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Bioorganic & Medicinal Chemistry Letters 18 (2008) 2574–2579
Bioorganic & Medicinal Chemistry Letters

5-Sulfonyl-benzimidazoles as selective CB2 agonists

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Received 9 February 2008; revised 14 March 2008; accepted 16 March 2008
Available online 20 March 2008

Abstract—A novel series of benzimidazole CB2-receptor agonists was synthesized and the structure–activity relationship explored. The results showed agonistic activities with an EC_{50} up to 0.5 nM and excellent selectivity (>4000-fold) over the CB1 receptor. The size of the substituent on the 2-position determined the level of agonism, ranging from inverse agonism to partial agonism to full agonism, which was more pronounced for the rat CB2 receptor. A wide variation of sulfonyl substituents at the benzimidazole 5-position was tolerated, which was used to optimize the drug-like properties. This resulted into lead compound **14j** that can be used to investigate the potential of a selective, peripherically acting CB2 agonist. The in vitro profile of key compounds is displayed using pie bar charts (VlaaiVis).

The endocannabinoid system has been known to mediate a complex array of biological effects. These effects are regulated through at least two distinct G-protein coupled receptors, the CB1- and CB2-receptor.[1] While the effects mediated by CB1, mostly in the CNS, have been thoroughly investigated, those mediated by CB2 are not well defined. CB2 selective agonists are claimed to be effective in the treatment of pain,[2] various inflammatory diseases,[3] osteoporosis[4], and atherosclerosis.[5] Separation between the therapeutically undesirable psychotropic effects mostly associated with the CB1 receptor, and the clinically desirable ones are considered to be essential for a cannabinoid agonist to be widely accepted as a useful drug. Two strategies can be envisaged to avoid possible CB1-mediated central side effects of cannabinoid receptor agonists. One could aim for compounds without central penetration, only exerting their pharmacological effects peripherically, or aim for selective CB2-compounds. Since cannabinoid ligands, in general, are rather lipophilic molecules, the complete avoidance of central penetration is not a straightforward task. Most of the described CB2 selective ligands possess a substantial potency for the CB1 receptor when tested in our protocols, which can contribute to their analgesic action.[6] The discrepancies between the reported potency values can be explained by differences in methodologies used as pointed out by Huffman with WIN-55,212-2.[7] Even combination experiments of CB2 agonist ligands and CB1 antagonists cannot always mask or suppress the CB1 mediated activity of the ligands due to the incompatibility of their pharmacokinetic properties and/or the degree of selectivity of the used antagonists.

Therefore, in order to investigate the true potential of a CB2 agonist, there is a need for drug-like CB2 ligands with an increased selectivity over the CB1 receptor.

A high throughput screening assay was initiated for the development of novel selective CB2 agonists. The benzimidazole derivative **1** was obtained as an initial hit compound. The pharmacological data are presented in a pie bar chart (VlaaiVis)[8] (Fig. 1 and Table 1).

The pie bar chart displays the profile of a compound and allows for an easy comparison of compounds. In this type of visualization, the preferred profile (Table 1) is represented as the grey outer rim and determines the properties of the dataset. Each property is represented by a colored wedge in the circle, and the width of these wedges hints at the relative importance of the property. An increase in coloring of the wedge corresponds to an improved value for the respective parameter: the fuller

Keyword: Cannabinoid receptor CB2 agonist benzimidazole VlaaiVis species difference.
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doi:10.1016/j.bmcl.2008.03.048

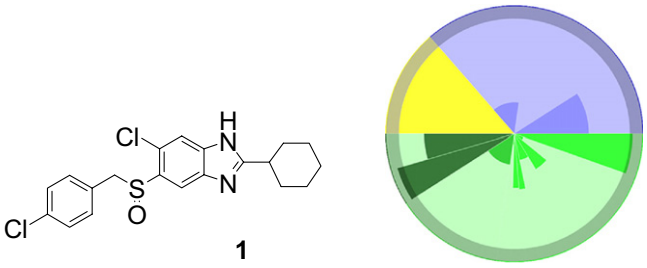


Figure 1. Pharmacological data: EC_{50} CB2 = 25 nM; ratio EC_{50} CB2/CB1 = 354.

