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The Pentafluorosulfanyl Group in Cannabinoid Receptor Ligands: Synthesis and Comparison with Trifluoromethyl and tert-Butyl Analogues

Stefano Altomonte, Gemma L. Baillie, Ruth A. Ross, Jennifer Riley, Matteo Zanda

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. LogP values) than the CF$_3$ counterparts and lower 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 vs. 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 improve both its metabolic stability and bioavailability$^2$. Fluorine could be incorporated by fluorination or alternatively via 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$_3$ group is often referred to as a “super-trifluoromethyl” group (Figure 1)$^{13-15}$.

![Steric demand of the three groups compared in this work: 'Bu > SF$_5$ >> CF$_3$](image)

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 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$^{30}$. 

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several cannabinoid ligands were developed. Among these ligands, the most studied is probably SR141716 (Rimonabant)\textsuperscript{31}, a pyrazole-core inverse agonist which was discovered by Sanofi-Synthelabo (now Sanofi-Aventis) in 1994 (Figure 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\textsubscript{5} group relative to its closest bioisosteric substituents, namely CF\textsubscript{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\textsubscript{5}-group, as well as CF\textsubscript{3} and the tert-butyl groups, and directly compare the SF\textsubscript{5} derivatives with their CF\textsubscript{3} and tert-Bu counterparts from the pharmacological viewpoint. SF\textsubscript{5}, CF\textsubscript{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\textsubscript{1}-affinity and selectivity vs. the CB\textsubscript{2}\textsuperscript{32} and (2) SF\textsubscript{5}-substituted anilines or nitro-anilines are accessible starting materials (see below).

Since, to the best of our knowledge, SF\textsubscript{5}-substituted cannabinoid receptor ligands have never been described in the literature, we decided to synthesise two different classes of pyrazole-core CB\textsubscript{1} receptor ligands: the former based on a Rimonabant-type structure and the latter based on ligands described in a Pharmaness’ patent\textsuperscript{33}, where the 4-chloro-phenyl ring is replaced by a 2-bromo-thiophene.

![Scheme 1: Reagents and conditions: Fe, HCl/EtOH, reflux, 2 h.](image)

Thiophenyl-compounds 5a-f and 9a-d were prepared according to the general synthetic methods shown in Schemes 2 and 3, respectively\textsuperscript{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 thionyl chloride and then reacted with several anilines to afford the desired products 5.

**Results and Discussion**

**Chemistry**

The starting para-SF\textsubscript{5}-substituted aniline is commercially available whereas meta-SF\textsubscript{5}-aniline was synthesised from the commercially available 3-nitro-SF\textsubscript{5} benzene (Scheme 1). 3-Nitro-SF\textsubscript{5}-benzene was treated with iron-powder in a refluxing HCl/Ethanol solution\textsuperscript{34}, affording the corresponding 3-(pentafluoro-λ\textsuperscript{2}-sulfanyl)aniline in good yield (83\%). The SF\textsubscript{5} group remained unreactive under these conditions, confirming the high chemical stability of this group.
Scheme 2: Reagents and conditions: (a) Diethyl oxalate, LiHMDS, THF/Et2O (2/1), −78 °C to r.t., 16 h; (b) EtOH, r.t., 24 h; (c) AcOH, 120 °C, 16 h; (d) NBS, CH3CN, 0 °C to r.t., 16 h; (e) KOH, MeOH, reflux, 3 h; (f) thionyl chloride, toluene, reflux, 3 h; (g) Et3N, DCM, 0 °C to r.t., 16 h.

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.

Scheme 3: 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) Et3N, DCM, 0 °C to room temp, 16 h

The synthesis of the Rimonabant-like derivatives 13, shown in Scheme 4, was analogously accomplished following the strategy described by Lan et al.12.
physicochemical molecular properties, including the LogP of pyrazole cannabinoid ligands too.

