Bioorganic & Medicinal Chemistry Letters 21 (2011) 1748–1753

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of potent and orally bioavailable heterocycle-based cannabinoid CB1 receptor agonists

Takao Kiyoi [a], Julia M. Adam [a,*], John K. Clark [a], Keneth Davies [a], Anna-Marie Easson [a], Darren Edwards [a], Helen Feilden [a], Ruth Fields [a], Stuart Francis [a], Fiona Jeremiah [a], Duncan McArthur [a], Angus J. Morrison [a], Alan Prosser [a], Paul D. Ratcliffe [a], Jurgen Schulz [a], Grant Wishart [a], James Baker [b], Robert Campbell [b], Jean E. Cottney [b], Maureen Deehan [b], Ola Epemolu [b], Louise Evans [b]

[a] *Department of Chemistry, Merck Research Laboratories, MSD, Newhouse, Lanarkshire ML1 5SH, UK*
[b] *Department of Pharmacology, Merck Research Laboratories, MSD, Newhouse, Lanarkshire ML1 5SH, UK*

ARTICLE INFO

Article history:
Received 7 December 2010
Revised 15 January 2011
Accepted 18 January 2011
Available online 22 January 2011

Keywords:
Cannabinoid
CB1 receptor agonist
Oral bioavailability

ABSTRACT

Novel 3-(1*H*-indol-3-yl)-1,2,4-oxadiazoles and -thiadiazoles were synthesized and found to be potent CB1 cannabinoid receptor agonists. The oral bioavailability of these compounds could be dramatically improved by optimization studies of the side chains attached to the indole and oxadiazole cores, leading to identification of a CB1 receptor agonist with good oral activity in a range of preclinical models of antinociception and antihyperalgesia.

The CB1 cannabinoid receptor is a member of G protein-coupled receptor (GPCR) superfamily, which is characterized by seven transmembrane helicies.[1] The CB1 receptor is located primarily in the central nervous system but is also expressed on peripheral neurones. Recently many studies revealed that the CB1 receptor is a potential therapeutic target against pain and several other diseases including glaucoma, traumatic brain injury and multiple sclerosis.[2] Several lines of evidence have been reported regarding the analgesic effects of CB1 agonists in both experimental animal models and clinical studies. Moreover, a couple of CB1 agonists including Δ^9-tetrahydrocannabinol (Δ^9-THC, Fig. 1), one of the major bioactive components of cannabis, are used clinically as antiemetics in cancer chemotherapy or appetite stimulants in AIDS patients.[3] Sativex™,[4] a medicinal cannabis extract containing a mixture of Δ^9-THC and cannabidiol, has been recently launched for treatment of multiple sclerosis (MS)- and cancer-associated neuropathic pain, and for MS-associated spasticity.

We have previously described indole-3-carboxamide **1**[5] and its structurally constrained analogue[6] as CB1 agonists which would be suitable for intravenous administration as peri-operative analgesics. We have also discovered that introduction of a bicyclic piperazine moiety on the amide side chain significantly improved the CB1 agonist activity.[7] Duration of action could be prolonged by replacement of the carboxamide by heterocycles as metabolically stable bioisosteres (Fig. 2, compounds **2** and **3**).[8,9] These heterocyclic indole derivatives displayed good antinociceptive activities in the mouse tail flick test after iv administration, however, they did not show potent antinociceptive activity after oral administration because of their poor oral bioavailability. In order to find compounds having potent CB1 agonist activity and improved oral bioavailability, we conducted a detailed SAR study based on **2** and **3** by the introduction of various hydrophilic groups into the heterocyclic indole core as well as the conversion of the heterocycle itself to a more hydrophilic one. Compounds were initially screened in an in vitro CB1 agonist assay and an aqueous solubility assay. The criteria for the initial phase of the lead optimization study were established as a CB1 agonist pEC_{50} greater than or equal to 7.0 together with aqueous solubility greater than 10 mg/l at pH 7.4, since drug solubility is one of the most important factors for oral absorption.[10]

The synthetic route to 3-(1*H*-indol-3-yl)-1,2,4-thiadiazole derivatives is summarized in Scheme 1. Regio-selective carboxylation of 7-chloroindole **4** was achieved by the reaction with trifluoroacetic anhydride followed by the hydrolysis of the trifluoromethylcarbonyl group. After the introduction of the tetrahydropyranylmethyl moiety and conversion of the carboxylic acid to the oxathiazolone, 1,2,4-thiadiazole ring was constructed by microwave assisted [3+2] cycloaddition in the presence of ethyl

