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4-Cyano-5-(2-Thiophenyl)-Pyrazoles are High Affinity CB_1 Receptor Ligands

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Pyrazoles bearing a 5-thiophenyl and a 4-cyano group were synthesised and tested for their affinity to the cannabinoid CB_1 receptor showing in many cases single digit nanomolar Ki values and moderate to good selectivity towards the CB_2 receptor. Some of these pyrazole ligands, such as **8g**, displayed relatively low lipophilicity (experimental LogP < 4) and high calculated Topological Polar Surface Area (TPSA) (> 90) suggesting that these compounds may behave as peripherally restricted CB_1 ligands. Furthermore, 2-fluoroethyl carboxamides **8d**, **8h** and **8l** are interesting candidates for further development into PET tracers.

Introduction

Cannabinoid receptors (CBRs) belong to the family of G-protein coupled receptors (GPCRs). There are at least two classes of CBRs known: cannabinoid receptors subtype-1 (CB_1), localised predominantly in the Central Nervous System (CNS) and cannabinoid receptors subtype-2 (CB_2), mostly present peripherally in the immune system. The CB_1 receptor has received considerable attention during the last 20 years due to its involvement in a number of disorders and pathologies, such as obesity, depression, schizophrenia and cancer. The rise and fall of the anti-obesity CNS-active CB_1 inverse agonist Rimonabant (SR141716) suggested that peripherally restricted ligands of the CB_1 receptor may be a better option, devoid of the severe side effects (mostly suicidal-tendencies and depression) of Rimonabant while maintaining the favourable biological and pharmacological properties of CB_1 ligands[1]. Therefore, a remarkable effort has been devoted to the design and development of CB_1 ligands unable to penetrate the brain, and to the identification of the most important physico-chemical and structural properties responsible for reducing or suppressing the brain uptake of these molecules. Lipophilicity, number of hydrogen-bond donor functions and polar surface area (PSA) are among the most used metrics for estimating the capacity of a molecule that is not actively transported into the brain to undergo passive diffusion across the blood-brain-barrier (BBB). Various recent reviews have been dedicated to the important topic of peripherally-restricted CB1 ligands and to the different strategies pursed for achieving selective peripheral blockade of CB1 receptors.[1–3]

In 2008, Tseng et al. reported CB_1 receptor-ligand SAR studies on 5-thiophenyl pyrazoles carrying different aliphatic functionalities in position 5' on the thiophene ring, such as either alkynyl or alkenyl chains, suggesting that lipophilic moieties in that position favourably interact with the binding site of the CB_1 receptor.[4] One of these analogues, TM38837, was shown to be a highly potent CB_1 antagonist having very limited brain penetration.[5]

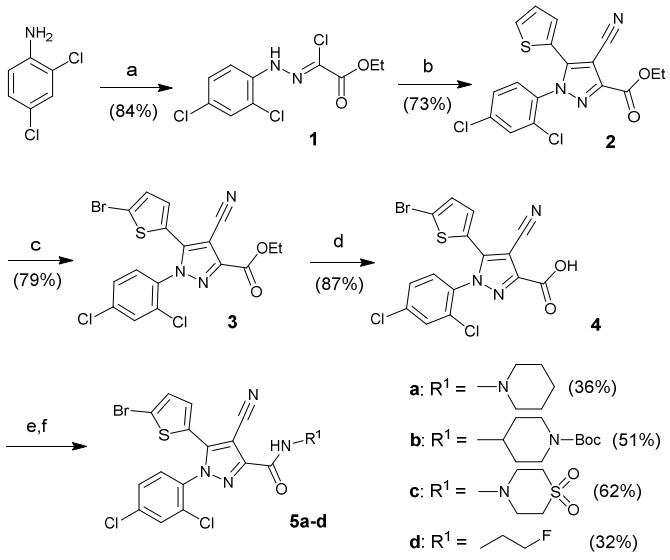
With the view of developing novel peripherally restricted CB_1 ligands having reduced lipophilicity and increased PSA relative to TM38837 and its analogues, we decided to replace the 4-methyl/ethyl pyrazole substituent featured by these compounds with a cyano group, and investigate the effect of this structural change on CB_1 and CB_2 binding affinity and selectivity. Indeed, although the cyano group has been previously used as a pyrazole-substituent in a CB_1 ligand,[6] to the best of our knowledge neither the full scope of this strategy nor the concomitant replacement of the rimonabant-type C-5 aryl group with a thiophenyl group have been reported yet. Furthermore, we decided to investigate the effect of incorporating a fluoroalkyl chain on 4-cyano-5-thiophenyl-pyrazoles with the view of producing, in future studies, a ^{18}F-labelled cyano-pyrazole for PET imaging.

