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The pentafluorosulfanyl group in cannabinoid receptor ligands: synthesis and comparison with trifluoromethyl and *tert*-butyl analogues†

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An array of cannabinoid ligands, bearing *meta*- and *para*-substituted pentafluorosulfanyl (SF_5) aniline groups in position 3 of the pyrazole ring, was efficiently synthesised and compared with the exact trifluoromethyl and *tert*-butyl analogues. In general, the SF_5 substituted ligands showed higher lipophilicity (*i.e.* log *P* values) than the CF_3 counterparts and lower lipophilicity than the *tert*-butyl ones. In terms of pharmacological activity, SF_5 pyrazoles generally showed slightly higher or equivalent CB_1 receptor affinity (K_i), always in the nanomolar range, and selectivity towards the CB_2 relative to both CF_3 and *tert*-butyl analogues. Functional β-arrestin recruitment assays were used to determine equilibrium dissociation constants (K_b) and showed that all of the tested SF_5 and CF_3 compounds are CB_1 neutral antagonists. These results confirm the possibility of successfully using an aromatic SF_5 group as a stable, synthetically accessible and effective bioisosteric analogue of the electron-withdrawing CF_3 group, and possibly also of bulky aliphatic groups, for drug discovery and development applications.

Introduction

Pentafluorosulfanyl group

Although only a few fluorinated natural compounds have been isolated,[1] it is well known that introduction of one or more fluorine atoms into a molecule can have profound effects on the binding to a receptor, and can improve both its metabolic stability and bioavailability.[2] Fluorine could be incorporated by fluorination or alternatively *via* a building-block approach. Among the fluorinated motifs, the trifluoromethyl group occupies a prominent role in drug discovery,[3] and several blockbuster drugs display a CF_3 substituent.[4]

In 1960 Sheppard reported for the first time the synthesis of an aromatic compound bearing a pentafluorosulfanyl group, SF_5.[5] However, due to of the inconvenient synthetic access to pentafluorosulfanyl arenes, the breakthrough in the commercialization first and then in the application of SF_5-compounds in drug discovery and materials science came in the late 90s, with the improvement of their synthesis.

The synthesis of pentafluorosulfanyl compounds, their biological applications and properties have been reviewed.[6–9] Importantly, the pentafluorosulfanyl group is often compared to the trifluoromethyl group, and because of its higher lipophilicity,[10] electronegativity,[11] chemical stability and greater steric demand, which is only slightly lower than that of the *tert*-butyl group,[12] the SF_5 group is often referred to as a "super-trifluoromethyl" group (Fig. 1).[13–15]

Cannabinoid receptors

Cannabinoid receptors belong to the G-protein coupled receptors family (GPCRs).[16,17] At least two cannabinoid receptor subtypes have been identified: CB_1 and CB_2,[18] furthermore, the CB_1 type has two splice variants, denominated CB_{1A} and CB_{1B}.[19,20] The distribution of CB_1 receptors is localised predominantly in the brain[21] whereas the CB_2 are present in the peripheral nervous system (PNS) cells.[22] However, recent studies have demonstrated the presence of CB_1 in the PNS[23] and, on the

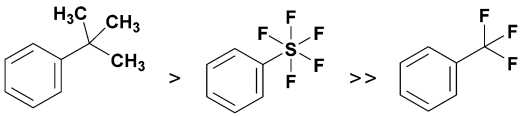

Fig. 1 Steric demand of the three groups compared in this work: ^{t}Bu > SF_5 ≫ CF_3.

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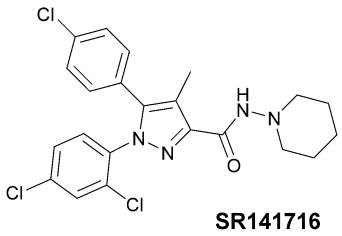


Fig. 2 Chemical structure of Rimonabant (SR141716).

other hand, of the CB_2 in the central nervous system, albeit in low density.[24] Since CB_1 receptors are associated with several disorders, such as depression,[25] anxiety,[26] stress,[27] schizophrenia,[28] chronic pain[29] and obesity,[30] several cannabinoid ligands were developed. Among these ligands, the most studied is probably SR141716 (Rimonabant),[31] a pyrazole-core inverse agonist which was discovered by Sanofi-Synthelabo (now Sanofi-Aventis) in 1994 (Fig. 2), marketed in Europe as anti-obesity drug and subsequently withdrawn from the market owing to its side-effects.

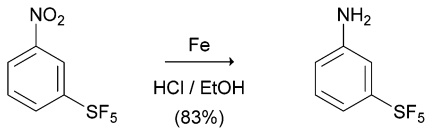
The scientific question we wanted to address in this work was about the position occupied by the SF_5 group relative to its closest bioisosteric substituents, namely CF_3 and *tert*-butyl groups, in terms of its effect on key pharmacological and physico-chemical properties, such as lipophilicity and solubility. To answer, we decided to use a Rimonabant-type scaffold as a model bioactive structure for incorporating an SF_5-group, as well as CF_3 and the *tert*-butyl groups, and directly compare the SF_5 derivatives with their CF_3 and *tert*-Bu counterparts from the pharmacological viewpoint. SF_5, CF_3 or *tert*-butyl groups were incorporated on a carboxy-aniline residue in position 3 of the pyrazole ring, since (1) 3-carboxy-aniline Rimonabant analogues were shown to have excellent CB_1-affinity and selectivity *vs.* the CB_2[32] and (2) SF_5-substituted anilines or nitro-anilines are accessible starting materials (see below).

Since, to the best of our knowledge, SF_5-substituted cannabinoid receptor ligands have never been described in the literature, we decided to synthesise two different classes of pyrazole-core CB_1 receptor ligands: the former based on a Rimonabant-type structure and the latter based on ligands described in a Pharmaness' patent,[33] where the 4-chloro-phenyl ring is replaced by a 2-bromo-thiophene.

Results and discussion

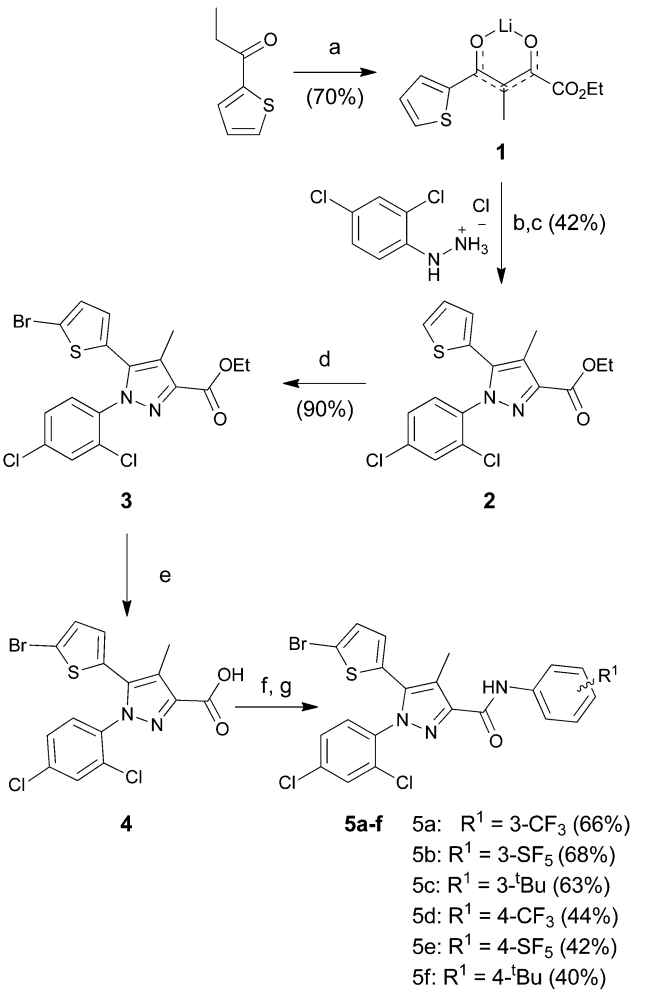
Chemistry

The starting *para*-SF_5-substituted aniline is commercially available whereas *meta*-SF_5-aniline was synthesised from the commercially available 3-nitro-SF_5 benzene (Scheme 1). 3-Nitro-SF_5-benzene was treated with iron-powder in a refluxing HCl–ethanol solution,[34] affording the corresponding 3-(pentafluoro-λ^6-sulfanyl)aniline in good yield (83%). The SF_5 group remained unreactive under these conditions, confirming the high chemical stability of this group.

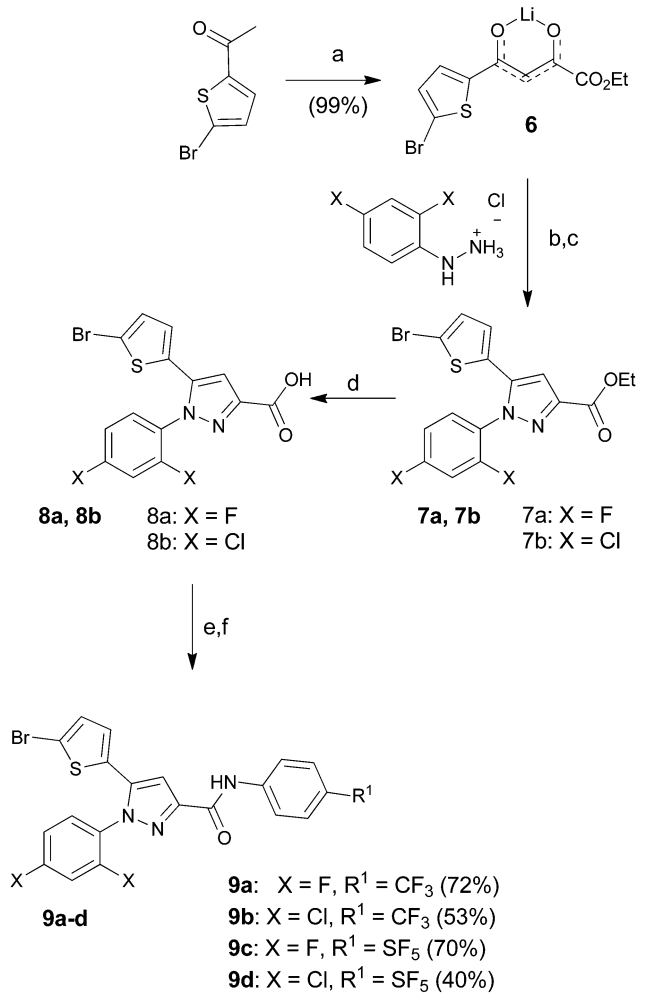


Scheme 1 Reagents and conditions: Fe, HCl–EtOH, reflux, 2 h.

