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5-Sulfonyl-benzimidazoles as selective CB2 agonists-Part 2

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ABSTRACT

In a previous communication, the SAR of a series of potent and selective 5-sulfonyl-benzimidazole CB2-receptor agonists was described. The lack of in vivo activity of compounds from this series was attributed to their poor solubility and metabolic stability. In this Letter, we report on the further optimization of this series, leading to the relatively polar and peripherically acting CB2 agonists **41** and **49**. Although both compounds were not active in acute pain models, the less selective compound **41** displayed good, sustained activity in a chronic model of neuropathic pain without the tolerance observed with morphine. In addition, both **41** and **49** delayed the onset of clinical symptoms in an experimental model for Multiple sclerosis.

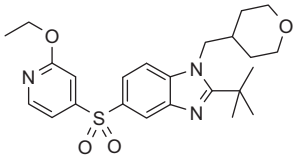
The two known cannabinoid receptors CB1 and CB2 play a modulatory role in a complex array of biological processes.[1] Activation of both receptors has been associated with analgesic and anti-inflammatory effects. However, while clear analgesic effects have been observed for CB1-agonists, the reports on the in vivo effects of CB2-agonists in animal pain[2] and inflammation[3] models are far less conclusive.[4] The undesirable psychotropic effects associated with activation of the CB1 receptor make the CB1 receptor a less attractive drug target for pain indications. The CB2 receptor does not carry this liability. It is mainly located in the periphery, and its abundance in immune cells underlines the anti-inflammatory potential.

Recently, we have reported on a series of 5-sulfonyl-benzimidazoles as potent, highly selective, and peripherically restricted CB2 agonists.[5] One of the key compounds arising from this series was compound **1** (Fig. 1), which combined selectivity with an acceptable drug-like profile. Although this translated in a decent pharmacokinetic profile, no analgesic effect could be demonstrated in pain models. Achieving high compound levels over a prolonged period of time proved to be difficult, which was attributed to the suboptimal metabolic stability of **1**, in combination with poor solubility. In this Letter we describe the further optimization of 5-sulfonyl-benzimidazole CB2 agonists with improved metabolic stability and solubility, which would allow us to investigate the potential use of peripherically restricted CB2 agonists in animal models.[6]

As reported by us previously,[5] and confirmed by other groups describing SAR on benzimidazole CB2 agonists,[7] the *t*-butyl group at the benzimidazole 2-position is optimal for achieving potency as well as full agonistic efficacy. Therefore, our exploration focused around further variation of the substituents at the benzimidazole 1- and 5-positions.

In **1**, the introduction of an *ortho*-substituted pyridyl sulfone substituent had resulted in an increased CB2/CB1 selectivity, as well as an acceptable CYP-inhibition profile.[5] Common approaches to improve solubility include the introduction of additional heteroatoms, or increasing the number of rotatable bonds.[8] We tried to combine this via the introduction of heteroatom containing elongated alkyl groups on the *ortho* pyridyl position (compounds **2**–**8**, Table 1).

Their synthesis is depicted in Scheme 1. Oxygen or nitrogen linked alkyl substituents were easily introduced on fluoropyridyl substituted **9**[5] to give **2**–**5**. Carbon linked alkyl groups were synthesized via a selective Shonogashira reaction on 2,4-dichloropyridine (**10**), followed by catalytic hydrogenation to give the 2-alkyl-4-chloropyridines **12**. These then underwent a Buchwald type



hCB2 EC_{50} 0.3nM
hCB1 EC_{50} 1280 nM
metabolism (hLM, 15 min.) 48%
kinetic solubility (pH 7.4) <1 µM

1

Figure 1. Structure and properties of previous lead and Pfizer analogue.

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§ Present affiliations: Abbott.

