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Article

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The Trifluoromethyl Group as a Bioisosteric Replacement of the Aliphatic Nitro Group in CB_1 Receptor Positive Allosteric Modulators (PAMs)

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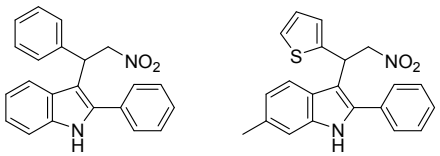
ABSTRACT

The first generation of CB_1 positive allosteric modulators (PAMs; e.g., ZCZ011) featured a 3-nitroalkyl-2-phenyl-indole structure. Although a small number of drugs include the nitro group, it is generally not regarded as being "drug-like", and this is particularly true for aliphatic nitro groups. There are very few case studies where an appropriate bioisostere replaced a nitro group that had a direct role in binding. This may be indicative of the difficulty of replicating its binding interactions. Herein we report the design and synthesis of ligands targeting the allosteric binding site on the CB_1 cannabinoid receptor, in which a CF_3 group successfully replaced the aliphatic NO_2. In general, the CF_3-bearing compounds were more potent than their NO_2 equivalents and also showed improved in vitro metabolic stability. The CF_3-analogue (**1**) with the best balance of properties was selected for further

pharmacological evaluation. Pilot in vivo studies showed that (±)-**1** has similar activity to (±)-ZCZ011, with both showing promising efficacy in a mouse model of neuropathic pain.

INTRODUCTION

The endocannabinoid system plays a major role in modulating neurotransmitter release in the central nervous system and thus its modulation elicits a broad range of physiological effects. Compounds activating cannabinoid receptors represent a promising therapeutic opportunity for the treatment of many disorders, including depression, anxiety and pain. However, global activation of the cannabinoid CB_1 receptor by direct agonism leads to psychoactive side effects, physical dependence and abuse liability. There is both an opportunity and a need to provide safer and more effective ways of harnessing the therapeutic benefits of CB_1 receptor activation. Recent reviews have highlighted the therapeutic potential of targeting the allosteric binding site on the CB_1 receptor using positive allosteric modulators (PAMs),[1,2] which have the advantage of enhancing the actions of endocannabinoids directly at the orthosteric receptors expressed within spinal and brain pain processing centres. Thus, PAMs are predicted to be free of the psychoactive side-effects associated with direct CB_1 agonists that globally activate these receptors. The impressive efficacy of small molecule tool CB_1 PAMs in models of neuropathic pain and post-traumatic stress disorder, without any evidence of addiction or psychotropic side-effects, support this hypothesis.



AZ-4 / GAT211 ZCZ-011 (**5**)

Figure 1

The activity of the first CB_1 PAM - AZ-4,[3] also known as F-087[4] and now commonly referred to as GAT-211[5] (Figure 1), was reported in a conference poster by researchers at AstraZeneca Montreal,[3] having been first synthesised by Noland and Lange in 1959.[6] The structure has since been modified to

improve potency (ZCZ011,[7] **5** in Table 1) and the enantiomers of AZ-4 separated (GAT228/229)[5], but no substantial development or comprehensive structure activity relationships have been reported. The primary problematic issue with these compounds is the presence of an aliphatic nitro group. Despite its presence in a small number of major drugs, including nifedipine, chloramphenicol, ranitidine and metronidazole, most academic and industrial medicinal chemists do not regard a nitro group as being "drug-like". However, surprisingly little published evidence exists to support this blanket exclusion of the nitro-group from drug-like space. Justification for this exclusion may be based upon accepted wisdom, or derived from personal experience (see *e.g*. Muegge and co-workers;[8] Beck and co-workers;[9] and discussions on internet forums, including the well-regarded "In the Pipeline"[10,11]); or based on a well-established (if only potential) toxic liability associated solely with the aromatic nitro and its propensity for formation of an aryl nitrenium ion, which can bind to DNA.[12] In additional examples of this general dismissal of the nitro group from drug-like space, the nitro was one of the groups eliminated prior to the studies leading to Baell's identification of the range of pan-assay interference compounds[13]; likewise, any structures containing a nitro are explicitly excluded by Rankovic and Morphy[14] as being acceptable for use in fragment-based lead discovery.

While little information exists on the toxicological issues associated with aliphatic nitro groups, the widely-held opinions described above usually require its removal or replacement early in the development of a bioactive compound. However, there are only a few case studies in which a nitro group has been successfully replaced by an appropriate bioisostere,[9] which may be indicative of the difficulty of replicating its binding interactions. Nitro groups may act as hydrogen bond acceptors and may also stack with other groups, including ketones and alcohols.[15] Additionally, Alston and co-workers[16] reported that the nitroalkyl group can serve as a one- and two-electron reductant, oxidant, ambident nucleophile, electrophile, ligand, and leaving group in reactions with enzymes and other biomolecules and suggested that "the richness of its reactivity may have hindered the application of the nitroalkyl group in medicinal chemistry." Much more commonly, nitro groups are initially present

in bioactive compounds as aromatic substituents, where their electron-withdrawing properties may polarise the ring, giving optimal interaction with electron-rich moieties within the biological target. In such cases, simple replacement with other strongly-electron-withdrawing substituents, e.g. halogens or haloalkyl groups, may be possible.

Because of this strong electron-withdrawing nature, the presence of a nitro group also facilitates many chemical reactions; thus, it is often found in screening collections (including the collection from which AZ-4 was originally identified). The reaction of an indole with nitrostyrene has been widely reported in the literature and there are a considerable range of methods available for this simple 1,4-conjugate addition reaction.[17–24] There is literature precedent for the nitro group to be replaced with a carboxylic acid in this series of compounds, which is achieved by initial reaction of an indole with the highly-electron deficient diethylbenzylidene malonate.[21,22] The limited similarity of nitro group binding to carboxylic acid binding, in spite of the near perfect isosterism, has been discussed by Kelly and Kim.[25] A small range of derivatives can be accessed from the carboxylic acid (e.g. CH_2OH, CO_2H, CO_2Et, $CONHNH_2$, $CONH_2$ and CONHMe, **Figure 2** - see Supporting Information); however, these were inactive as CB_1 PAMs.

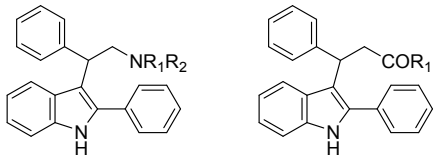


Figure 2.

Likewise, simple derivatives based on reduction of the nitro to an amine (**Figure 2**), with or without subsequent substitution, gave inactive compounds (see Supporting Information).

Modelling studies on GAT228[26] indicate that the nitro group may form polar interactions with a serine, a methionine and a cysteine residue in the absence of the orthosteric agonist (CP55,940). However, this case appears to apply to an orthosteric agonist. Although not stated as a conclusion by the

authors, the modelling suggests that, in the presence of an agonist, GAT228 moves deeper into the receptor; accordingly, the nitro group may be too far from these binding groups to retain a strong interaction. Thus, it remains uncertain that specific binding interactions provided by the nitro group are required for its activity as a PAM. However, the lack of activity shown by any of the compounds in **Figure 2** suggests that such interactions will be necessary for PAM activity.

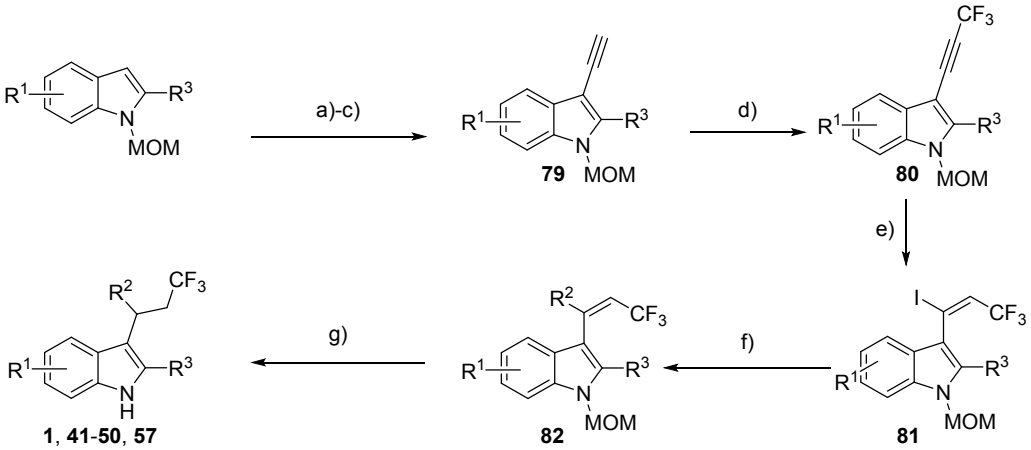
Herein we report that the CF_3 offers a viable bioisosteric replacement for an aliphatic nitro group, demonstrating the removal of the non-drug-like nitro group from the most-commonly-studied and utilised CB_1 PAMs, to achieve compounds with improved potency and metabolic stability.

RESULTS AND DISCUSSION

Chemistry.

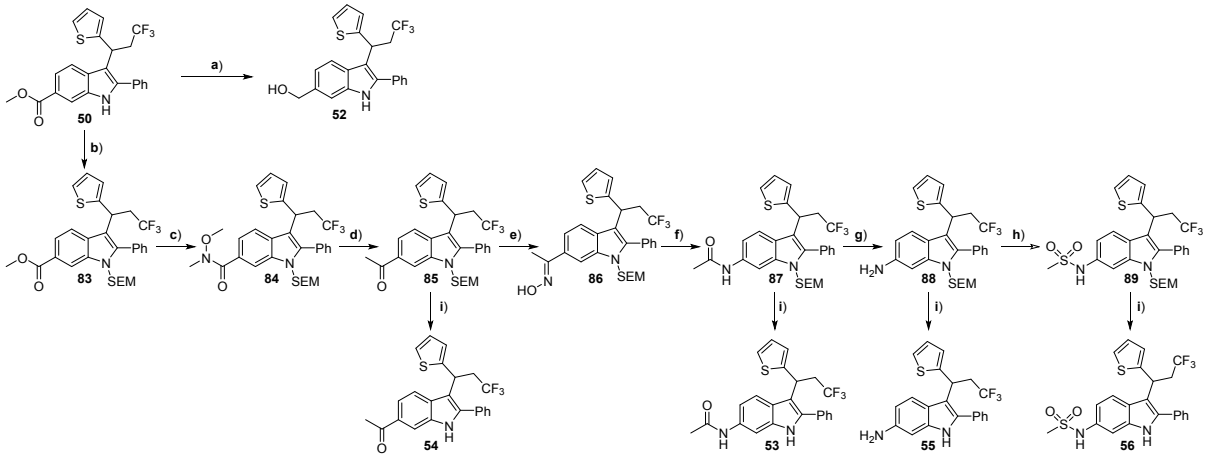
A library of indole derivatives **1**-**57** (Table 1) was synthesised using a variety of methods. Nitro-indole derivatives **2**-**25** were obtained by $InBr_3$-catalyzed Michael addition of 2-(2-nitrovinyl)-aryl/heteroaryl derivatives to the corresponding indoles, upon microwave heating (see Supporting Information Scheme SI-1 for details). Dicyano derivatives **29** and **32**-**34** having R^2 = Ph and R^4 = $CH(CN)_2$ were obtained by addition of lithium indolyl-cuprate(I) to benzylidene-malononitriles (Supporting Information, Scheme SI-4). Compounds having R^2 = Ph and R^4 = CH_2CN were obtained either via Fischer indole syntheses (**28**, **30**), or via $InBr_3$-promoted addition of phenyl-oxirane to an indole, followed by one-carbon homologation (**31**). A similar strategy, followed by one-carbon homologation using TMS-CF_3 and Barton-McCombie dehydroxylation was used to access the CF_3-indoles **37** and **39** (Supporting Information, Scheme SI-5). The cyclopropyl CF_3-indole **40** was also prepared using a multi-step process involving TMS-CF_3 homologation and Barton-McCombie dehydroxylation (Supporting Information, Scheme SI-7). However, the most versatile method for efficiently preparing a wide range of CF_3-indoles is shown in Scheme 1. The starting indole was iodinated at the 3-position with NIS to be then subjected to Sonogashira coupling to give **79**. Terminal alkyne trifluoromethylation with in-situ generated "Cu-

