenzimidazoles **6a** and **6b** in moderate yields. Reduction of the pyridine ring

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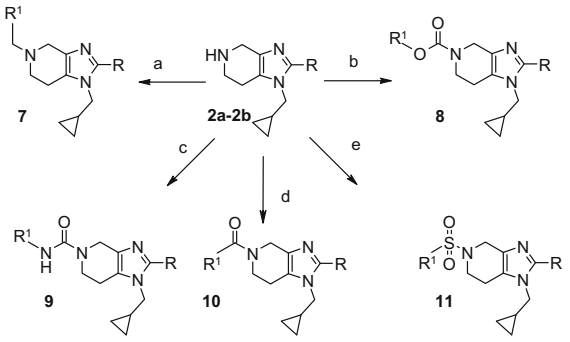

Scheme 2. Library design around templates **2a**–**b**. Alkyls **7**: (a) Aldehydes, DCM, $NaBH(OAc)_3$, 30 °C, 16 h success rate 46%. Carbamates **8**: (b) DCM, TEA CDI 30 °C 16 h, then alcohols 30 °C 16 h success rate 77%. Ureas **9**: (c) DCM, TEA, Triphosgene 30 °C 16 h, then amines 30 °C 16 h success rate 35%. Amides **10**: (d) Acids, DIEA, HATU, DMA 30 °C 16 h success rate 70%. Sulfonamides **11**: Sulfonyl chlorides, Dichloroethane, TEA, DMAP 30 °C 16 h success rate 71%. All final compounds were purified by preparative HPLC.

was performed using PtO_2 affording the fused piperidines **2a** and **2b** in 35 and 50% yields respectively.

Scaffolds **2a** and **2b** were functionalized by reductive amination and formation of carbamates, ureas, amides and sulfonamides by parallel chemistry using standard procedures. Overall 126 compounds distributed in 5 classes were prepared (Scheme 2).

There is general consensus in the medicinal chemistry community that high molecular weight and high lipophilicity are correlated with poor oral drug-like properties. For example within a series, increased microsomal clearance and pharmacological promiscuity[8] are often associated with higher *c*Log *P* values, while limited cell permeation and absorption are linked with low lipophilicity. To achieve a compromise between absorption and first-pass clearance, a *c*Log *P* value between 2 and 3 is often considered optimal in an oral drug program.

On the other hand, protein–ligand binding is partially driven by lipophilic interactions, and the optimal *c*Log *P* for a class of ligands will depend on the nature of target protein. Since the endogenous ligands for the CB_2 receptor are highly lipophilic fatty acid derivatives (endocannabinoids[9]), a higher range of *c*Log *P* values was considered, despite the increased risk of metabolic instability.

Monomers were selected based on similarity with previously established SAR, and our library design criteria included *c*Log *P* and molecular weight filters (*c*Log *P* between 0 and 5, mean value 3.0 and molecular weight (MW) below 500, mean value 377). Figure 2a shows the distribution of *c*Log *P* versus MW for each class of compounds, and Figure 2b the distribution of compounds by *c*Log *P* bins. 84 compounds (66%) had a *c*Log *P* between 2 and 4. Distribution across bins was series dependent, for example, most alkyl derivatives **7** (yellow) had a *c*Log *P* above 4 and most sulfonamides **11** (blue) had a *c*Log *P* value between 2 and 3.

High in vitro potency is a desirable attribute in drug candidates, as it reduces the risk of non-specific, off-target pharmacology. Associated with low clearance, high potency also allows for low total dose and thus lower risk of idiosyncratic toxicity.[10,11] Given the aforementioned link between lipophilicity and clearance there is a greater likelihood of achieving good in vivo performance when potency can be increased within a series by making changes that keep log *P* or log *D* fixed or even reduced.