---

* Corresponding author. Fax: +44(0)1698 736187.
E-mail addresses: julia.adam@merck.com, juliaadam@blueyonder.co.uk (J.M. Adam).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.01.082

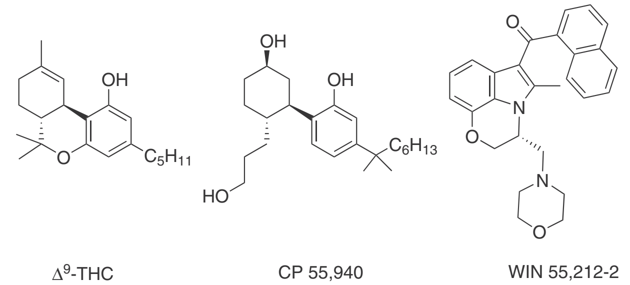


Figure 1. Representative classical cannabinoid agonists.

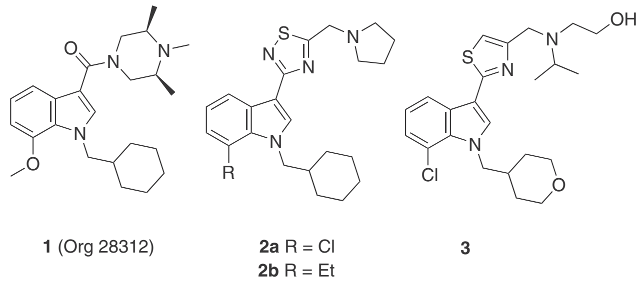
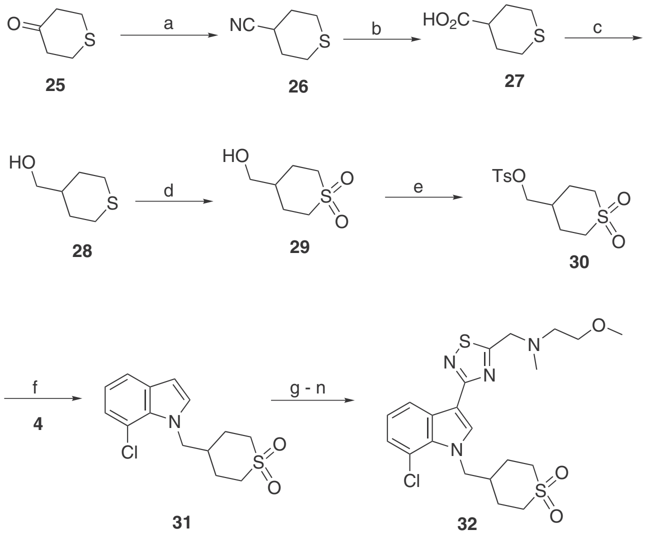
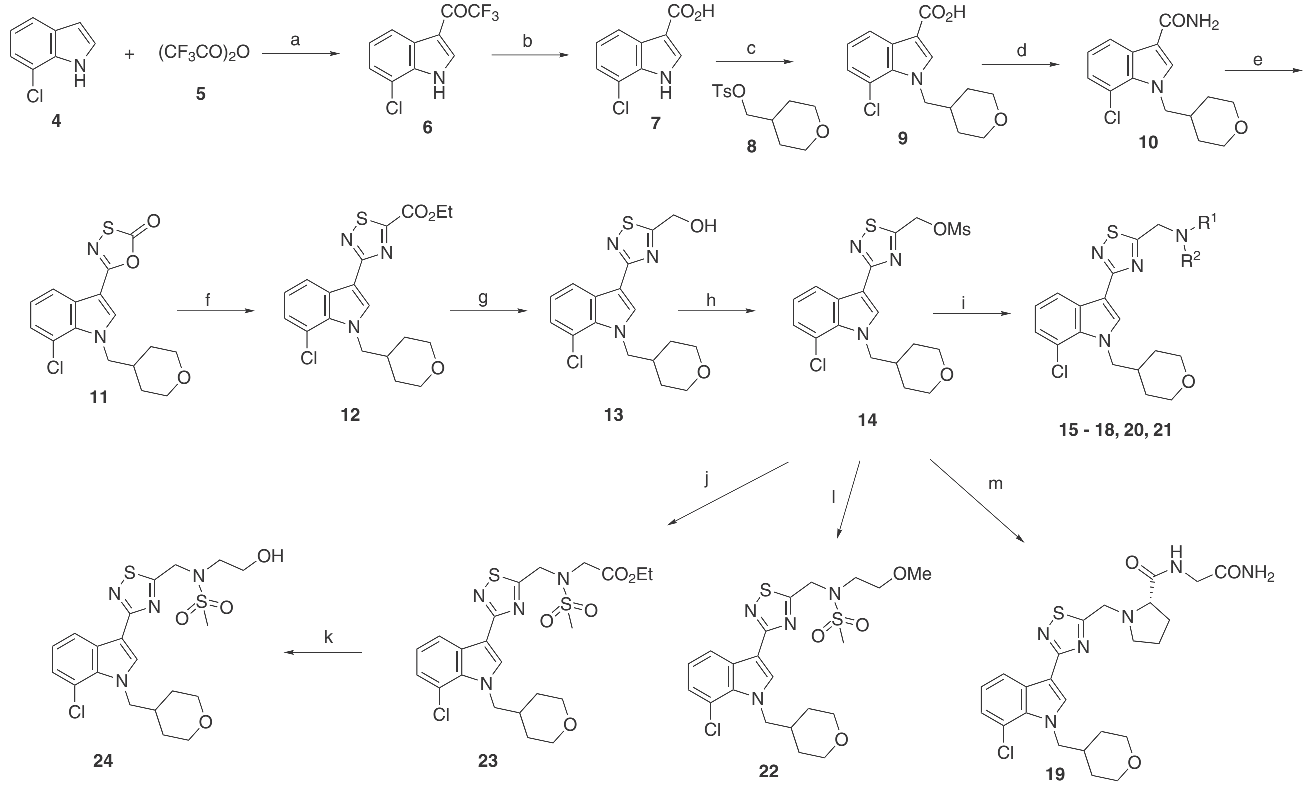