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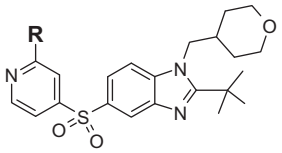
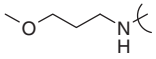
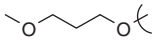
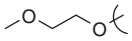
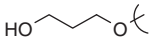
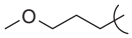
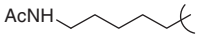
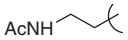
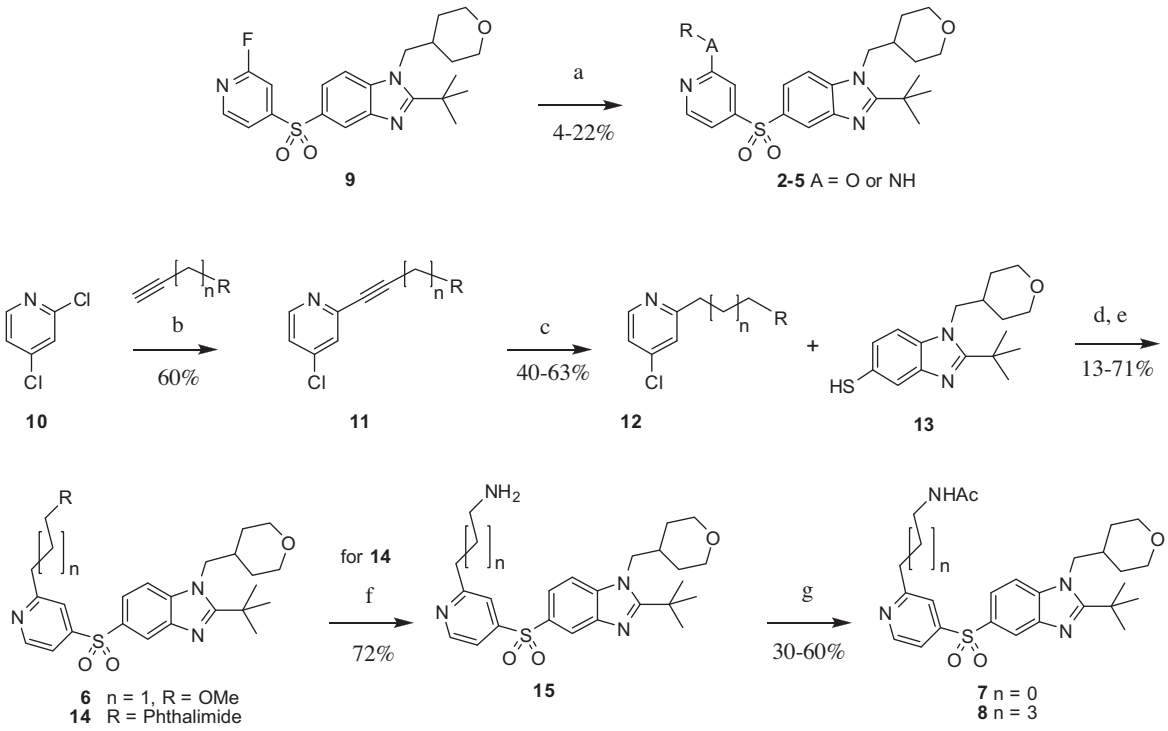
Table 1
Elongated analogues of ethoxy substituted **1**



Compound	R	hCB2 EC_{50} (nM)	hCB1 EC_{50} (nM)	% Metabolism in hLM (15 min)
1		0.3	1280	48
2		0.8	955	99
3		0.5	3850	100
4		1.3	804	53
5		1.2	3630	40
6		1.1	>10000	93
7		5.6	>10000	86
8		1.4	8320	44