CF_3" under aerobic conditions gave CF_3-alkynes **80**,[27] which were transformed into iodo-alkenes **81** with lithium iodide under mild acidic aonditions,[28] which were then submitted to Stille coupling with the corresponding aryl stannanes leading to N-MOM-protected indoles **82**. The latter were first de-protected with HCl and then reduced with Et_3SiH in the presence of TFA to afford the target CF_3-indoles **1**, **41**-**50** and **57**.



Scheme 1. Synthesis of CF_3-PAMs via Stille coupling/ionic hydride reduction strategy.
Reagents and conditions: a) NIS, 0 °C, CH_2Cl_2; b) TMSCCH, $PdCl_2(PPh_3)_2$, CuI, ET_3N, 23 °C, DMF; c) TBAF, 0 °C, THF; d) $TMSCF_3$, CuI, TMEDA, K_2CO_3, air, 23 °C, DMF; e) LiI, HOAc, CH_2Cl_2, 0 – 23 °C; f) R^2SnBu_3, $PdCl_2(MeCN)_2$, P(*o*-Tol)$_3$, CuI, 55 °C, DMF; g) 6 M HCl, 60 °C, THF then Et_3SiH, TFA, 0 – 23 °C, CH_2Cl_2.

Further late-stage functional groups manipulations, shown in Scheme 2, expanded the library of CF_3-indole derivatives to include compounds **52**-**56** and **83**-**89**.



Scheme 2. Late-stage functional group interconversions strategy.

Reagents and conditions: a) Super-Hydride®, THF; b) SEMCl; c) HNMe(OMe)-HCl, *i*PrMgCl, THF, 0 °C; d) MeLi, THF, -78 °C; e) NH_2OH-HCl; f) cyanuric chloride, DMF, 23 °C; g) N_2H_4 hydrate, EtOH, 100 °C; h) $MeSO_2Cl$, Et_3N, 23 °C; i) HF_{aq}-TFA.

All the compounds above were obtained in racemic form, but pure enantiomers could be obtained for a number of derivatives, such as **1**, **8**, **26** and **50**, using preparative HPLC separation on chiral column (see Supporting Information for details). Compound (+)-**1** was crystallized from EtOAc/Hexane solution and its structure was determined by X-ray crystallography,[29] which showed that the stereogenic centres C9 and C31 had (*S*)-configurations (Figure 3). Note, as a consequence of the change of priority of the substituents, (*R*)-(–)-1 and (*S*)-(–)GAT229 have different descriptors but share the same stereocentre's topicity.

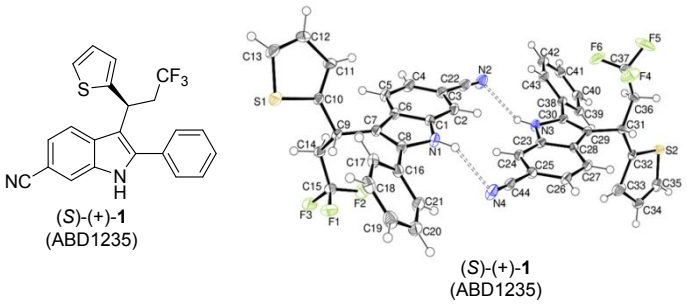


Figure 3. X-ray crystal structure of (*S*)-(+)-**1**.

In vitro pharmacology. Modification of the ZCZ011 structure indicated that either a 6-Me (**5**), 6-Cl (**6**), 6-CF_3 (**7**) or 6-CN (**8**) substitution led to moderately potent and efficacious CB_1 PAMs, as determined by enhancement of β-arrestin recruitment, induced by the CB1 agonist CP55,940 at its EC_{20} in the PathHunter® β-Arrestin assay (Eurofins DiscoverX), by a variation of the methods described previously.[7] The results are shown in **Table 1**. Substitutions on the 5-position of the indole gave compounds with poor potency [5-Me (**2**), 5-CN (**3**), 5-NO_2 (**4**)]. However, results from the same assay demonstrated that, alongside their PAM activity, most of these compounds were also allosteric agonists, giving receptor activation in the absence of the orthosteric ligand. This compares with previous reports[5] that the enantiomers of AZ-4 (GAT228 and 229) show considerable differences in their properties, with the (+) enantiomer being an allosteric agonist and the (–) being a pure PAM.

Separation of the enantiomers of (±)-**8** (6-CN) confirmed that (–)-**8** was the more potent PAM but, contrary to GAT228/229,[5] did not indicate that the (+)-**8** was an agonist: both (–)- and (+)-**8** showed enhancement of CP55,940-induced β-arrestin recruitment (E_{max} = 117% and 51% for the (–) and (+) respectively, $p<0.005$ between enantiomers) with EC_{50} values that were not significantly different (664 and 863 nM, for the (–) and (+) respectively). However, both showed very low agonism (activation in the absence of CP55,940) (E_{max} = 21 and 6% for the (–) and (+) respectively) with very poor potency (EC_{50} >10 μM) making them both effectively pure PAMs (**Table 1**). Replacement of the 2-phenyl group (R^3) with different aryl or cycloalkyl groups as in **9**-**25** was not tolerated. Other researchers[30] have noted that, in the absence of any (R^1) substitutions on the indole, 4-fluoro, 4-chloro or 3-chloro-4-fluoro substitution on the 2-phenyl (R^2) appeared to be beneficial for potency in a cAMP assay; however, no examples featuring both an R^1 and R^2 substitution were shown.

In addition to the potential toxicological concerns, the methylene group adjacent to the nitro group was expected to be acidic and potentially a metabolic liability, contributing to the short half-life. ZCZ011 (**5**) was shown to be highly unstable in the rat and mouse liver microsomal assays ($t_{½} \leq 10$ min), with moderate stability in human liver microsomes ($t_{½}$ = 41 min) (Cyprotex Ltd). No significant increase in stability was seen with the 6-Cl (**6**) and 6-CF_3 (**7**) derivatives or with the placement of an electron-withdrawing 4-Cl on the 2-phenyl group (**16**) (see **Table 1**). We therefore concluded that further development of the nitro-bearing compounds was unlikely to lead to drug-like compounds (suitable either for preclinical development or as tool compounds for in vivo target validation studies), and set about developing the synthetic methods required to permit replacement of the nitro group with a range of bioisosteres.

Modelling software (FieldStere and FieldAlign, products available from Cresset Biomolecular at the time) was used to suggest moieties that might mimic the electronic properties of the nitro group, with the primary feature modelled being that of a hydrogen bond acceptor. Many structures were identified, but synthetic methods were generally lacking. Various 5-membered heterocycles were

investigated (Fig. 4); however, none of these were active and the syntheses were not straightforward. Thus, this line of investigation was terminated because of unproductivity.

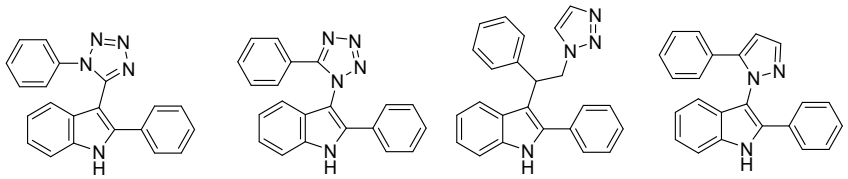
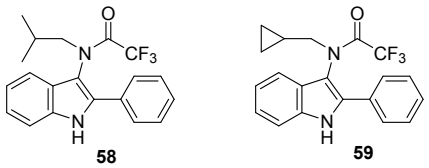


Figure 4. Inactive heterocyclic analogues of ZCZ011 investigated in this work (see Supporting Information: **Het-1** – **Het-4**).

The cyano group is sometimes proposed as being a bioisostere for a non-aromatic nitro group;[31] although perhaps the most widely known example of this is in fact the use of nitro as a bioisostere of a cyano group, as seen in the development of ranitidine from cimetidine.[32] This bioisosterism was affirmed by the (albeit modest) potency of compounds **30** and **32**, which featured replacement of the CH_2NO_2 with either a CH_2CN or a $CH(CN)_2$ moiety. Compound **31** showed trends for somewhat reduced potency and efficacy as compared to its nearest nitro analogue (not significant), and showed similarly-poor metabolic stability in rat liver microsomes, possibly again related to the acidity of the proton adjacent to the CN group(s).

Studies aimed at replacement of the stereogenic carbon atom with an amine led to the preparation of a range of compounds bearing a $-COCF_3$ substituent, some of which (such as compounds **58** and **59**, Fig. 5) showed a modest degree of enhancement (~50% at 1 μM).

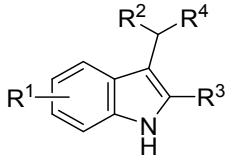


58 **59**

Figure 5. Trifluoroacetamide derivatives tested in this work.

It was assumed that these compounds would be highly susceptible to hydrolysis and they were not regarded as development candidates or even appropriate for use as tool compounds. However, these

results suggested that the $-CF_3$ group might be a suitable bioisostere. Consequently, we set about developing synthetic methodologies to allow access to target compounds.