Results and Discussion

Chemistry

The synthesis of 5-(5-Bromo)thiophenyl-pyrazoles **5a-d** and 5-(5-alkynyl)thiophenyl-pyrazoles **8a-l** was performed according to an improved synthetic pathway previously described for the preparation of the 4-cyano pyrazole JHU75528.[7] Compounds **5a-d** and **8a-l** were synthesised in 6 and 7 steps with 13-25% and 10-23% overall yields, respectively (Schemes 1 and 2). The hydrazone **1** was obtained in good yield by Japp-Klingemann reaction of an arenediazonium salt, prepared in situ from the commercially available 2,4 dichloro aniline, with ethyl 2-chloro-3-oxobutanoate. The condensation of the 2,4 dichlorophenyl hydrazone **1** with the 3-oxo-3-(2-thienyl)propionitrile to give the 4-cyano pyrazole **2** was performed employing triethyl amine as base in tert-butanol as solvent at 30 °C. Gao reported this combination was the best in order to limit by-products due to hydrolysis and to obtain the

pyrazole ring in acceptable yields;[7] however, since to the best of our knowledge, this was the first time that a keto-thiophene was employed as a nucleophile in this reaction, we decided to investigate whether different conditions could lead to higher yields (Table 1).



Scheme 1. Reagents and conditions: a) $NaNO_2$, aq. HCl (7% v/v), at 0°C for 1 h; sodium acetate, ethyl 2-chloro-3-oxobutanoate, EtOH, room temperature for 2 h b) 3-oxo-3-(2-thienyl)propionitrile, Et_3N, 30°C, 16 h c) NBS, DMF, 0 °C to 80 °C, 16 h; d) KOH, MeOH, room temperature, 3 h; e) thionyl chloride, toluene, reflux, 3 h; f) Et_3N, DCM, amine, 0 °C to room temperature, 16 h.

Table 1. Conditions for the synthesis of 5-thiophene-pyrazole **2**.

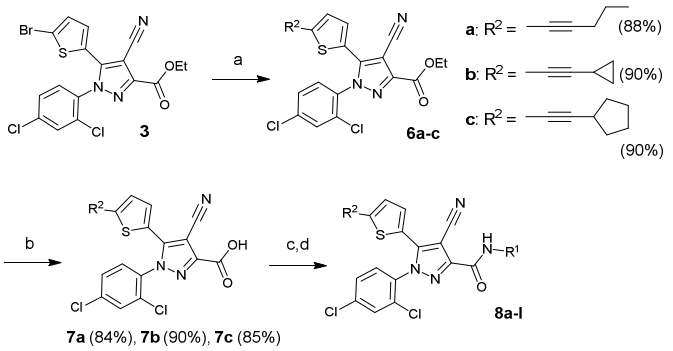
Entry	Base	Solvent	Temperature	Yield[a]
1	NaOEt	EtOH (abs)	rt	21
2	^{t}BuOK	THF (dry)	rt	22
3	LiHMDS	THF (dry)	-78 °C to rt	decomposition
4	NaH	THF (dry)	0 °C to rt	39
5[b]	NaH	THF (dry)	rt	36
6	Et_3N	^{t}BuOH	30 °C	57
7[c]	Et_3N	^{t}BuOH	30 °C	73

[a]Yields refer to the major product, isolated and purified by flash chromatography. [b]18-crown-6 was employed to increase the availablity of the "naked" nucleophile. [c]The hydrazone was added portionwise to the reaction mixture.

The base LiHMDS in THF consumed rapidly almost all the β-cyano ketone giving a mixture of by-products (entry 3) whereas alkoxide bases, such as sodium ethoxide (entry 1) and potassium *tert*-butoxide (entry 2) gave the pyrazole **2** in poor yields. The yield slightly improved by using the inorganic base sodium hydride in THF (entry 4); it is worth noting that there was no significant difference when the reaction was carried out in the presence of a crown ether (entry 5). In agreement with the literature results,[7] the use of triethyl amine in *tert*-butanol at 30 °C (entry 6) turned out to be the best choice; however, we noticed that the portion-wise addition of hydrazone **1** to the reaction mixture significantly increased the yield of **2** from 57 to 73% (entry 7).