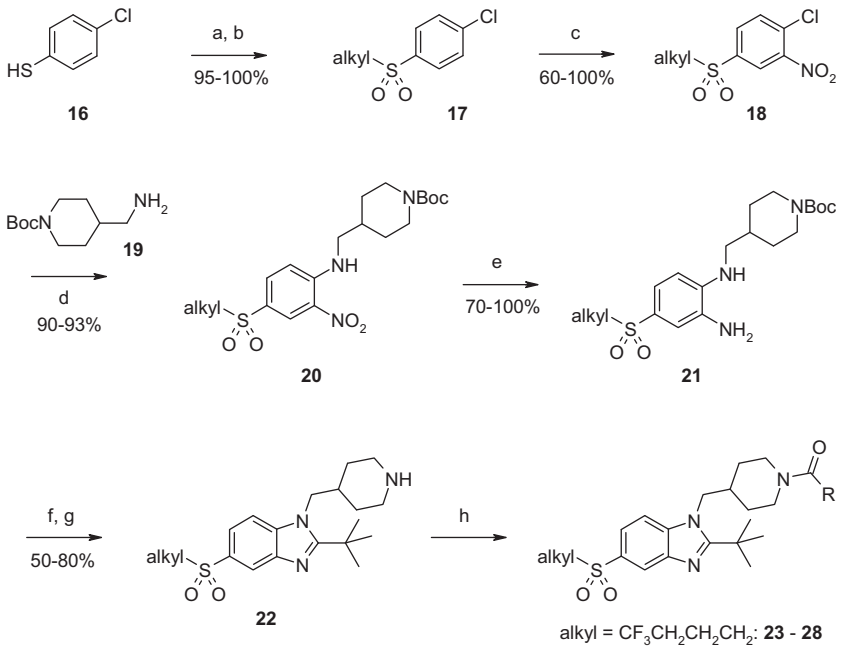
Scheme 1. Reagents and conditions: (a) R-OH, NaH, dioxane;,or R-NH_2, dioxane, 100 °C; (b) $PdCl_2(PPh_3)_2$ (cat.), CuI, Et_3N, 70 °C; (c) Pt/C 5% (cat.), H_2, thiophene sol., Et_3N, MeOH; (d) Pd_2dba_3 (cat.), Xantphos, Cs_2CO_3, dioxane, 80–100 °C; (e) mCPBA, $CHCl_3$; (f) hydrazine, EtOH; (g) AcCl, Et_3N, CH_2Cl_2.

coupling with **13**, whose synthesis has been described before.[5] Subsequent oxidation gave sulfones **6** and **14**. The latter were deprotected and acetylated to give **7** and **8**.

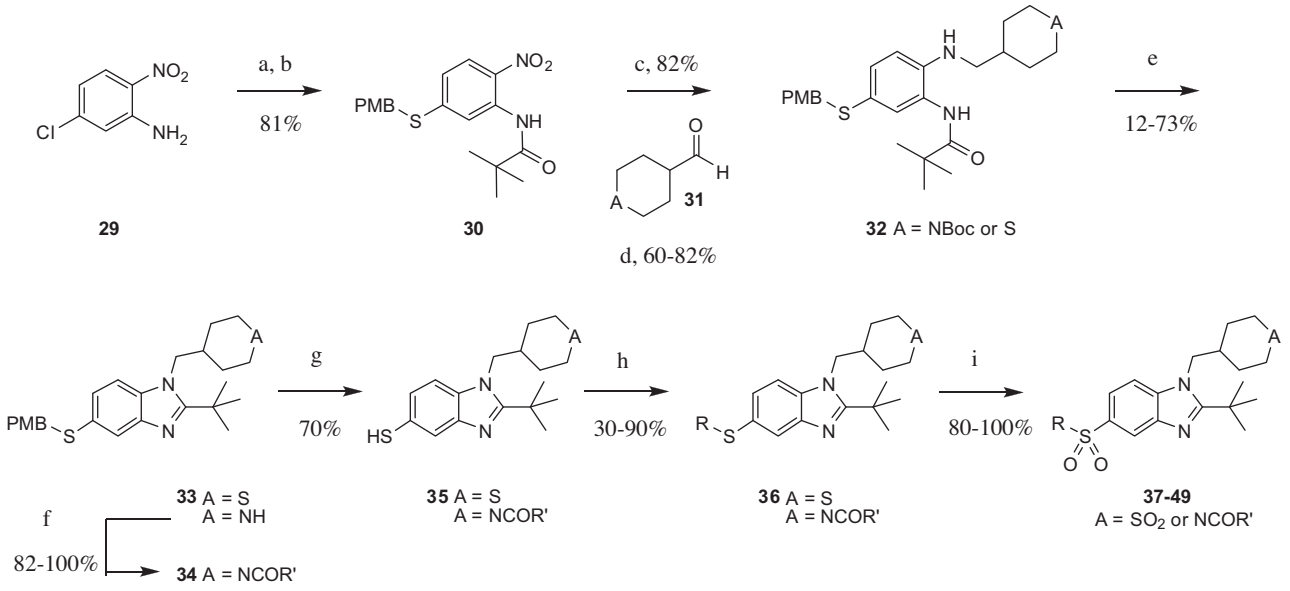
In general, the introduction of diversely substituted alkyl groups was well tolerated, and maintained the potency and good to excellent selectivity of **1** (Table 1).[9] Unfortunately, the compounds displayed a worse, or at best similar, metabolic stability compared to **1**. More unexpectedly, the solubility of these compounds did not greatly improve either (data not shown).

