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Design, synthesis and structure–activity relationships of (indo-3-yl) heterocyclic derivatives as agonists of the CB1 receptor. Discovery of a clinical candidate

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ABSTRACT

We report an expansion of the structure–activity relationship (SAR) of a novel series of indole-3-heterocyclic CB1 receptor agonists. Starting from the potent but poorly soluble lead, **1**, a rational approach was taken in order to balance solubility, hERG activity and potency while retaining the desired long duration of action within the mouse tail flick test. This led to the discovery of compound **38** which successfully progressed into clinical development.

The CB1 cannabinoid receptor is a member of the G-protein coupled receptor (GPCR) superfamily, which is characterized by seven-transmembrane receptors. The CB1 receptor is located primarily in the central nervous system but is also expressed on peripheral neurones. Activation of the CB1 receptor has been suggested as a potential strategy for the treatment of pain and several other diseases including glaucoma, traumatic brain injury and multiple sclerosis,[1] while inhibition of CB1 receptors has been explored as a strategy for treatment of obesity and addiction.[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-THC, one of the major bioactive components of cannabis, are used clinically as antiemetics in cancer chemotherapy or appetite stimulants in AIDS patients. However, the classical cannabinoids represented by Δ^9-THC are highly lipophilic and the administration methods are still limited. We have previously described indole-3-carboxamide derivatives as water soluble CB1 agonists, suitable for intravenous administration as potential post-operative analgesics, with a fast onset and short duration of action.[3] Recently, we followed up these compounds with a series of indole 3-heterocyclic derivatives culminating in the discovery of compound **1**, Table 1, found to have an improved duration of action.[4] Unfortunately, **1** suffered from poor aqueous solubility an attribute that appears almost ubiquitous within the CB1 agonist pharmacophore. In this Letter, we report the further optimisation of the indole 3-heterocyclic series and, in particular, our approaches taken to improve the physicochemical profile.

In an effort to introduce solubility we focussed initially on the structure–activity relationships established around the N-1 substituent. The compounds were synthesised according to Scheme 1, or in the case of compound **1** as previously described.[3,4] Starting from the 7-substituted indole **4** the carboxylic acid was introduced using trifluoroacetic anhydride followed by aqueous sodium hydroxide. Treatment of the commercially available methyl tetrahydro-2*H*-pyran-4-carboxylate **7** with lithium aluminium hydride furnished the requisite alcohol **8**, which was converted to the tosylate **9** using

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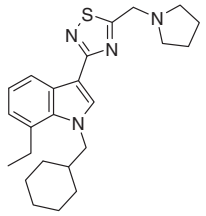
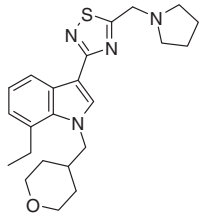
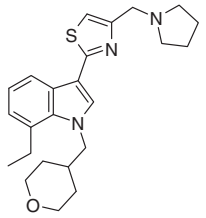
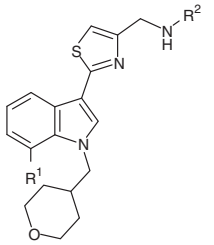
Table 1
CB1 activities and Solkin solubility comparisons for THP substituted indole-3-heterocycles

Entry	Compound	pEC_{50}[b]	Coeff. Var. (%)	Solkin[7] ($mg\ L^{-1}$)
1[a]		8.4	1.9	<1
2[b]		8.3	2.3	<1
3[c]		7.1	4.1	76