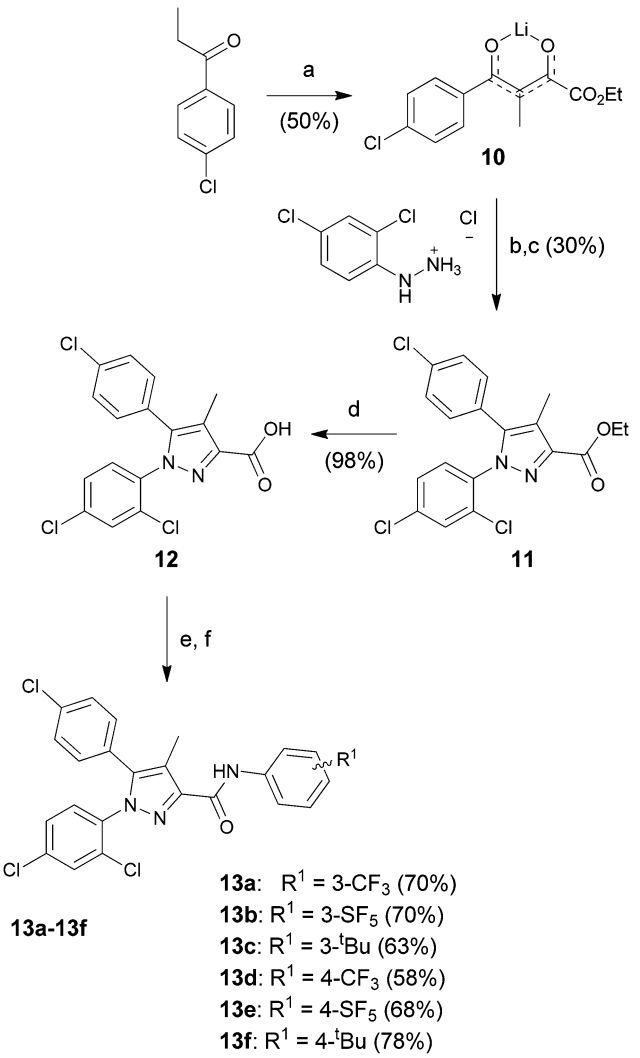
Thiophenyl-compounds **5a–f** and **9a–d** were prepared according to the general synthetic methods shown in Schemes 2 and 3, respectively.[35] 4-Methyl-pyrazole-substituted compounds **5** were synthesised starting with a reaction of 2-propionyl-thiophene with diethyl oxalate using LHMDS as a base which provided the stable lithium salt **1** in moderate yields. The latter was allowed to react first with 2,4-dihalo-phenylhydrazine hydrochloride in ethanol, followed by intramolecular cyclization in refluxing acetic acid to provide the pyrazole ester **2**. Treatment of **2** with NBS afforded the 2-bromothiophene **3** in 90% yield *via* regioselective bromination in position 5 of the thiophene ring. The ester **3** was hydrolysed under basic conditions to give the carboxylic acid **4** in *very good* yield. The acid **4** was first converted into the corresponding acyl chloride with



Scheme 2 Reagents and conditions: (a) diethyl oxalate, LiHMDS, THF/Et_2O (2/1), −78 °C to room temp., 16 h; (b) EtOH, room temp., 24 h; (c) AcOH, 120 °C, 16 h; (d) NBS, CH_3CN, 0 °C to r.t., 16 h; (e) KOH, MeOH, reflux, 3 h; (f) thionyl chloride, toluene, reflux, 3 h; (g) Et_3N, DCM, 0 °C to r.t., 16 h.



Scheme 3 Reagents and conditions: (a) diethyl oxalate, LiHMDS, THF/Et_2O (2/1), −78 °C to room temp, 16 h; (b) EtOH, room temp, 24 h; (c) AcOH, 120 °C, 16 h; (d) KOH, MeOH, reflux, 3 h; (e) thionyl chloride, toluene, reflux, 3 h; (f) Et_3N, DCM, 0 °C to room temp, 16 h.



Scheme 4 Reagents and conditions: (a) diethyl oxalate, LiHMDS, THF/Et2O (2/1), −78 °C to room temp, 16 h; (b) EtOH, room temp, 24 h; (c) AcOH, 120 °C, 16 h; (d) KOH, MeOH, reflux, 3 h; (e) thionyl chloride, toluene, reflux, 3 h; (f) Et_3N, DCM, 0 °C to room temp, 16 h.

thionyl chloride and then reacted with several anilines to afford the desired products **5**.

Similar procedure was used for the synthesis of the pyrazoles **9** (Scheme 3), having no substitution in position 4. In this case, however, the overall synthesis was one-step shorter thanks to the commercial availability of 2-bromo-5-acetyl-thiophene, which allowed us to skip the bromination reaction.

The synthesis of the Rimonabant-like derivatives **13**, shown in Scheme 4, was analogously accomplished following the strategy described by Lan *et al.*[32]

log *P* measurement

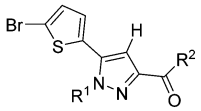
Streich *et al.* showed that an SF_5 substituent on phenoxyacetic acid imparts higher lipophilicity than a CF_3 group.[10] We therefore investigated whether this was the case for our pyrazole cannabinoid ligands too.

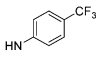
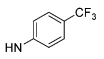
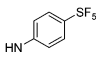
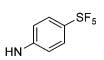
Several software packages allow the prediction of physicochemical molecular properties, including the log *P* (octanol/water partition coefficient). However, there are often significant discrepancies among the calculated values. We therefore decided to set up an experimental method for determining the log *P* of these CB_1 ligands *via* reverse phase-HPLC analysis.

The retention times obtained (which are proportional to the lipophilic character of the molecules) confirmed that molecules bearing the SF_5 moiety are more lipophilic than the CF_3 counterparts and, in general, a substituent in *para* position influences the hydrophobicity of the entire molecule to a greater extent ($\Delta\log P_{(SF_5-CF_3)p} = 0.3$) than in *meta* ($\Delta\log P_{(SF_5-CF_3)m} = 0.2$) (Tables 1 and 2). Not surprisingly, replacement of these fluorinated functions with a strongly lipophilic *tert*-butyl group further increased the log *P* of the molecules.

Replacement of the 4-methyl group on the pyrazole, as in compounds **5** and **13**, with a hydrogen, as in compounds **9**, reduced the lipophilicity of both SF_5 and CF_3 derivatives by $\Delta\log P_{(CH_3-H)SF_5} = 0.7$ and $\Delta\log P_{(CH_3-H)CF_3} = 0.6$ units respectively. A further hydrophilicity enhancement was observed by

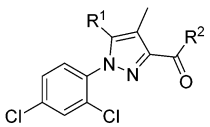
Table 1

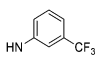
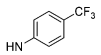
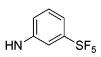
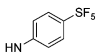
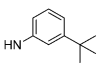
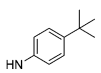
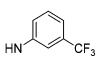
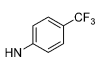
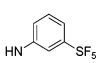
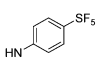
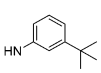
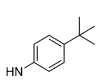


Compound	R^1	R^2	log P^a (±SEM)	CB_1 K_i (nM) (±SEM)	CB_2 K_i (nM) (±SEM)
9a			4.67 (±0.20)	817.8 (±247.2)	2991.0 (±988.2)
9b			5.60 (±0.27)	137.7 (±36.9)	528.4 (±204.8)
9c			4.97 (±0.22)	302.7 (±72.1)	1009.0 (±349.2)
9d			5.89 (±0.30)	58.8 (±13.4)	588.2 (±229.9)

[a] Determined experimentally by means of RP-HPLC (see experimental section for details).

Table 2



Compound	R^1	R^2	log P^a (±SEM)	CB_1 K_i (nM) (±SEM)	CB_2 K_i (nM) (±SEM)
5a			6.19 (±0.32)	50.9 (±6.1)	362.2 (±67.6)
5d			6.34 (±0.34)	65.3 (±13.0)	706.6 (±309.7)
5b			6.42 (±0.35)	66.1 (±15.5)	991.2 (±231.7)
5e			6.61 (±0.37)	7.9 (±3.3)	306.4 (±140.3)
5c			7.09 (±0.40)	19.9 (±4.6)	102.1 (±25.0)
5f			7.04 (±0.41)	106.9 (±30.1)	906.2 (±361.0)
13a			5.97 (±0.30)	26.5 (±6.4)	609.6 (±195.5)
13d			6.14 (±0.32)	31.3 (±9.8)	392.8 (±91.4)
13b			6.21 (±0.33)	11.2 (±2.4)	637.3 (±205.2)
13e			6.41 (±0.35)	17.1 (±3.4)	537.2 (±210.3)
13c			6.95 (±0.38)	8.5 (±1.5)	1950.0 (±852.6)
13f			6.81 (±0.40)	47.9 (±19.8)	2053.0 (±949.8)

[a] Determined experimentally by means of RP-HPLC (see Experimental section for details).

substitution of the 2,4 difluoro aryl group in **9a,c** with the chlorinated analogue in **9b,d** ($\Delta\log P_{(F-Cl)} = 0.9$).