We then turned our attention to the benzimidazole-1-substituent. This had been shown to be crucial for achieving good potency, but the tetrahydropyranyl ring is also a potential metabolic soft spot. Earlier explorations had indicated a preference for lipophilic groups at the benzimidazole 1-position to achieve high potency, but this also resulted in low metabolic stability, as well as a reduced selectivity of CB2 versus CB1.[5] Now, we replaced the tetrahydropyranyl moiety with more polar acylated piperidinyl and tetrahydro-1,1-dioxo-thiopyranyl groups to improve both the metabolic stability and the solubility.

The synthesis of the target compounds is depicted in Schemes 2 and 3. Introduction of a variety of acylated piperidinyl methyl groups was most easily achieved via a nucleophilic aromatic substitution



Scheme 2. Reagents and conditions: (a) alkyl-halide, Cs_2CO_3, acetone; (b) mCPBA, $CHCl_3$; (c) HNO_3, H_2SO_4; (d) K_2CO_3, dioxane or *i*PrOH, 75–100 °C, 3–6 h; (e) H_2, Pd/C (cat.) MeOH; (f) PivCl, pyridine, CH_2Cl_2; (g) conc. HCl, HOAc, reflux, 3 h; (h) RCOCl or $(RCO)_2O$, Et_3N, CH_2Cl_2.



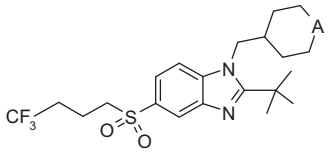
Scheme 3. Reagents and conditions: (a) PMBSH, KOH, EtOH, reflux, 2 h; (b) PivCl, pyridine, CH_2Cl_2; (c) Fe, aq HOAc, reflux, 4 h; (d) $NaBH_3CN$, HOAc, CH_2Cl_2; (e) HOAc, reflux; (f) $(R'CO)_2O$, THF or (for R' = COH) MeOCOH, reflux, 24 h. (g) TFA, MW 120 °C, 25–60 min; (h) R-halide, Cs_2CO_3, acetone or Pd_2dba_3 (cat.), Xantphos, Cs_2CO_3, dioxane, 80–100 °C; (i) mCPBA, $CHCl_3$.

with **19** on halobenzenes, activated by the *para*-sulfonyl and *ortho*-nitro-groups such as in **18** (Scheme 2). Reduction of the nitro group in **20** to aniline **21** was followed by pivaloylation to introduce the *t*-butyl group. Subsequent condensation to the benzimidazole nucleus under acidic conditions, with concomitant debocylation, led to **22**. Finally, acylation of **22** yielded compounds **23**–**28**.

An alternative synthetic route allowed for a later introduction of 5-sulfonyl substituents, and is shown in Scheme 3. Starting from 5-chloro-2-nitro-aniline **29**, 4-methoxybenzylthiol (PMBSH) was introduced as a protecting group via nucleophilic aromatic substitution and subsequent reaction with pivaloylchloride gave **30**. Reduction of the nitro group was followed by reductive amination with aldehyde **31** to introduce the desired benzimidazole-1-substituent. Condensation to the benzimidazole under acidic conditions again resulted in debocylation, which allowed for subsequent introduction of various acyl groups to give **34**. Deprotection of the *para*-methoxybenzyl group with TFA gave intermediates **35**. In the next steps the various 5-sulfonyl groups could be introduced. Alkyl substituents were introduced via nucleophilic substitution, (hetero)aromatic substituents were introduced via Pd-catalyzed reactions.[10] Finally, the sulfides **36** were oxidized to the sulfones **37–49** using *meta*-chloroperoxybenzoic acid.