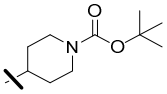
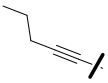
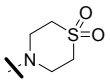
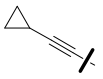
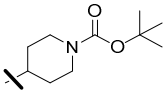
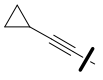
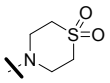
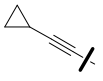
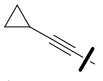
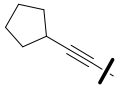
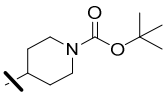
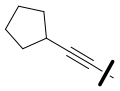
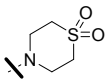
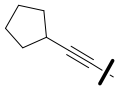
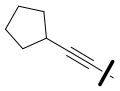
The regioselective bromination of thiophene **2** at position 5 (Scheme 1) was accomplished using *n*-bromosuccinimide (NBS), in DMF. The reaction solvent was optimised as well and, although there were no significant differences in yields between ACN and DMF, the latter was preferred since the product **3** could be isolated by precipitation. It is worth noting that although relatively harsh conditions were employed in this step, no bromination of other positions on the thiophene ring was noticed. The bromo-thiophene **3** was then hydrolysed to the corresponding acid **4** with potassium hydroxide in methanol; to avoid the formation of by-products resulting from hydrolysis of the cyano group, the reaction was carried out at room temperature. The carboxylic acid **4** was then converted into the corresponding acyl chloride by thionyl chloride in toluene and then coupled to different amines to afford the amides **5a-d** (see Table 2 for the yields).

Alternatively (Scheme 2), bromo-thiophene **3** was coupled with three different alkynes via a Sonogashira reaction to afford the 5-alkynyl-pyrazoles **6a-c** in very good yields (88-90 %). Since the starting alkynes were volatile, all the reactions were carried out in sealed vessels. Compounds **6a-c** were hydrolysed to the corresponding acids **7a-c** and then coupled to several amines, via the corresponding acyl chlorides, affording the amides **8a-l** (Table 2). It is worth noting that it was possible to perform the syntheses without any chromatographic purification of **5a-d** until the final acylation of the amines and of **8a-l** until the Sonogashira coupling step.



Scheme 2. Reagents and conditions: a) $(Ph_3)_2PdCl_2$, CuI, DIPA, 40 °C for 40 min; alkyne, 80 °C, 16 h b) KOH, MeOH, room temperature, 3 h; c) thionyl chloride, toluene, reflux, 3 h; d) Et_3N, DCM, amine, 0 °C to room temperature, 16

Table 2. Synthesis of pyrazoles **8a-i**.

Compound	R^1	R^2	Yield[a]
8a			47%
8b			65%
8c			45%
8d			33%
8e			48%
8f			53%
8g			39%
8h			28%
8i			41%
8j			46%
8k			37%
8l			31%

[a] From **7** over two steps.

Binding affinity and SAR

Binding affinity tests for the cannabinoid receptors were performed on all compounds **5** and **8** by means of radio-receptor binding assays using the protocol previously described.[8] The results are summarised in Table 3.