When a 4-chloro-phenyl moiety (compounds **13**) was replaced by a 5-bromo-thiophene group (compounds **5**), the log *P* decreased by $\Delta\log P_{(Thy-Ph)} = 0.2$, despite the benzene group (log *P* benzene = 2.15)[36] is reported to be more hydrophobic than the thiophene group (log *P* thiophene = 1.81).[36] These results are presumably influenced by the presence on the thiophene ring of the bromine atom, which is softer and more hydrophobic than chlorine.

Most of the tabulated log *P* values for the CB_1 inverse agonist Rimonabant (SR141716) were determined *in silico*, and the two experimental values reported in literature, obtained through the flask-shake technique, are quite different ($\log D_{7.4} = 4.6 \pm 0.8$[37] and $\log D_{7.4} = 3.8$[38]). In order to obtain a new experimental value we decided to test SR141716 using the previously described RP-HPLC method, which provided a log *P* value of 4.73 ± 0.20 for Rimonabant. This confirmed that all the new ligands presented in this article exhibit higher hydrophobicity than SR141716.

Binding affinity and SAR

Equilibrium binding assays. The binding affinities for the cannabinoid receptors of all the compounds **5**, **9** and **13** were determined by radio-receptor binding assays using the protocol previously described.[39] In this assay compounds **5**, **9** and **13** were subjected to equilibrium binding studies with the orthosteric agonist probe [^{3}H]CP55940, and the ligands were assayed for their capacity to displace [^{3}H]CP55940 from mouse brain membranes which express high levels of CB_1 receptors and from hCB_2 transfected CHO cells.

Compounds **9a–d** lack a substituent in position 4 of the pyrazole ring, incorporate a (5-bromo-thiophene) residue in position 5 and carry different aryl residues in position 1 (2,4-difluorophenyl for **9a,c** and 2,4-dichlorophenyl for **9b,d**). In this series, we compared the *para*-phenyl substituted compounds **9a** and **9b**, bearing a CF_3 group, with, respectively, **9c** and **9d**, carrying an SF_5 group (Table 1). In both cases, the SF_5 compounds **9c,d** showed higher affinity (lower K_i) than the CF_3 counterparts. In terms of CB_1/CB_2 selectivity, while **9a,c** were comparable, **9d** showed modest but higher CB_1/CB_2 selectivity (10-fold) than **9b** (4-fold).

It has been previously demonstrated that an aliphatic substituent in position 4 of the pyrazole ring imparts higher CB_1 selectivity in pyrazole-based ligands; in particular Chen *et al.* performed a 3D quantitative structure–activity relationship (QSAR) of 5-aryl pyrazole structures using the comparative molecular field analysis (CoMFA),[40] highlighting the importance of the 4-methyl group on pyrazole ring to achieve a better CB_1/CB_2 ratio.

We therefore investigated also the effect of SF_5, CF_3 and *tert*-butyl groups as 3-phenyl-carboxamide substituents in two series of 4-methyl-pyrazole cannabinoid ligands: 5-(5-bromo-thiophene)-pyrazoles **5a–f** and Rimonabant-type 5-(4-chlorophenyl)-pyrazoles **13a–f**.

As expected, the lower apparent K_is of all compounds **5** and **13** (Table 2) relative to **9a–d** confirmed that introduction of a methyl group in position 4 of the pyrazole ring results in an affinity increase *versus* CB_1 and, on the other hand, decreased the E_{max} measured by the displacement assay on CB_2 CHO cells (see Table S1, ESI†).

In the *para*-phenyl substituted series of compounds **5d–f**, the SF_5-compound **5e** showed the highest CB_1 affinity, whereas the presence of the *tert*-butyl group in **5f** caused a substantial drop both in affinity and CB_1/CB_2 selectivity. Interestingly, the CB_1/CB_2 selectivity was higher for the SF_5 derivative **5e** relative to the CF_3 analogue **5d**. In the *meta*-substituted series of compounds **5a–c**, the *tert*-butyl derivative **5c** featured the highest CB_1 affinity, whereas the SF_5 compound **5b** and the CF_3-analogue **5a** showed comparable CB_1 affinities.

For the 4-chlorophenyl Rimonabant-type series of compounds **13a–f** (Table 2), we also observed nanomolar affinities for the CB_1, in the same range of compounds **5a–f**, but the CB_1/CB_2 selectivity was generally higher. In the *meta*-substituted series of compounds **13a–c**, the SF_5 derivative **13b** and the *tert*-butyl **13c** displayed the highest CB_1 affinity, and **13b** showed 2-fold higher affinity than the CF_3 analogue. The situation was somewhat reversed for the *para*-substituted compounds **13d–f** where the SF_5 compound **13e** displayed the lowest apparent CB_1 K_i, whereas **13d** and **13f** showed similar affinities.

However, importantly, all of the 4-methyl-substituted pyrazoles bearing the SF_5 or ^{t}Bu groups in *para* position, namely **5e**, **5f**, **9d**, **9c** and **13f**, displaced [^{3}H]CP55940 only partially at the maximum concentration of 10^{-5} M with E_{max} values ranging from 35.3 to 43.2 (see Fig. 3 and Table S1, ESI†). Considering that the 4-trifluomethyl arene analogue **5d** produced a nearly full displacement of [^{3}H]CP55940, we initially hypothesised that this behaviour could be explained by the binding of the compound to a topographically distinct site on CB_1 (an 'allosteric binding site'). We therefore investigated the effect of thiophenyl **5e** on the dissociation of [^{3}H]CP55940 from mouse brain membranes; as this is the gold standard method of assessing an allosteric interaction.[41] However, compound **5e** had no significant effects on CB_1 agonist dissociation indicating that it is not an allosteric modulator.

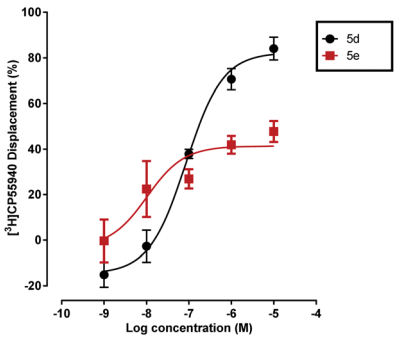


Fig. 3 Effect of compounds **5d,e** on equilibrium binding of [^{3}H]CP55940 to mouse brain membranes. Data shown are mean ± SEM 3–4 independent experiments. Data were best fitted by a sigmoidal concentration–response curve.

At that point, we hypothesised that the partial displacement might be due to a solubility issue at the highest concentrations tested, *i.e.* 10^{-5} M. In this context, Jackson *et al.* had previously shown that, although the S–F bond is longer and more polarizable than C–F one, the entropic cost that derives from dissolving in water a larger group played a major role, resulting in lower S_w (water solubility) values for most of the pentafluorosulfanyl analytes relative to their CF_3 analogues.[42] Laser nephelometry has become the method of choice for measuring solubility of molecules in a drug discovery setting.[43]

We therefore submitted compounds **5e**, **5d** and **5b** to a laser nephelometry assay, for assessing the solubility of each compound at different concentrations. However, these experiments showed that all of the compounds above were soluble at the highest concentration of 10^{-5} M (99.9% aqueous buffer, 0.1% DMSO) (see Table S2, ESI,† for details).

Functional assays. β-Arrestin recruitment assays, such as the PathHunter® β-arrestin assay, can be used for the identification of compounds with an agonistic, antagonistic, inverse agonistic, or allosteric modulation profile for GPCR ligands.[44] Increasing concentrations of an agonist binding to the orthosteric binding site on the receptor will result in a dose response curve where the EC_{50} value is determined as the half maximal response. The addition of a competitive antagonist will result in a significant rightward shift in this dose response curve. This is due to the antagonist competing for the same site on the receptor as the agonist; therefore a higher concentration of agonist is required to reach the same maximal response. This will result in a significant increase in the EC_{50} value obtained.

Using the PathHunter® β-arrestin assay in hCB_1 cells, [^{3}H]CP55940 stimulated β-arrestin recruitment with an EC_{50} of 16.9 nM (11–27 nM) (95% confidence limits for all of the β-arrestin experiments herein described). The fluorinated *para*-substituted CB_1 receptor ligands, **5e**, **5d**, **13e** and **13d** (Table 2) caused a significant rightward shift in the dose response curve of [^{3}H]CP55940, with an EC_{50} of 111.0 nM (82–151 nM), 235.8 nM (157–355 nM), 245.8 nM (194–311 nM) and 439.6 nM (298–649 nM) respectively (see ESI† for the graphics). Analogously, with the fluorinated *meta*-substituted CB_1 receptor ligands **13b**, **13a**, **5b**, and **5a**, [^{3}H]CP55940 stimulated beta-arrestin recruitment with an EC_{50} of 17.2 nM (14–22 nM) and the SF_5 compounds again, caused a significant rightward shift in the dose response curve of [^{3}H]CP55940 with EC_{50} values of 932.6 nM (451–1927 nM), 1528 nM (336–6953 nM), 158.6 nM (116–218 nM) and 57.0 nM (42–77 nM), respectively.

All of the tested compounds produced a significant increase in the EC_{50} values with a rank order of efficacy of **13a**, **13b**, **13d**, **13e**, **5d**, **5b**, **5e**, and **5a**, and should be therefore considered competitive antagonists of [^{3}H]CP55940 for the CB_1 receptor.