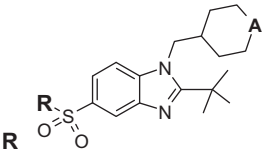
To investigate the change in properties by going from a tetrahydropyranyl to acylated piperidinyl substituents, the

Table 2
Effect of N substitution on hCB_2 agonist activity and selectivity



	A	hCB2 EC_{50} (nM)	hCB1 EC_{50} (nM)	% Metabolism in hLM (15 min)
50	O	1.1	320	36
22	NH	240	>10000	nd
23	NCOH	2.4	5623	17
24	$NCOCH_3$	13	>10000	17
25	$NCOCH_2CH_3$	19	>10000	4
26	$NCOCF_3$	23	>10000	20
27	$NCOOCH_3$	17	>10000	54
28	$NCOCH_2OCH_3$	1585	>10000	nd

Table 3
Variation of C-5- substitution with selected N-substituents



	R	A	hCB2 EC_{50} (nM)	hCB1 EC_{50} (nM)	% Metabolism in hLM (15 min)
37	Ethyl	NCOH	23	>10000	7
38	Ethyl	$NCOCH_3$	8.4	>10000	13
39	Ethyl	SO_2	28	>10000	nd
40	*c*Propylmethyl	NCOH	2.2	2512	16
41	*c*Propylmethyl	$NCOCH_3$	8.2	>10000	18
42	*c*Propylmethyl	SO_2	8.4	2676	2
43	*c*Pentyl	$NCOCH_3$	3.2	>10000	87
44	Methoxyethyl	$NCOCH_3$	26	>10000	nd
45	Hydroxypropyl	$NCOCH_3$	145	>10000	nd
46	4-*F*-phenyl	$NCOCH_3$	2.9	>10000	8
47	4-Pyridyl	$NCOCH_3$	4.6	>10000	29
48	2-Thiazolyl	NCOH	8.4	321	nd
49	2-Thiazolyl	$NCOCH_3$	7.7	3428	9

trifluorobutylsulfonyl group was selected as the benzimidazole 5-substituent (Table 2). As reported previously, this particular substituent had led to a potent, but moderately selective CB2 agonist when combined with the tetrahydropyranylmethyl group at the 1-position (compound **50**).[5] Replacement of the THP-group in this compound, would allow us to determine the influence of this structural change at the 1-position on CB2/CB1 selectivity, in addition to potency, metabolic stability, and solubility.

All the acylated piperidinyl analogues **23**–**28** displayed an improved CB2/CB1 selectivity profile (Table 2), with EC_{50} values for CB1 activation for most derivatives above 10 μmol. Metabolic stability and solubility (data not shown) were also improved for most compounds. The non-acylated piperidinyl analogue **22** appeared to have lost most of the CB_2 activity. In comparison to **50**, a drop in potency was also observed for the acylated piperidinyl compounds, but the 2–20 nM potency, in combination with the overall improved properties, encouraged us to explore this variation further.

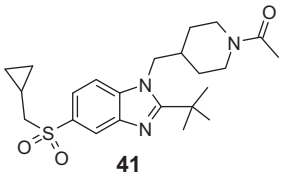
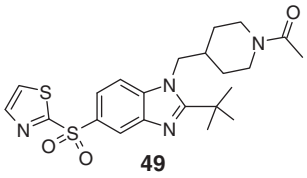
A set of analogues was prepared and tested containing formylated and acetylated piperidinyl methyl N-substituents, combined with various 5-sulfonyl groups. In addition, the tetrahydro-1,1-dioxo-thiopyranyl methyl group was investigated as a potential alternative to the acyl piperidinyl methyl group (Table 3).