Streich at al. showed that an SF group imparts higher lipophilicity than a CF group. 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 LogP (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 LogP of these CB1 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 SF5 moiety are more lipophilic than the CF3 counterparts and, in general, a substituent in para position influences the hydrophobicity of the entire molecule to a greater extent (\(\Delta \text{LogP}_{(\text{SF5-CF3})p} = 0.3\)) than in meta (\(\Delta \text{LogP}_{(\text{SF5-CF3})m} = 0.2\)) (Tables 1 and 2). Not surprisingly, replacement of these fluorinated functions with a strongly lipophilic tert-butyl group further increased the LogP 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 SF5 and CF3 derivatives by \(\Delta \text{LogP}_{(\text{CH3-HSF5})} = 0.7\) and \(\Delta \text{LogP}_{(\text{CH3-HCF3})} = 0.6\) units respectively. A further hydrophilicity enhancement was observed by substitution of the 2,4 difluoroaryl group in 9a,c with the chlorinated analogue in 9b,d (\(\Delta \text{LogP}_{(F-CH)} = 0.9\)).

When a 4-bromo-thiophene group (compounds 13) was replaced by a 5-bromo-thiophene group (compounds 5), the LogP decreased by \(\Delta \text{LogP}_{(\text{Br})} = 0.2\), despite the benzene group (LogP benzene = 2.15) is reported to be more hydrophobic than the thiophene group (LogP thiophene = 1.81). 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 LogP values for the CB1 inverse agonist Rimonabant (SR141716) were determined in silico, and the two experimental values reported in literature, obtained through the flash-shake technique, are quite different (LogD3,4 = 4.6 ± 0.8 and LogD7,4 = 3.8). In order to obtain a new experimental value we decided to test SR141716 using the previously described RP-HPLC method, which provided a LogP 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. In this assay compounds 5, 9 and 13 were subjected to equilibrium binding studies with the orthosteric agonist probe \(^{[3]H}\text{CP55940}\), and the ligands were assayed for their capacity to displace \(^{[3]H}\text{CP55940}\) from mouse brain membranes which express high levels of CB1 receptors and from hCB2 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 CF3 group, with, respectively, 9e and 9d, carrying an SF5 group (Table 1). In both cases, the SF5 compounds 9e,d showed higher affinity (lower Kd) than the CF3 counterparts. In terms of CB1/CB2 selectivity, while 9a,c were comparable, 9d showed modest but higher CB1/CB2 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 CB1 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)\(^6\), 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, 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\(_D\)'s 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\(_{\text{max}}\) measured by the displacement assay on CB\(_2\) CHO cells (see Table S1, Supporting Information).

In the para-phenyl substituted series of compounds 5d-f, the SF\(_3\)-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\(_3\) derivative 5e relative to the CF\(_3\) analogue 5d. In the meta-substituted series of compounds 5a-c, the tert-butyl derivative 5e featured the highest CB\(_1\) affinity, whereas the SF\(_3\) 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\(_3\) 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\(_3\) compound 13e displayed the lowest apparent CB\(_1\) K\(_D\), whereas 13d and 13f showed similar affinities.

However, importantly, all of the 4-methyl-substituted pyrazoles bearing the SF\(_3\) or '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\(_{\text{max}}\) values ranging from 35.3 to 43.2 (see Figure 3 and Table S1, Supporting Information). Considering that the 4-trifluoromethyl 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.

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 pentafluoro-sulfanyl analogues\(^42\). Laser nephelometry has become the method of choice for measuring solubility of molecules in a drug discovery setting\(^43\).

![Figure 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.](image-url)

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, Supporting Information, for details).