Comp	R^1	R^2	R^3	R^4	[a]PAM Activity: >50 % enhancement * at 1000 nM ** at 500 nM *** at 200 nM	EC_{50}		E_{max}		[f]$T_{1/2}$ (min)		
						[b]PAM	[c]Ago	[d]PAM	[d]Ago	HLM	RLM	MLM
2	5-Me	2-Thienyl	Ph	CH_2NO_2	*	-	-	-	-	-	-	-
3	5-CN	2-Thienyl	Ph	CH_2NO_2	NA	-	-	-	-	49.4	8.2	-
4	5-NO_2	2-Thienyl	Ph	CH_2NO_2	NA	-	-	-	-	-	-	-
5	6-Me	2-Thienyl	Ph	CH_2NO_2	**	283±140	777±363	106±18	55±24	41.2±3.1	6.4±1.2	10.4±2.1
6	6-Cl	2-Thienyl	Ph	CH_2NO_2	**	462±363	1530±1128	154±10	82±48	32.7	13.8	-
7	6-CF_3	2-Thienyl	Ph	CH_2NO_2	**	284±211	974±780	163±17	106±47	30.7	15.6	-
(±)-**8**	6-CN	2-Thienyl	Ph	CH_2NO_2	*	-	-	-	-	-	-	-
(+)-**8**	6-CN	2-Thienyl	Ph	(+)CH_2NO_2	*	863±211	>10000	51±18##	9±4#	-	-	-
(-)-**8**	6-CN	2-Thienyl	Ph	(-)CH_2NO_2	*	664±215	>10000	117±19##	21±6#	-	-	-
9	6-Cl	2-Thienyl	4-OMe-C_6H_4-	CH_2NO_2	NA	-	-	-	-	-	-	-
10	6-Cl	2-Thienyl	4-NMe_2-C_6H_4-	CH_2NO_2	NA	-	-	-	-	-	-	-
11	6-Cl	2-Thienyl	4-CN-C_6H_4-	CH_2NO_2	NA	-	-	-	-	-	-	-
12	6-Cl	2-Thienyl	4-CO_2Me-C_6H_4-	CH_2NO_2	NA	-	-	-	-	-	-	-
13	6-Cl	2-Thienyl	4-SO_2Me-C_6H_4-	CH_2NO_2	NA	-	-	-	-	-	-	-
14	6-Cl	2-Thienyl	4-COOH-C_6H_4-	CH_2NO_2	NA	-	-	-	-	-	-	-
15	6-Cl	2-Thienyl	4-Ac-C_6H_4-	CH_2NO_2	NA	-	-	-	-	-	-	-
16	6-Me	2-Thienyl	4-Cl-C_6H_4-	CH_2NO_2	NA	-	-	-	-	24.2	9.8	-
17	6-Cl	2-Thienyl	4-Cl-C_6H_4-	CH_2NO_2	NA	-	-	-	-	-	-	-
18	6-Cl	2-Thienyl	4-F-C_6H_4-	CH_2NO_2	NA	-	-	-	-			-
19	6-Me	2-Thienyl	4-F-C_6H_4-	CH_2NO_2	NA	-	-	-	-	-	-	-
20	6-Cl	2-Thienyl	3-OMe-C_6H_4-	CH_2NO_2	NA	-	-	-	-			-
21	6-Cl	2-Thienyl	3-CN-C_6H_4-	CH_2NO_2	NA	-	-	-	-			-

22	6-Me	2-Thienyl	Cyclopropyl	CH_2NO_2	*	-	-	-	-			-
23	6-Me	2-Thienyl	Cyclopentyl	CH_2NO_2	*	-	-					-
24	6-Me	2-Thienyl	Cyclohexyl	CH_2NO_2	NA	-	-	-	-	-	-	-
25	5-CN	2-Thienyl	Cyclohexyl	CH_2NO_2	NA	-	-	-	-	21.4	3.8	-
(±)-**26**	-	Phenyl	Ph	CH_2CO_2H	NA	-	-	-	-	62.7	-	-
(+)-**26**		Phenyl	Ph	CH_2CO_2H	NA	-	-	-	-	-	-	-
(-)-**26**		Phenyl	Ph	CH_2CO_2H	NA	-	-	-	-	-	-	-
27	-	Phenyl	Ph	CH_2OH	NA	-	-	-	-	-	-	-
28	-	Phenyl	Ph	CH_2CN	NA	-	-	-	-	42.7	14.5	-
29	-	Phenyl	Ph	$CH(CN)_2$	NA	-	-	-	-	26.2	14.8	-
30	6-Me	Phenyl	Ph	CH_2CN	*	-	-	-	-	45.7	18.7	-
31	6-Cl	Phenyl	Ph	CH_2CN	*	1113±401	2354±601	100±36	24±7	43.2	22.5	-
32	6-Me	Phenyl	Ph	$CH(CN)_2$	*	-	-	-	-	39.3	12.4	-
33	6-Me	2-Thienyl	Ph	$CH(CN)_2$	*	-	-	-	-	31.2	4.9	-
34	6-Cl	2-Thienyl	Ph	$CH(CN)_2$	NA	-	-	-	-	29.6	13.5	-
35	-	Phenyl	Ph	$COCF_3$	NA	-	-	-	-	51.1	41.4	-
36	-	Phenyl	Ph	$CHOHCF_3$	NA	-	-	-	-	39.4	18.2	-
37	-	Phenyl	Ph	CH_2CF_3	*	-	-	-	-	85.2	40.7	-
38	6-Me	Phenyl	Ph	$COCF_3$	**	-	-	-	-	267	24.8	-
39	6-Me	Phenyl	Ph	CH_2CF_3	**	-	-	-	-	87.2	28.2	-
40	6-Me	Cycloprop -yl methyl	Ph	CH_2CF_3	*	-	-	-	-	74.4	19.8	-
41	6-Me	2-Thienyl	Ph	CH_2CF_3	**	-	-	-	-	108	17.5	-
42	6-Me	2-Furyl	Ph	CH_2CF_3	*	-	-	-	-	59.1	10.3	-
43	6-Cl	2-Furyl	Ph	CH_2CF_3	**	-	-	-	-	63.9	46.3	-
44	6-Cl	2-Thienyl	Ph	CH_2CF_3	***	-	-	-	-	157	54.3	-
45	6-Cl	Phenyl	Ph	CH_2CF_3		-	-	-	-	98.6	78.0	-
46	6-CF_3	Phenyl	Ph	CH_2CF_3	***	-	-	-	-	-	-	-
47	6-CF_3	2-Thienyl	Ph	CH_2CF_3	**	322±252	801±554	203±54	95±15	49.7	36.7	-
48	5-Me	Phenyl	Ph	CH_2CF_3	NA	-	-	-	-	-	-	-
49	5-Me	2-Thienyl	Ph	CH_2CF_3	NA	-	-	-	-	-	-	-
(±)-**50**	6-CO_2Me	2-Thienyl	Ph	CH_2CF_3	***	-	-	-	-	-	-	-
(+)-**50**	6-CO_2Me	2-Thienyl	Ph	CH_2CF_3	***	160±147‡‡	682±211‡	88±28##	26±5##	-	-	-
(-)-**50**	6-CO_2Me	2-Thienyl	Ph	CH_2CF_3	***	113±62‡	416±167‡	156±41##	51±11##	-	-	-
51	6-CO_2H	2-Thienyl	Ph	CH_2CF_3	NA	-	-	-	-	-	-	-

52	6-CH_2OH	2-Thienyl	Ph	CH_2CF_3	NA	-	-	-	-	-	-	-
53	6-NHAc	2-Thienyl	Ph	CH_2CF_3	NA	-	-	-	-	-	-	-
(±)-**1**	6-CN	2-Thienyl	Ph	CH_2CF_3	***	177±66	647±270	100±25	30±14	-	-	-
(*S*)-(+)-**1**	6-CN	2-Thienyl	Ph	CH_2CF_3	**	342±142‡	1206±345‡	79±32 #	17±4	-	-	-
(*R*)-(-)-**1**	6-CN	2-Thienyl	Ph	CH_2CF_3	**	363±169‡‡	828±310‡‡	125±29 #	25±6	72.3±30.5	34.9±7.4	26.5±1.5
54	6-Ac	2-Thienyl	Ph	CH_2CF_3	***	158±60	741±362	121±23	39±9	51.2	12.2	-
55	6-NH_2	2-Thienyl	Ph	CH_2CF_3	NA	-	-	-	-	-	-	-
56	6-$NHSO_2Me$	2-Thienyl	Ph	CH_2CF_3	NA	-	-	-	-	-	-	-
57	6-CF_3	2-Thienyl	Cyclopentyl	CH_2CF_3	**	301±66	755±237	162±47	68±14	47.1	44.4	-

Table 1: Enhancement of β-arrestin recruitment induced by the CB1 agonist CP55,940 and metabolic stability data from human, rat and mouse liver microsomal stability studies (HLM, RLM and MLM). [a]Pilot data were generated as described previously[7] and represents data from a single experiment. [b]Selected compounds were evaluated by Eurofins DiscoverX in three independent experiments, using the PathHunter® β-Arrestin assay. Positive allosteric modulation was assessed using EC_{20} CP55,940. The EC_{50} value represents the compound concentration at which 50% of the maximal achievable EC_{20} CP55,940-induced β-arrestin recruitment is seen (± standard deviation) for each specific compound. [c]Agonism was evaluated in the same assay and EC_{50} and E_{max} values were generated as described for positive allosteric modulation, using compound alone in the absence of agonist. [d]The E_{max} for each compound is the maximum EC_{20} CP55,940-induced β-arrestin recruitment relative to the maximum achievable with CP55,940. [e]Human, rat and mouse liver microsomal stability studies were conducted Cyprotex Ltd. Half-life values represent data from a single experiment (5 timepoints), except where ± stdev is given (three independent experiments). ## P<0.005; # P<0.05, between enantiomers (Student's t-test). ‡‡ P< 0.005 ‡ P<0.05, between IC_{50} values as PAM and agonist (two-tailed t-test). NA = not active (defined as <50% enhancement in CP55,940-induced β-arrestin recruitment at 1 μM).

The unsubstituted derivatives (**35**, **36** and **37**) featuring the CH_2CF_3, $COCF_3$ and $CH(OH)CF_3$ showed a small degree of enhancement (30 – 50%) at 1 μM (see **Table 1**). Incorporation of the 6-methyl group from ZCZ011 (compounds **38** and **39**) gave a further increase in potency and efficacy (50 – 100% enhancement at 300 nM). Compounds showed good stability in human liver microsomes, and potential improvement in rat liver microsomes (RLM) compared to their nitro analogues (see **Table 1**).