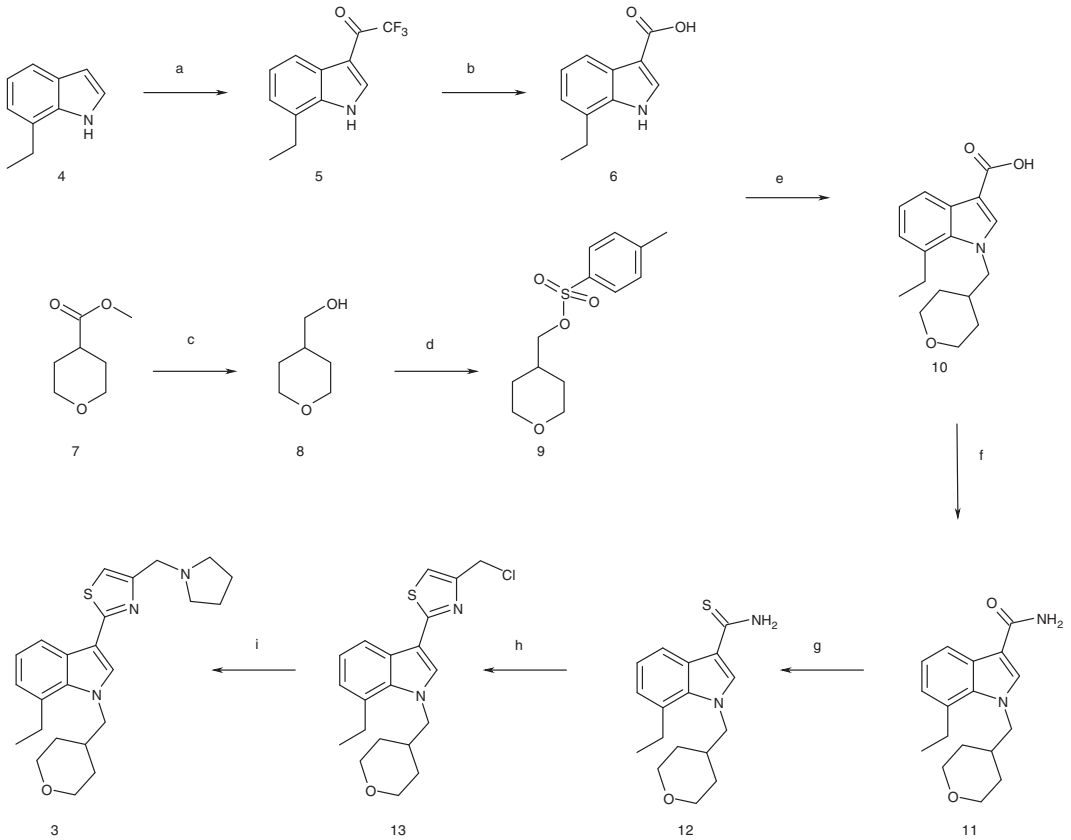
[a] Compound was tested as the HCl salt.
[b] pEC_{50} values are shown as mean and coefficient of variation from $n = 2–5$ separate experiments performed in triplicate.