Figure 2. The structures of CB1 agonists suitable for iv administration.



Scheme 2. Reagents and conditions: (a) $CNCH_2OTs$, *t*-BuOK, DME, *t*-BuOH, 0 °C to rt, 3 h, 91%; (b) NaOH, EtOH/water, 90 °C, 2 h; (c) $BH_3 \cdot Me_2S$, THF, 70 °C, 2 h; (d) $NaIO_4$, MeOH/water, 60 °C, 4 h; (e) TsCl, pyridine, cat. DMAP, $CHCl_3$, rt, 72 h, 63% from **26**; (f) NaH, DMF, rt, 72 h, 90%; (g) $(CF_3CO)_2O$, DMF, 5 °C to rt, 2 h; (h) 4 N NaOH, EtOH, reflux, 2 h, 77% from **31**; (i) oxalyl chloride, THF, 0 °C to rt, 18 h, then aqueous ammonia, K_2CO_3, DCM, 0 °C, 1 h, 95%; (j) ClSCOCl, THF, reflux, 3 h, 74%; (k) $NCCO_2Et$, xylenes, reflux, 3 h, 87%; (l) $NaBH_4$, MeOH/DCM, rt, 15 min, 85%; (m) MsCl, DIPEA, DCM, rt, 15 min, 79%; (n) excess (2-methoxyethyl)methylamine, DIPEA, NMP, 40 °C, 3 h, 55%.



Scheme 1. Reagents and conditions: (a) DMF, 5–10 °C, 2 h; (b) 4 N NaOH, reflux, 1 h, 81% from **4**; (c) NaH, DMF, 50 °C, 6 h; (d) oxalyl chloride, DCM, 0 °C to rt, 18 h, then aqueous ammonia, K_2CO_3, DCM, rt, 1 h, 45% from **7**; (e) ClSCOCl, THF, reflux, 3 h, 85%; (f) $NCCO_2Et$, *m*-xylene, 180 °C, 15 min, microwave, 85%; (g) $NaBH_4$, MeOH/THF, 0 °C to rt, 18 h, 59%; (h) MsCl, Et_3N, DCM, 0 °C, 1 h, quant; (i) excess NHR^1R^2, THF, 150 °C, 15 min, microwave or NHR^1R^2, K_2CO_3, NMP (*N*-methylpyrrolidone), rt, 18 h, 21–65%; (j) glycine ethyl ester, Et_3N, THF, 160 °C, 10 min, microwave, 47%, then MsCl, Et_3N, DCM, rt, 2 h, 54%; (k) $NaBH_4$, MeOH/THF, rt, 18 h, 59%; (l) excess 2-methoxyethylamine, THF, 160 °C, 10 min, microwave, then MsCl, Et_3N, DCM, rt, 18 h, 53%; (m) H-Pro-Gly-OH, K_2CO_3, NMP, 100 °C, 5 min, microwave, then oxalyl chloride, DCM, rt, 18 h, followed by aqueous ammonia, DCM, rt, 1 h, 22%.