**FUNCTIONAL ASSAYS**

\(\beta\)- Arrestin recruitment assays, such as the PathHunter® \(\beta\)-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® \(\beta\)-arrestin assay in hCB\(_1\) cells, \([^3]H\)CP55940 stimulated \(\beta\)-arrestin recruitment with an EC\(_{50}\) of 16.9 nM (11-27 nM) (95% confidence limits for all of the \(\beta\)-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 Supporting Information 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 SF₃ compounds again, caused a significant rightward shift in the dose response curve of |³H|CP55940 with EC₅₀ 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 EC₅₀ 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 |³H|CP55940 for the CB₁ receptor.
Table 1:

<table>
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<th>Compound</th>
<th>R&lt;sup&gt;1&lt;/sup&gt;</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;</th>
<th>LogP&lt;sup&gt;a&lt;/sup&gt; (±SEM)</th>
<th>CB&lt;sub&gt;1&lt;/sub&gt; Ki (nM) (±SEM)</th>
<th>CB&lt;sub&gt;2&lt;/sub&gt; Ki (nM) (±SEM)</th>
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<tr>
<td>9a</td>
<td></td>
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<td>4.67 (±0.20)</td>
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<td>9b</td>
<td></td>
<td></td>
<td>5.60 (±0.27)</td>
<td>137.7 (±36.9)</td>
<td>528.4 (±204.8)</td>
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<td>9c</td>
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<td></td>
<td>4.97 (±0.22)</td>
<td>302.7 (±72.1)</td>
<td>1009.0 (±349.2)</td>
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<tr>
<td>9d</td>
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<td>5.89 (±0.30)</td>
<td>58.8 (±13.4)</td>
<td>588.2 (±229.9)</td>
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* Determined experimentally by means of RP-HPLC (see experimental section for details)
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<th>Compound</th>
<th>R¹</th>
<th>R²</th>
<th>LogP (±SEM)</th>
<th>CB¹ Kᵢ (nM) (±SEM)</th>
<th>CB² Kᵢ (nM) (±SEM)</th>
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* Determined experimentally by means of RP-HPLC (see experimental section for details)
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 property since CB$_1$ binding to the orthosteric binding pocket would be expected to also exclude by means of nephelometry assays, so we are no change in agonist dissociation. Solubility problems were observation could be that the compounds are binding to an allosteric pocket. However, this seems unlikely as we observed 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 $^1$HCP5S940 in the functional assays (Emax 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 substituent 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 1H at 400.13 MHz, for 13C at 100.58 MHz and for 19F at 376.45 MHz. $^1$H NMR chemical shifts are reported relative to TMS, and the solvent resonance was employed as the internal standard (CDCl$_3$ δ = 7.26). $^{13}$C NMR spectra were recorded with complete proton decoupling, and the chemical shifts are reported relative to TMS as the internal standard (CDCl$_3$ δ = 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; 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 x 4.60 mm L x 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 (λ= 254 nm or λ= 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.

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

3-(Pentafluoro-1,4-sulfanyl)aniline

To a stirred acidic warm solution (50 °C) of pentafluoro(3-nitrophenyl)-1,4-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 X 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, $^{13}$C and $^{19}$F NMR spectra matched to those reported by Bowden et al.

Lithium Salt of Ethyl 3-Methyl-2,4-dioxo-4-(thiophen-2-yl)butanoate (1).

To a magnetically stirred solution of lithium bis(tri-methylsilyl)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_{2}S 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, 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 1H, 12C 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, 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: 1H 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); 13C NMR (101 MHz, CDCl3) δ 162.5, 142.9, 136.9, 136.6, 135.7, 133.8, 130.9, 130.2, 130.1, 129.3, 127.9, 120.4, 115.0, 61.1, 14.5, 10.0. ESMS, calculated m/z C_{11}H_{11}BrCl_{2}N_{2}O_{2}S 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: 1H NMR (400 MHz, DMSO-d6) δ 130.5 (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); 13C 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, 129.0, 129.9, 119.6, 114.6, 10.2. ESMS, calculated m/z C_{15}H_{14}BrCl_{2}N_{2}O_{2}S 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-carboxamide (5a).

