Bioorganic & Medicinal Chemistry Letters 21 (2011) 4284–4287

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Optimisation of a novel series of selective CNS penetrant CB_2 agonists

Christine Watson *, Dafydd R. Owen, Denise Harding, Kana Kon-I, Mark L. Lewis, Helen J. Mason, Miyako Matsumizu, Takasuke Mukaiyama, Margarita Rodriguez-Lens, Akiko Shima, Mifune Takeuchi, Isabelle Tran, Tim Young

Pfizer Global Research and Development, Ramsgate Road, Sandwich, Kent CT13 9NJ, United Kingdom

ARTICLE INFO

Article history:
Received 13 April 2011
Revised 17 May 2011
Accepted 18 May 2011
Available online 25 May 2011

Keywords:
CB2 agonist
CNS penetration
Benzimidazole
CB1 selective

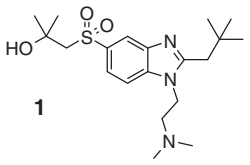
ABSTRACT

A series of benzimidazole CB_2 receptor agonists were prepared and their properties investigated. Optimisation of the three benzimidazole substituents led to the identification of compound **23**, a potent CB_2 full agonist (EC_{50} 2.7 nM) with excellent selectivity over the CB_1 receptor (>3000-fold). Compound **23** demonstrated good CNS penetration in rat. Further optimisation led to the identification of compound **34** with improved selectivity over hERG and excellent CNS penetration in rat.

Cannabinoid receptors are involved in a broad range of processes including appetite, anxiety, memory, cognition, immune regulation and inflammation.[1] In particular, the association of cannabinoids with pain pathology has long been recognised and indeed agonists of the CB_1 receptor are analgesic.[2] However, their use is severely limited by the associated hypomobility, hypothermia and catalepsy.[3] CB_2 receptors were first reported to be located only in the periphery, mainly in immune cells, but it has now been established that they are also expressed in glial cells in the central nervous system (CNS). Indeed, in pre-clinical models of neuropathic pain, CB_2 receptors are upregulated in the CNS.[4] While there is evidence that CB_2 agonists are efficacious in animal models of pain,[5] recent reports have highlighted that some highly selective CB_2 agonists are not efficacious in rat models of analgesia.[6]

The debate about the effectiveness of selective CB_2 agonists as analgesics prompted us to report our efforts in this field. Our aim was to identify a centrally penetrant, selective CB_2 agonist as a potential analgesic agent devoid of the side-effects associated with the CB_1 receptor.

Previous efforts in our laboratories had resulted in the discovery of compound **1** (Fig. 1) as a potent CB_2 agonist.[7] Compound **1** and all the compounds in this communication proved to be full agonists in our CB_2 functional assay[8] unless otherwise stated. **1** was also selective over CB_1 (CB_1 binding K_i >10 μM) and was metabolically stable in human and rat liver microsomes (HLM and RLM, respectively). However, the in vivo clearance in rat proved to be high (Cl 75 mL/min/kg) and the oral bioavailability was a modest 21%. In addition, the compound exhibited very poor CNS penetration—the ratio of unbound drug in the brain to unbound drug in the plasma ($[brain]_u$:$[plasma]_u$) was <1%.[9]



hCB_2 EC_{50} 12 nM
hCB_1 Ki >19.4 μM
HLM Cl_{int} <7.0 μL/min/mg
RLM Cl_{int} <11.2 μL/min/mg
TPSA 84, clogP 2.8
Bioavailability in rat 21%
$[brain]_u$:$[plasma]_u$ <1%

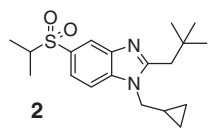
Figure 1. Structure and properties of project lead.