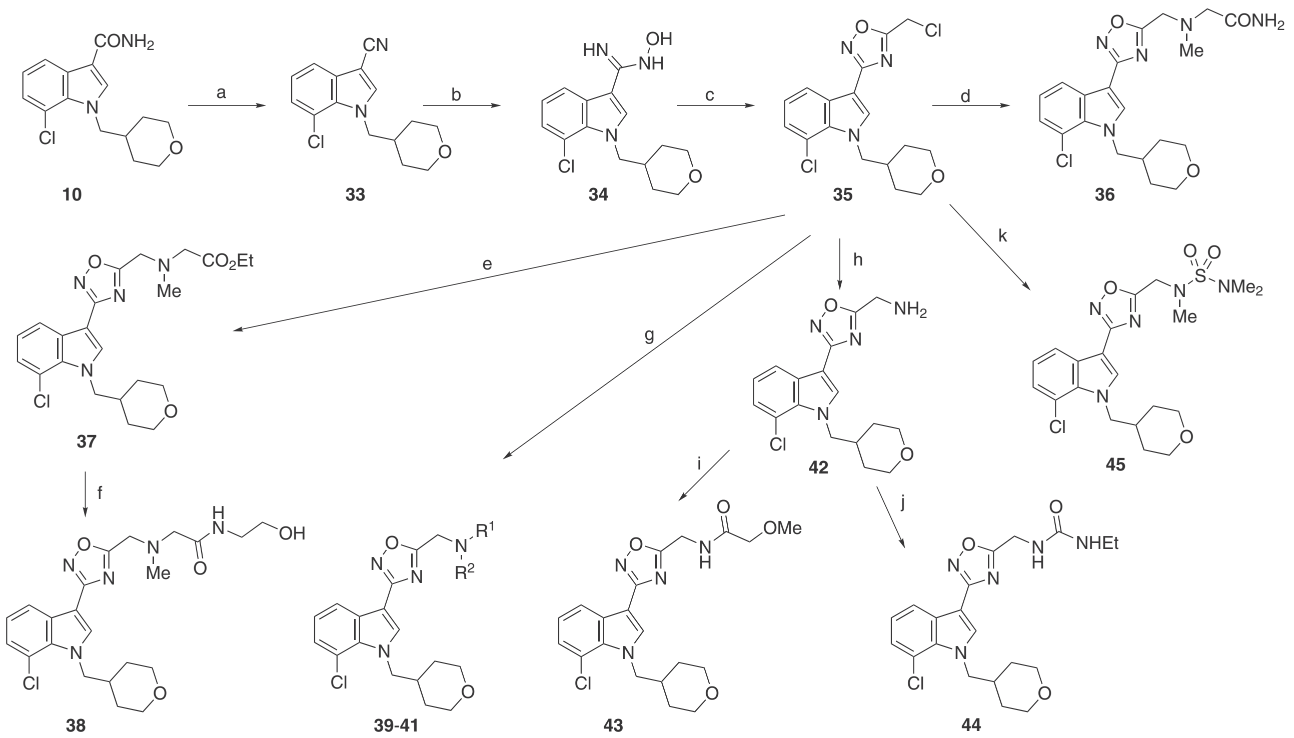
cyanoformate.[11] Reduction, mesylation and displacement with secondary amines afforded the thiadiazoles **15**–**21**. **22** and **24** were synthesized by the reaction of **14** with primary amines followed by sulfonylation of the basic nitrogen atom. Synthesis of **32** having a six-membered sulfone on the indole-1-position was achieved by the reaction of **4** with the tosylate **30**, followed by regioselective carboxylation on the indole 3-position, thiadiazole ring formation and modification of the side chain. The intermediate **30** was synthesized by cyanation reaction of **25** using tosylmethyl isocyanide,[12] hydrolysis of the nitrile to carboxylic acid, reduction, oxidation of the thio ether and tosylation (Scheme 2).