Summary and conclusions

We have shown that the pentafluorosulfanyl group can effectively replace a trifluoromethyl group in pyrazole-type cannabinoid ligands. The resulting SF_5-compounds behaved as competitive CB_1 receptor antagonists, which is an interesting property since CB_1 inverse agonists have been reported to produce severe adverse effects that limit their clinical applications, whereas CB_1 neutral antagonists might have improved clinical utility.[45] Furthermore SF_5-compounds showed nanomolar inhibition (K_i) and equilibrium dissociation constants (K_b) for the CB_1 receptor, with moderate CB_1/CB_2 selectivity.

Both affinity and selectivity of SF_5-compounds were generally slightly superior or comparable to that of CF_3 and *tert*-butyl analogues. Experimental lipophilicity (log *P*) was found to follow the trend *tert*-butyl > SF_5 > CF_3. Functional assays showed that all of the tested compounds, belonging to the SF_5 and CF_3 series, are CB_1 neutral antagonists. It is worth noting that some of the compounds, incorporating *para*-SF_5- or *tert*-butyl-aryl groups on the C-3 pyrazole ring, displayed an apparent partial displacement of [^{3}H]CP55940 in the functional assays (E_{max} values ranging from 35.3 to 43.2), while ligands binding to the orthosteric binding pocket would be expected to fully displace the radioligand. One explanation for this observation could be that the compounds are binding to an allosteric pocket. However, this seems unlikely as we observed no change in agonist dissociation. Solubility problems were also excluded by means of nephelometry assays, so we are currently unclear as to the explanation for this observation. Overall, the data collected in this work confirm that (1) an aromatic SF_5 group is an effective bioisosteric analogue of the CF_3 group, and possibly also of bulky aliphatic groups like the *tert*-butyl, (2) it can be successfully used as a substitutent in biologically active compounds and drug candidates.

Experimental section

Chemistry

Solvents, reagents and apparatus. Reagent-grade commercially available solvents and reagents were used without further purification.

NMR data were recorded on Bruker ADVANCE III for ^{1}H at 400.13 MHz, for ^{13}C at 100.58 MHz and for ^{19}F at 376.45 MHz. ^{1}H NMR chemical shifts are reported relative to TMS, and the solvent resonance was employed as the internal standard (CDCl3 $\delta = 7.26$). ^{13}C NMR spectra were recorded with complete proton decoupling, and the chemical shifts are reported relative to TMS with the solvent resonance as internal standard (CDCl3 $\delta = 77.0$). ^{19}F NMR spectra were referenced to $CFCl_3$ as the external standard. All chemical shift (δ) are reported in parts per million (ppm) downfield from TMS and coupling constant (*J*) in Hertz. Splitting patterns are reported as follows: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublet of doublets; t, triplet; td, triplet of doublets; appt, apparent triplet; q, quadruplet; qd, quadruplet of doublets; m, multiplet; br, broad signal.

Mass Analysis was performed using an Agilent 1200 HPLC system coupled to an Agilent G6120 single quadrupole detector equipped with electrospray ionization (ESI) source in direct infusion modality.

Lipophilicities were determined using a Reverse Phase (RP)-HPLC with an Agilent 1200 HPLC system equipped with a DAD, analytical Phenomenex Luna C-18 column (250 × 4.60 mm L × ID, particle size: 5 μ) and an ESI-MS detector.

HRMS analysis were performed using an LTQ Orbitrap XL MS spectrometer.

All reactions were carried out in oven- or flame-dried glassware under nitrogen atmosphere, unless stated otherwise, and were magnetically stirred and monitored by TLC on silica gel (60 F254 pre-coated glass plates, 0.25 mm thickness).

Visualization was accomplished using irradiation with a UV lamp ($\lambda = 254$ nm or $\lambda = 365$ nm), and/or staining with potassium permanganate or ceric ammonium molybdate solution.

Purification of reaction products was performed using flash chromatography on silica gel (60 Å, particle size 40–63 μm) according to the procedure of Still and co-workers.[46]

Yields refer to chromatographically and spectroscopically pure compounds, unless stated otherwise.

3-(Pentafluoro-λ^6-sulfanyl)aniline. To a stirred acidic warm solution (50 °C) of pentafluoro(3-nitrophenyl)-λ^6-sulfane (1.00 g, 3.93 mmol) in ethanol–HCl (11 mL:0.8 mL, 37% v/v), iron powder (1.22 g, 21.63 mmol) was added portionwise. The reaction was refluxed for 2 h. After cooling, the solid was removed by means of filtration; the filtered was diluted with dichloromethane. The organic phase was acidified with HCl 2N. The aqueous phase was separated, basified and extracted with dichloromethane (3 × 50 mL). The combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated to give the crude aniline as a yellow oil (0.72 g, 83% yield): the obtained ^{1}H, ^{12}C and ^{19}F NMR spectra matched to those reported by Bowden *et al.*[34]

*Lithium salt of ethyl 3-methyl-2,4-dioxo-4-(thiophen-2-yl)butanoate (**1**).* To a magnetically stirred solution of lithium bis(trimethylsilyl)amide (61 mL, 60.60 mmol, 1.0 M in THF) in diethyl ether (110 mL) at −78 °C, 1-(2-thienyl)-1-propanone (7 mL, 55.10 mmol) in diethyl ether (42 mL) was added dropwise under a nitrogen atmosphere. After the mixture was stirred at the same temperature for an additional 45 min, diethyl oxalate (9 mL, 66.11 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for another 16 h. The reaction precipitate was filtered, washed with diethyl ether, and dried under vacuum to afford the crude lithium salt **1** (9.50 g, 70%) as a pale-yellow solid. The product was used without further purification. ESMS, calculated *m*/*z* $C_{11}H_{11}LiO_4S$ 246.05 $[M]^+$, found *m*/*z* (relative intensity) 247.0 $[M + H]^+$ (100), 265.0 $[M + H + Na - Li]^+$ (100), 279.0 $[M + H + K - Li]^+$ (100).

*1-(2,4-Dichlorophenyl)-4-methyl-5-thiophen-2-yl-1H-pyrazole-3-carboxylic acid ethyl ester (**2**).* To a solution of lithium salt **1** (8.50 g, 33.83 mmol) in ethanol (26 mL) was added 2,4-dichlorophenylhydrazine hydrochloride (8.85 g, 40.60 mmol) in one portion at room temperature under nitrogen. The resulting mixture was stirred at the same temperature for 24 h. After the reaction was complete, the precipitate was filtered, washed with ethanol and diethyl ether, and dried under vacuum to give a light-yellow solid (7.47 g). This crude solid, without purification, was dissolved in acetic acid (68 mL) and heated to reflux for 16 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate (3 × 50 mL). The combined extracts were washed with water, saturated aqueous sodium bicarbonate, and brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Purification by flash column chromatography on silica gel with DCM gave ester **2** (5.33 g, 42% over two steps) as a white solid: the obtained ^{1}H, ^{12}C and spectra matched to those reported by Tseng *et al.*[35]

*5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid ethyl ester (**3**).* To a magnetically stirred solution of **2** (2.60 g, 6.61 mmol) in acetonitrile (23 mL) was added NBS (1.43 g, 7.94 mmol) in small portions under nitrogen at 0 °C. The resulting mixture was then warmed to room temperature and stirred for 16 h. The reaction was quenched with saturated aqueous sodium thiosulfate and concentrated under reduced pressure to remove acetonitrile. The aqueous layer was extracted with ethyl acetate (2 × 40 mL). The organic layers were combined, washed with water, brine, dried over anhydrous sodium sulfate, and concentrated to give the crude residue, which was subjected to purification by flash chromatography on silica gel with *n*-hexane–ethyl acetate (8 : 2) to afford bromo ester **3** (2.70 g, 90%) as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 7.46 (d, $J = 2.0$ Hz, 1H), 7.36 (d, $J = 8.5$ Hz, 1H), 7.33 (dd, $J = 8.4$, 2.0 Hz, 1H), 6.95 (dd, $J = 3.9$, 0.8 Hz, 1H), 6.64 (dd, $J = 3.9$, 0.8 Hz, 1H), 4.44 (q, $J = 7.1$ Hz, 2H), 2.42 (s, 3H), 1.42 (t, $J = 7.1$ Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.5, 142.9, 136.9, 136.6, 135.7, 133.8, 130.9, 130.2, 130.1, 130.1, 129.3, 127.9, 120.4, 115.0, 61.1, 14.5, 10.0. ESMS, calculated *m*/*z* $C_{17}H_{13}BrCl_2N_2O_2S$ 459.92 $[M]^+$, found *m*/*z* (relative intensity) 460.9 $[M + H]^+$ (100), 482.9 $[M + Na]^+$ (100).

*5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid (**4**).* To a solution of bromo ester **3** (2.64 g, 5.62 mmol) in methanol (26 mL) was added potassium hydroxide (0.73 g, 11.24 mmol) in methanol (8 mL) dropwise at room temperature. The resulting mixture was heated to reflux for 3 h. After hydrolysis was complete, the reaction mixture was cooled to room temperature, poured into ice-water, and acidified with 2N hydrochloric acid. The precipitate was filtered, washed with water, and dried under vacuum to give the thiophene carboxylic acid **4** (2.19 g, 90%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.05 (s, 1H), 7.89 (d, $J = 2.3$ Hz, 1H), 7.73 (d, $J = 8.5$ Hz, 1H), 7.63 (dd, $J = 8.5$, 2.3 Hz, 1H), 7.23 (d, $J = 3.9$ Hz, 1H), 6.88 (d, $J = 3.9$ Hz, 1H), 2.32 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 163.9, 143.3, 136.7, 136.2, 135.9, 133.1, 132.3, 131.5, 130.8, 130.2, 130.0, 129.0, 119.6, 114.6, 10.2. ESMS, calculated *m*/*z* $C_{15}H_9BrCl_2N_2O_2S$ 431.9 $[M]^+$, found *m*/*z* (relative intensity) 470.9 $[M + K]^+$ (100).