Table 1. Preferred profile as used in the pie bar chart representation

	Properties	Preferred profile
Blue[a]	hCB2-agonism-EC_{50}	<1 nM
	Ratio EC_{50} CB2/CB1	>1000
	hCB2-agonism-% efficacy	⩾100%
Green	CYP-450 1A2/2C19/2C9/2D6 3A4 BFC, BQ and DBF inhibition[9]	<50%
	%Metabolism in human liver microsomes after 15 min	<50%
	Solubility at pH 7.4 and pH 4	>20 μM
Yellow	Rule of five violation	⩽1

[a] Start from the first blue value and read clockwise.

the better. The blue value represents the in vitro pharmacological activity of a compound. The green wedges contain the ADME data and the yellow bar corresponds to in silico calculations.

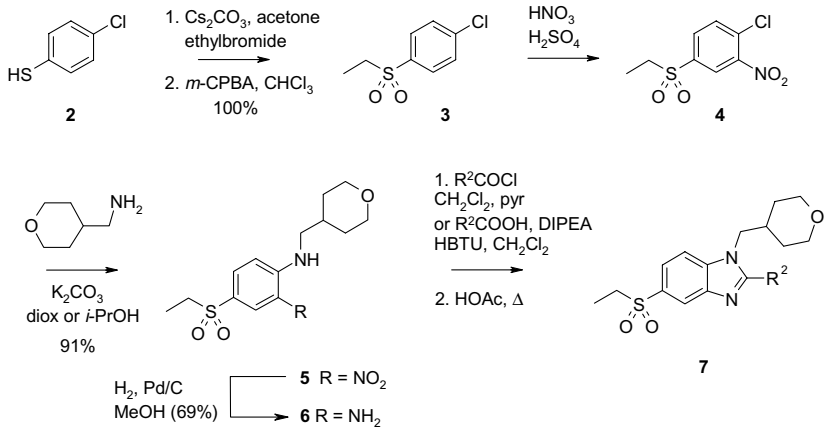
Several different intermediates were synthesized to enable variation at particular sites of the molecule. For the variation of the right-hand side of the molecule synthesis began with 4-chlorothiophenol **2** where the desired R^5-substituent was introduced via nucleophilic substitution (Scheme 1). The resulting sulfide was oxidized to sulfone **3** using *meta*-chloroperoxybenzoic acid. Nitration under standard conditions afforded compound **4**, then R^1 was introduced via a nucleophilic aromatic substitution making use of R^1NH_2, mostly tetrahydro-4-pyranylmethylamine, to give **5**. Reduction of the nitro group to aniline **6** was followed by acylation to introduce substituent R^2. The desired benzimidazoles **7** were obtained via final condensation in hot acetic acid. This synthesis route was ideal for the exploration of the SAR around R^1 and R^2 since they were introduced at a late stage of the synthesis.

A synthetic route where the R^5-substituent was introduced in one of the last steps started from 5-chloro-2-nitro-aniline **8** (Scheme 2). 4-Methoxybenzylthiol (PMBSH) was introduced as a protecting group via nucleophilic aromatic substitution and subsequent reaction with pivaloylchloride gave **9**. Reduction of the nitro group followed by reductive amination with tetrahydropyran-4-carbaldehyde introduced the most favorable R^1-substituent. Condensation to the benzimidazole under acidic conditions and subsequent deprotection of the *para*-methoxybenzyl group with TFA gave intermediate **11**. In the next step, the various R^5 groups could be introduced. Alkyl substituents were introduced via nucleophilic substitution making use of R^5-Br or R^5-Cl. (Hetero)aromatic substituents were introduced via Pd-catalyzed reactions.[10] Finally, the sulfides were oxidized to sulfones **12**–**14** using *meta*-chloroperoxybenzoic acid.

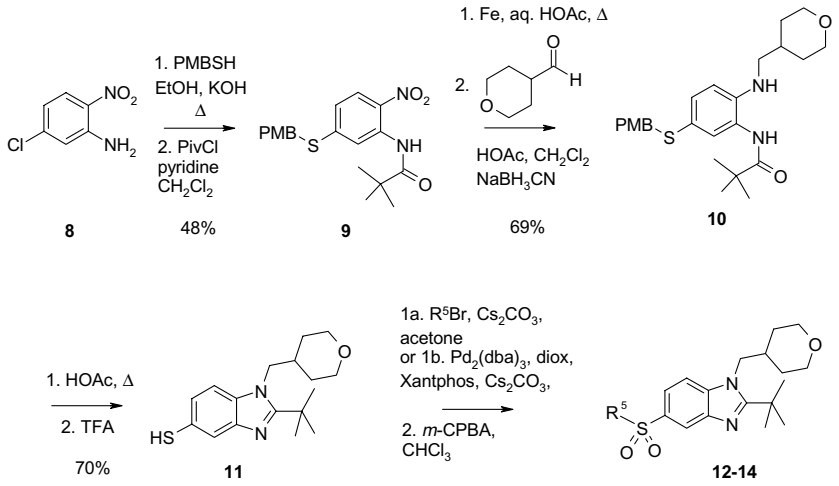
The first exploration of the SAR around **1** focused on the variation of the substituents at the benzimidazole 2- and 5-positions, but did not lead to any improvement in potency or selectivity for the CB2 receptor.