We prepared a range of CF_3-derivatives (**40**-**57**) with the aim of SAR elucidation and further improvements in potency and metabolic stability. Several compounds showed excellent potency and efficacy, with enhancement of over 100% at <200 nM and HLM/RLM $T_{1/2}$ >50 min; however, results from these compounds tended to be variable, due to solubility issues not seen with the nitro derivatives. In order to address these solubility issues, we prepared analogues with a range of polar substituents on the indole. In parallel, we used the nitro series of compounds as a model system for rapid preparation of the analogues bearing substituents on the 2-phenyl group. Unfortunately, these studies showed an unforgiving SAR (see Supporting Information for observations on the SAR) in that none of the substitutions on the 2-phenyl were tolerated and none of 6-CO_2H (**51**), 6-CH_2OH (**52**), 6-NHAc (**53**), 6-NH_2 (**55**) or 6-$NHSO_2Me$ (**56**) gave active compounds. The 6-Ac derivative (**54**) was potent and efficacious but showed poor stability in the RLM assay ($T_{1/2}$ = 12 min). Results are shown in **Table 1**.

Aside from those compounds with solubility issues, the most potent derivative was the 6-CO_2Me (**50**) for which the enantiomers were separated. As expected from GAT228/229[5] and our own observations from nitro compound **8**, as shown in **Table 1**, the (–) enantiomer was significantly more efficacious ($p<0.005$) than the (+) enantiomer both as a PAM (E_{max} = 156±41 vs 88±28%, for the (–) and (+) respectively; and as an agonist (E_{max} = 51±11 vs 26±5%, for the (–) and (+) respectively). Both the (–) and (+) enantiomers were more potent ($p<0.05$) as PAMs than as agonists (EC_{50} = 113±62 / 160±147 nM for the (–) and (+) respectively as PAMs vs EC_{50} = 416±167 / 682±211 nM for the (–) and (+)

respectively as agonists. However, as an ester, compound **50** was assumed to be unsuitable for further evaluation, due to metabolic instability and, unfortunately, the likely metabolite (the 6-CO_2H analogue **51**) was inactive.

The compound with the best balance of properties was the 6-CN analogue (**1**). As with the 6-CO_2Me analogue **50**, both the (–) and (+) enantiomers were significantly ($p<0.05$) more efficacious as PAMs (E_{max} = 125±29 / 79±32% for the (–) and (+) respectively) than as agonists (E_{max} = 25±6 / 17±4% for the (–) and (+) respectively); with the (–) significantly more efficacious than the (+) enantiomer ($p<0.05$) as a PAM. Likewise, both the (–) and (+) enantiomers were more potent ($p<0.05$) as PAMs than agonists (EC_{50} = 363±169 / 342±142 nM as PAMs, for the (–) and (+) respectively; and IC_{50} = 828±310 / 1206±345 nM as agonists, for the (–) and (+) respectively). The 6-CN derivative (–)**-1** was considerably more stable than ZCZ011, with HLM/RLM/MLM $T_{1/2}$ of 72.3±30.5 (*N.S.* in comparison with ZCZ011) / 34.9±7.4 ($p<0.005$ to ZCZ011) / 26.5±1.5 min ($p<0.005$ to ZCZ011) respectively, compared to 41.2±3.1 / 6.4±1.2 / 10.4±2.1 min respectively for ZCZ011. Results are shown in **Table 1**.

Thus, compound (–)-**1** was selected for further pharmacological evaluation. The enantiomers of CF_3-derivative **1** and the enantiomers of its nitro analogue **8** were compared to ZCZ011 in several assays, to assess further the bioisosterism of the -CF_3 and -NO_2 groups.

A similar pattern for compound potency, but not efficacy, was seen in the DiscoverX Hithunter® cAMP assay (Eurofins DiscoverX): -CF_3-bearing compounds **1** showed a trend for greater potency, than their -NO_2 equivalents **8**, and the (–) enantiomers also showed a trend for greater potency than their (+) analogues. The results are shown in **Table 2**. However, contrary to the situation seen with β-arrestin recruitment, the compounds possessed similar efficacy, with all compounds acting as both PAMs and full agonists, completely inhibiting forskolin-stimulated cAMP production. The significance of this apparent difference between the effects on β-arrestin and cAMP is unclear. However, it must be noted that, as neither ZCZ011 or (±)-**1** showed any activity in the tetrad or psychoactive effects (see

Supporting Information), agonism, with regard to cAMP signalling, does not appear to translate to agonism at CB_1 *in vivo*.

	cAMP EC_{50} (nM)		cAMP E_{max} (%)	
	PAM	Agonist	PAM	Agonist
ZCZ011	18 ± 1	32 ± 10	98 ± 0	94 ± 4
(+)-**8**	181 ± 95	251 ± 75	102 ± 5	96 ± 2
(−)-**8**	36 ± 10	46 ± 7	99 ± 2	97 ± 2
(*S*)-(+)-**1**	40 ± 17	76 ± 8	96 ± 3	89 ± 6
(*R*)-(−)-**1**	17 ± 5	29 ± 6	90 ± 1	84 ± 8

Table 2. Effects of compounds on cAMP generation, either alone (Agonist) or induced by EC_{20} CP55,940 (PAM) in the presence of EC_{80} forskolin in the DiscoverX Hithunter® cAMP assay, using a variation of the methodology described previously.[7] Data is the result of three independent experiments.

Binding studies were also conducted (Eurofins Cerep) using a variant of the methodology described previously[7] and demonstrated that, like ZCZ011, 1 μM (+)-**1** and (−)-**1**, showed a significant enhancement in binding of the CB_1 agonist, [^{3}H]CP55940. At this concentration, no enhancement was noted with either (+)-**8** and (−)-**8**. The results are shown in **Table 3**.

	% Enhancement of [^{3}H]CP55,940 binding
ZCZ011	127.1 ± 13.3
(+)-**8**	99.0 ± 10.1
(−)-**8**	108.4 ± 8.3
(*S*)-(+)-**1**	144.4 ± 3.5
(*R*)-(−)-**1**	131.4 ± 4.3

Table 3. Effect of 1 μM ZCZ011, (+)-**8**, (−)-**8**, (+)-(*S*)-**1** or (*R*)-(−)-**1** on [^{3}H]CP55,940 binding, using a variation of the methodology described previously.[7] Data is the result of three independent experiments.

In vivo efficacy. Using the methodology previously described for the racemic ZCZ011,[7] (±)-**1** was evaluated in several CB_1 receptor-sensitive in vivo models and was shown to possess a similar activity profile as ZCZ011.

(±)-**1** (40 mg/kg) did not elicit pharmacological effects in the tetrad assay when administered alone. (±)-**1** did not affect either the distance travelled or immobility time during assessment of locomotor behavior, did not produce antinociception in the tail withdrawal test, did not produce hypothermia or cause immobility in the catalepsy bar test (see Supporting Information). Thus, even at a high dose, (±)-**1** did not elicit in vivo effects indicative of CB_1 receptor orthosteric agonism. In contrast, (±)-**1** (40 mg/kg) augmented a subset of pharmacological effects of the CB_1 receptor orthosteric agonist CP55,940. Importantly, (±)-**1** produced significant leftward shifts in the dose-response relationships of CP55,940 in producing antinociception in the tail withdrawal test (see Supporting Information) and thus was further evaluated for its potential in the mouse chronic constrictive injury of the sciatic nerve (CCI) model of neuropathic pain.

As shown in **Figure 6**, mice displayed profound mechanical allodynia seven days post CCI surgery in both the ipsilateral (Panel **A**) and contralateral (Panel **B**) paws. At 40 mg/kg (the same dose at which ZCZ011 produces anti-allodynic effects[7]) (±)-**1** significantly reversed CCI-induced mechanical allodynia in a time-dependent manner in the ipsilateral [$F_{time}(6, 168)=6.411$; $F_{drug}(3, 28)= 101.5$; $P<0.0001$; $F_{interaction}(18, 168) = 7.394$; $P<0.0001$, **Figure 6A**] and contralateral [$F_{time}(6, 168)=14.81$; $F_{drug}(3, 28) = 182.2$; $F_{interaction}(18, 168) = 4.901$; $P<0.0001$, **Figure 6B**] paws. In particular, (±)-**1** produced a significant anti-allodynic effect at 0.5 h post-administration and achieved maximal effectiveness by 1 h.

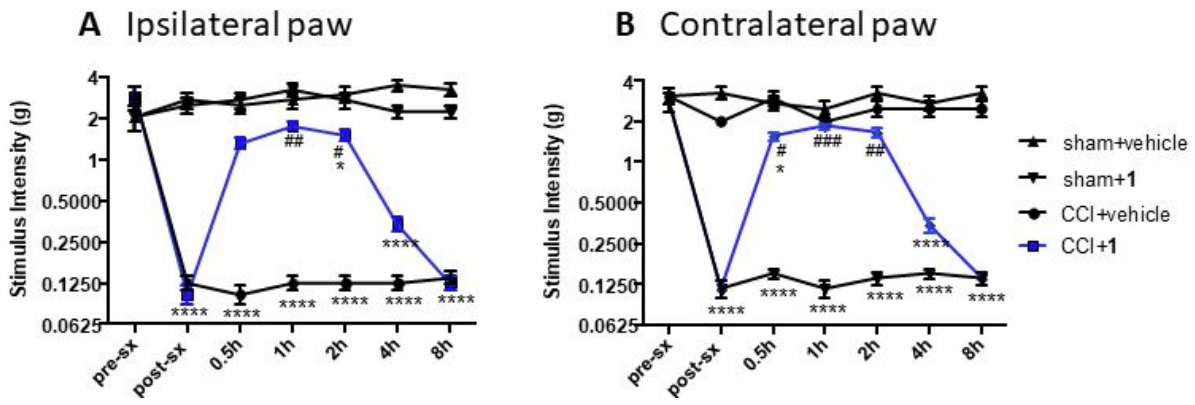


Figure 6. Acute administration of 40 mg/kg (±)-**1** time-dependently reverses CCI-induced mechanical sensitivity in ipsilateral and contralateral paw. Data are expressed as mean ± SEM (n = 8/group). ****p<0.0001, *p<0.05 vs sham+vehicle; ###p<0.001 ##p<0.01, #p<0.05 vs CCI+vehicle. pre-sx (pre-surgery); post-sx (post-surgery).

CONCLUSION

In conclusion, we have demonstrated that a -CF_3 serves as a viable bioisosteric replacement for an aliphatic nitro group. Thus, we have shown that it is possible to remove the "non-drug-like" nitro group from the most-commonly-studied CB_1 PAMs to give compounds with improved potency and metabolic stability, which retain the activity profile of the parent nitro compound. These findings offer major assistance in the development of a preclinical candidate suitable for evaluation of the therapeutic potential of CB_1 PAMs in a range of unmet medical needs. We hypothesise that the CF_3 group might be a bioisosteric replacement for a nitro group also in other classes of bioactive compounds, especially when the nitro group is not involved in highly stabilizing hydrogen-bonds with the receptor, as the CF_3 group is known to be a weak hydrogen-bond acceptor.