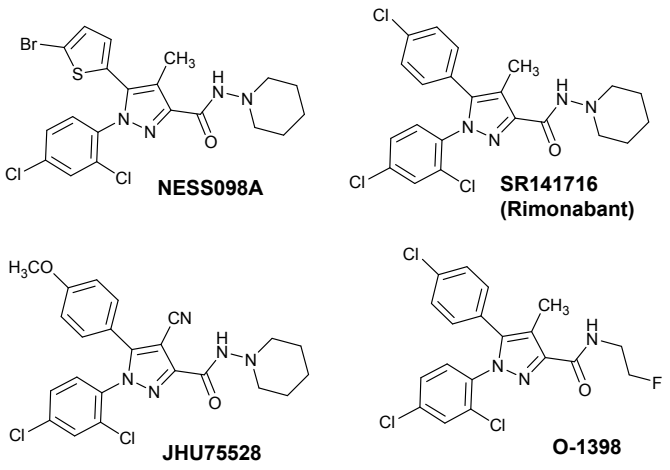


Figure 1. Structures of CB_1 ligands NESS098A, Rimonabant, JHU75528 and O-1398

The cyano pyrazole **5a** is an analogue of NESS098A (Fig. 1) and is structurally related to the inverse agonist SR141716 (Rimonabant, Fig. 1). Replacement of the methyl in position 4 on the pyrazole ring with the more polar cyano group increased 4-fold the CB_1/CB_2 selectivity of **5a** relative to NESS098A (Table 3), while maintaining a similar affinity. Furthermore, **5a** showed a reduced lipophilicity (experimental LogP) relative to that displayed by Rimonabant. Slightly lower CB_1 affinity and significantly lower CB_1/CB_2 selectivity were observed for **5b**, having R^1 = N-Boc-4-piperidyl as carboxamide substituent. A significant drop of CB_1 affinity was measured for compounds **5c,d**, having respectively a dioxo-thiomorpholine and 2-fluoroethyl carboxamide residues as R^1. The introduction of alkynyl substituents on the 5-thiophenyl ring in compounds **8** brought about a significant general improvement of the CB_1 affinity relative to the corresponding precursors **5a-d**, as demonstrated by the fact that all compounds **8a-l** have CB_1 K_i in the 1.0-13.0 nM range. On the other hand, the nature of this alkynyl residue R^2 had little effect on CB_1 affinity and CB_1/CB_2 selectivity. In contrast, the nature of the carboxamido residue R^1 had a more profound effect on both CB_1 affinity and CB_1/CB_2 selectivity. In fact all the compounds having a Rimonabant-type R^1 = piperidyl (**8a, 8e, 8i**) showed higher CB_1 affinity (K_i ca. 1 nM) and CB_1/CB_2 selectivity than their analogues having the same alkynyl R^2 and different carboxamides R^1 within the three series **8a-d**, **8e-h** and **8i-l**. It is worth noting that 2-fluoroethyl carboxamides **8d**, **8h** and **8l**, which should be considered candidate PET tracers as the fluorine atom is potentially amenable to ^{18}F-radiofluorination, all showed K_i CB_1 ca. 10 nM, which is essentially equal to that of the CB_1 PET tracer **JHU75528**. Furthermore **8h** displayed a good CB_1/CB_2 selectivity = 42, combined with a significantly lower experimental LogP than that of Rimonabant. All together, the data above suggest that **8h** is an interesting candidate for further development into an experimental PET tracer for CB_1 imaging. It is also noteworthy that the optimised fluoroethylamide compounds **8h,d,l** displayed much higher CB_1 affinity than that reported for the rimonabant-type fluoroethylamide analogue **O-1398** (Fig. 1 and Table 3),[9] and even the bromothiophenyl precursor **5d** showed 4-fold higher CB1 activity than **O-1398**. Considering the nature of the CB

receptors, which are transmembrane receptors and generally prefer ligands with high lipophilicity,[10] it is not surprising that compound **5c**, which has the lowest lipophilicity (LogP= 3.3±0.2) displayed one of the lowest CB_1 affinities whereas, for instance, the N-BOC protected **8j** with its high LogP value of 5.7±0.3 showed one of the best CB_1 affinities. Finally, as shown in Tables 1 and 2, compounds having R^1 = N-Boc-4-piperidyl (**5b, 8b,f,j**) or dioxo-thiomorpholine (**5c, 8c,g,k**) display calculated Topological Polar Surface Area (TPSA) values[11] >90, which is the generally accepted cut-off value for brain penetration,[11] whereas Rimonabant has a TPSA ~ 50, so these compounds might be periferally restricted.

Table 3. Pharmacological evaluation of pyrazoles **5a-d** and **8a-l**.