A recent publication by Pagé et al. on benzimidazole derivatives as selective CB2 inverse agonists illustrated the importance of a substituent on the benzimidazole-N^1-nitrogen.[11] In their series, a cyclohexylmethyl or a tetrahydro-4-pyranylmethyl substituent at this position was well tolerated. When implemented to our initial set of compounds, we realized their added value as demonstrated in Table 2.

In general, the cyclohexylmethyl group (**17**) led to the most potent compounds but the tetrahydro-4-pyranylm-

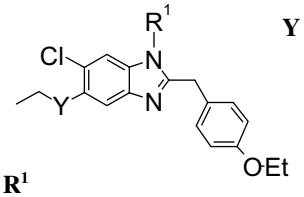


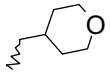
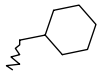
Scheme 1.



Scheme 2.

Table 2. Influence of benzimidazole-N^1-nitrogen substituent and oxidation state of sulfur



R^1	S (a)	SO (b)	SO_2 (c)
H **15**	EC_{50}: 2 μM	EC_{50}: >10 μM	
 **16**	EC_{50}: 4 nM CB2/CB1: **75** %Metab[a]: 98%	EC_{50}: 20 nM CB2/CB1: **484** %Metab: 95%	EC_{50}: 20 nM CB2/CB1: **537** %Metab: 66%
 **17**	EC_{50}: 125 nM CB2/CB1: 1.2 %Metab: 85%	EC_{50}: 6.3 nM CB2/CB1: 8.2	EC_{50}: 5 nM CB2/CB1: 50 %Metab: 74%

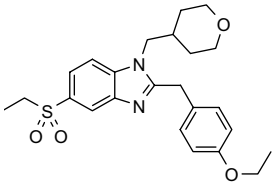
[a] %Metabolism as measured after 15 min incubation with human liver microsomes (hLM).

ethyl group (**16**) showed the best selectivity window with only a slight decrease in potency. In Table 2, the influence of the oxidation state of the sulfur atom is also demonstrated. Although not yet optimal, the sulfone derivatives **16c**–**17c** show the highest metabolic stability as well as the best CB2/CB1 selectivity.

Removal of the chlorine atom at the 6-position led to compound **7a** (Fig. 2). With an improved potency, selectivity, and stability, **7a** became our new starting point for the development of a highly selective CB2 agonist.

As shown in the pie bar chart (Fig. 2), compound **7a** fulfills most of the criteria as set in our preferred profile; however it acts as a partial agonist as indicated with the third blue wedge. The percentage of agonism efficacy reached only 44%.

A benzimidazole series with a 2,3-dihydrobenzo-furanylmethyl substituent at the 2-position has been reported as inverse agonists.[11] Another article describing a 2-oxoquinoline series as CB2 receptor inverse agonists uses similar *para*-alkoxybenzyl substituents to elicit a full CB2 inverse agonist response.[12] We decided to focus



7a

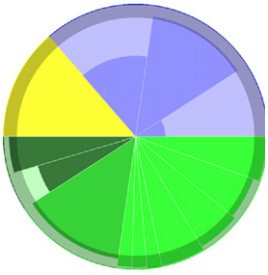
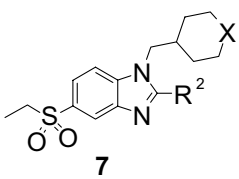


Figure 2. Pharmacological data: EC_{50} CB2 = 4.0 nM; ratio EC_{50} CB2/CB1 = 1597; metabolism_hLM = 34%.