Apolar alkyl or (hetero)aryl sulfonyl substituents resulted in potent and selective CB2 agonism. Introduction of more polar substituents such as in **45**, or an overall decreased lipophilicity as in **37**, **39**, or **44** led to a decrease in potency. The combination of N-formyl piperidinyl methyl 1-substituents with apolar 5-sulfonyl groups such as in **23**, **40**, and **48** led to the most potent CB2 agonists in this series, but also <10 μM CB1 agonism. Bis-sulfonyl containing compounds **39** and **42** showed a comparable activity profile compared to their acylpiperidinyl analogues, but suffered from low solubility. Most compounds of interest were subsequently subjected to a modified tetrad test in rats with doses up to 40 mg/kg (intraperitoneal) for typical CNS mediated CB1 side-effects.[11] In this test even compounds displaying weak CB1 agonism are active. For example, in this assay, **50** had a lowest active dose (LAD) for body temperature decrease at 10 mg/kg and flat body posture at 2.5 mg/kg. Of all current compounds tested from Table 3, only compounds **46** and **48** were positive with a LAD of 10 mg/kg on all CB1 mediated observations. While these effects can be explained for **48** based on its limited CB2/CB1 selectivity, for **46** this is less obvious. Species differences or increased brain penetration could offer a potential explanation, but this was not investigated.

Compounds **41** and **49** were selected for further in vivo profiling, respectively as a highly selective and moderately selective CB2 agonist, both without CB1 mediated side-effects up to 40 mg/kg.

Physicochemical and key in vitro and in vivo DMPK properties for **41** and **49** are shown in Table 4. Compared to the high lipophilicity of most reported cannabinoid ligands, **41** and **49** are relatively polar, with a Log*P* of 1.67 and 1.90, respectively. For formulation purposes, a good solubility was observed for **41**, but **49** was only

Table 4
Physicochemical and key in vitro and in vivo DMPK properties for **41** and **49**

	 **41**		 **49**	
Log *P*	1.67		1.90	
TPSA	81		122	
Solubility (mg/mL)	1.5 (H_2O) 25 (10% CD)		0.02 (H_2O) 8 (20% CD)	
Metabolic stability[a]	5% (h), 0% (r) 12% (m), 4% (d)		12% (h), 0% (r), 6% (m), 0% (d)	
In vivo *PK*[b]	*Rat*	*Dog*	*Rat*	*Dog*
Cl (L/h/kg)	2.5	0.8	3.2	0.7
V_d (L/kg)	4.4	1.9	1.3	3.7
$T_{1/2}$ (iv)	1.3 h	3 h	3.5 h	4 h
$T_{1/2}$ (po)	4 h	3.2 h	3.1 h	5–19 h
Fabs	56%	86%	35%	100%
Brain/plasma	0.18		0.05	

[a] % Metabolism as measured after 60 min incubation at 5 μM with liver microsomes (h = human, r = rat, m = mouse, d = dog).
[b] Rat (Sprague–Dawley): 2.5 mg/kg iv, 10 mg/kg po Dog (Beagle): 1 mg/kg iv, 5 mg/kg po.

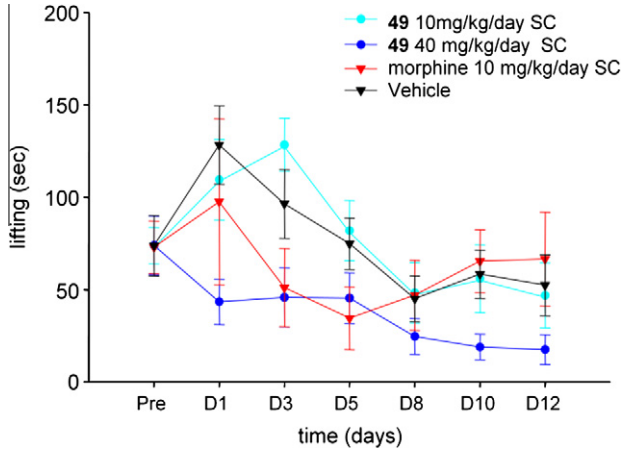


Figure 2. Effect on CCI induced cold allodynia: lifting on a cold plate (0 °C for 4 min) in CCI rats ($n = 7$) during chronic treatment with **42** versus morphine.[13]