Table 2
SAR around indole 3-thiazole

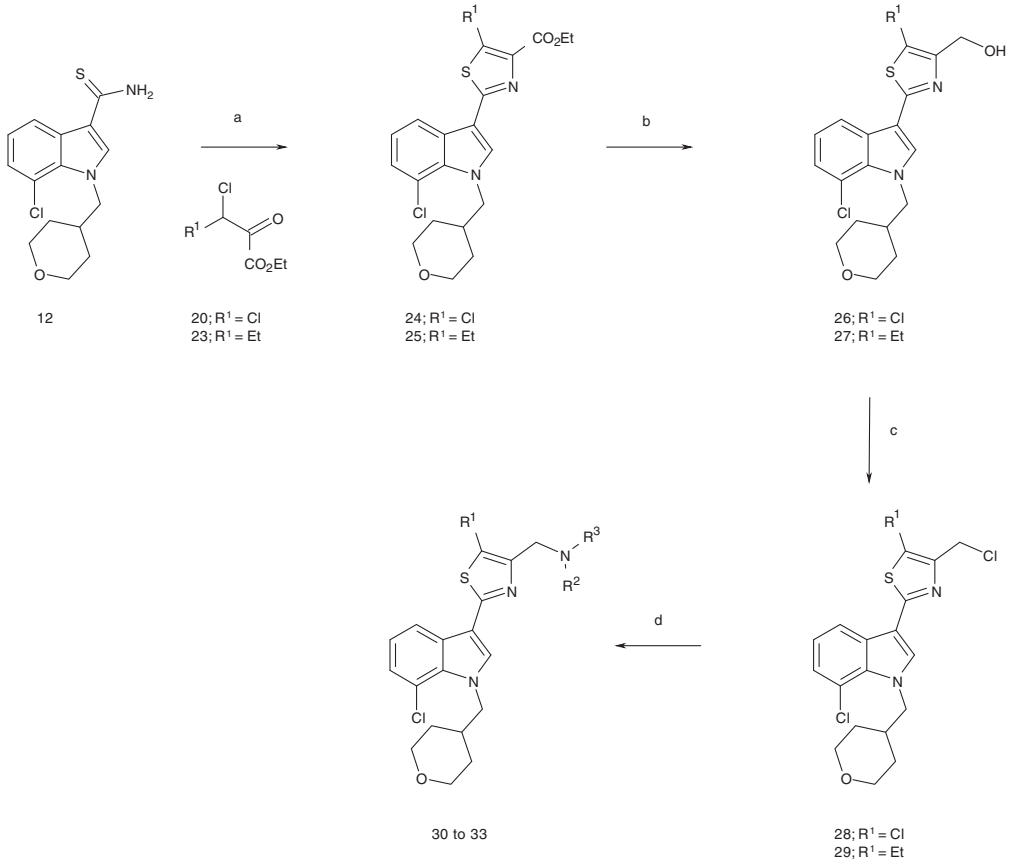


Entry	R1	Amine	pEC_{50}[b]	Coeff. Var. (%)	Solkin ($mg\ L^{-1}$)	hERG pK_i
3[a]	Et		7.1	4.1	76	6.0
14[a]	Et		7.8	4.0	71	5.9
15	F		6.2[c]	N/A	68	5.8
16	F		7.1[c]	N/A	72	6.1
17[a]	Cl		6.4	6.0	72	6.1
18[a]	Cl		7.6	7.5	66	6.2

[a] Compound was tested as the HCl salt.
[b] pEC_{50} values are shown as mean and coefficient of variation from $n = 2–5$ separate experiments performed in triplicate (except where indicated with [c] where $n = 1$).



Scheme 1. Preparation of indole analogs. Reagents and conditions: (a) Trifluoroacetic anhydride, DMF, 0 °C to rt, 1 h; (b) NaOH (4 M, aq), reflux, 1 h, (60%) [over steps a and b]; (c) lithium aluminium hydride, THF, 0 °C to 45 °C, 1 h, (81%); (d) para toluene sulfonylchloride, pyridine, 0 °C to rt, 1.5 h, (92%); (e) NaH, DMF, 50 °C, o/n, (82%); (f) oxalylchloride, 0 °C to rt, NH_3, K_2CO_3, DCM, o/n, (87%); (g) P_2S_5, THF, 55 °C, 3 h; (h) 1,3-dichloroacetone, EtOH, 60 °C, 1.5 h, (48%) [over steps g and h]; (i) pyrrolidine, K_2CO_3, MeCN, microwave 150 °C for 600s, (64%).



Scheme 2. Preparation of indole analogs. Reagents and conditions: (a) DMF, microwave 140 °C, 30 min; (b) lithium borohydride, THF, 0 °C for 1 h then 70 °C for 1 h; (c) methanesulfonylchloride, triethylamine, DCM, 1 h, (26–38%) [over steps a–c]; (d) amine, K_2CO_3, NaI, acetonitrile, microwave 160 °C, 5 min, (32–93%).

standard conditions of para toluenesulfonyl chloride in pyridine. Subsequent N-alkylation was achieved via double deprotonation of the 7-substituted indole carboxylic acid **6** using sodium hydride, and reaction with (tertahydro-2*H*-pyran-4-yl)methyl4-methylbenzenesulfonate **9**. Conversion to the primary amide **11** was achieved via the acid chloride, and subsequent treatment with Lawesson's reagent furnished the thioamide **12**. Reaction with 1,3-dichloroacetone gave the desired chloromethyl thiazole **13**,[5] which then left only a simple displacement with the desired amine, for example, pyrrolidine, to deliver the final compound.