Our expectation was that hydrogen bonding groups in **1**, in particular the hydrogen bond donor alcohol, had limited its CNS penetration. Thus we re-evaluated our structure activity relationship (SAR) data, looking for starting points with the potential for improved brain penetration which also retained good CB_2 potency and selectivity over the CB_1 receptor. By removing polar functionality, we expected to improve CNS penetration but risked compromising metabolic stability through increased lipophilicity.

Compound **2**, Figure 2, had an encouraging profile. It showed good CB_2 potency and selectivity and the lower TPSA[10] was expected to result in improved CNS penetration. As expected, the increased lipophilicity (measured by *c* log *P*) had increased the rate of clearance in vitro, hence we embarked on a programme of SAR exploration aimed at addressing this.

* Corresponding author. Tel.: +44 1304646390; fax: +44 1304651817.
E-mail address: christine.watson100@gmail.com (C. Watson).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.05.063



hCB_2 EC_{50} 3.2 nM
hCB_1 Ki >10.0 μM
HLM Cl_{int} 23 μL/min/mg
RLM Cl_{int} 144 μL/min/mg
TPSA 60, clogP 4.3

Figure 2. Structure and properties of lead compound **2**.

A general route to these benzimidazoles is shown in Scheme 1. The synthesis started with alkylation of the sulfide formed by reduction of disulfide **3** followed by oxidation to give sulfone intermediate **5**. Elaboration to the diamine intermediate **8** generally proceeded in good yield via nitration, chloride displacement and nitro reduction. Benzimidazole ring formation was achieved by reaction with the appropriate acid chloride then cyclisation using *p*-toluenesulfonic acid.

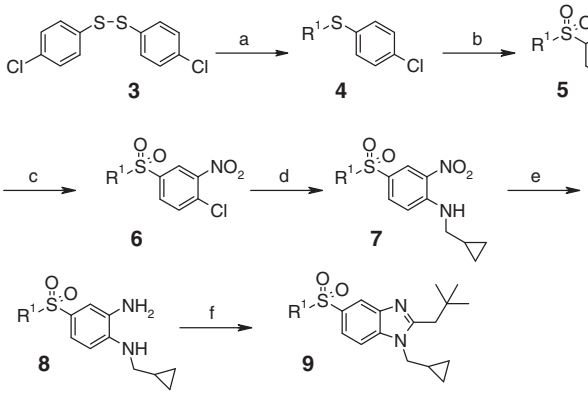
Alternatively, late stage modification of the sulfone was enabled using the route shown in Scheme 2. Starting from 1,4-dibromo-2-nitrobenzene (**10**), introduction of the amine was followed by nitro reduction and benzimidazole formation. A range of thiols could be introduced by a palladium catalysed coupling.[11] Oxidation to the sulfone then delivered the final compound.

A range of substituents on the sulfone was prepared using these routes and good CB_2 potency could be readily achieved, as shown in Table 1. Unfortunately, any gains in potency tended to be offset by increased metabolic instability (as measured by HLM and RLM). However, we were encouraged by the data for compound **16**. This appeared to be as potent as compound **2** but had lower *c* log *P* (4.0) and an improved metabolic profile.

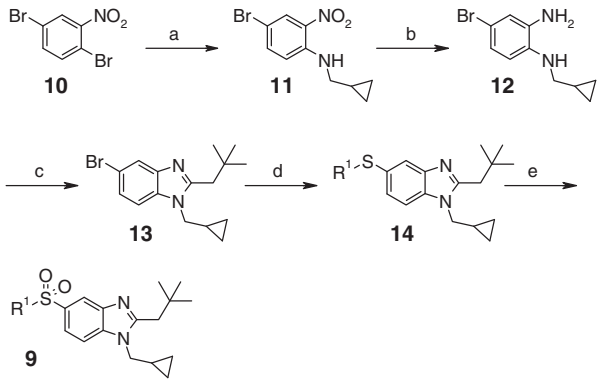
Optimisation of the C-2 position was enabled by library chemistry (Scheme 3) from diamine intermediate **21**. Benzimidazole formation was achieved directly in parallel from a range of acids using either T_3P[12] or HATU to activate the acid, giving a synthesis success rate of 54% for the library.