EXPERIMENTAL SECTION

General Methods: All reactions were carried out under a nitrogen atmosphere, with dry solvents under anhydrous conditions, unless otherwise mentioned. **Solvents and reagents:** Anhydrous tetrahydrofuran (THF), diethyl ether (Et_2O), methylene chloride (CH_2Cl_2), and toluene were purchased from commercial suppliers. Reagents were purchased at the highest commercial quality, used without

further purification and handled in accordance with COSHH regulations. **Chromatography:** Flash chromatography (FC) was carried out on Silica gel (Merck Silica gel Si 60, 40-63 μm). Thin-layer chromatography (TLC) was carried out on glass-based 0.25 mm Merck silica gel plates (60F-254) which were developed with UV irradiation (254 nm and 365 nm), an aqueous solution of $KMnO_4$ and an ethanolic solution of ammonium molybdate, and heat as developing agents. **^{1}H NMR spectra:** These were recorded at 400 MHz on a Bruker ADVANCEIII 400 instrument. Chemical shifts (δ_H) are given in parts per million (ppm) as referenced to the appropriate residual solvent peak. **^{13}C NMR spectra:** These were recorded at 100.6 MHz on a Bruker ADVANCE III 400 instrument. **^{19}F NMR spectra**: These were recorded at 376 MHz on a Bruker ADVANCE III 400 instrument without 1H decoupling. Chemical shifts (δ_C) are given in parts per million (ppm) as referenced to $CFCl_3$ as 0 ppm. All NMR spectra were recorded at 298 K unless otherwise stated. Chemical shifts (δ_C) are given in parts per million (ppm) as referenced to $CHCl_3$. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad. **Purity analysis:** purities of all final compounds were determined by reverse phase HPLC-MS using an Agilent 1200 series chromatograph system coupled with an Agilent G6120 signal quadrupole detector equipped with an electrospray ionisation source and detection in positive mode. **HPLC conditions for purity analysis**: Phenomenex Luna C18(2) 100Å column, 4.6 × 250 mm, 5 μm; mobile phase: 75% MeOH / 25% H_2O with 0.1% formic acid; flow rate: 1mL/min. All tested compounds were found to be at least 96% purity. **Chiral separation:** semi-preparative enantiomeric separations were carried out using an Agilent Technologies 1200 Series HPLC system equipped with a DAD and a normal phase ChiralPak® IA (10.0 × 250 mm, 5 μm) chiral column from Daicel Chemical Industries Ltd. **Optical rotations:** These were measured on an AA-65 Angular Scale automatic polarimeter from Optical Activity Limited with a 1 dm cell at the sodium D line.

The following procedures for the preparation of CF_3-PAMs **1**, **41** and **52**-**56** are representative examples.

2-Phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1H-indole-6-carbonitrile (1)

To a solution of compound **82l** (147 mg, 0.335 mmol) in THF (5 mL) was added 6 M HCl (0.5 mL). The mixture was stirred at 60 °C for 16 h prior to quenching with a saturated solution of $NaHCO_3$ (10 mL) and diluting with CH_2Cl_2 (10 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (4 × 5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was passed through a short plug of silica gel (CH_2Cl_2) and concentrated *in vacuo* prior to use.

To a solution of the crude product in CH_2Cl_2 (5 mL) was added Et_3SiH (233 mg, 320 µL, 2.0 mmol) and trifluoroacetic acid (193 mg, 130 µL, 1.689 mmol) at 0 °C. The resulting red solution was stirred at 23 °C for 16 h before it was quenched with a saturated solution of $NaHCO_3$ (10 mL) and diluted with CH_2Cl_2 (10 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (4 × 5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes:EtOAc 10:1) to give compound **1** (102 mg, 77% for 2 steps) as pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.58 (s, 1H), 7.77 (dd, *J* = 1.3, 0.6 Hz, 1H), 7.61 – 7.46 (m, 6H), 7.34 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.22 (dd, *J* = 5.1, 0.8 Hz, 1H), 6.98 (dd, *J* = 5.1, 3.6 Hz, 1H), 6.93 (dt, J = 3.6, 1.2 Hz, 1H), 4.96 (dd, *J* = 9.5, 4.8 Hz, 1H), 3.35 – 2.93 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 146.9, 140.2, 134.9, 131.2, 129.8, 129.3, 129.1, 128.8, 127.0, 126.0 (q, *J* = 277.9 Hz), 124.5, 124.2, 123.0, 120.7, 120.5, 116.0, 113.6, 104.6, 39.2 (q, *J* = 37.5 Hz), 31.8 (q, *J* = 3.1 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ = -64.34 (t, *J* = 10.2 Hz, 3F). *m/z* (ESI) 397.1 [M+H$^+$].

6-Methyl-2-phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1H-indole (41)

Using the method analogous to prepare compound **1**, with Et_3SiH (176 mg, 242 µL, 1.51 mmol), compound **82a** (108 mg, 0.25 mmol) and trifluoroacetic acid (144 mg, 97 µL, 1.26 mmol) were employed. Purification by flash column chromatography (silica gel, hexanes:EtOAc 10:1) to give compound **41** (64 mg, 66% for 2 steps) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.95 (s, 1H), 7.62 – 7.36 (m, 6H), 7.23 (s, 1H), 7.20 (dd, *J* = 4.2, 2.4 Hz, 1H), 7.04 – 6.91 (m, 3H), 4.97 (dd, *J* = 9.2, 4.8 Hz, 1H), 3.30 – 3.16 (m, 1H), 3.16 – 3.00 (m, 1H), 2.53 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 148.0, 136.7, 135.4, 132.6, 132.3, 128.9 128.7, 128.3, 126.8, 126.3 (q, *J* = 278.1 Hz), 124.5, 124.2, 124.1, 121.7, 119.9,

112.8, 111.3, 39.6 (q, *J* = 27.1 Hz), 32.1 (q, *J* = 3.2 Hz), 21.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -64.42 (t, *J* = 10.7 Hz, 3F). *m/z* (ESI) 386.1 [M+H$^+$].

(2-Phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1H-indol-6-yl)methanol (52)

To a solution of compound **50** (15 mg, 0.035 mmol) in THF (2 mL) was added a solution of Super-Hydride® (105 µL, 0.105 mmol, 1.0 M in THF) at 0 °C. The resulting mixture was stirred at 0 °C and allowed to warm to 23 °C for 16 h before it was quenched with a saturated solution of NH_4Cl (10 mL) and diluted with CH_2Cl_2 (10 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl (4 x 5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes:EtOAc 5:1 → 1:1) to give compound **52** (13 mg, 94%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.13 (s, 1H), 7.69 – 7.35 (m, 7H), 7.19 (dd, *J* = 4.7, 1.5 Hz, 1H), 7.12 (d, *J* = 8.1 Hz, 1H), 7.02 – 6.83 (m, 2H), 4.95 (dd, *J* = 9.3, 4.8 Hz, 1H), 4.81 (s, 2H); ^{19}F NMR (376 MHz, $CDCl_3$) δ = -64.54 (t, *J* = 10.6 Hz, 3F). *m/z* (ESI) 424.1 [M+Na$^+$].

N-(2-Phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1H-indol-6-yl)acetamide (53)

To a solution of compound **87** (15 mg, 0.027 mmol)in acetonitrile (2 mL) in a PTFE container was added an aqueous solution of HF (1 mL, 48 % wt) at 0 °C. The resulting solution was allowed to warm to 23 °C and stirred for 16 h before it was quenched with a saturated solution of $NaHCO_3$ (20 mL). The mixture was stirred at 23 °C for 10 min before EtOAc (20 mL) was added. The layers were separated and the organic layer was extracted with H_2O (2 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*.

The residue was re-dissolved in MeOH (2 mL) and K_2CO_3 was added at 0 °C. The mixture was stirred at 0 °C for 30 min prior to quenching with a saturated solution of NH_4Cl and diluted with EtOAc (30 mL). The layers were separated and the organic layer was extracted with H_2O (2 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes:EtOAc 3:1) to give compound **53** (9 mg, 78% for 2 steps) as pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.56 (brs, 1H), 8.14 (d, *J* = 1.5 Hz, 1H), 7.57 – 7.45 (m, 4H), 7.45 – 7.36 (m, 2H), 7.31 (s, 1H), 7.18 (dd, *J* = 5.0, 1.1 Hz, 1H), 6.97 – 6.89 (m, 2H), 6.79 (dd, *J* = 8.5, 1.8 Hz, 1H), 4.92 (dd, *J* = 8.9, 4.9 Hz, 1H), 3.22 – 2.93 (m, 2H), 2.17 (s, 3H); ^{13}C NMR (101 MHz,

$CDCl_3$) δ = 168.3, 148.2, 142.3, 137.8, 134.2, 132.9, 129.0, 128.6, 128.2, 126.9, 126.4 (q, *J* = 278.5 Hz), 124.3, 121.1, 120.4, 113.1, 110.9, 97.0, 39.1 (q, *J* = 26.8 Hz), 32.2 (q, *J* = 2.8 Hz), 24.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -64.42 (t, *J* = 10.4 Hz, 3F). *m/z* (ESI) 429.1 [M+H$^+$].

1-(2-Phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1H-indol-6-yl)ethan-1-one (54)

Using the method analogous to prepare compound **53**, with compound **85** (22 mg, 0.04 mmol) was employed. Purification by flash column chromatography (silica gel, hexanes:EtOAc 3:1) to give compound **54** (12 mg, 72% for two steps) as a colourless oil. ^1H NMR (400 MHz, $CDCl_3$) δ = 8.59 (s, 1H), 8.13 (s, 1H), 7.74 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.59 – 7.42 (m, 6H), 7.20 (d, *J* = 4.8 Hz, 1H), 7.02 – 6.86 (m, 2H), 4.96 (dd, *J* = 9.5, 4.6 Hz, 1H), 3.18 – 3.03 (m, 2H), 2.67 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 198.2, 147.3, 140.1, 135.7, 131.7, 131.6, 130.4, 129.1, 128.7, 126.9, 126.2 (q, *J* = 276.2 Hz), 124.4, 124.2, 120.4, 119.7, 113.3, 112.2, 39.4 (q, *J* = 27.1 Hz), 31.9 (q, *J* = 2.8 Hz), 26.8; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -64.39 (t, *J* = 10.4 Hz, 3F). *m/z* (ESI) 414.1 [M+H$^+$].