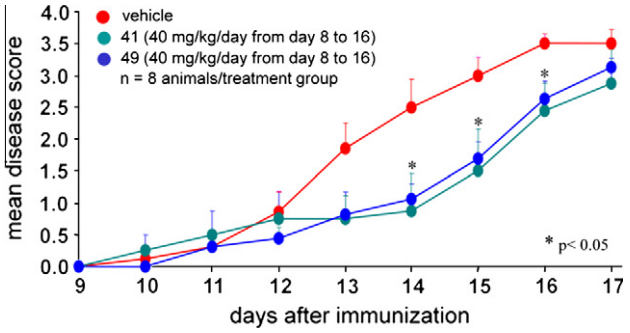


Figure 3. Effect on EAE disease scores in mice after chronic treatment with **41** and **49**.

soluble at 20 μg/mL in water. After addition of 20% cyclodextrin (CD), formulations could be prepared of **49** with a concentration up to 8 mg/mL. In all species tested, the compounds were found to be metabolically stable in vitro.

For **41**, in vivo plasma kinetics studies in rat and dog showed a fairly high clearance and short half life in rat after iv dosing, but the profile after oral dosing was acceptable with a half life of 4 h and an absolute oral bioavailability (Fabs) of 56%. For **49**, a similar profile was observed in rat, with a slightly lower absolute oral bioavailability of 35%. In a rat tissue distribution study, **41** and **49** gave a brain to plasma ratio of 0.18 and 0.05, respectively, indicating poor blood brain barrier penetration. For both compounds, an overall improvement of the PK profile was observed in dogs.

Compounds **41** and **49** were then tested in a battery of analgesic and anti-inflammatory models.[12] Both **41** and **49** did not show appreciable efficacy at 10 mg/kg in a number of acute nociception models such as the hot plate, tail withdrawal reflex, or paw flick tests. They were also ineffective in myco- and carrageenan-induced thermal hyperalgesia. Significant effects were only observed after chronic treatment, and some preliminary results are shown in Figures 2 and 3. In the chronic constriction injury (CCI) model **of** neuropathic pain in rats,[13] **49** did reverse CCI induced cold allodynia[14] after daily subcutaneous (SC) administration of 40 mg/kg (Fig. 2). This effect was maintained after 12 days of dosing, in contrast to treatment with morphine for which tolerance was observed after 5–8 days of dosing. In the same test, no significant effect was observed for the more CB2 selective **41** at doses of 10 and 40 mg/kg SC. The activity observed for **49** can therefore not be attributed with certainty to CB2 agonism. The activity could also be the result of the weak CB1 activity of **49**.[4] Very low occupancy of CB1 receptors has been shown to be sufficient to ellicit CB1-mediated effects,[4a] and the current difference in activity for the highly CB2 selective **41** versus the less selective **49** could be in support of this.

In an experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS), chronic oral administration of **41** and **49** (40 mg/kg) in mice significantly delayed neurological signs in EAE (Fig. 3).[15] Since in this model for MS, both **41** and **49** are equally active, this effect is more likely to be related to CB2 agonism, than the effects observed in the CCI model. It should be noted that the delay in neurological signs is only temporary. For an optimal approach to treat MS, an increased brain penetration may be required.[16]

Despite the activity observed in both of these chronic models, the limited analgesic and anti-inflammatory effects of both compounds question the therapeutic usefulness of peripherically

restricted CB2 agonists. Recently, Pfizer has reported on the optimization of the same series of 5-sulfonyl benzimidazole CB2-agonists, leading to selective and brain penetrant CB2-agonist.[7c] A direct comparison of the peripherically restricted **41** or **49** with a brain penetrant analogue from the same series in relevant animal models could offer further insight into the need for brain penetration in order to be efficacious.

Acknowledgement

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