The SAR for the C-2 position is shown in Table 2. Although a large number of benzimidazoles with high structural diversity could be synthesised in this way, the SAR for CB_2 agonist activity proved very tight. In addition, we found that extending the size of R^2 could impact on efficacy, for example, compound **31** had an E_{max} of 58%,[13] further limiting the scope for optimisation at this position.

In fact only *tert*-butyl retained similar potency to neopentyl (compound **23** vs compound **16**).

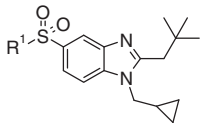


Scheme 1. Reagents and condition: (a) (i) $NaBH_4$, EtOH, THF, 0 °C, 30 min; (ii) R^1-I, 0 °C to rt, 3 h; (b) mCPBA, CH_2Cl_2, 0 °C to rt, 2 h; (c) KNO_3, H_2SO_4, 80 °C, 2 h; (d) (aminomethyl)cyclopropane, DIPEA, IPA, 75 °C, 18 h; (e) H_2, 10% Pd/C, THF, 25 °C, 4 h; (f) (i) 3,3-dimethyl-butyryl chloride, EtOAc, rt, 1 h; (ii) *p*-TsOH·H_2O, reflux, 3 h.

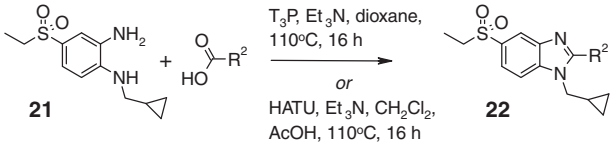


Scheme 2. Reagents and conditions: (a) (aminomethyl)cyclopropane, EtOH, 80 °C, 18 h, 100%; (b) Fe, NH_4Cl, EtOH, H_2O, reflux, 4 h, 98%; (c) (i) $^tBuCH_2COCl$, DIPEA, EtOAc, rt, 1 h; (ii) pTsOH·H_2O, reflux, 9 h, 49%; (d) R^1SH, $Pd_2(dba)_3$, xantphos, 1,4-dioxane, reflux, 21 h; (e) mCPBA, CH_2Cl_2, 0 °C, 1 h.

Table 1
Effect of C-5 substituent on hCB_2 agonist activity



Compound	R^1	hCB_2 EC_{50} nM	HLM Cl_{int}	RLM Cl_{int}
2	Isopropyl	3.2	23	144
15	*t*-Butyl	0.43	38	ND
16	Ethyl	2.9	8.4	38
17	$(CH_3)_2NCH_2CH_2$–	32	79	170
18	$Me_2C(OH)CH_2$–	3.1	<7	244
19	$HOCH_2$–$C(CH_3)_2$–	1.2	<7	54
20	$PhCH_2$–	6.5	276	369



Scheme 3. Parallel modification of R^2 substituent.

Encouragingly, **23** showed low levels of metabolism in HLM and RLM. The physicochemical properties (*c* log *P* 3.5; TPSA 60) were also expected to give improved CNS penetration relative to compound **1**.

Further profiling showed that **23** exhibited excellent selectivity over the hCB_1 receptor in binding and functional assays (Fig. 3). It was therefore progressed to an in vivo pharmacokinetic (PK) study in rat.

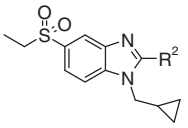
Rat PK (1 mg/kg i.v.) demonstrated that it was a low clearance compound and oral dosing (2 mg/kg) led to excellent bioavailability (F = 100%). Encouragingly, the CNS penetration in rat had also improved significantly with a $[brain]_u$:$[plasma]_u$ ratio of 30%.

Whilst compound **23** demonstrated that we could achieve our desired potency, selectivity and PK profile, it suffered from significant hERG ion channel activity (hERG patch clamp IC_{50} 2.6 μM).