Lipophilic Efficiency (LipE) has recently been introduced[8,12] as a parameter that combines both potency and lipophilicity (*c*Log *P*, *c*Log *D* or measured Log *D*, if appropriate), and is defined as

$$\mathrm{LipE} = \mathrm{pIC_{50}}(\text{or } \mathrm{pEC_{50}}) - c\mathrm{Log}P \quad (1)$$

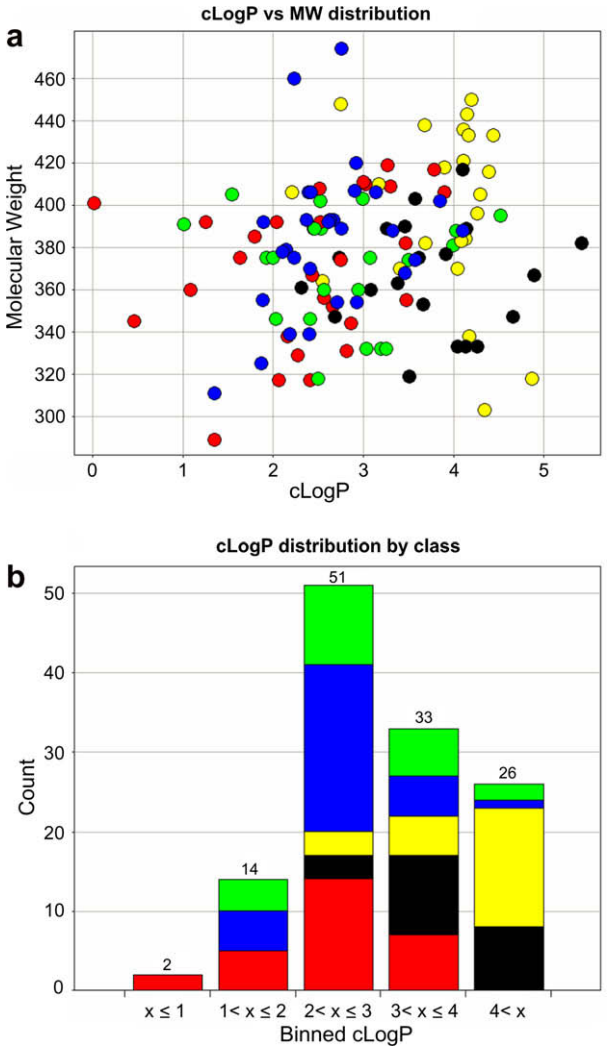

Figure 2. (a) *c*Log *P* versus molecular weight distribution of compounds, colored by class. (b) Distribution of compounds in each *c*Log *P* bins. Yellow: alkyls **7**; black: carbamates **8**; green: ureas **9**; red: amides **10**; blue: sulfonamides **11**.

LipE is increased when the pIC_{50} is increased by more than the increase in *c*Log *P*. For example, a potent (EC50 9) compound with a *c*Log *P* of 2 will have a LipE of 7. This value of *c*Log *P* is often consistent with reasonable in vivo clearance, solubility and protein binding. These factors contribute positively to the chances of such a compound achieving good efficacy and duration in vivo through a combination of good PK and potency.[13] High LipE compounds should outperform low LipE compounds as these will be compromised by reduced potency or increased lipophilicity. Furthermore, keeping the log *P* or log *D* of clinical candidates low will reduce the chances of seeing toxicity or side effects.[8] A recent analysis of animal safety studies[14] showed an increased risk of adverse outcome for compounds with *c*Log *P* > 3. In fact, we have found that it is very common for a clinical candidate to have the highest, or near highest, LipE for the series.[12] Whilst achievable LipE values are target and series specific, compounds with LipE above 5 are usually considered highly optimized.

Modestly potent CB_2 full agonists were found in the alkyl, carbamate, urea and amide classes. Representative examples from each class are represented in Figure 3. The nature of the alkyl side chain in the imidazole 2-position had a moderate if unpredictable impact on binding affinity (**7b** and **7c** vs **9b** and **9c**). LipE values were higher for amides **10a**–**b** (Table 1).

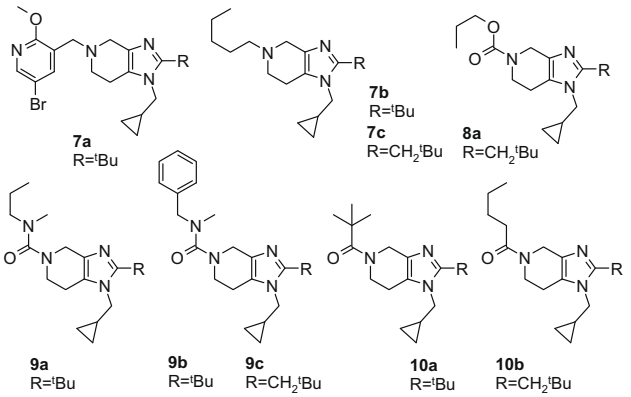

Figure 3. Most potent alkyl, carbamate, urea and amide compounds.