2-Phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1H-indol-6-amine (55)

Using the method analogous to prepare compound **53**, with compound **88** (30 mg, 0.058 mmol) was employed. Purification by flash column chromatography (silica gel, hexanes:EtOAc 1:1) gave compound **55** (18 mg, 80% for 2 steps) as pale yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ = 7.86 (brs, 1H), 7.53 – 7.42 (m, 4H), 7.41 (dd, *J* = 5.2, 3.6 Hz, 1H), 7.31 (s, 1H), 7.20 – 7.13 (m, 1H), 6.94 (d, *J* = 3.6 Hz, 2H), 6.72 (d, *J* = 1.8 Hz, 1H), 6.56 (dd, *J* = 8.4, 2.0 Hz, 1H), 4.91 (dd, *J* = 8.8, 5.1 Hz, 1H), 3.28 – 2.55 (m+brs, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 148.0, 142.2, 137.6, 134.0, 132.7, 128.9, 128.5, 128.0, 126.7, 126.3 (q, *J* = 277.1 Hz), 124.1, 120.9, 120.2, 112.9, 110.7, 96.8, 39.6 (q, *J* = 26.8 Hz), 32.0 (q, *J* = 3.2 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ = -64.45 (t, *J* = 10.6 Hz, 3F). *m/z* (ESI) 387.1 [M+H$^+$].

N-(2-Phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1H-indol-6-yl)methanesulfonamide (56)

Using the method analogous to prepare compound **53**, with compound **89** (32 mg, 0.054 mmol) was employed. Purification by flash column chromatography (silica gel, hexanes:EtOAc 1:1) gave compound **56** (20 mg, 81% for 2 steps) as pale yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ = 8.29 (brs, 1H), 7.64 – 7.40 (m, 7H), 7.19 (dd, *J* = 5.0, 1.2 Hz, 1H), 7.02 – 6.91 (m, 2H), 6.89 (dd, *J* = 8.5, 2.0 Hz, 1H), 6.67 (brs, 1H), 4.94 (dd, *J* = 9.1, 4.9 Hz, 1H), 3.22 – 3.01 (m, 2H), 3.00 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$)

δ = 147.5, 137.1, 136.5, 132.0, 131.1, 129.0, 128.7, 126.8, 126.3 (q, *J* = 276.8 Hz), 125.0, 124.3, 124.1, 120.9, 115.3, 112.8, 105.7, 39.4 (q, *J* = 27.2 Hz), 39.0, 31.9 (q, *J* = 3.1 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ = -64.38 (t, *J* = 10.4 Hz, 3F). *m/z* (ESI) 464.1 [M+H$^+$].

3-Ethynyl-1-(methoxymethyl)-6-methyl-2-phenyl-1H-indole (79a)

To a solution of 1-(methoxymethyl)-6-methyl-2-phenyl-1*H*-indole (575 mg, 2.29 mmol) in CH_2Cl_2 (10 mL) was added *N*-iodosuccinimide (525 mg, 2.33 mmol) at 0 °C. The reaction mixture was stirred for 3 h before it was quenched with a saturated solution of Na_2SO_3 (5 mL) and diluted with H_2O (10 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to give the corresponding 3-iodo-indole, which was used without further purification.

To a solution of the crude 3-iodo-indole in DMF (5 mL) was added trimethylsilylacetylene (337 mg, 475 µL, 3.43 mmol), $PdCl_2(PPh_3)_2$ (80 mg, 0.114 mmol), CuI (26 mg, 0.137 mmol) and Et_3N (695 mg, 957 µL, 6.87 mmol) at 23 °C. The reaction mixture was stirred for 6 h at 23 °C before it was quenched with a saturated solution of NH_4Cl (10 mL) and diluted with EtOAc (50 mL). The layers were separated, and the organic layer was extracted with H_2O (3 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was passed through a short plug of silica gel (CH_2Cl_2) and concentrated *in vacuo* prior to use.

To a solution of the crude product was added a solution of tetra-*n*-butylammonium fluoride (2.8 mL, 2.8 mmol, 1.0 M in THF) at 0 °C. The reaction mixture was stirred for 2 h at 23 °C before it was it was quenched with a saturated solution of NH_4Cl (10 mL) and diluted with EtOAc (50 mL). The layers were separated, and the organic layer was extracted with H_2O (3 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes:EtOAc 5:1) to give compound **79a** (566 mg, 90% for 3 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.76 – 7.66 (m, 1H), 7.59 – 7.44 (m, 5H), 7.40 – 7.33 (m, 1H), 7.15 (d, *J* = 8.0 Hz, 1H), 5.39 (s, 2H), 3.28 (s, 3H), 3.18 (s, 1H), 2.56 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 144.4, 137.1, 133.6, 131.2, 130.5, 130.3, 128.8, 128.5, 127.2, 123.4, 119.7, 110.5, 79.7, 78.00, 74.9, 56.0, 22.0. *m/z* (ESI) 276.1 [M+H$^+$].

3-Ethynyl-1-(methoxymethyl)-2-phenyl-1H-indole-6-carbonitrile (79g)

Using the method analogous to prepare compound **79a**, with compound **94d** (700 mg, 2.67 mmol), *N*-iodosuccinimide (616 mg, 2.74 mmol), trimethylsilylacetylene (394 mg, 555 µL, 4.01 mmol), $PdCl_2(PPh_3)_2$ (94 mg, 0.134 mmol), CuI (25 mg, 0.131 mmol), Et_3N (813 mg, 1.12 mL, 8.03 mmol) and tetra-*n*-butylammonium fluoride (3.40 mL, 3.40 mmol, 1.0 M in THF) were used. Purification by flash column chromatography (silica gel, hexanes:EtOAc 5:1) gave compound **79g** (596 mg, 78% for 3 steps) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.88 (d, *J* = 0.5 Hz, 1H), 7.85 (dd, *J* = 8.2, 0.5 Hz, 1H), 7.72 (d, *J* = 1.8 Hz, 1H), 7.70 (d, *J* = 1.4 Hz, 1H), 7.61 – 7.49 (m, 4H), 5.41 (s, 2H), 3.30 (s, 3H), 3.19 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 147.8, 135.5, 132.5, 130.2, 129.7, 129.1, 129.0, 128.8, 124.6, 120.9, 120.2, 115.5, 106.1, 80.9, 76.2, 75.3, 56.3. *m/z* (ESI) 309.1 [M+Na^+].

1-(Methoxymethyl)-6-methyl-2-phenyl-3-(3,3,3-trifluoroprop-1-yn-1-yl)-1H-indole (80a)

Using a method described by Blanchard,[27] to a solution of *N,N,N´,N´*-tetramethylethylenediamine (324 mg, 418 µL, 2.79 mmol) in DMF (5 mL) was added CuI (531 mg, 2.79 mmol) and K_2CO_3 (771 mg, 5.58 mmol) at 23 °C. The resulting blue mixture was stirred vigorously at 23 °C under air (1 atm) for 20 min before trimethyl(trifluoromethyl)silane (529 mg, 550 µL, 3.72 mmol) was added and the resulting mixture was stirred for further 15 min under air. After which time, the deep green mixture was cooled to 0 °C prior to adding a mixture of compound **79a** (512 mg, 1.86 mmol) and trimethyl(trifluoromethyl)silane (529 mg, 550 µL, 3.72 mmol) in DMF (2 mL). The reaction mixture was stirred at 0 °C for 30 min and allowed to warm to 23 °C for 6 h before it was quenched with a saturated solution of NH_4Cl (10 mL) and diluted with EtOAc (50 mL). The layers were separated, and the organic layer was extracted with H_2O (3 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes:EtOAc 4:1) to give compound **80a** (556 mg, 87%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.75 – 7.64 (m, 3H), 7.64 – 7.47 (m, 3H), 7.39 (s, 1H), 7.19 (d, *J* = 8.0 Hz, 1H), 5.40 (s, 2H), 3.32 (s, 3H), 2.58 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 147.0 (q, *J* = 1.7 Hz), 137.1, 134.3, 130.1, 129.50, 129.47, 128.8, 126.5, 124.1, 119.4, 115.5 (q, *J* = 256.2 Hz), 110.9, 93.6 (q, *J* = 2.5 Hz), 83.3 (q, *J* = 6.2

Hz), 78.7 (q, *J* = 52.2 Hz), 75.0, 56.1, 22.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -48.59 (s, 3F). *m/z* (ESI) 344.1 [M+H$^+$].

1-(Methoxymethyl)-2-phenyl-3-(3,3,3-trifluoroprop-1-yn-1-yl)-1H-indole-6-carbonitrile (80g)

Using the method analogous to prepare compound **80a**, with compound **79g** (300 mg, 1.05 mmol), *N,N,N´,N´*-tetramethylethylenediamine (185 mg, 255 µL, 1.57 mmol), CuI (300 mg, 1.58 mmol), K_2CO_3 (435 mg, 3.15 mmol) and trimethyl(trifluoromethyl)silane (596 mg, 620 µL, 4.19 mmol) were used. Purification by flash column chromatography (silica gel, hexanes:EtOAc 6:1) gave compound **80g** (352 mg, 95%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.92 (s, 1H), 7.83 (d, *J* = 8.2 Hz, 1H), 7.72 – 7.65 (m, 2H), 7.64 – 7.59 (m, 3H), 7.56 (dd, *J* = 8.2, 1.2 Hz, 1H), 5.44 (s, 2H), 3.33 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 150.5 (q, *J* = 1.7 Hz), 135.6, 131.8, 130.4, 130.0, 129.0, 128.2, 125.3, 120.7, 119.7, 115.9, 115.1 (q, *J* = 256.4 Hz), 106.9, 94.5 (q, *J* = 2.2 Hz), 81.1 (q, *J* = 6.2 Hz), 79.5 (q, *J* = 52.4 Hz), 75.4, 56.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ = -49.13 (s, 3F). *m/z* (ESI) 355.1 [M+H$^+$].

(E)-1-(Methoxymethyl)-6-methyl-2-phenyl-3-(3,3,3-trifluoro-1-iodoprop-1-en-1-yl)-1H-indole (81a)

Using a modified method described by Zhong,[28] to a solution of compound **80a** (420 mg, 1.22 mmol) in CH_2Cl_2 (4 mL) was added LiI (180 mg, 1.35 mmol) and HOAc (1 mL) at 0 °C. The resulting mixture was stirred at 23 °C for 16 h before it was quenched with a saturated solution of $NaHCO_3$ (10 mL) and diluted with CH_2Cl_2 (10 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes:EtOAc 10:1) to give compound **81a** (558 mg, 97%) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.65 – 7.48 (m, 6H), 7.38 (s, 1H), 7.24 – 7.14 (m, 1H), 6.71 (q, *J* = 7.1 Hz, 1H), 5.38 (s, 2H), 3.25 (s, 3H), 2.58 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 137.5, 137.0, 133.6, 132.0 (q, *J* = 33.9 Hz), 130.4, 130.2, 129.0, 128.6, 124.0, 123.3, 121.3 (q, *J* = 274.4 Hz), 119.4, 115.8, 110.6, 104.4 (q, *J* = 6.2 Hz), 74.8, 55.9, 22.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -60.80 (d, *J* = 7.1 Hz, 3F). *m/z* (ESI) 472.0 [M+H$^+$].