We now assessed the potential to optimise the cyclopropylmethyl substituent (R^3, Table 3) aiming to identify a compound with a similar profile to **23** but reduced hERG affinity.

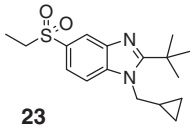
A broader range of substituents could be tolerated at R^3 with cyclic heterocycles, small alkyl groups and polar functionality all showing promising activity. Compounds **32** and **34** looked promising—they both had good potency and low levels of metabolism in

Table 2
Effect of C-2 substituent on hCB_2 agonist activity



Compound	R^2	hCB_2 EC_{50} nM	HLM Cl_{int}	RLM Cl_{int}
16		2.9	8.4	38
23		2.7	9	12
24		132	ND	ND
25		199	43	34
26		425[a]	ND	ND
27		82	8.0	33
28		25	11	106
29		51	8.0	11
30		9.6	42	106
31		67[b]	ND	ND

[a] E_{max} 79%.
[b] E_{max} 58%.

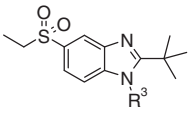


hCB_2 EC_{50} 2.7 nM
hCB_1 Ki >20.0 μM
hCB_1 EC_{50} >10.0 μM
Rat PK (1mg/kg i.v.):
Cl 20 mL/min/kg
Vd 3.2 L/kg
$T_{1/2}$1.9h

23

Figure 3. Structure and properties of compound **23**.

Table 3
Effect of N substituent on hCB_2 agonist activity



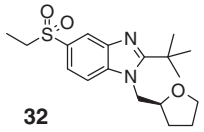
Compound	R^3	hCB_2 EC_{50} nM	HLM Cl_{int}	RLM Cl_{int}
32		11	12	17
33		106	8.0	8.5
34		5.3	7.0	19
35		39	8.0	8.5

HLM and RLM. Their selectivity over CB_1 and hERG is shown in Table 4.

Compound **34** had similar a *c* log *P* to compound **23** and although the hERG activity was slightly reduced, this was negated by weaker CB_2 activity, resulting in a similar selectivity window for both compounds. Pleasingly, compound **32** was a potent CB_2 agonist and selective with respect to CB_1 with significantly reduced hERG activity compared to **23**. The in vivo clearance in rat was similar to compound **23** and the oral bioavailability was once again 100% (Fig. 4). Encouragingly, the $[brain]_u/[plasma]_u$ in rat was 1:1, that is, **32** was fully CNS penetrant.

Table 4
hERG activity in the optimisation of compound **23**

Compound	hCB_2 EC_{50} nM	hCB_1 EC_{50} nM	hERG IC_{50} nM	*c* log *P*
23	2.7	>10000	2600	3.5
32	11	15000	63000	2.7
34	5.3	ND	6200	3.4



HLM Cl_{int} 12 μl/min/mg
RLM Cl_{int} 17 μl/min/mg

Rat PK (1mg/kg i.v):
Cl 27.1 ml/min/kg
Vd 1.0 L/kg
$T_{1/2}$0.4h
$[brain]_u$:$[plasma]_u$ 1.0

32

Figure 4. PK properties of compound **32**.

In summary, the benzimidazole scaffold enabled identification of novel, CNS penetrant CB_2 agonists with excellent selectivity over the CB_1 receptor. We have identified compounds with similar potency to lead compound **2** but with an improved PK profile. Compound **23** is a useful tool compound to investigate the pharmacology of brain penetrant selective CB_2 agonists. Further optimisation led to the discovery of compound **32**, a CNS penetrant selective CB_2 agonist with reduced hERG activity. Further studies are underway to assess the in vivo pharmacology of this compound in pain models.

Acknowledgments

We would like to acknowledge the Pfizer Nagoya project team for their discovery of the benzimidazole sulfone scaffold and their work in this series.