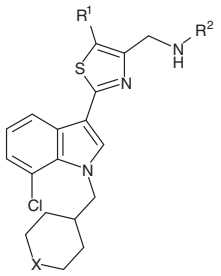
The prepared compounds were tested for CB1 agonist activity using CHO cells doubly transfected with human CB1 and luciferase reporter gene.[6] As shown in Table 1, replacement of the cyclohexyl group with the tetrahydropyranyl (THP) moiety lead to compounds with good CB1 agonist potency. However, it was clear that the role of the heterocycle in combination with this more polar group did have an impact on the intrinsic physical chemical properties of the molecule, as while the thiadiazole containing compound **2** exhibited <1 mg/L solubility, compound **3** demonstrated excellent levels of solubility while maintaining good potency. It was for this reason that we focussed our attention on the thiazole moiety, and pursued exploration of the structure–activity relationships while keeping this group constant.

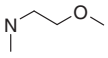
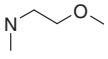
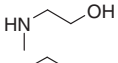
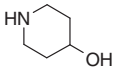
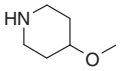
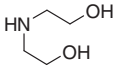
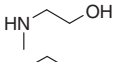
With this in mind, we sought to probe changes to the 7-position on the indole core while also making subtle changes to the amine moiety, Table 2. In all cases the THP substituted indole 3-thiazoles retained good levels of solubility. Overall, the 7-substituent appeared to have only a minor effect on CB1 agonist potency, while replacement of the pyrrolidine with a diethylamine moiety consistently led to improved activity. However, of concern was the observed binding affinity at the hERG channel,[8] as exemplified by compound **15**, which demonstrated similar levels of activity at both receptors.

It is well documented that lipophilic compounds containing a basic amine are prime candidates for hERG binding.[9] A number of strategies for improving selectivity over hERG have been reported in the literature, ranging from discrete structural modifications such as; altering the electronics of aryl groups by incorporation of electron withdrawing groups or removal of electron donating groups, control of log *P*, and manipulation of the pK_a of the basic nitrogen.[9] In this case, we felt we could target both improved solubility and selectivity over hERG, through lowering log *P* by the introduction of increased polarity in the amine region of the molecule, hence utilising an analogous route to that described in Scheme 1 compounds **34–42** were synthesised.[10] In parallel with this we also felt that substitution on the thiazole ring, which had to date remained untouched, should also become an area of focus. Compounds **30**–**33** were synthesised from the thioamide **12** according to Scheme 2. Ethyl 3,3-dichloropyruvate **20** and ethyl 3-chloro-2-oxopentanoate **23** were prepared as described by Garcia-Alles[11] and Tsuboi.[12] Reaction with the thioamide **12** provided the 5-substituted thiazole 4-carboxylate units, which were reduced with lithium borohydride to give the primary alcohols. These were converted to the chloride, using methanesulfonylchloride and triethylamine, which was subsequently displaced with a range of amines to produce the final compounds **30**–**33**.

Pleasingly, the introduction of a polar moiety did not lead to a loss of CB1 activity. Furthermore, open chain amines bearing both alkoxy and hydroxy substituents were preferred, compounds **35**–

Table 3
SAR of amine unit and thiazole substitution



Entry	X	R1	Amine	pEC_{50}[b]	Coeff. Var. (%)	Solkin (mg L^{-1})	hERG pK_i	RLM CL_{int} (μl min^{-1} mg^{-1})	HLM CL_{int} (μl min^{-1} mg^{-1})
34[a]	C	H		7.2[c]	N/A	<1	6.6	22	36
35[a]	O	H		8.2	3.9	65	6	111	95
36	O	H		7.4	8	55	5.7	167	<16
37	O	H		7.9	4.3	ND	6.1	ND	ND
38	O	H		8.0	2.9	1	6.0[a]	>160	161
39	O	H		7.2	3.8	ND	5.6	16	12
40	O	H		6.6	5.8	1	5.8	>270	80.4
41	O	H		7.3	2.3	3	5.4	>270	97.9
42[a]	O	H		7.3	2.6	61	5.6	>270	111
30[a]	O	Et		8.2	0.7	25	6.2	ND	79
31[a]	O	Et		8.7	2.2	<1	6.5	>270	114
32[a]	O	Et		8.6	1.5	<1	6.6	ND	ND
33[a]	O	Cl		8.0	1.3	<1	6.2	>270	>270

[a] Compound was tested as the HCl salt.
[b] pEC_{50} values are shown as mean and coefficient of variation from $n = 2–5$ separate experiments performed in triplicate (except where indicated with [c] where $n = 1$).