(E)-1-(methoxymethyl)-2-phenyl-3-(3,3,3-trifluoro-1-iodoprop-1-en-1-yl)-1H-indole-6-carbonitrile (81g)

Using the method analogous to prepare compound **81a**, with compound **80g** (330 mg, 0.931mmol) and LiI (150 mg, 1.12 mmol). The residue was passed through a short plug of silica gel (CH_2Cl_2) and concentrated *in vacuo* to give compound **81g**, which was used without further purification.

(E)-1-(Methoxymethyl)-6-methyl-2-phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)prop-1-en-1-yl)-1H-indole (82a)

To a solution of compound **81a** (146 mg, 0.31 mmol) in DMF (5 mL) was added tri-*n*-butyl(thiophen-2-yl)stannane (175 mg, 0.47 mmol), P(*o*-Tol)$_3$ (14 mg, 0.046 mmol), $PdCl_2(MeCN)_2$ (7.8 mg, 0.03 mmol) and CuI (9 mg, 0.046 mmol) at 23 °C. The resulting mixture was stirred at 23 °C for 20 min before it was heated to 55 °C for 3 h. After which time, the reaction mixture was quenched with a saturated solution of NH_4Cl (10 mL) and diluted with Et_2O (50 mL). The layers were separated, and the organic layer was extracted with H_2O (3 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes:EtOAc 10:1) to give compound **82a** (120 mg, 90%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.50 – 7.31 (m, 7H), 7.27 (d, *J* = 5.0 Hz, 1H), 7.06 (d, *J* = 8.1 Hz, 1H), 6.98 (d, *J* = 3.4 Hz, 1H), 6.91 (dd, *J* = 4.9, 3.8 Hz, 1H), 6.31 (q, *J* = 8.0 Hz, 1H), 5.46 (s, 2H), 3.24 (s, 3H), 2.57 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 144.3, 138.4 (q, *J* = 5.5 Hz), 137.3, 132.9, 130.8, 130.3, 128.4, 128.3, 128.1, 127.7, 127.4, 126.2, 123.1 (q, *J* = 270.6 Hz), 123.0, 119.4, 115.1 (q, *J* = 33.4 Hz),111.1, 110.5, 74.8, 55.6, 22.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -58.23 (d, *J* = 8.0 Hz, 3F). *m/z* (ESI) 428.1 [M+H$^+$].

(E)-1-(methoxymethyl)-2-phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)prop-1-en-1-yl)-1H-indole-6-carbonitrile (82l)

Using the method analogous to prepare compound **82a**, with compound **81g** (449 mg, 0.931 mmol), tri-*n*-butyl(thiophen-2-yl)stannane (522 mg, 1.40 mmol), P(*o*-Tol)$_3$ (43 mg, 0.141 mmol), $PdCl_2(MeCN)_2$ (24 mg, 0.093 mmol) and CuI (18 mg, 0.095 mmol) were used. Purification by flash column chromatography (silica gel, hexanes:EtOAc 5:1) gave compound **82l** (334 mg, 82%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.93 (s, 1H), 7.48 (d, *J* = 8.3 Hz, 1H), 7.45 – 7.32 (m, 6H), 7.31 – 7.23 (m,

1H), 6.93 – 6.83 (m, 2H), 6.30 (q, *J* = 8.2 Hz, 1H), 5.47 (s, 2H), 3.26 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 143.5, 142.6, 137.1 (q, *J* = 6.0 Hz), 135. 6, 131.1, 130.1, 129.5, 129.3, 128.5, 128.2, 127.89, 127.86, 124.2, 123.3 (q, *J* = 268.2 Hz), 121.5, 120.3, 115.9 (q, *J* = 34.2 Hz), 115.7, 111.4, 105.3, 75.1, 56.0; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -58.18 (d, *J* = 8.2 Hz, 3F). *m/z* (ESI) 439.1 [M+H$^+$].

Methyl 2-phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1-((2-(trimethylsilyl)-ethoxy)methyl)-1H-indole-6-carboxylate (83)

To a solution of compound **50** (540 mg, 1.257 mmol) in THF (3 mL) was added NaH (75 mg, 1.886 mmol, 60 % in mineral oil) at 0 °C. After stirring at 0 °C for 30 min, 2-(trimethylsilyl)ethoxymethyl chloride (SEM-Cl, 282 mg, 300 µL, 1.51 mmol, 90% technical grade) was added. The resulting reaction mixture was stirred at 0 °C and allowed to warm to 23 °C for 3 h before it was quenched with a saturated solution of NH_4Cl (5 mL) and diluted with EtOAc (50 mL). The layers were separated and the organic layer was extracted with H_2O (4 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes:EtOAc 5:1) to give compound **83** (680 mg, 97%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.33 (s, 1H), 7.85 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.59 – 7.39 (m, 6H), 7.16 (d, *J* = 5.0 Hz, 1H), 6.93 (dd, *J* = 5.1, 3.6 Hz, 1H), 6.86 (d, *J* = 3.5 Hz, 1H), 5.41 (s, 2H), 4.65 (dd, *J* = 9.9, 4.3 Hz, 1H), 3.97 (s, 3H), 3.34 (ABq, *J* = 7.6 Hz, 2H), 3.26 – 3.08 (m, 1H), 3.08 – 2.92 (m, 1H), 0.80 (ABq, *J* = 7.6 Hz, 2H), -0.07 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 167.9, 147.3, 142.0, 136.7, 130.8, 130.2, 129.6, 129.3, 128.6, 126.7, 126.1 (q, *J* = 277.1 Hz), 124.2, 124.0, 123.8, 121.5, 119.4, 114.7, 113.1, 94.3, 72.9, 65.8, 52.0, 39.0 (q, *J* = 27.5 Hz), 32.1 (q, J = 2.6 Hz), 17.8, -1.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -64.20 (t, *J* = 10.4 Hz, 3F). *m/z* (ESI) 560.2 [M+H$^+$].

N-Methoxy-N-methyl-2-phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indole-6-carboxamide (84)

Using a modified method described by Williams,[33] to a solution of compound **83** (408 mg, 0.729 mmol) and *N,O*-dimethylhydroxylamine hydrochloride (108 mg, 1.11 mmol) in THF (3 mL) was added a solution of isopropylmagnesium chloride (1.10 mL, 2.19 mmol, 2.0 M in THF) at 0 °C. After stirring at 0 °C for 30 min, the solution was allowed to warm to 23 °C and stirred for further 3 h before it was

quenched with a saturated solution of NH_4Cl (5 mL) and diluted with EtOAc (50 mL). The layers were separated and the organic layer was extracted with H_2O (4 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes:EtOAc 3:1) to give compound **84** (326 mg, 76%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.00 (s, 1H), 7.61 – 7.39 (m, 7H), 7.16 (dd, *J* = 5.1, 0.9 Hz, 1H), 6.92 (dd, *J* = 5.1, 3.6 Hz, 1H), 6.87 (dt, *J* = 3.5, 1.2 Hz, 1H), 5.38 (ABq, *J* = 12.7 Hz, 2H), 4.64 (dd, *J* = 9.7, 4.3 Hz, 1H), 3.65 (s, 3H), 3.42 (s, 3H), 3.33 (ddd, *J* = 9.5, 6.2, 1.9 Hz, 2H), 3.25 – 3.09 (m, 1H), 3.12 – 2.89 (m, 1H), 0.80 (ddd, *J* = 9.5, 6.2, 1.9 Hz, 2H), -0.07 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.7, 147.4, 140.9, 136.4, 130.9, 130.4, 129.2, 128.6, 127.9, 127.8, 126.7, 126.2 (q, *J* = 278.5 Hz), 124.2, 123.8, 120.7, 119.3, 114.5, 111.8, 73.0, 65.7, 61.0, 39.0 (q, *J* = 27.2 Hz), 34.4, 32.1 (q, *J* = 2.8 Hz), 17.8, -1.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -64.19 (t, *J* = 10.3 Hz, 3F). *m/z* (ESI) 589.2 [M+H$^+$].

1-(2-Phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indol-6-yl)ethan-1-one (85)

To a solution of compound **84** (408 mg, 0.54 mmol) in THF (3 mL) was added a solution of methyllithium (860 µL, 0.860 mmol, 1.6 M in Et_2O) at -78 °C. After stirring at -78 °C for 30 min, the solution was allowed to warm to 0 °C and stirred for further 3 h before it was quenched with a saturated solution of NH_4Cl (5 mL) and 0.1 M HCl solution (5 mL). The mixture was stirred at 23 °C for 10 min before EtOAc (50 mL) was added. The layers were separated and the organic layer was extracted with H_2O (4 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes:EtOAc 5:1) to give compound **85** (252 mg, 86%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.26 (s, 1H), 7.80 (dd, *J* = 8.4, 1.4 Hz, 1H), 7.64 – 7.41 (m, 6H), 7.17 (dd, *J* = 5.1, 1.0 Hz, 1H), 6.94 (dd, *J* = 5.1, 3.6 Hz, 1H), 6.90 – 6.84 (m, 1H), 5.44 (s, 2H), 4.66 (dd, *J* = 10.0, 4.2 Hz, 1H), 3.41 – 3.30 (m, 2H), 3.25 – 3.10 (m, 1H), 3.09 – 2.95 (m, 1H), 2.71 (s, 3H), 0.85 – 0.74 (m, 2H), -0.06 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 198.0, 147.3, 142.5, 136.8, 131.7, 130.8, 130.1, 129.8, 129.4, 128.6, 126.8, 126.1 (q, *J* = 277.4 Hz), 124.3, 123.8, 120.8, 119.5, 114.8, 111.8, 73.0, 65.9, 38.9 (q, *J* = 27.6 Hz), 32.1 (q, *J* = 3.3 Hz), 26.82, 17.78, -1.49; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -64.14 (t, *J* = 10.3 Hz, 3F). *m/z* (ESI) 544.2 [M+H$^+$].

(E)-/(Z)-1-(2-Phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1-((2-(trimethyl-silyl)ethoxy)methyl)-1H-indol-6-yl)ethan-1-one oxime (86)

To a solution of compound **85** (220 mg, 0.405 mmol) in MeOH (5 mL) was added hydroxylamine hydrochloride (34 mg, 0.489 mmol) and sodium acetate (50 mg, 0.61 mmol). The resulting mixture was heated to 70 °C for 4 h before it was cooled to 23 °C and quenched with a saturated solution of NH_4Cl (10 mL) and then diluted with CH_2Cl_2 (10 mL). The layers were separated and the aqueous layer was extracted with CH_2Cl_2 (4 x 5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to give oxime **86** as a mixture of (*E*)- and (*Z*)-isomers. Oxime **86** was used without further purification.