*General procedure for the synthesis of compounds **5a–5f**.* The general procedure is illustrated below for compound **5a**.

5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carbonyl chloride. A solution of the crude acid **4** (0.80 g, 1.81 mmol) and thionyl chloride (399 μL, 5.44 mmol) in toluene (12 mL) was refluxed for 3 h. Solvent was evaporated under reduced pressure, and the residue was then re-dissolved in toluene (8 mL) first and then in hexane (5 mL); after evaporation the crude acyl chloride (0.80 g, 98% yield) was obtained as a white solid.

*5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-N-(3-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (**5a**).* A solution in dichloromethane of the carboxylic chloride (1 mL, 0.36 M) obtained previously from **4**, was added dropwise to a solution of 3-(trifluomethyl)aniline (50 μL, 0.40 mmol) and

triethylamine (50 μL, 0.36 mmol) in dichloromethane (0.5 mL) at 0 °C. After stirring at room temperature for 16 h, to the reaction mixture was added water and the organic phase was extracted with dichloromethane (3 × 3 mL). The combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Flash column chromatography on silica gel with *n*-hexane–ethyl acetate (8 : 2) gave carboxamide **5a** (150 mg, 66% yield) as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.87 (s, 1H), 8.01 (s, 1H), 7.85 (d, J = 7.8 Hz, 1H), 7.54 (d, J = 2.1 Hz, 1H), 7.46 (t, J = 8.0 Hz, 1H), 7.39 (dd, J = 8.5, 2.1 Hz, 1H), 7.36 (d, J = 8.4 Hz, 2H), 6.98 (d, J = 3.9 Hz, 1H), 6.68 (d, J = 3.9 Hz, 1H), 2.52 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.4, 144.3, 138.3, 137.7, 136.8, 135.5, 133.7, 131.42 (q, J = 32.4 Hz), 130.6, 130.4, 130.2, 129.9, 129.5, 129.4, 128.1, 125.2, 123.87 (q, J = 272.6 Hz), 122.57, 120.55 (q, J = 3.9 Hz), 119.6, 116.34 (q, J = 4.0 Hz), 115.2; ^{19}F NMR (376 MHz, chloroform-d) δ −62.72. ESMS, calculated m/z $C_{22}H_{13}BrCl_2F_3N_3OS$ 574.93 $[M]^+$, found m/z (relative intensity) 575.9 $[M + H]^+$ (100). HRMS m/z M^+H^+ calcd for $C_{22}H_{13}BrCl_2F_3N_3OS$: 573.9370; found: 573.9361%.

*5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-N-(3-(pentafluoro-λ^6-sulfanyl)phenyl)-1H-pyrazole-3-carboxamide (**5b**).* **5b** (163 mg, 68% yield) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.87 (s, 1H), 8.13 (t, J = 2.0 Hz, 1H), 7.87 (dd, J = 8.1, 1.9 Hz, 1H), 7.54 (d, J = 2.1 Hz, 1H), 7.50 (d, J = 8.3 Hz, 1H), 7.44 (d, J = 8.1 Hz, 1H), 7.40 (dd, J = 8.5, 2.1 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 3.9 Hz, 1H), 6.68 (d, J = 3.9 Hz, 1H), 2.52 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.4, 154.25 (appt, J = 17.1 Hz), 144.2, 138.2, 137.8, 136.9, 135.4, 133.8, 130.6, 130.4, 130.3, 129.8, 129.4, 129.2, 128.1, 122.5, 121.28 (p, J = 4.7 Hz), 119.6, 117.25 (p, J = 4.0 Hz), 115.2, 9.7; ^{19}F NMR (377 MHz, chloroform-d) δ 84.17 (p, J = 149.2 Hz), 62.73 (d, J = 150.0 Hz). ESMS, calculated m/z $C_{21}H_{13}BrCl_2F_5N_3OS_2$ 632.90 $[M]^+$, found m/z (relative intensity) 631.9 $[M - H]^-$ (100). HRMS m/z M^+H^+ calcd for $C_{21}H_{13}BrCl_2F_5N_3OS_2$: 631.9059; found: 631.9052%.

*5-(5-Bromothiophen-2-yl)-N-(3-(tert-butyl)phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (**5c**).* **5c** (87 mg, 63% yield) was obtained as a white solid ^{1}H NMR (400 MHz, chloroform-d) δ 8.71 (s, 1H), 7.64–7.57 (m, 1H), 7.59 (d, J = 2.0 Hz, 1H), 7.54 (dd, J = 1.9, 0.7 Hz, 1H), 7.38 (dd, J = 1.9, 1.3 Hz, 2H), 7.30 (d, J = 8.6 Hz, 1H), 7.18–7.12 (m, 1H), 6.97 (d, J = 3.9 Hz, 1H), 6.67 (d, J = 3.8 Hz, 1H), 2.52 (s, 3H), 1.33 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.2, 152.2, 144.9, 137.5, 137.4, 136.7, 135.6, 133.8, 130.7, 130.4, 130.2, 130.1, 129.2, 128.6, 128.0, 121.2, 119.4, 117.0, 116.9, 115.0, 34.8, 31.3 (x 3), 9.8. ESMS, calculated m/z $C_{25}H_{22}BrCl_2N_3OS$ 563.00 $[M]^+$, found m/z (relative intensity) 564.0 $[M + H]^+$ (100). HRMS m/z M^+H^+ calcd for $C_{25}H_{22}BrCl_2N_3OS$: 562.0122; found: 562.0111%.

*5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-N-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (**5d**).* **5d** (97 mg, 44% yield) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.89 (s, 1H), 7.80 (d, J = 8.5 Hz, 2H), 7.60 (d, J = 8.6 Hz, 2H), 7.54 (d, J = 2.1 Hz, 1H), 7.40 (dd, J = 8.5, 2.1 Hz, 1H), 7.36 (d, J = 8.4 Hz, 1H), 6.98 (d, J = 3.9 Hz, 1H), 6.68 (d, J = 3.9 Hz, 1H), 2.52 (d, J = 0.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.4, 144.3, 140.9, 137.7, 136.9, 135.4, 133.7, 130.6, 130.4, 130.2, 129.8, 129.4, 128.2, 126.25 (q, J = 3.6 Hz), 125.57 (q, J = 32.5 Hz, ×2), 124.13 (q, J = 272.2), 119.6, 119.2 (×2), 115.2, 9.7; ^{19}F NMR (376 MHz, chloroform-d) δ −62.07. ESMS, calculated m/z $C_{22}H_{13}BrCl_2F_3N_3OS$ 574.93 $[M]^+$, found m/z (relative intensity) 575.8 $[M + H]^+$ (100). HRMS m/z M^+H^+ calcd for $C_{22}H_{13}BrCl_2F_3N_3OS$: 573.9370; found: 573.9361%.

*5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-N-(4-(pentafluoro-λ^6-sulfanyl)phenyl)-1H-pyrazole-3-carboxamide (**5e**).* **5e** (100 mg, 42% yield) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.92 (s, 1H), 7.81–7.68 (m, 4H), 7.53 (d, J = 2.1 Hz, 1H), 7.42–7.31 (m, 2H), 6.98 (d, J = 3.9 Hz, 1H), 6.68 (d, J = 3.9 Hz, 1H), 2.51 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.4, 149.03 (appt, J = 17.6 Hz), 144.1, 140.5, 137.8, 136.9, 135.4, 133.7, 130.6, 130.4, 130.2, 129.7, 129.4, 128.1, 126.99 (p, J = 4.7 Hz, ×2), 119.7, 118.8 (×2), 115.2, 9.7; ^{19}F NMR (377 MHz, chloroform-d) δ 85.35 (p, J = 150.1, 149.4 Hz), 63.55 (d, J = 150.0 Hz). ESMS, calculated m/z $C_{21}H_{13}BrCl_2F_5N_3OS_2$ 632.90 $[M]^+$, found m/z (relative intensity) 631.8 $[M - H]^-$. HRMS m/z M^+H^+ calcd for $C_{21}H_{13}BrCl_2F_5N_3OS_2$: 631.9059; found: 631.9048%.

*5-(5-Bromothiophen-2-yl)-N-(4-(tert-butyl)phenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (**5f**).* **5f** (84 mg, 40% yield) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.67 (s, 1H), 7.58 (d, J = 8.7 Hz, 2H), 7.53 (dd, J = 1.9, 0.6 Hz, 1H), 7.41–7.32 (m, 4H), 6.97 (d, J = 3.9 Hz, 1H), 6.67 (d, J = 3.8 Hz, 1H), 2.52 (s, 3H), 1.32 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.2, 147.0, 144.9, 137.4, 136.7, 135.6, 135.2 (×2), 133.8, 130.7, 130.4, 130.2, 129.2, 128.0, 125.8 (×2), 119.6 (×2), 119.4, 115.0, 34.4, 31.4 (×3) 9.8. ESMS, calculated m/z $C_{25}H_{22}BrCl_2N_3OS$ 563.0 $[M]^+$, found m/z (relative intensity) 564.0 $[M + H]^+$ (100). HRMS m/z M^+H^+ calcd for $C_{25}H_{22}BrCl_2N_3OS$: 562.0122; found: 562.0113%.