Table 4
Potency and duration of action of compounds in the mouse tail flick test following iv administration

Compound	ED_{50} (μmol kg^{-1})	DofA (min)
1	0.8	180
30	0.02	>60
32	0.04	<60
35	0.08	120
38	0.2	150

38, to alkoxy and hydroxyl substituted cyclic amines compounds **39–41**, Table 3. Substitution on the 5-position of the thiazole again led to compounds with good levels of CB1 potency, however, it also resulted in a trend towards reduced solubility. Importantly, compounds **30**, **32**, **35** and **38** all showed good CB1 agonist activity ($pEC_{50} \geqslant 8.0$), a tolerable level of selectivity over hERG binding and a measurable level of aqueous solubility.

These compounds were progressed into the mouse tail flick test and assayed for both in vivo potency and duration of action (DofA). All compounds tested demonstrated improved levels of in vivo potency as compared to compound **1** however, of the thiazole containing compounds tested, compound **38** maintained the longest duration of analgesic efficacy in this test, Table 4.

The in vitro and in vivo DMPK profile of **38** is summarised below. As demonstrated in Table 3, compound **38** is very rapidly metabolised in vitro by rat and human hepatic microsomes, which is also observed in mouse and dog hepatic microsomes (CL_{int} values of 176 and 131 μl min^{-1} mg^{-1}, respectively). The high in vitro intrinsic clearance in mouse, rat and dog is in agreement with the observed in vivo clearance, Table 5. However, despite the high degree of metabolism compound **38** achieves a relatively long plasma half-life, perhaps as a consequence of the extensive volume of distribution. Importantly, compound **38** demonstrates increased levels of brain penetration as compared to the lead compound **1** with a Brain/Plasma (B:P) ratio at C_{max} of 3.9 and 0.05, respectively.

Table 5
Comparison of in vivo pharmacokinetics of compounds **1** and **38**

Compound	Species	CL (ml min^{-1} kg^{-1})	V_{ss} (L/Kg)	$T_{1/2}$ (min)	B:P ratio (C_{max})	%ppb
1	Mouse ICR	28	1.4	72	0.05	99.9
38	Mouse ICR	159	11.3	83	3.9	98.9
38	Rat Wistar	127	15	96	ND	98.5
38	Dog (Beagle)	28	5	180	ND	99.2

Table 6
Cytochrome P450 inhibition profile of compound **38**

CYP	1A1	1A2	2C8	2C9	2C19	2D6	2E1	3A4 _BQ[a]	3A4_DB[b]
IC_{50} (μM)	1.4	>100	3.3	2.8	7.2	97.8	>100	>100	>100

[a] BQ—7-Benzyloxyquinoline used as substrate.
[b] DB—Dibenzylfluorescein used as substrate.

Table 7
Micro-shake-flask solubility data for compounds **1** and **38** compared with CP 55,940[13]

Compound	Buffer	Initial pH	Final pH	Solubility (mg L^{-1})	Comment
CP 55,940	Water	5.3	6.2	<1	
	Citrate buffer pH 5.0	5.0	5.1	<1	
1	Water	5.7	2.2	25	
	Citrate buffer pH 5.0	5.0	4.7	<1	
38	Water	5.3	2.3	>4000	Over solubility limit Solubility >4 mg ml^{-1}
	Citrate buffer pH 5.0	5.0	4.7	3611	

Further investigation of the CYP enzymes involved in the metabolism of **38** indicates that hepatic metabolism of **38** is mediated by CYP3A4. Additionally there is rapid metabolism in vitro by CYP1A1, an enzyme found in tissues such as the lung and kidney, but not expressed in the human liver (data not shown). Compound **38** was found to be an inhibitor of the major human P450 enzymes CYP1A1, 2C8, 2C9, 2C19 and 2D6, Table 6.

Despite having low solubility at neutral pH, compound **38** had a solubility of 3611 mg L^{-1} using a pH 5 citrate buffer as the vehicle, a vast improvement upon **1** and known CB1 agonist CP 55,940 both of which exhibited <1 mg L^{-1} solubility under the same conditions, Table 7.