Table 3. Influence of substituent in position 2 on partial agonism[a]

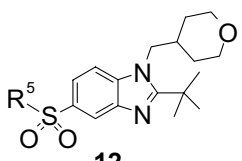


7

R^2	EC_{50} (nM)	Human CB2 agonism %Eff	Rat CB2 agonism %Eff
a $CH_2C_6H_4OEt$	4.0	44	
b CH_3 (X = CH_2)	31		
c $CH(CH_3)_2$ (X = CH_2)	1.6	101	99
d $C(CH_3)_3$ (X = CH_2)	0.8	104	
e $C(CH_3)_3$	1.2	120	102
f $CH_2CH(CH_3)_2$	4.0	101	43
g *c*-pentyl	4.0	109	0
h CH_2-benzo[1,3]dioxole[b]	$>10^4$		

[a] X is equal to oxygen unless mentioned.
[b] **7h** EC_{50} = 6.3 nM; human CB2 inverse agonism %Eff = 92.[13]

Table 4. 5-Alkylsulfonyl substitution



12

R^5	EC_{50} (nM)	Ratio EC_{50} CB2/CB1	%Metabolism in hLM (15 min)
a $CF_3(CH_2)_3$	1	285	36
b $CF_3(CH_2)_2$	1.3	394	13
c CH_3CH_2	1.2	551	0
d *c*-PropCH_2	1.3	121	14
e $CN(CH_2)_2$	4.0	2114	24
f $HO(CH_2)_2$	2.5	3846	6
g $H_2N(CH_2)_2$	10	912	
h $EtHN(CH_2)_3$	158	4.3	
i $AcHN(CH_2)_3$	100	109	

on the 2-position to understand the partial agonist nature of compound **7a** (Table 3). The influence of the substituent at the 2-position was retrieved from a set of compounds **7a**–**h** possessing a cyclohexylmethyl or a tetrahydro-4-pyranylmethyl group in the benzimidazole 1-position.

Increasing the bulkiness of the moiety at the 2-substituent resulted in increased CB2 potency as demonstrated by comparing compounds **7b**, **c**, and **d**. Any further increase in bulkiness led to partial agonism as observed for **7a** or even inverse agonism as demonstrated by **7h**.[13] Further SAR studies around this template enabled us to conclude that the effect of bulkiness on agonism is even more pronounced for the rat CB2-receptor. Compound **7f** showed partial agonism in rat and **7g** showed no agonism at all, while both retained full agonism for the human receptor. Similar differences between rat and human CB2-receptors have been observed regarding binding potencies.[14] Any effort to increase the polarity on this site of the molecule resulted in a dramatic decrease of potency. Overall, the *tert*-butyl group had the best balance between percentage effective agonism and potency.

The right-hand side of the molecule was now fully optimized and rather lipophilic. Substituents at the 1-position should fit a large hydrophobic pocket on the receptor and only a hydrogen acceptor moiety such as an ether functionality is tolerated. A *tert*-butyl group at the 2-position seemed to be the most optimal substituent. Compound **7e** emerged as the preferred candidate, but despite a reasonable CB2/CB1 EC_{50} ratio of ~500 it still induced the typical psychotropic effects when dosed in rat, commonly referred to as the tetrad test.[15]

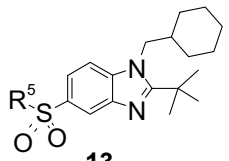
We aimed for a CB2/CB1 selectivity ratio of at least 1000 to avoid these psychotropic effects. In order to improve the selectivity, we also explored the left-hand side of the molecule. As learned from our initial series, there was a certain degree of freedom for variation at the 5-position. First, the influence of an alkylsulfonyl group was investigated (Table 4) (**12a**–**i**).

There was no significant effect on the parameters by changing the chain length as demonstrated with a trifluoroalkyl group in **12a**–**b**. Simple alkyl groups in **12a**–**d** showed good activity but moderate selectivity. Introduction of polar groups such as cyano- (**12e**) and hydroxyl groups (**12f**) improved the selectivity but diminished the potency toward the CB2 receptor. Amine (**12g**, **h**) or amide substituents (**12i**) decreased the potency considerably. Further SAR exploration pointed toward benzylsulfonyl substituted compounds **13** (Table 5). This variation led to a favorable potency but suffered severely from metabolic cleavage of the benzylic moiety within **13a**. Difluorination as in **13b** could solve this, but again selectivity remained an issue.

Further SAR studies were focused on aryl- and heteroarylsulfonyl substituents in 5-position (**14a**–**l**, Table 6).