The 1,2,4-oxadiazoles were synthesized by initial formation of the amidoxime **34**, which was prepared by dehydration of the primary amide **10** to the nitrile **33** and subsequent treatment with hydroxylamine hydrochloride. With the amidoxime **34** in hand the 5-chloromethyl-[1,2,4]-oxadiazole **35** was readily obtained by reaction with chloroacetyl chloride under basic conditions. A series of amino groups could be introduced by nucleophilic substitution reactions of **35**. Further modifications on the side chain are illustrated in Scheme 3.

The prepared compounds were tested for CB1 receptor agonist activity using CHO cells doubly transfected with human CB1 and a luciferase reporter gene.[13] As shown in Table 1, introduction of hydrophilic moieties into the first lead compound **2a** retained the CB1 agonist activity in several cases. Conversion of the cyclohexyl group of **2a** to a tetrahydropyran ring was tolerated with no reduction in the CB1 agonist activity (**2a** vs **15**). As regards the pyrrolidinyl moiety, 2-hydroxymethyl pyrrolidine **17** showed comparable activity to the unsubstituted pyrrolidine. However, introduction of amide or carboxyl groups diminished the activity (**16**, **18** and **19**). Methoxy- and hydroxy-ethylamine, **20** and **21** retained good CB1 agonist activity. The corresponding sulfonamide derivatives **22** and **24** showed good CB1 agonist activity, especially **24** had high activity despite its low lipophilicity ($c\log P = 1.8$). On the other hand, the sulfone derivative **32**, in which the polar group is appended to the indole nitrogen, exhibited moderate activity, but had decreased metabolic stability in mouse and human liver microsomes. In general, although aqueous solubility could be improved by introduction of some kinds of hydrophilic groups such as amide or carboxylic moieties onto the pyrrolidine ring, all of the thiadiazoles having pEC_{50} greater than 7.0 still did not have sufficient aqueous solubility to meet the criteria.

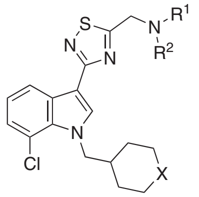
We have previously reported that a 3-(1,2,4-oxadiazol-3-yl)indole derivative showed comparable CB1 agonist activity to the corresponding thiadiazole,[8] and since improved aqueous solubility was observed by introducing an amide moiety onto the thiadiazole core as mentioned above, we started to synthesize the oxadiazole derivatives bearing an amide side chain. As a result, the simple primary amide **36** was found to have good CB1 agonist activity and aqueous solubility (Table 2). Modification of the amide with a further hydroxyethyl group retained the activity and improved aqueous solubility (compound **38**), although serine (**39**) and piperidine carboxamide (**40**) derivatives had lower activity. Conversion of the basic nitrogen atom to amide or urea decreased both CB1 agonist activity and aqueous solubility. The sulfonamide (**45**) exhibited good CB1 agonist activity.

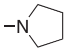
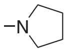
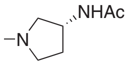
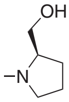
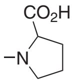
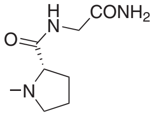
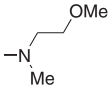
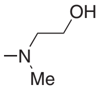
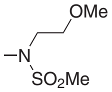
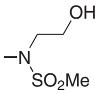
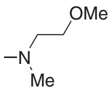
With these results in hand, we focused our attention on compound **36**, because of its good CB1 agonist activity, aqueous solubility and metabolic stability in microsomes. As shown in Table 3, compound **36** exhibited high affinity for both CB1 and CB2 cannabinoid receptors, as determined by radioligand competition binding assays using [^{3}H]CP 55,940 binding to either hCB1 or hCB2 receptors expressed in insect Sf9 membranes. The in vitro and in vivo DMPK profiles of **36** are summarized in Table 4. Perme-