Compound	CB_1 K_i (nM)	CB_2 K_i (nM)	K_i CB_2/ K_i CB_1	Experimental LogP[a]
5a[b]	13,7± 4,5	2307,0± 528,7	177	4,0± 0,2
5b[c]	30,3± 8,2	628,8± 252,1	20	4,4± 0,2
5c[d]	296,1± 99,9	5641,0± 2600,0	19	3,3± 0,2
5d[e]	304,0± 104,5	1245,0± 532,3	4	3,6± 0,2
8a[b]	1,0± 0,3	59,3± 18,7	59	4,8± 0,2
8b[c]	6,5± 1,2	206,7± 61,5	32	5,2± 0,3
8c[d]	5,3± 2,2	97,0± 38,1	18	4,0± 0,2
8d[e]	5,9± 2,1	156,0± 30,2	26	4,4± 0,2
8e[b]	1,8± 0,8	96,8± 18,4	53	4,5± 0,2
8f[c]	5,4± 1,6	102,8± 32,2	20	4,9± 0,3
8g[d]	11,4± 3,5	157,1± 48,7	14	3,7± 0,2
8h[e]	13,0± 4,5	550,6± 197,1	42	4,1± 0,2
8i[b]	1,1± 0,2	36,1± 10,8	36	5,3± 0,3
8j[c]	1,3± 0,4	34,4± 12,5	26	5,7± 0,3
8k[d]	5,3± 1,6	55,7± 13,5	10	4,5± 0,2
8l[e]	10,3± 3,2	104,5± 30,2	10	4,8± 0,2
SR141716[f]	2.2[f]	4900[f]	2227	4,7± 0,2
NESS098A[f]	17.4[12]	781[12]	45	n.d.
JHU75528	11± 7	n.d.	n.d.	3.3 [g]
O-1398	852± 175	n.d.	n.d.	n.d.

[a]Determined experimentally by means of RP-HPLC (see experimental section for details); [b]TPSA[13] (Å^2) = 73.95; [c]TPSA[13] (Å^2) = 100.25; [d]TPSA[13] (Å^2) = 108.09; [e]TPSA[13] (Å^2) = 70.71; [f] TPSA[13] (Å^2) = 50.16; [g] LogD7.4 value reported by the authors.[14]

Functional assays

Two of the novel ligands, **8d** and **8h** which are the most suitable for prospective PET imaging applications, were investigated using a functional assay, the [^{35}S]GTPγS binding assay (for details see Supporting Information). **8d** caused a significant increase in [^{35}S]GTPγS binding to 42.1% (95% confidence limits, 30 – 55) with an EC_{50} value of 255nM (95% confidence limits, 28 – 2351) when investigated alone. When 100nM was used to antagonize the CB_1 receptor agonist CP55940 there was an increase in the EC_{50} value from 21.3nM (95% confidence limits, 4 – 104) in the presence of vehicle, to 313nM (95% confidence limits, 18 – 5536) in the presence of 100nM **8d**.

8h also caused a significant increase in [^{35}S]GTPγS binding to 44.7% (95% confidence limits, 40 – 58) with an EC_{50} value of 123nM (95% confidence limits, 13 – 1131) when investigated alone. When 100nM was used to antagonize the CB_1 receptor agonist CP55940 there was an increase in the EC_{50} value from 3.7nM (95% confidence limits, 0.3 – 43) in the presence of vehicle, to 268nM (95% confidence limits, 62 – 1159) in the presence of 100nM **8h**.

These data show that both the tested compounds showed a significant increase in the EC_{50} values and should be therefore considered antagonists of CP55940 for the CB_1 receptor. Given the structural similarity of **8d,h** with all of the other novel CB_1 ligands **5** and **8** we confidently assume that all of these compounds are also CB_1 antagonists.

Conclusion

Replacement of the Rimonabant-type C-5 aryl ring with a thiophenyl bioisostere and of the C-3 methyl group with a more polar cyano group generated a novel class of pyrazolyl cannabinoid receptor subtype 1 ligands, that were synthesised in 6 (**5a-d**) and 7 steps (**8a-l**) with 13-25% and 10-23% overall yields, respectively. Among the 5-bromothiophenyl derivatives **5a-d**, only **5a** displayed high CB_1 affinity and CB_1/CB_2 selectivity and modification of the 1-piperidyl carboxamide residue R^1 caused a decrease of both affinity and selectivity. Replacement of the bromine atom with an alkynyl residue as in compounds **8a-l** resulted in a generally increased CB_1 affinity (1.0-13.0 nM) and maintained good CB_1/CB_2 selectivity (ratios in the range 10 to 59). 2-Fluoroethyl carboxamides **8d**, **8h** and **8l** are interesting candidates for further development into PET tracers, particularly **8h** that showed CB_1 K_i = 13.0 nM and CB_1/CB_2 selectivity = 42. Both lipophilicity and TPSA could be efficiently tuned in these class of CB1 ligands. In fact, compound **8g** showed low lipophilicity (experimental LogP = 3.7) relative to most known CB_1 ligands, combined with a good affinity (CB_1 K_i = 11.4 nM), whereas **8j** had very high lipophilicity (LogP = 5.7), very high affinity (CB_1 K_i = 1.1 nM) and good selectivity (CB_1/CB_2 = 36). However, the same compound **8j** and other analogues (**8b,c,f,g,k**) feature high calculated TPSA (>100) so they may behave as peripherally restricted CB_1 ligands. In particular, the pentynyl thiophene **8c** showed similar biological and physico-chemical properties relative to the well-studied peripherally restricted cannabinoid ligand AM6545.[15] Functional binding assays performed on two of the novel ligands, **8d** and **8h**, showed that these compounds are competitive antagonists of CP55940 for the CB_1 receptor.