*Lithium salt of ethyl 4-(5-bromothiophen-2-yl)-2,4-dioxobutanoate (**6**).* To a magnetically stirred solution of lithium bis(trimethylsilyl)amide (23 mL, 22.56 mmol, 1.0 M in THF) in diethyl ether (41 mL) at −78 °C, 1-(5-bromothiophen-2-yl)ethanone (4.25 g, 20.51 mmol) in diethyl ether (16 mL) was added dropwise under a nitrogen atmosphere. After the mixture was stirred at the same temperature for an additional 45 min, diethyl oxalate (3.4 mL, 24.62 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for another 16 h. The reaction precipitate was filtered, washed with diethyl ether, and dried under vacuum to afford the crude lithium salt **6** (6.31 g, 99%) as a pale-yellow solid. The product was used without further purification. ESMS, calculated m/z $C_{10}H_8BrLiO_4S$ 309.9 $[M]^+$, found m/z (relative intensity) 326.9 $[M - Li + H + Na]^+$ (63), 343 $[M - Li + H + K]^+$ (31).

*General procedure for the synthesis of compounds **7a,7b**.* The general procedure is illustrated below for compound **7a**.

*Ethyl 5-(5-bromothiophen-2-yl)-1-(2,4-difluorophenyl)-1H-pyrazole-3-carboxylate (**7a**).* To a solution of lithium salt **6** (3 g, 9.55 mmol) in ethanol (26 mL) was added 2,4-difluorophenylhydrazine hydrochloride (2.143 g, 11.46 mmol) in one portion at room temperature under nitrogen. The resulting mixture was stirred at the same temperature for 24 h. After reaction was complete, the precipitate was filtered, washed with ethanol and diethyl ether, and dried under vacuum to give a light-yellow solid (2.66 g). This crude solid, without purification, was

dissolved in acetic acid (20 mL) and heated to reflux for 16 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate (3 × 70 mL). The combined extracts were washed with water, saturated aqueous sodium bicarbonate, and brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Purification by flash column chromatography on silica gel with DCM gave ester **7a** (2.08 g, 58% over two steps) as a white solid. ^{1}H NMR (400 MHz, chloroform-d) δ 7.51 (td, J = 8.5, 5.7 Hz, 1H), 7.08 (s, 1H), 7.08–6.92 (m, 2H), 6.93 (d, J = 3.9 Hz, 1H), 6.69 (d, J = 3.9 Hz, 1H), 4.44 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.54 (dd, J = 254.1, 11.0 Hz), 161.6, 157.88 (dd, J = 256.9, 12.8 Hz), 145.3, 139.5, 130.83 (d, J = 10.1 Hz), 130.8, 130.4, 127.4, 123.29 (dd, J = 12.7, 4.1 Hz), 114.4, 112.25 (dd, J = 22.7, 3.8 Hz), 108.4, 105.29 (dd, J = 26.5, 23.0 Hz), 61.3, 14.3; ^{19}F NMR (376 MHz, chloroform-d) δ −104.59 (qd, J = 8.4, 5.7 Hz), −115.10 to −115.23 (m). ESMS, calculated m/z $C_{16}H_{11}BrF_2N_2O_2S$ 414.0 $[M]^+$, found m/z (relative intensity) 415.0 $[M + H]^+$ (100), 435.0 $[M + Na]^+$ (100).

*Ethyl 5-(5-bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carboxylate (**7b**).* **7b** (2.36 g, 60% over two steps) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 7.54 (d, J = 2.1 Hz, 1H), 7.45 (d, J = 8.4 Hz, 1H), 7.41 (dd, J = 8.5, 2.1 Hz, 1H), 7.08 (s, 1H), 6.92 (d, J = 3.9 Hz, 1H), 6.66 (d, J = 3.9 Hz, 1H), 4.44 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.7, 145.2, 139.5, 137.2, 135.3, 134.0, 131.0, 130.8, 130.5, 130.4, 128.2, 127.3, 114.5, 108.1, 61.4, 14.4. ESMS, calculated m/z $16H_{11}BrCl_2N_2O_2S$ 445.9 $[M]^+$, found m/z (relative intensity) 446.9 $[M + H]^+$ (100).

*General procedure for the synthesis of compounds **8a,8b**.* The general procedure is illustrated below for compound **8a**.

*5-(5-Bromothiophen-2-yl)-1-(2,4-difluorophenyl)-1H-pyrazole-3-carboxylic acid (**8a**).* To a solution of bromo ester **7a** (1.72 g, 4.07 mmol) in methanol (19 mL) was added potassium hydroxide (0.80 g, 12.21 mmol) in methanol (9 mL) dropwise at room temperature. The resulting mixture was heated to reflux for 3 h. After hydrolysis was complete, the reaction mixture was cooled to room temperature, poured into ice-water, and acidified with 2N hydrochloric acid. The precipitate was filtered, washed with water, and dried under vacuum to give thiophene carboxylic acid **8a** (1.34 g, 85%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (bs, 1H), 7.83 (td, J = 8.8, 5.9 Hz, 1H), 7.64 (ddd, J = 10.3, 9.0, 2.8 Hz, 1H), 7.42–7.32 (m, 1H), 7.28 (s, 1H), 7.23 (d, J = 4.0 Hz, 1H), 7.11 (d, J = 4.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ 163.66 (dd, J = 251.4, 11.7 Hz), 163.0, 157.92 (dd, J = 253.6, 13.7 Hz), 146.0, 139.7, 132.28 (d, J = 10.7 Hz), 131.6, 130.8, 129.3, 123.43 (dd, J = 12.6, 3.8 Hz), 114.0, 113.45 (dd, J = 22.9, 3.6 Hz), 108.6, 106.16 (dd, J = 27.4, 23.6 Hz); ^{19}F NMR (376 MHz, DMSO-d_6) δ −104.80 (qd, J = 8.7, 5.8 Hz), −117.33 (q, J = 9.4 Hz). ESMS, calculated m/z $C_{14}H_7BrF_2N_2O_2S$ 383.9 $[M]^+$, found m/z (relative intensity) 422.9 $[M + K]^+$ (100).

*5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carboxylic acid (**8b**).* **8b** (1.42 g, 79%) was obtained as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.18 (s, 1H), 8.00 (d, J = 2.3 Hz, 1H), 7.82 (d, J = 8.5 Hz, 1H), 7.71 (dd, J = 8.5, 2.3 Hz, 1H), 7.31 (s, 1H), 7.22 (d, J = 4.0 Hz, 1H), 7.09 (d, J = 4.0 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ 163.0, 145.8, 139.5, 136.9, 135.5, 133.6, 132.5, 131.6, 130.8, 130.6, 129.4, 129.1, 114.0, 108.3. ESMS, calculated m/z $C_{14}H_7BrCl_2N_2O_2S$ 417.9 $[M]^+$, found m/z (relative intensity) 456.9 $[M + K]^+$ (100).

*General procedure for the synthesis of compounds **9a–9d**.* The general procedure is illustrated below for compound **9a**.

5-(5-Bromothiophen-2-yl)-1-(2,4-difluorophenyl)-1H-pyrazole-3-carbonyl chloride. A solution of the crude acid **8** (0.20 g, 0.51 mmol) and thionyl chloride (112 μL, 1.53 mmol) in toluene (3 mL) was refluxed for 3 h. The solvent was evaporated under reduced pressure, and the residue was then re-dissolved in toluene (3 mL) first and then in hexane (4 mL); after evaporation the crude acyl chloride (0.19 g, 95% yield) was obtained as a white solid.

*5-(5-Bromothiophen-2-yl)-1-(2,4-difluorophenyl)-N-(4-(trifluoromethyl)-phenyl)-1H-pyrazole-3-carboxamide (**9a**).* A solution in dichloromethane of the acyl chloride obtained previously (0.9 mL, 0.24 M), was added dropwise to a solution of 4-(trifluomethyl)aniline (30 μL, 0.23 mmol) and triethylamine (33 μL, 0.23 mmol) in dichloromethane (0.2 mL) at 0 °C. After stirring at room temperature for 16 h, to the reaction mixture was added water and the organic phase was extracted with dichloromethane (3 × 3 mL). The combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Flash column chromatography on silica gel with *n*-hexane–ethyl acetate (9 : 1) gave carboxamide **9a** (88 mg, 72% yield) as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.81 (s, 1H), 7.81 (d, J = 8.5 Hz, 2H), 7.61 (d, J = 8.6 Hz, 2H), 7.51 (td, J = 8.5, 5.7 Hz, 1H), 7.17 (s, 1H), 7.12–7.00 (m, 2H), 6.95 (d, J = 3.9 Hz, 1H), 6.74 (d, J = 3.9 Hz, 1H); ^{13}C NMR (101 MHz, CDCl3) δ 163.74 (dd, J = 254.8, 10.9 Hz), 159.1, 158.03 (dd, J = 257.3, 12.8 Hz), 147.8, 140.6, 130.7, 130.64 (d, J = 10.2 Hz), 130.5, 127.7, 126.32 (q, J = 3.8 Hz, ×2), 125.99 (q, J = 32.6 Hz), 124.09 (q, J = 271.8 Hz), 123.15 (dd, J = 13.0, 4.2 Hz), 122.74, 119.3 (×2), 114.8, 112.48 (dd, J = 22.8, 3.9 Hz), 107.0, 105.66 (dd, J = 26.5, 23.0 Hz); ^{19}F NMR (376 MHz, chloroform-d) δ −62.11, −104.02 (qd, J = 8.3, 5.7 Hz), −115.07 to −115.16 (m). ESMS, calculated m/z $C_{21}H_{11}BrF_5N_3OS$ 529 $[M]^+$, found m/z (relative intensity) 530 $[M + H]^+$ (100). HRMS m/z M^+H^+ calcd for $C_{21}H_{11}BrF_5N_3OS$: 527.9805; found: 527.9794%.