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 of dichloromethane of the carboxylic chloride (1 mL, 0.36 M) obtained previously from 4, was added dropwise to a solution of 3-(trifluoromethyl)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: 1H 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); 13C NMR (101 MHz, CDCl3) 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; 19F NMR (376 MHz, Chloroform-d) δ -62.72. ESMS, calculated m/z C_{22}H_{13}BrCl_{2}F_{3}N_{2}OS 574.93 [M]^+, found m/z (relative intensity) 575.9 [M+H]^+ (100). HRMS m/z M^+ calcd. for C_{22}H_{13}BrCl_{2}F_{3}N_{2}OS: 573.9370; found: 573.9361
5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-N-(3-(pentafluoro-2-sulfanyl)phenyl)-1H-pyrazole-3-carboxamide (5b)

5b (163 mg, 68% yield) was obtained as a white solid: 1H NMR (400 MHz, Chloroform-d) δ 8.87 (s, 1H), 7.64 – 7.57 (m, 1H), 7.59 (d, J = 2.0 Hz, 1H), 7.54 (d, J = 1.9 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); 13F 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}BrClF_{2}N_{3}O_{2}S_2 632.90 [M]^+; found m/z (relative intensity) 631.9 [M-H]^ – 100). HRMS m/z M^+ calcd. for C_{21}H_{13}BrClF_{2}N_{3}O_{2}S_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 (5e)

5e (87 mg, 63% yield) was obtained as a white solid: 1H 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 (d, J = 1.9 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); 13C 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_{32}BrCl_{2}N_{3}O_{4}S 562.00 [M]^+; found m/z (relative intensity) 100. HRMS m/z M^+ calcd. for C_{25}H_{32}BrCl_{2}N_{3}O_{4}S : 562.0122; found: 562.0111.

Lithium Salt of ethyl 4-(5-bromothiophen-2-yl)-2,4-dioxobutanoate (6)

To a magnetically stirred solution of lithium bis(triethylsilyl)amide (23 mL, 22.56 mmol, 1.0 M in THF) in diethyl ether (41 mL) at -78 °C, 1-(5-bromothiophen-2-yl)ethaneone (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_{21}H_{23}BrLiO_{3}S 309.9 [M]^+; found m/z (relative intensity) 326.9 [M-Li+H+Na]^+. (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 x 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. ¹H 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, 1H), 1.41 (t, J = 7.1 Hz, 3H); ¹³C NMR (101 MHz, CDCl₃) 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, 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; ¹⁹F NMR (376 MHz, Chloroform-d) δ -104.59 (qd, J = 8.4, 5.7 Hz), -115.10 – -115.23 (m). ESMS, calculated m/z 446.9 [M+H]⁺, found m/z 446.9 [M+H]⁺ (100).

**General Procedure for the Synthesis of Compounds 8a, 8b.**

The general procedure is illustrated below for compound 8a.

5-(Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carboxylic acid (8a).

To a solution of bromo ester 7a (1.72 g, 4.07 mmol) in methanol (9 mL) was added potassium hydroxide (0.80 g, 13.6 mmol) and triethylamine (33 µl, 0.23 mmol) and heated to reflux for 3 h. The resulting mixture was heated to reflux for 3 h. The solution was then cooled to room temperature, poured into ice-water, washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Flash column chromatography on silica gel with Hexane / ethyl acetate (9:1) gave carboxylic acid 8a (88 mg, 72% yield) as a white solid: ¹H NMR (400 MHz, Chloroform-d) δ 8.81 (s, 1H), 7.81 (d, J = 8.5 Hz, 1H), 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); ¹³C 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 (dd, J = 10.2 Hz), 130.5, 127.7, 126.32 (q, J = 3.8 Hz, x2), 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 (x2), 114.8, 112.48 (dd, J = 22.8, 3.9 Hz), 107.0, 105.66 (dd, J = 26.5, 23.0 Hz); ¹⁹F NMR (376 MHz, Chloroform-d) δ -62.11 , -104.02 (qd, J = 8.3, 5.7 Hz), -115.07 – -115.16 (m). ESMS, calculated m/z C₁₃H₁₁BrF₂N₂O₃ 529 [M⁺], found m/z (relative intensity) 530.
Lithium Salt of ethyl 4-(4-chlorophenyl)-3-methyl-2,4-dioxobutanoate (10).