Scheme 3. Reagents and conditions: (a) P_2O_5, DMF, 5 °C to rt, 30 min, 69%; (b) $NH_2OH{\cdot}HCl$, DIPEA, EtOH, reflux, 20 h, 91%; (c) $ClCH_2COCl$, NaH, THF, −70 °C to rt, 4 h, 65%; (d) $HNMeCH_2CONH_2{\cdot}HCl$, K_2CO_3, NMP, rt, 18 h, 67%; (e) $HNMeCH_2CO_2Et$, DIPEA, CH_3CN, 60 °C, 18 h, quant.; (f) NaOH, MeOH/water, 60 °C, 2 h, 67%, then ethanolamine, 1-propylphosphonic acid cyclic anhydride, DIPEA, DCM, rt, 1 h, 50%; (g) NHR^1R^2, K_2CO_3, CH_3CN, rt, 72 h, 56–67%; (h) 2 M ammonia in MeOH, 120 °C, 20 min, microwave, quant.; (i) $MeOCH_2CO_2H$, HATU, DIPEA, DCM, rt, 16 h, 17%; (j) ethyl isocyanate, DCM, rt, 16 h, quant.; (k) 8 M, methylamine in EtOH, 40 °C, 2 h, then Me_2NSO_2Cl, Et_3N, DMAP, DCM, rt, 16 h, 27%.

Table 1
CB1 agonist activity, $c \log P$, Solkin[14] aqueous solubility and microsomal stability of 3-(1,2,4-thiadiazol-3-yl)indole derivatives



Compound	X	-NR^{1}R^2	$c \log P$	pEC$_{50}$[a]	Solubility[b]	MLM[c]	HLM[d]
CP 55,940			5.8	7.7			
WIN 55,212-2			5.4	7.3			
1			5.6	7.6	NT[e]	>270	>270
2a	CH_2		6.2	7.8	<1	17	35
15	O		3.8	7.7	<1	42	53
16	O		2.4	6.1	16	18	NT[e]
17	O		3.1	7.8	<1	NT[e]	NT[e]
18	O		1.4	6.8	108	NT[e]	NT[e]
19	O		2.0	6.4	15	<12	72
20	O		3.4	7.1	<1	85	87
21	O		2.6	7.1	<1	14	35
22	O		2.6	7.1	<1	125	206
24	O		1.8	7.7	<1	96	51
32	SO_2		2.0	6.7	<1	262	123

[a] Values are means of three experiments.
[b] Aqueous solubility (mg/L) was measured in pH 7.4 buffer using the Solkin assay.
[c] MLM; mouse microsomal clearance (μL/min/mg).
[d] HLM; human microsomal clearance (μL/min/mg).
[e] NT; not tested.

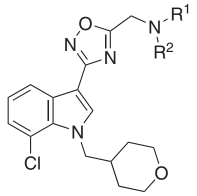
ability across a Caco-2 (human intestinal carcinoma cell line) monolayer was used as an in vitro model of intestinal absorption. The value of the apparent permeability coefficient (P_{app}) predicted moderate oral absorption with no evidence of active efflux. Indeed, the compound showed good oral bioavailability ($F = 53\%$) after po administration in rat, while the oral bioavailability of the lead compound **3** was only 4%.

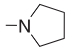
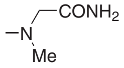
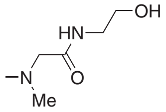
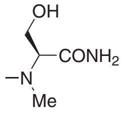
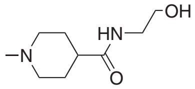
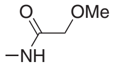
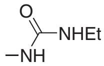
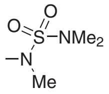
Based on its promising DMPK profile, **36** was progressed for testing in several animal models to evaluate its antinociceptive activity. As shown in Table 5 and **36** significantly increased tail flick latency[15] after both iv and po administration; the ED$_{50}$ values were 1.6 and 7.9 μmol/kg, respectively. Moreover, oral administration of **36** demonstrated anti-hyperalgesic effects in both heat stimulus (Chung Hargreaves) and mechanical stimulus (Chung von Frey) neuropathic pain models.[16]

In preliminary studies of in vitro toxicity, blockade of the hERG channel was assessed in a [^{3}H]dofetilide competition binding assay,[17] which showed that **36** had very weak hERG binding activity (pK_i = 4.5). Furthermore, the compound was clean in the Vitotox™ assay,[18] which can rapidly assess genotoxicity, at a concentration of 10^{-4} M.