*5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-N-(4-(trifluoromethyl)-phenyl)-1H-pyrazole-3-carboxamide (**9b**).* **9b** (69 mg, 53%) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.81 (s, 1H), 7.80 (d, J = 8.5 Hz, 2H), 7.65–7.56 (m, 3H), 7.46 (d, J = 1.9 Hz, 2H), 7.18 (s, 1H), 6.94 (d, J = 3.9 Hz, 1H), 6.71 (d, J = 3.9 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.1, 147.7, 140.6, 140.5, 137.5, 135.1, 134.1, 130.7, 130.7, 130.7, 130.5, 128.4, 127.6, 126.33 (q, J = 3.7 Hz, ×2), 125.98 (q, J = 32.8 Hz), 124.08 (q, J = 271.3 Hz), 119.3 (×2), 114.8, 106.6; ^{19}F NMR (376 MHz, chloroform-d) δ −62.10. ESMS, calculated m/z $C_{21}H_{11}BrCl_2F_3N_3OS$ 560.9 $[M]^+$, found m/z (relative intensity) 583.8 $[M + Na]^+$ (100). HRMS m/z M^+H^+ calcd for $C_{21}H_{11}BrCl_2F_3N_3OS$: 559.9214; found: 559.9203%.

*5-(5-Bromothiophen-2-yl)-1-(2,4-difluorophenyl)-N-(4-(pentafluoro-λ^6-sulfanyl)phenyl)-1H-pyrazole-3-carboxamide (**9c**).* **9c** (110 mg, 70%) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.81 (s, 1H), 7.81–7.72 (m, 4H), 7.51 (td, J = 8.5, 5.7 Hz, 1H), 7.18 (s, 1H), 7.13–7.01 (m, 2H), 6.96 (d, J = 3.9 Hz, 1H), 6.74 (d, J = 3.9 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.76 (dd,

J = 254.8, 11.0 Hz), 159.1, 158.02 (dd, J = 257.2, 12.8 Hz), 149.14 (appt, J = 17.5 Hz), 147.6, 140.7, 140.3, 130.63 (d, J = 10.4 Hz), 130.5, 127.7, 127.04 (p, J = 4.6 Hz, ×2), 123.11 (dd, J = 12.6, 4.2 Hz), 118.9 (×2), 114.9, 112.50 (dd, J = 22.8, 3.9 Hz), 107.0, 105.67 (dd, J = 26.4, 22.9 Hz), 100; ^{19}F NMR (377 MHz, chloroform-d) δ 85.18 (p, J = 150.6, 149.7 Hz), 63.49 (d, J = 150.0 Hz), −103.91 (qd, J = 8.3, 5.7 Hz), −115.12 (q, J = 8.9 Hz). ESMS, calculated m/z $C_{20}H_{11}BrF_7N_3OS_2$ 586.9 $[M]^+$, found m/z (relative intensity) 585.9 $[M - H]^-$ (100). HRMS m/z M^+H^+ calcd for $C_{20}H_{11}BrF_7N_3OS_2$: 585.9493; found: 585.9484%.

*5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-N-(4-(pentafluoro-λ^6-sulfanyl)phenyl)-1H-pyrazole-3-carboxamide (**9d**).* **9d** (60 mg, 40%) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.82 (s, 1H), 7.82–7.70 (m, 4H), 7.61 (d, J = 1.9 Hz, 1H), 7.52–7.40 (m, 2H), 7.19 (s, 1H), 6.95 (d, J = 3.9 Hz, 1H), 6.71 (d, J = 3.9 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.1, 149.13 (p, J = 17.9 Hz), 147.5, 140.6, 140.2, 137.6, 135.1, 134.1, 130.7, 130.7, 130.6, 130.5, 128.4, 127.6, 127.07 (p, J = 4.3 Hz, ×2), 118.9 (×2), 114.9, 106.6; ^{19}F NMR (377 MHz, chloroform-d) δ 85.17 (p, J = 150.7, 149.7 Hz), 63.48 (d, J = 150.0 Hz). ESMS, calculated m/z $C_{20}H_{11}BrCl_2F_5N_3OS_2$ 618.9 $[M]^+$, found m/z (relative intensity) 617.8 $[M - H]^-$ (100). HRMS m/z M^+H^+ calcd for $C_{20}H_{11}BrCl_2F_5N_3OS_2$: 617.8902; found: 617.8894%.

*Lithium salt of ethyl 4-(4-chlorophenyl)-3-methyl-2,4-dioxobutanoate (**10**).* To a magnetically stirred solution of lithium bis(trimethylsilyl)amide (64 mL, 63.93 mmol, 1.0 M in THF) in diethyl ether (63 mL) at −78 °C, 1-(2-thienyl)-1-propanone (10 g, 58.12 mmol) in diethyl ether (73 mL) was added dropwise under a nitrogen atmosphere. After the mixture was stirred at the same temperature for an additional 45 min, diethyl oxalate (9 mL, 63.93 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for another 16 h. The reaction precipitate was filtered, washed with diethyl ether, and dried under vacuum to afford the crude lithium salt **10** (8.05 g, 50%) as a pale-yellow solid. The product was used without further purification.

*Ethyl 5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylate (**11**).* To a solution of lithium salt **10** (8.05 g, 28.73 mmol) in ethanol (99 mL) was added 2,4- dichlorophenylhydrazine hydrochloride (6.88 g, 31.60 mmol) in one portion at room temperature under nitrogen. The resulting mixture was stirred at the same temperature for 24 h. After reaction was complete, the precipitate was filtered, washed with ethanol and diethyl ether, and dried under vacuum to give a light-yellow solid (6.1 g). This crude solid, without purification, was dissolved in acetic acid (45 mL) and heated to reflux for 16 h. The reaction mixture was poured into ice-water and extracted with ethyl acetate (3 × 50 mL). The combined extracts were washed with water, saturated aqueous sodium bicarbonate, and brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Purification by flash column chromatography on silica gel with hexane–acetate (8 : 2) gave ester **11** (3.44 g, 30% over two steps) as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 7.38 (d, J = 2.2 Hz, 1H), 7.34 (d, J = 8.4 Hz, 1H), 7.32–7.27 (m, 3H), 7.07 (d, J = 8.6 Hz, 2H), 4.45 (q, J = 7.1 Hz, 2H), 2.33 (s, 3H), 1.43 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.2, 143.4, 143.3, 136.5, 136.3, 135.4, 133.5, 131.3 (×2), 131.2, 130.5, 129.3 (×2), 128.2, 127.5, 119.6, 61.4, 14.9, 10.1. ESMS, calculated m/z $C_{19}H_{15}Cl_3N_2O_2$ 408.0 $[M]^+$, found m/z (relative intensity) 409.0 $[M + H]^+$ (100).

*5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid (**12**).* To a solution of bromo ester **3** (2.32 g, 5.54 mmol) in methanol (26 mL) was added potassium hydroxide (0.72 g, 11.09 mmol) in methanol (8 mL) dropwise at room temperature. The resulting mixture was heated to reflux for 3 h. After hydrolysis was complete, the reaction mixture was cooled to room temperature, poured into ice-water, and acidified with 2N hydrochloric acid. The precipitate was filtered, washed with water, and dried under vacuum to give the carboxylic acid **12** (2.10 g, 98%) as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.73 (d, J = 2.3 Hz, 1H), 7.67 (d, J = 8.5 Hz, 1H), 7.54 (dd, J = 8.5, 2.3 Hz, 1H), 7.42 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 2.20 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ 164.1, 143.4, 142.9, 136.1, 135.6, 134.3, 132.3, 132.1, 131.8 (×2), 130.0, 129.2 (×2), 128.8, 127.4, 118.4, 9.9. ESMS, calculated m/z $C_{17}H_{11}Cl_3N_2O_2$ 380.0 $[M]^+$, found m/z (relative intensity) 381.0 $[M + H]^+$ (100).

*General procedure for the synthesis of compounds **13a–13g**.* The general procedure is illustrated below for compound **13a**.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carbonyl chloride. A solution of the crude acid **12** (1 g, 2.57 mmol) and thionyl chloride (565 μL, 7.70 mmol) in toluene (17.1 mL) was refluxed for 3 h. Solvent was evaporated under reduced pressure, and the residue was then re-dissolved in toluene (10 mL) first and then in hexane (10 mL); after evaporation the crude acyl chloride (0.98 g, 95% yield) was obtained as a white solid.

*5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-(3-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (**13a**).* A solution in dichloromethane of the acyl chloride obtained previously (0.96 mL, 0.41 M), was added dropwise to a solution of 3-(trifluomethyl)aniline (55 μL, 0.44 mmol) and triethylamine (61 μL, 0.44 mmol) in dichloromethane (0.4 mL) at 0 °C. After stirring at room temperature for 16 h, to the reaction mixture was added water and the organic phase was extracted with dichloromethane (3 × 3 mL). The combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Flash column chromatography on silica gel with n-hexane–ethyl acetate (8 : 2) gave carboxamide **13a** (160 mg, 70% yield) as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.91 (s, 1H), 8.03 (t, J = 1.9 Hz, 1H), 7.86 (d, J = 8.3 Hz, 1H), 7.51–7.41 (m, 2H), 7.40–7.27 (m, 5H), 7.12–7.07 (d, J = 8.5 Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.6, 144.4, 143.7, 138.4, 136.3, 135.7, 135.2, 133.0, 131.40 (q, J = 32.3 Hz), 130.8 (×2), 130.5, 130.4, 129.5, 129.0 (×2), 128.0, 126.8, 122.55, 122.53 (q, J = 272.0 Hz), 120.50 (q, J = 3.9 Hz), 118.4, 116.32 (q, J = 4.0 Hz), 9.5; ^{19}F NMR (376 MHz, chloroform-d) δ −62.74. ESMS, calculated m/z $C_{24}H_{15}Cl_3F_3N_3O$ 523 $[M]^+$, found m/z (relative intensity) 546.0 $[M + Na]^+$ (100). HRMS m/z M^+H^+ calcd for $C_{24}H_{15}Cl_3F_3N_3O$: 524.0311; found: 524.0301%.