To a magnetically stirred solution of lithium bis(tri-methylsilyl)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 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: ¹H 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); ¹³C NMR (101 MHz, CDCl₃) δ 163.2, 144.3, 143.3, 136.5, 136.3, 135.4, 133.5, 131.3 (x2), 131.2, 130.5, 129.3(x2), 128.2, 127.5, 119.6, 61.4, 14.9, 10.1. ESMS, calculated m/z C₁₉H₁₅Cl₂N₂O₂ 408.0 [M⁺], found m/z (relative intensity) 409.0 [M⁺]⁺ (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: ¹H NMR (400 MHz, DMSO-d₆) 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), 2.71 (d, J = 8.4 Hz, 2H), 2.20 (s, 3H); ¹³C NMR (101 MHz, DMSO) δ 164.1, 143.4,
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-carboxamide (13a)

A solution of the crude acid 12 (1.0 g, 2.57 mmol) and thionyl chloride (565 μl, 7.70 mmol) in toluene (171 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-(trifluoromethylaniline (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 x 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: 1H 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); 13C NMR (101 MHz, CDCl3) 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 (x2), 130.5, 130.4, 129.5, 129.0 (x2), 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; 19F NMR (376 MHz, Chloroform-d) δ -62.74. ESMS, calculated m/z C26H15Cl3F3N5O [M]+ (relative intensity) 546.0 [M+Na]+ (100). HRMS m/z M H+ calcd. for C25H15Cl3F3N5O: 524.0311; found: 524.0301.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-N-(3-(pentafluoro-λ6-sulfanyl)phenyl)-1H-pyrazole-3-carboxamide (13b)

13b (182 mg, 70% yield) was obtained as a white solid: 1H 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); 13C NMR (101 MHz, CDCl3) 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 (x2), 130.5, 130.4, 129.2, 129.0 (x2), 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; 19F NMR (377 MHz, Chloroform-d) δ 84.17 (p, J = 150.4 Hz), 62.72 (d, J = 150.0 Hz). ESMS, calculated m/z C27H15Cl3F3N5 OS 583.0 [M]+, found m/z (relative intensity) 582.0 [M-H]- (56). HRMS m/z M H+ calcd. for C27H15Cl3F3N5O : 582.0000; found: 581.9991.
Prepared mixing methanol with water in proportions of 85:15 and the flow rate was 1 ml/min. A solution of urea in a methanol
1.10 to 5.70 (Benzyl alcohol, LogP 1.10; Benzene, LogP 2.10; Phenanthrene, LogP 4.50; Triphenylamine, LogP 5.70) were
chosen as a “standard” calibration mixture for the determination. 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 uM 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 uM) indicate insolubility.

Solubility tests

Equilibrium Binding Assays

Binding assays were performed with the CB1 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 CB2 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 uM of the corresponding unlabelled ligand and was 70 - 80% of the total binding.

Notes and references


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The Pentafluorosulfanyl Group in Cannabinoid Receptor Ligands: Synthesis and Comparison with Trifluoromethyl and \(\text{tert}\)-Butyl Analogues

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Competitive CB\(_1\) receptor antagonists carrying an aromatic SF\(_5\) group in position 3 of a pyrazole ring were synthesised and compared with their CF\(_3\) and \(\text{tert}\)-butyl analogues, showing (1) LogP values in the order \(\text{tert}\)-butyl > SF\(_5\) > CF\(_3\); (2) CB\(_1\) \(K_i\)s (in the nanomolar range) and CB\(_1\)/CB\(_2\) selectivities slightly higher or equivalent than the CF\(_3\) and \(\text{tert}\)-butyl counterparts. This confirms that an aromatic SF\(_5\) group can be used as a bioisosteric analogue of a CF\(_3\) group and possibly of a bulky aliphatic group too.