Furthermore, compound **38** was shown to be potent in the rat tail flick test with an ED_{50} of 0.55 μmol kg^{-1} and maintained its analgesic efficacy for 140 min, following an intravenous bolus dose of 1.1 μmol kg^{-1}. As a consequence of both the sustained duration of action within the rat tail flick test and the excellent solubility at pH 5, compound **38** was chosen in preference to compound **1** for subsequent clinical development and pharmacokinetic evaluation in a Phase 1 clinical trial. The predicted human clearance, volume of distribution and terminal half-life were calculated[14] and resulted in anticipated values of 18 ml min^{-1} kg^{-1}, 9.7 L kg^{-1} and 6.1 h, respectively. The human PK prediction worked well, with the observed figures from the Phase 1 clinical trial being 14 ml min^{-1} kg^{-1}, 6.9 L kg^{-1} and 9.6 h, respectively, for a 6 μg kg^{-1} intravenous bolus dose.

In summary, a series of indole-3-thiazole derivatives was optimised through a systematic SAR study of the indole N-1 position, thiazole substitution and the amine moiety. These efforts resulted in novel ligands with good potency, selectivity and in vivo efficacy and ultimately a clinical candidate. Compound **38** is a potent CB1 receptor agonist, which retained the desired long duration of action after intravenous administration in the mouse and rat tail flick tests, while also demonstrating improved aqueous solubility. Furthermore, the observed human pharmacokinetic parameters for compound **38** were well predicted from the pre-clinical data using simple scaling models.

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References and notes

1. Recent reviews for cannabinoid receptor agonists: (a) 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, 2006; Vol. 44, pp 207–329; (b)*Handbook of Experimental Pharmacology*; Pertwee, R. G., Ed.; Springer, 2005; Vol. 168, (c) Huffman, J. W.; Padgett, L. W. *Curr. Med. Chem.* **2005**, *12*, 1395.
2. (a) Jagerovic, N.; Fernandez-Fernandez, C.; Goya, P. *Curr. Top. Med. Chem.* **2008**, *8*, 205; (b) Bermudez-Silva, F. J.; Viveros, M. P.; McPartland, J. M.; Rodriguez de Fonseca, F. *Pharmacol. Biochem. Behav.* **2010**, *95*, 375.
3. (a) Adam, J.; 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.; Houghton, A.; Westwood, P.; Walker, G. *Med. Chem. Commun.* **2010**, *1*, 54; (b) Kiyoi, T.; et al. *Bioorg. Med. Chem. Lett.* **2010**, doi:10.1016/j.bmcl.2010.06.067.; (c) Moir, E. M.; Yoshiizumi, K.; Cairns, J.; Cowley, P.; Ferguson, M.; Jeremiah, F.; Kiyoi, T.; Morphy, R.; Tierney, J.; Wishart, G.; York, M.; Baker, J.; Cottney, J. E.; Houghton, A. K.; McPhail, P.; Osprey, A.; Walker, G.; Adam, J. A. *Bioorg. Med. Chem. Lett.* **2010**, *24*, 7327.
4. Morrison, A. J.; Adam, J. A.; 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.* **2011**, *21*, 506.
5. Einsiedel, J.; Thomas, C.; Hübner, H.; Gmeiner, P. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2041.
6. 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.
7. 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.
8. Inhibition of [^{3}H]-dofetilide binding to hERG K^+ channels stably expressed in HEK293 cells following a 10 μM dose of test compound. Data represents means of two experiments performed in duplicate.
9. Jamieson, C. et al *J. Med. Chem.* **2006**, *49*, 5029.
10. Compound **38** was synthesised, starting from 7-Cl indole, in an analogous way to that described in Scheme 1 with an overall yield of 25%.
11. Garcia-Alles, L. F.; Erni, B. *Eur. J. Biochem.* **2002**, *269*, 3226.
12. Tsuboi, S.; Furutani, H.; Takeda, A. *Synthesis* **1987**, *3*, 292.
13. Micro-shake-flask solubility was measured as described in Ref. 3a using Milli-Q water and 25 mM Citrate buffer, pH 5.0.
14. The clearance in man was estimated using in vitro microsomal stability data and standard scaling techniques (non-restricted well-stirred model) which predicted well in pre-clinical species. The volume of distribution in man was estimated from the mean unbound volume in mouse, rat and dog. Correction for human plasma protein binding gave the estimate for volume of distribution.