Table 1
Agonist activity[a] on the hCB2 receptor

Compound	EC_{50} (nM)[15]	*c*Log *P*	LiPE[b]
7a	295	4.4	2.1
7b	878	4.3	1.6
7c	3000	4.9	0.7
8a	560	4.3	2.0
9a	380	3.2	3.2
9b	637	4.0	2.2
9c	523	4.5	1.8
10a	320	2.1	4.4
10b	1020	2.4	3.6

[a] Values are means of two experiments.
[b] LiPE is calculated as LiPE = pEC_{50} − *c*Log *P* (Eq. 1).

In contrast, the sulfonamides **11a–n** (Fig. 4) proved to be highly potent full agonists at the hCB_2 receptor, the isoxazoles **11a** and **11b** being the most potent. These results warranted further in vitro profiling of sulfonamides on the hCB_1 receptor and on rat liver microsomes (RLM). When tested at 10 μM on the hCB_1 receptor, only compounds **11a**, **11c** and **11l** displayed noticeable agonist activity. Determination of the hCB_1 EC_{50} for these compounds showed that the compounds were weak hCB_1 agonists (EC_{50} **11a** 7.5 μM; **11c** 18 μM, **11l** > 14 μM) (Table 2).

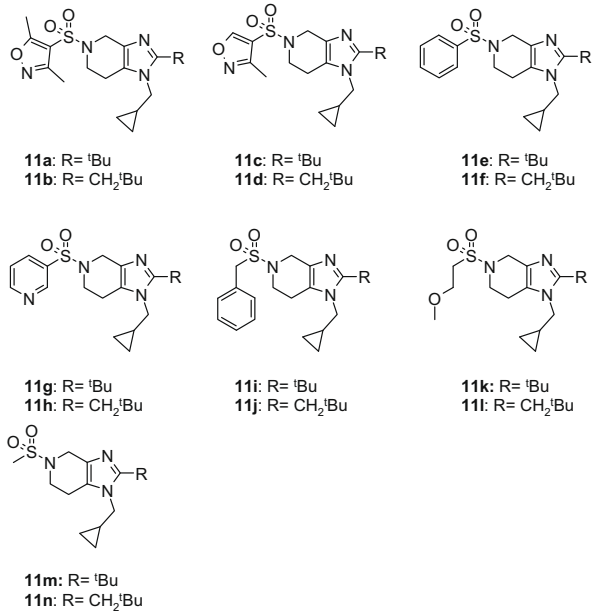

Figure 4. Most potent sulfonamide compounds.

Table 2
Agonist activity of sulfonamides **11a–n** on the hCB_2 and hCB1 receptors

Compound	hCB2 EC_{50} (nM)	hCB_1% resp. at 10[a] (μM)	RLM Cl_{int}[c]	*c*Log *P*	LiPE
11a	5.1	66%[b]	>50	2.4	5.9
11b	7.0	24%	>50	2.9	5.3
11c	55.4	38%[b]	<8.5	2.1	5.2
11d	18.5	9%	46	2.6	5.1
11e	7.9	10%	>50	3.6	4.5
11f	9.1	9%	>50	4.1	3.9
11g	14.2	11%	>50	2.2	5.6
11h	24.5	4%	>50	2.8	4.9
11i	21.8	13%	>50	3.3	4.3
11j	13.4	15%	>50	3.9	4.0
11k	26.7	25%	>50	1.9	5.7
11l	21.5	37%[b]	>50	2.4	5.3
11m	403	10%	<8.5	1.4	5.1
11n	439	9%	13	1.9	4.5

[a] % response at 10 μM (CP-55940 = 100%).[16]
[b] **11a** EC_{50} 7.5 μM **11c** EC_{50} 18 μM **11l** EC_{50} > 14 μM.
[c] A corrective factor taking microsomal binding into account was used.[16]

Despite controlled *c*Log *P* values and good LipE's, most compounds displayed high metabolic clearance. Removal of one potentially metabolically vulnerable methyl group in isoxazoles **11a** and **11b** led to the more stable compounds **11c** and **11d. 11a** and **11c** have similar *c*Log *P* values, and the discrepancy in the rate of microsomal clearance clearly indicates the specific effect of the isoxazole methyl group on metabolic stability.