Substitution on the aryl group improved the potency, independent of the site of substitution (**14b**–**d**). In the case of *meta*-substituents (**14c**) an excellent selectivity was obtained. Unfortunately, *ortho*- and *meta*-substituted phenylsulfones displayed poor metabolic stability. A decrease in activity was observed by introducing polarity (**14e**–**f**) in line with the alkyl substituents, though this was less pronounced. Heteroaryl substituents (**14g**–**i**) were mostly well tolerated. Pyridine **14g**

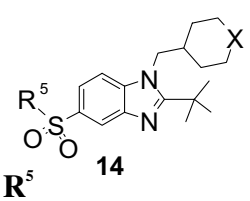
Table 5. 5-Benzylsulfonyl substitution



13

R^5	EC_{50} (nM)	Ratio EC_{50} CB2/CB1	%Metabolism in hLM (15 min)
a *p*-CN-Ph-CH_2	0.5	436	73
b *p*-CN-Ph-CF_2	1.6	62	28

Table 6. 5-(Hetero)aryl substitution[a]



	EC_{50} (nM)	Ratio EC_{50} CB2/CB1	%Metabolism in hLM (15 min)
a Ph (X = CH_2)	1.2	14	23
b 2,6-Cl-Ph	0.4	292	96
c 3-CN-Ph	0.5	1462	61
d 4-CN-Ph	0.6	191	42
e 4-H_2NCH_2-Ph	5.0	363	
f 4-AcNMeCH_2-Ph	1.6	550	50
g 4-Pyridine	1.2	215	28
h 2-thiazole	0.8	4	25
i 2-furan	0.4	40	
j 3-OEt-4-pyridine	0.3	4266	48
k 3-F-4-pyridine	0.8	495	40
l 3-Me-4-pyridine	1.3	1622	40

[a] X is equal to oxygen unless mentioned.

gave a reasonable potency but suffered from the known problem of severe CYP-interactions.[16] However, these interactions could be reduced to acceptable levels by the introduction of a pyridine *ortho*-substituent (**14j–l**), and this also increased the selectivity for the CB2 receptor in analogy with **14c**. In addition, compared to *meta*-substituted arylsulfone **14c**, the metabolic stability of *ortho*-substituted pyridines was improved to a reasonable level which led to acceptable plasma concentrations when dosed orally in vivo (Table 7).

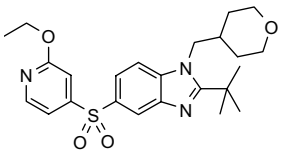
Overall, 2-*tert*-butyl-5-(2-ethoxypyridin-4-sulfonyl)-1-tetrahydropyran-4-ylmethyl)-1H-benzimidazole **14j** showed an optimal potency, selectivity and ADME-profile as illustrated in Figure 3.

In vivo plasma kinetics study (Table 7) for **14j** in rat (10 mg/kg po) showed an absolute oral bioavailability of 43% and a brain to plasma ratio of 0.1, indicating poor blood brain barrier penetration. Compound **14j** did not show any CB1 mediated side effects in the tetrad test (40 mg/kg po). This highly selective and almost exclusive peripheral acting compound will be used to investigate the true potential of a peripheric CB2 agonist. Full in vivo characterization will be published in pharmacological journals in due course.

Although compound **14j** possesses a promising profile, the solubility and the inhibition of CYP-450 isoforms 2C9 and 2C19 remain an issue, and further optimization is currently ongoing.

Table 7. Plasma kinetics after oral administration (10 mg/kg) of **14j** in male Sprague–Dawley rat

	C_{max} (ng/ml)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{inf} (ng h/ml)	Fabs (%)
14j	731 ± 353	0.5	3.4 ± 0.4	2501 ± 706	⩽1



14j

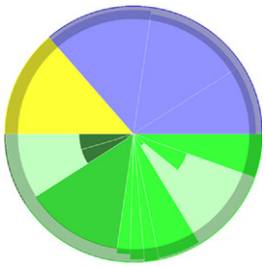


Figure 3. ADME-data: inhibition of Cyp-450 isoforms 2C19 = 76% inhibition at 10^{-5} M and 2C9 = 94% inhibition at 10^{-5} M.

Acknowledgment

We thank Dr. P. Stanislawski for the useful comments during the preparation of this manuscript.

Supplementary data

The details of the functional assay and the synthesis of **14j** are described. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.048.

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