In summary, a series of oxadiazolyl- and thiadiazolyl-indole derivatives were synthesized and found to be agonists of CB1 receptor. In addition, introduction of hydrophilic moieties to these

Table 2
CB1 agonist activity, *c* log *P*, Solkin[14] aqueous solubility and microsomal stability of 3-(1,2,4-oxadiazol-3-yl)indole derivatives



Compound	-NR^{1}R^2	c log *P*	pEC$_{50}$[a]	Solubility[b]	MLM[c]	HLM[d]
41		3.5	7.0	<1	NT[e]	NT[e]
36		1.8	7.4	21	<12	36
38		1.8	7.3	88	43	66
39		1.1	6.6	95	51	34
40		1.4	6.9	115	<12	16
43		1.9	6.7	4	<12	<12
44		2.7	5.7	<1	40	26
45		1.8	7.5	<1	69	65

[a] Values are means of three experiments.
[b] Aqueous solubility (mg/L) was measured in pH 7.4 buffer using the Solkin assay.
[c] MLM; mouse microsomal clearance (mL/min/mg).
[d] HLM; human microsomal clearance (mL/min/mg).
[e] NT; not tested.

Table 3
Profiles of the CB1 agonist **36** in in vitro hCB1 and hCB2 binding assays

Compound	CB1 p*K*$_i$	CB2 p*K*$_i$
CP 55,940	9.5	9.5
WIN 55,212-2	7.9	8.6
1	8.9	9.1
36	8.4	8.2

Table 4
The in vitro and in vivo DMPK profiles of **36**

Caco-2 permeability	
$P_{\text{app AP to BL}}$ (nm/s)	83
$P_{\text{app BL to AP}}$ (nm/s)	127
PK (rat, 0.4 mg/kg, iv)	
AUC$_{\text{plasma}}$ (h ng/ml)	244
Clearance (ml/min/kg)	28
$T_{1/2}$ elimination (h)	1.4
V_{ss} (L/kg)	3.0
PK (rat, 5.0 mg/kg, po)	
AUC$_{\text{plasma}}$ (h ng/ml)	1570
$C_{\max}$ (ng/ml)	173
$T_{\max}$ (h)	1.0
F (%)	53

Table 5
The in vivo activities of **36**

	ED$_{50}$ (mol/kg)
Rat tail flick model (iv)	1.6
Rat tail flick model (po)	7.9
Rat Chung Hargreaves model (po)	5.5
Rat Chung von Frey model (po)	26

heterocycle derivatives improved the aqueous solubility and oral bioavailability. The selected compound **36**[19] exhibited good activity in animal models of antinociception and antihyperalgesia after oral administration.

References and notes

1. Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. *Nature* **1990**, *346*, 561.
2. Recent reviews for cannabinoid agonists: (a) Pertwee, R. G. *Exp. Opin. Invest. Drugs* **2000**, *9*, 1553; (b) Adam, J.; Cowley, P. M.; Kiyoi, T.; Morrison, A. J.; Mort, C. J. W. In *Progress in Medicinal Chemistry*; King, F. D., Lawton, G., Eds.; Elsevier: Amsterdam, 2006; Vol. 44, pp 207–329; (c)*Handbook of Experimental Pharmacology*; Pertwee, R. G., Ed.; Springer: Heidelberg, 2005; Vol. 168, (d) Huffman, J. W.; Padgett, L. W. *Curr. Med. Chem.* **2005**, *12*, 1395; (e) Turcotte, D.; Dorze, J. A.; Esfahani, F.; Frost, E.; Gomori, A.; Namaka, M. *Expert Opin. Pharmacother.* **2010**, *11*, 17.