N-(2-Phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1-((2-(trimethylsilyl)-ethoxy)methyl)-1H-indol-6-yl)acetamide (87)

Using a modified method described by Giacomelli,[34] 2,4,6-trichloro-[1,3,5]triazine (cyanuric chloride, 32 mg, 0.445 mmol) was added DMF (1 mL) at 23 °C and stirred for 30 min before a solution of oxime **86** (226 mg, 0.404 mmol) in DMF (1 mL) was added. The resulting mixture was stirred for 4 h at 23 °C prior to quenching with a saturated solution of $NaHCO_3$ (10 mL) and diluted with EtOAc (50 mL). The layers were separated and the organic layer was extracted with H_2O (4 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (silica gel, hexanes:EtOAc 1:1) to give compound **87** (208 mg, 92% for 2 steps) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.01 (d, *J* = 1.0 Hz, 1H), 7.59 (s, 1H, NH), 7.55 – 7.42 (m, 5H), 7.40 (d, *J* = 8.5 Hz, 1H), 7.14 (d, *J* = 5.0 Hz, 1H), 7.05 (dd, *J* = 8.5, 1.5 Hz, 1H), 6.96 – 6.88 (m, 1H), 6.86 (d, *J* = 3.4 Hz, 1H), 5.31 (s, 2H), 4.62 (dd, *J* = 9.5, 4.3 Hz, 1H), 3.32 (ddd, *J* = 9.7, 5.2, 3.0 Hz, 2H), 3.21 – 3.07 (m, 1H), 3.07 – 2.91 (m, 1H), 2.19 (s, 3H), 0.82 – 0.73 (m, 2H), -0.07 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 168.4, 147.8, 138.9, 137.5, 133.1, 131.0, 130.7, 128.9, 128.5, 126.7, 126.2 (q, *J* = 279.0 Hz), 124.1, 123.7, 123.0, 120.0, 114.3, 114.0, 103.1, 72.8, 65.6, 39.0 (q, *J* = 27.1 Hz), 32.1 (q, *J* = 2.7 Hz), 24.6, 17.8, -1.5; ^{19}F NMR (376 MHz, $CDCl_3$) δ = -64.19 (t, *J* = 10.3 Hz, 3F). *m/z* (ESI) 559.2 [$M+H^+$].

2-Phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1-((2-(trimethylsilyl)ethoxy)-methyl)-1H-indol-6-amine (88)

To a solution of compound **87** (106 mg, 0.19 mmol) in EtOH (2 mL) in a sealable tube was added hydrazine hydrate (50 µL, 1.03 mmol). The tub was sealed and heated to 100 °C for 16 h before the reaction mixture was quenched with 0.1 M HCl (1 mL) and diluted with EtOAc (50 mL). The layers were separated, and the organic layer was washed with a saturated solution of $NaHCO_3$ (2 x 10 mL) and H_2O (2 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to give compound **32** as a pale yellow solid. Compound **88** was used without further purification.

N-(2-Phenyl-3-(3,3,3-trifluoro-1-(thiophen-2-yl)propyl)-1-((2-(trimethylsilyl)-ethoxy)methyl)-1H-indol-6-yl)methanesulfonamide (89)

To a solution of compound **88** (37 mg, 0.072 mmol) in CH_2Cl_2 (2 mL) was added pyridine (10 µL, 0.124 mmol) at 0 °C followed by methanesulfonyl chloride (6 µL, 0.078 mmol). The resulting mixture was stirred at 0 °C before it was quenched by a saturated solution of $NaHCO_3$ (5 mL) and diluted with EtOAc (20 mL). The layers were separated and the organic layer was extracted with H_2O (2 x 10 mL). The combined organic layers were dried over Na_2SO_4 and concentrated *in vacuo* to give compound **89** as a yellow oil. The residue was used without further purification.

CCI mouse model of neuropathic pain: Subjects consisted of male C57BL/6J mice (6-8 weeks old, body mass of 27–32 g) obtained from the Virginia Commonwealth University Transgenic Mouse Core (Richmond, Virginia). Mice were group-housed (four per cage) for at least one week before the beginning of experiments on a 12/12 light/dark cycle (lights on at 0600 h), with an ambient temperature of 20–22 °C and humidity of 55–60%. Standard rodent chow and water were available ad libitum. Animal experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Virginia Commonwealth University and followed the NIH Guidelines for the Care and Use of Laboratory Animals. (±)-**1** was dissolved in ethanol (5% of total volume), alkamuls-620 (Sanofi-Aventis, Bridgewater, NJ) (5% of total volume), and saline (0.9 % NaCl) (90% of total volume) and administered intraperitoneally in a volume of 10 mL/kg.

The CCI model of neuropathic pain was used to assess the anti-allodynic effects of (±)-**1**. Surgical procedure for chronic constriction of the sciatic nerve was performed as previously described,[35] but modified for the mouse.[36] In brief, the mice were anesthetized with isoflurane (induction 5% vol. followed by 2.0% in oxygen), and the right hind leg was shaved and cleaned with betadine and ethanol. Using aseptic procedures, the sciatic nerve was carefully isolated and loosely ligated using three segments of 5–0 chromic gut sutures (Ethicon, Somerville, NJ, USA). Sham surgery was identical to CCI surgery, but the nerve was neither ligated nor manipulated. The overlying muscle was sutured closed with (1) 4–0 sterile silk suture (Ethicon), and animals recovered from anaesthesia within approximately 5 min. Mice were randomly assigned into the CCI or sham surgical groups.

Behavioral assessment of mechanical allodynia

Following 15/20 minutes of habituation to the testing environment, von Frey filaments were used to determine baseline paw withdrawal responses, as previously described.[37] Mice were unrestrained and were singly placed under an inverted wire mesh basket to allow for unrestricted air flow and the mechanical allodynia was assessed with von Frey filaments (North Coast Medical, Morgan Hill, CA) applied randomly to the left and right plantar surfaces of the hind paw. Lifting, licking or shaking the paw in response to three stimulations was coded as a positive response. Basal von Frey paw withdrawal responses were assessed prior to CCI or sham surgery (pre-sx) and again on post-surgery day 7 prior to drug administration (post-sx). Following the assessment of von Frey thresholds on day 7, CCI and sham mice were assigned to different treatment groups and were given a singular i.p. injection of vehicle or (±)-**1** (40 mg/kg). Each mouse was tested for paw withdrawal thresholds 0.5 h, 1 h, 2 h, 4 h, and 8 h after the injections.

Statistical analysis

For all in vivo data, statistical analyses were performed using the computer program GraphPad Prism version 6.0 (GraphPad Software, Inc., San Diego, CA). Data were analyzed by two-way analysis of variance (ANOVA) with drug treatment as a between subject factor and time as a within subject factor.

A *P* value of <0.05 was considered statistically significant. Following significant ANOVAs, the Bonferroni post-hoc test was used to ascertain further differences for both ipsilateral and contralateral paws. All data are expressed as the mean ± SEM.

PathHunter® β-Arrestin assay.

Beta-arrestin recruitment studies were conducted by Eurofins DiscoverX, using the PathHunter® β-arrestin assay. Briefly, this assay monitors the activation of CB_1 using enzyme fragment complementation, with β-galactosidase as the functional reporter. When the CB_1 receptor is activated and β-arrestin is recruited to the receptor, enzyme complementation occurs, restoring β-galactosidase activity, effecting substrate transformation. Product formation is detected by chemiluminescence. PathHunter cell lines were expanded from freezer stocks according to standard procedures. Cells were seeded in a total volume of 20 µL into white walled, 384-well microplates and incubated at 37 °C for the appropriate time prior to testing. Cells were pre-incubated with sample followed by agonist induction at the EC_{20} concentration of CP55,940 (2 nM). Intermediate dilution of sample stocks was performed to generate 5X sample in assay buffer. 5 µL of 5x sample was added to cells and incubated at 37 °C or room temperature for 30 min. Vehicle concentration was 1%. 5 µL of 6X EC_{20} agonist in assay buffer was added to the cells and incubated at 37 °C or room temperature for 90 or 180 min. Assay signal was generated through a single addition of 12.5 or 15 µL (50% v/v) of PathHunter Detection reagent cocktail, followed by a 1 h incubation at room temperature. Microplates were read following signal generation with a PerkinElmer EnvisionTM instrument for chemiluminescent signal detection. Compound activity was analyzed using CBIS data analysis suite (ChemInnovation, CA). Percentage modulation was calculated using the following formula: % Modulation =100% x ((mean RLU of test sample - mean RLU of EC_{20} control) / (mean RLU of MAX control ligand - mean RLU of EC_{20} control)).

Hit Hunter® cAMP assay

cAMP generation studies were conducted by Eurofins DiscoverX as described previously.[7] Briefly, cells were pre-incubated with sample followed by agonist induction at the EC_{20} concentration (0.09 nM

CP55,940). Media was aspirated from cells and replaced with 10 µL 1:1 HBSS/10 mM Hepes : cAMP XS+ Ab reagent. Intermediate dilution of sample stocks was performed to generate 4X sample in assay buffer. 5 µL of 4X compound was added to the cells and incubated at room temperature or 37 °C for 30 minutes. 5 µL of 4X EC_{20} agonist was added to the cells and incubated at room temperature or 37 °C for 30 or 60 minutes. EC_{80} forskolin (20 µM) was included. After appropriate compound incubation, assay signal was generated through incubation with 20 µL cAMP XS+ ED/CL lysis cocktail for 1 h followed by incubation with 20 µL cAMP XS+ EA reagent for 3 h at room temperature. Microplates were read following signal generation with a PerkinElmer EnvisionTM instrument for chemiluminescent signal detection. Compound activity was analyzed using CBIS data analysis suite (ChemInnovation, CA). Percentage modulation is calculated using the following formula: % Modulation =100% x (1-(mean RLU of test sample - mean RLU of MAX control) / (mean RLU of EC_{20} control - mean RLU of MAX control)).

Pharmacokinetics Assays. Routine in vitro stability studies were conducted by Cyprotex Ltd. (Macclesfield, U.K.)

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at DOI: XXXXX. Experimental procedures, characterization of all intermediates and target compounds, and copies of NMR spectra of compounds **1**, **39-57**. Molecular formula strings of target compounds are available.

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R.A.R., M.Z. and I.R.G. are co-founders of Signal Pharma Limited, which owns the I.P. of some of the molecules described in this article.

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ABBREVIATIONS USED

HLM, human liver microsomes; RLM, rat liver microsomes; MLM, mouse liver microsomes SEM, Standard Error of Mean.

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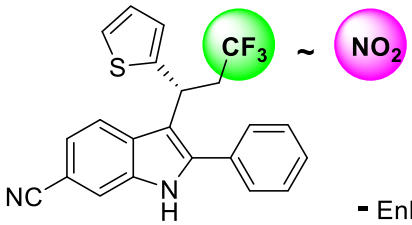
CF_3 as a Bioisostere of Aliphatic NO_2 in CB_1 Positive Allosteric Modulation



(*R*)-(–)-**1**
(ABD1236)

Bioisosteric replacement

- Enhanced potency and metabolic stability
- Retained in vivo activity