References and notes

1. Grotenhermen, F. *Curr. Drug Targets: CNS Neurol. Disord.* **2005**, *4*, 507.
2. Pertwee, R. G. *Prog. Neurobiol.* **2001**, *63*, 569.
3. Chaperon, F.; Thiebot, M.-H. *Crit. Rev. Neurobiol.* **1999**, *13*, 243.
4. (a) Beltramo, M.; Bernardini, N.; Bertorelli, R.; Campanella, M.; Nicolussi, E.; Fredduzzi, S.; Reggiani, A. *Eur. J. NeuroSci.* **2006**, *23*, 1530; (b) Zhang, J.; Hoffert, C.; Vu, H. K.; Groblewski, T.; Ahmad, S.; O'Donnell, D. *Eur. J. NeuroSci.* **2003**, *17*, 2750.
5. (a) Ibrahim, M. M.; Rude, M. L.; Stagg, N. J.; Mata, H. P.; Lai, J.; Vanderah, T. W.; Porreca, F.; Buckley, N. E.; Makriyannis, A.; Malan, T. P., Jr. *Pain* **2006**, *122*, 36; (b) Bingham, B.; Jones, P. G.; Uveges, A. J.; Kotnis, S.; Lu, P.; Smith, V. A.; Sun, S. C.; Resnick, L.; Chlenov, M.; He, Y.; Strassle, B. W.; Cummons, T. A.; Piesla, M. J.; Harrison, J. E.; Whiteside, G. T.; Kennedy, J. D. *Br. J. Pharmacol.* **2007**, *151*, 1061; (c) Beltramo, M. *Mini-Rev. Med. Chem.* **2009**, *9*, 11.
6. (a) Trotter, B. W.; Nanda, K. K.; Burgey, C. S.; Potteiger, C. M.; Deng, J. Z.; Green, A. I.; Hartnett, J. C.; Kett, N. R.; Wu, Z.; Henze, D. A.; Della Penna, K.; Desai, R.; Leitl, M. D.; Lemaire, W.; White, R. B.; Yeh, S.; Urban, M. O.; Kane, S. A.; Hartman, G. D.; Bilodeau, M. T. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2354; (b) Manley, P. J.; Zartman, A.; Paone, D. V.; Burgey, C. S.; Henze, D. A.; Della Penna, K.; Desai, R.; Leitl, M. D.; Lemaire, W.; White, R. B.; Yeh, S.; Urban, M. O.; Kane, S. A.; Hartman, G. D.; Bilodeau, M. T.; Trotter, B. W. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2359.
7. Kon-I, K.; Matsumizu, M.; Shima, A. U.S. 2006/0094750.
8. Ryckmans, T.; Edwards, M. P.; Horne, V. A.; Correia, A. M.; Owen, D. R.; Thompson, L. R.; Tran, I.; Tutt, M. F.; Young, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4406.
9. Kalvass, J. C.; Maurer, T. S.; Pollack, G. M. *Drug Metab. Distrib.* **2007**, *35*, 660.

10. Hitchcock, S. A.; Pennington, L. D. *J. Med. Chem.* **2006**, *49*, 7559.
11. Itoh, T.; Mase, T. *Org. Lett.* **2004**, *24*, 4587.
12. Chesworth, R.; Wessel, M. D.; Heyden, L.; Mangano, F. M.; Zawistoski, M.; Gegnas, L.; Galluzzo, D.; Lefker, B.; Cameron, K. O.; Tickner, J.; Lu, B.; Castleberry, T. A.; Petersen, D. N.; Brault, A.; Perry, P.; Ng, O.; Owen, T. A.; Pan, L.; Ke, H.-A.; Brown, T. A.; Thompson, D. D.; DaSilva-Jardine, P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5562.
13. Similar E_{max} SAR has been reported in Verbist, B. M. P.; De Cleyn, M. A. J.; Surkyn, M.; Fraiponts, E.; Aerssens, J.; Nijsen, M. J. M. A.; Gijsen, H. J. M. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2574.