3. Mackie, K. *Annu. Rev. Pharmacol. Toxicol.* **2006**, *46*, 101.
4. Peat, S. *Int. J. Palliat. Nurs.* **2010**, *16*, 481.
5. Adam, J. M.; Cairns, J.; Caulfield, W.; Cowley, P.; Cumming, I.; Easson, M.; Edwards, D.; Ferguson, M.; Goodwin, R.; Jeremiah, F.; Kiyoi, T.; Mistry, A.; Moir, E.; Morphy, R.; Tierney, J.; York, M.; Baker, J.; Cottney, J. E.; Houghton, A. K.; Westwood, P. J.; Walker, G. *Med. Chem. Commun.* **2010**, *1*, 54.
6. Kiyoi, T.; York, M.; Francis, S.; Edwards, D.; Walker, G.; Houghton, A. K.; Cottney, J. E.; Baker, J.; Adam, J. M. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4918.
7. Moir, E. M.; Yoshiizumi, K.; Ferguson, M.; Walker, G.; Houghton, A. K.; Cottney, J.; Baker, J.; Osprey, A.; Adam, J. A. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 7327.
8. Morrison, A. J.; Adam, J. M.; Baker, J.; Campbell, R.; Clark, J. K.; Cottney, J.; Deehan, M.; Easson, A.-M.; Fields, R.; Francis, S.; Jeremiah, F.; Keddie, N.; Kiyoi, T.; McArthur, D. R.; Meyer, K.; Ratcliffe, P. D.; Schulz, J.; Wishart, G.; Yoshiizumi, K. *Bioorg. Med. Chem. Lett.*, in press, doi:10.1016/j.bmcl.2010.10.093.
9. Ratcliffe, P.; et al. *Bioorg. Med. Chem. Lett.*, Submitted for publication.
10. Metcalfe, P. D.; Thomas, S. *Curr. Opin. Drug. Discov. Dev.* **2010**, *13*, 104.
11. (a) Morrison, A. J.; Paton, R. M.; Sharp, R. D. *Synth. Commun.* **2005**, *35*, 807; (b) Fordyce, E. A. F.; Morrison, A. J.; Sharp, R. D.; Paton, R. M. *Tetrahedron* **2010**, *66*, 7192.
12. Bosch, J.; Rubiralta, M.; Moral, M.; Ariño, J. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1533.
13. Price, M. R.; Baillie, G. L.; Thomas, A.; Stevenson, L. A.; Easson, M.; Goodwin, R.; McLean, A.; McIntosh, L.; Goodwin, G.; Walker, G.; Westwood, P.; Marrs, J.; Thomson, F.; Cowley, P.; Christopoulos, A.; Pertwee, R. G.; Ross, R. A. *Mol. Pharmacol.* **2005**, *68*, 1484.
14. Solkin aqueous solubilities were determined using a medium-throughput adaptation of a shake-flask methodology. A 10 mM solution of the test compound in DMSO was added to 0.05 M phosphate buffered saline pH 7.4 such that the final concentration of DMSO was 2%. The resultant mixture was then vortex mixed (1500 rpm) for 24 ± 0.5 h at 21±2 °C. After mixing, the resultant solution/suspension was filtered under vacuum using a filter plate (Millipore Multiscreen HTS, 0.4 μM). The concentration of the compound in the filtrate was determined by High Performance Liquid Chromatography (HPLC) running a generic acid gradient method with UV detection at 230 nm. Peak areas from analysis of the diluted filtrates were quantified by comparison to a calibration line prepared by injecting onto the HPLC three different volumes of a 50 μM solution of the test compound in DMSO. Solubilities were determined in duplicate for each test compound and average values reported.
15. Whiteside, G. T.; Gottshall, S. L.; Boulet, J. M.; Chaffer, S. M.; Harrison, J. E.; Pearson, M. S.; Turchin, P. I.; Mark, L.; Garrison, A. E.; Valenzano, K. *J. Eur. J. Pharmacol.* **2005**, *528*, 65.
16. (a) Honore, P. *Drug Dev. Res.* **2006**, *67*, 302; (b) Joshi, S. K.; Honore, P. *Expert Opin. Drug Discov.* **2006**, *14*, 323.
17. Murphy, S. M.; Palmer, M.; Poole, M. F.; Padegimas, L.; Hunady, K.; Danzig, J.; Gill, S.; Gill, R.; Ting, A.; Sherf, B.; Brunden, K.; Stricker-Krongrad, A. *J. Pharmacol. Toxicol. Methods* **2006**, *54*, 42.
18. Westerink, W. M.; Stevenson, J. C.; Lauwers, A.; Griffioen, G.; Horbach, G. J.; Schoonen, W. G. *Mutat. Res.* **2009**, *676*, 113.
19. Data for the HCl salt of **36**; ^{1}H NMR (400 MHz, CD_3OD) δ 1.40–1.50 (4H, m), 2.16–2.29 (1H, m), 3.19 (3H, s), 3.30–3.37 (4H, m), 3.92 (1H, td, *J* = 9, 3 Hz), 4.28 (2H, s), 4.93 (2H, s), 7.19 (1H, t, *J* = 8 Hz), 7.30 (1H, dd, *J* = 8, 1 Hz), 8.07 (1H, s), 8.13 (1H, dd, *J* = 8, 1 Hz); EsIMS: *m*/*z* 418.3 $[M+H]^+$.