As expected, within matched pairs (**11c–d** and **11m–n**), the more lipophilic compounds displayed higher clearance.

While the tetrahydro-1*H*-imidazo[4,5-*c*]pyridin-5-ium sulfonamides represent a new class of potent and selective CB_2 full agonists, these compounds display relatively high in vitro clearance for their *c*Log *P* range. In the sulfonamide series, potency was only observed for compounds with a *c*Log *P* value above 1.9, possibly owing to the intrinsically lipophilic nature of the CB_2 receptor binding site. Since reducing the microsomal clearance solely by lowering the *c*Log *P* is likely to lead to compounds with lower potency, the removal of specific metabolically vulnerable groups is predicted to be a better strategy to further advance this series.

Plotting *c*Log *P* against pEC_{50} (Fig. 5) for compounds **7–11** shows a distribution along lines of identical LipE values. Compounds **7–9**

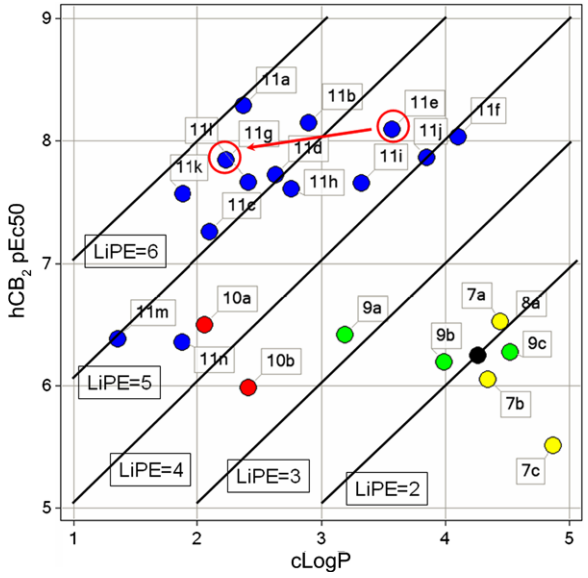

Figure 5. *c*Log *P* versus hCB2 pEC_{50} plot.

have low LipE values (low potency and relatively high *c*Log *P*). Amides **10a–b** have a much lower lipophilicity for the same potency range and thus have better lead-like properties than analogs **7–9.** Interestingly, comparing phenyl- and pyridyl-sulfonamide (**11e** and **11g**, red circles) indicates that a reduction of *c*Log *P* by almost 1.5 units is achievable without appreciable loss of potency. This hints at an area in the binding site where polarity may be tolerated, or even beneficial. Indeed, most compounds with a LipE greater than 5 possess an H-bond acceptor in the same region of space.

In conclusion, we have designed and rapidly prepared a range of alkyl, carbamate, urea, amide and sulfonamide derivatives of the tetrahydro-1*H*-imidazo[4,5-*c*]pyridin-5-ium scaffold using in-silico filters and parallel chemistry. LipE analysis of the dataset shows the higher intrinsic quality of the sulfonamide derivatives, and suggests that polar sulfonamides derivatives lacking metabolically vulnerable groups may provide us with a new class of potent, selective and metabolically stable CB_2 agonists.

Acknowledgment

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Supplementary data

Supplementary data (in vitro metabolism on Rat Liver Microsomes (RLM), Measurement of agonist activity at the human CB_1 receptor) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.062.

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15. Agonist activity of compounds on the CB_2 receptor was measured in CHO cells expressing human recombinant CB_2. Cells were incubated for 60 min at 37 °C with compounds in an 11 point, third log unit decrement concentration range and 20 μM forskolin. The cells were subsequently freeze thawed and cyclic AMP levels determined using the DiscoveRx enzyme fragment complementation method. An EC_{50} was determined via a sigmodal plot of the cAMP response versus log compound concentration.
16. See Supplementary data.