*5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-(3-(pentafluoro-λ^6-sulfanyl)phenyl)-1H-pyrazole-3-carboxamide (**13b**).* **13b** (182 mg, 70% yield) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.92 (s, 1H), 8.16 (t, J = 2.1 Hz, 1H), 7.91 (d, J = 9.0 Hz, 1H), 7.55–7.43 (m, 3H), 7.39–7.31 (m, 4H), 7.12 (d,

$J = 8.5$ Hz, 2H), 2.45 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.6, 154.25 (appt, J = 17.4 Hz), 144.2, 143.7, 138.3, 136.3, 135.7, 135.2, 133.0, 130.8 (×2), 130.5, 130.4, 129.2, 129.0 (×2), 128.0, 126.8, 122.4, 121.24 (p, $J = 3.9$, 3.4 Hz), 118.4, 117.22 (p, $J = 4.9$ Hz), 9.5; ^{19}F NMR (377 MHz, chloroform-d) δ 84.17 (p, $J = 150.4$ Hz), 62.72 (d, J = 150.0 Hz). ESMS, calculated m/z $C_{23}H_{15}Cl_3F_5N_3OS$ 583.0 $[M]^+$, found m/z (relative intensity) 582.0 $[M - H]^-$ (56). HRMS m/z M^+H^+ calcd for $C_{23}H_{15}Cl_3F_5N_3OS$: 582.0000; found: 581.9991%.

*N-(3-(tert-Butyl)phenyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (**13c**).* **13c** (88 mg, 63% yield) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.76 (s, 1H), 7.64–7.59 (m, 2H), 7.46 (t, $J = 1.3$ Hz, 1H), 7.35–7.25 (m, 5H), 7.18–7.13 (m, 1H), 7.10 (d, $J = 8.5$ Hz, 2H), 2.44 (s, 3H), 1.33 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.4, 152.2, 145.0, 143.4, 137.6, 136.2, 135.8, 135.0, 133.1, 130.9 (×2), 130.6, 130.4, 128.9 (×2), 128.6, 128.0, 127.1, 121.1, 118.2, 117.1, 116.9, 34.8, 31.4 (×3), 9.6. ESMS, calculated m/z $C_{27}H_{24}Cl_3N_3O$ 511.1 $[M]^+$, found m/z (relative intensity) 512.1 $[M + H]^+$ (100). HRMS m/z M^+H^+ calcd for $C_{27}H_{24}Cl_3N_3O$: 512.1063; found: 512.1052%.

*5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-(4-(trifluoromethyl)phenyl)-1H-pyrazole-3-carboxamide (**13d**).* **13d** (141 mg, 58% yield) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.93 (s, 1H), 7.82 (d, $J = 8.3$ Hz, 2H), 7.60 (d, $J = 8.3$ Hz, 2H), 7.47 (s, 1H), 7.37–7.28 (m, 4H), 7.09 (d, $J = 8.5$ Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.6, 144.4, 143.7, 141.0, 136.3, 135.7, 135.2, 133.0, 130.8 (×2), 130.5, 129.0 (×2), 128.2, 128.0, 126.8, 126.27 (q, $J = 3.7$ Hz, ×2), 125.55 (q, J = 31.9 Hz), 124.93 (q, J = 272.0 Hz), 119.2 (×2), 118.5, 9.5. ESMS, calculated m/z $C_{24}H_{15}Cl_3F_3N_3O$ 523 $[M]^+$, found m/z (relative intensity) 524.0 $[M + H]^+$ (100). HRMS m/z M^+H^+ calcd for $C_{24}H_{15}Cl_3F_3N_3O$: 524.0311; found: 524.0300%.

*5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-(4-(pentafluoro-λ^6-sulfanyl)phenyl)-1H-pyrazole-3-carboxamide (**13e**).* **13e** (177 mg, 68% yield) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.93 (s, 1H), 7.79 (d, $J = 9.0$ Hz, 2H), 7.77–7.69 (m, 2H), 7.47 (d, $J = 2.1$ Hz, 1H), 7.37–7.28 (m, 4H), 7.09 (d, J = 8.4 Hz, 2H), 2.43 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.6, 148.97 (appt, J = 17.0 Hz), 144.2, 143.8, 140.6, 136.4, 135.6, 135.2, 133.0, 130.8 (×2), 130.43, 130.42, 129.0 (×2), 128.0, 126.97 (p, J = 4.6 Hz, ×2), 126.72, 118.8 (×2), 118.5, 9.5; ^{19}F NMR (377 MHz, chloroform-d) δ 85.34 (p, $J = 150.1$, 149.1 Hz), 63.53 (d, $J = 149.9$ Hz). ESMS, calculated m/z $C_{23}H_{15}Cl_3F_5N_3OS$ 5823.0 $[M]^+$, found m/z (relative intensity) 582.0 $[M - H]^-$ (64). HRMS m/z M^+H^+ calcd for $C_{23}H_{15}Cl_3F_5N_3OS$: 582.0000; found: 581.9990%.

*N-(4-(tert-Butyl)phenyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (**13f**).* **13f** (162 mg, 78% yield) was obtained as a white solid: ^{1}H NMR (400 MHz, chloroform-d) δ 8.71 (s, 1H), 7.60 (d, $J = 8.7$ Hz, 2H), 7.46 (dd, $J = 1.8$, 0.8 Hz, 1H), 7.36 (d, $J = 8.7$ Hz, 2H), 7.33–7.29 (m, 4H), 7.09 (d, J = 8.5 Hz, 2H), 2.43 (s, 3H), 1.32 (s, 9H); ^{13}C NMR (101 MHz, CDCl3) δ 160.4, 147.0, 145.0, 143.4, 136.1, 135.9, 135.2, 135.0, 133.0, 130.9 (×2), 130.6, 130.4, 128.9 (×2), 127.9, 127.1, 125.8 (×2), 119.5 (×2), 118.2, 34.4, 31.4 (×3), 9.5. ESMS, calculated m/z $C_{27}H_{24}Cl_3N_3O$ 511.1 $[M]^+$, found m/z (relative intensity) 534.1 $[M + Na]^+$ (100). HRMS m/z M^+H^+ calcd for $C_{27}H_{24}Cl_3N_3O$: 512.1063; found: 512.1053%.

Determination of the partition coefficients (log P). Measurement of chromatographic capacity factor (k') by C-18 HPLC method was performed using the equation $k' = (t_r - t_0)/t_0$,[47,48] where t_0 is the retention time of unretained substance and t_r is the compound's retention time. The mobile phase was prepared mixing methanol with water in proportions of 85 : 15 and the flow rate was 1 mL min^{-1}. A solution of urea in a methanol–water solvent (85 : 15) was used for measurement of the column dead time ($t_0 = 2.673 \pm 0.002$, $n = 3$). Seven compounds having known log P values, tabulated and in the range from 1.10 to 5.70 (benzyl alcohol, log P 1.10; benzene, log P 2.10; toluene, log P 2.70; naphthalene, log P 3.60; biphenyl, log P 4; phenanthrene, log P 4.50; triphenylamine, log P 5.70) were chosen as a "standard" calibration mixture for the determination of retention times (t_r). Every measure was obtained in triplicate and at controlled temperature of 25 °C. Capacity factors (k') were calculated. The log k' value for each of seven compounds was plotted against its relative lipophilicity value reported in literature, based on the established linear relationship ($\log P = 3.64 \log k' + 3.39$, correlation coefficient = 0.95). The capacity factor of each compound was determined (the value was obtained on average of three experiments) and the relative log P value was obtained by extrapolation.

In vitro assays

Equilibrium binding assays. Binding assays were performed with the CB_1 receptor agonist, [3H]CP55940 (0.7 nM), 1 mg mL^{-1} bovine serum albumin (BSA) and 50 mM Tris buffer containing 0.1 mM EDTA and 0.5 mM MgCl2 (pH 7.4), total assay volume 500 μL. Binding was initiated by the addition of mouse brain membranes (30 μg) or CB_2 transfected CHO cells (5 μg). Assays were carried out at 37 °C for 60 minutes before termination by addition of ice-cold wash buffer (50 mM Tris buffer, 1 mg mL^{-1} BSA) and vacuum filtration using a 24-well sampling manifold (Brandel Cell Harvester) and Whatman GF/B glass-fibre filters that had been soaked in wash buffer at 4 °C for 24 h. Each reaction tube was washed five times with a 4 mL aliquot of buffer. The filters were oven-dried for 60 min and then placed in 5 mL of scintillation fluid (Ultima Gold XR, Packard), and radioactivity quantitated by liquid scintillation spectrometry. Specific binding was defined as the difference between the binding that occurred in the presence and absence of 1 μM of the corresponding unlabelled ligand and was 70–80% of the total binding.

β-Arrestin assays

PathHunter® HEK293 CB_1 beta-arrestin cells were plated 48 hours before use and incubated at 37 °C, 5% CO_2 in a humidified incubator. Compounds were dissolved in DMSO and diluted in OCC media to the required concentrations. 5 μL of compound or vehicle solution was added to each well and incubated for 60 minutes at 37 °C, 5% CO_2 in a humidified incubator. 5 μL of increasing concentrations of CP55940 was added to each well followed by a 90 minute incubation at 37 °C, 5% CO_2 in a humidified incubator. 55 μL of detection reagent is

then added followed by a further 90 minute incubation at room temperature in the dark. Chemiluminescence, indicated as RLU, was measured on a standard luminescence plate reader.

Solubility tests

The aqueous solubility was measured using laser nephelometry (BMG Labtech Nephelometer) following serial dilution of DMSO stocks into Tris Buffer (50 mM Tris–HCL, 50 mM Tris base and 0.1% BSA) to give final concentrations of 0.01, 0.1,1 and 10 μM and a final DMSO concentration of 0.1%. The amount of laser scatter caused by insoluble particulates (relative nephelometry units) was measured. RFU values 3-fold greater than control (0 μM) indicate insolubility.

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