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 δ = 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 δ = 77.0). ^{19}F NMR spectra were referenced to $CFCl_3$ as the external standard. All chemical shift (σ) 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 x 4.60 mm L x ID, particle size: 5 µ) and an ESI-MS detector.

HRMS analysis were performed by the EPSRC National Mass Spectrometry Service Centre (Swansea, UK).

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.[16]

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

*Ethyl 2-chloro-2-(2-(2,4-dichlorophenyl)hydrazono)acetate (**1**)*.2,4-Dichloroaniline (5.00 g, 30.56 mmol) was stirred at room temperature in an aqueous solution of HCl (7% v/v, 187 ml) until complete dissolution. The reaction mixture was cooled to 0 °C with ice and a solution of sodium nitrite (2.22 g, 31.17 mmol) in water (15 ml) was added dropwise for 1 hour. This cold orange mixture was cannulated into a cold solution of sodium acetate (2.43 g, 29.33 mmol) and ethyl 2-chloro-3-oxobutanoate (5.29 g, 30.56 mmol) in ethanol (305 ml). The temperature was allowed to reach the room temperature and the reaction was stirred for 2 h. The yellow precipitate formed was filtered, washed with water and dried overnight in a desiccator containing P_2O_5 to give the desired solid product **1** (84%). 1H NMR (400 MHz, Chloroform-d) δ 8.74 (s, 1H), 7.56 (d, J = 8.8 Hz, 1H), 7.36 (d, J = 2.3 Hz, 1H), 7.26 (ddd, J = 8.8, 2.3, 0.6 Hz, 1H), 4.40 (q, J = 7.1 Hz, 2H), 1.41 (t, J = 7.1 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 159.2, 136.6, 129.0, 128.4, 127.7, 119.4, 119.2, 116.6, 63.1, 14.2. ESMS, calculated m/z $C_{10}H_9Cl_3N_2O_2$ 295 [M+H]+, found m/z (relative intensity) 317 [M+Na]+ (95).

*Ethyl 4-cyano-1-(2,4-dichlorophenyl)-5-(thiophen-2-yl)-1H-pyrazole-3-carboxylate (**2**)*. A solution of 3-oxo-3-(2-thienyl)propionitrile (0.95 g, 6.11 mmol) and Et3N (3.1 ml, 22.23 mmol) in tert-butanol (15 ml) was magnetically stirred at 30° C for 30 min. To this reaction mixture, compound **1** (2.00 g, 5.56 mmol) was added portionwise in 3 h. The reaction was kept at 30 °C and stirred for 16 h. The brown precipitate was filtered, dissolved in DCM and washed with water (3 X 20 ml). The organic phase was dried over anhydrous Na2SO4 and evaporated. The crude product was purified by column chromatography on silica gel (Hex/EtOAc 70:30) to afford the product **2** as a pale yellow solid (73%). 1H NMR (400 MHz, Chloroform-d) δ 7.62 (dd, J = 3.8, 1.2 Hz, 1H), 7.56 (dd, J = 1.8, 0.7 Hz, 1H), 7.49 – 7.41 (m, 3H), 7.11 (dd, J = 5.1, 3.8 Hz, 1H), 4.52 (q, J = 7.1 Hz, 2H), 1.47 (t, J = 7.1 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 159.5, 145.9, 145.7, 138.1, 134.3, 134.0, 131.0, 130.7, 130.6, 130.1, 128.5, 128.0, 125.4, 112.7, 93.3, 62.4, 14.2. ESMS, calculated m/z $C_{17}H_{11}Cl_2N_3O_2S$ 392 [M+H]+, found m/z (relative intensity) 392.0 [M+H]+ (100).

*Ethyl 5-(5-bromothiophen-2-yl)-4-cyano-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carboxylate (**3**)*. N-Bromosuccinimide (4.13 g, 22.95 mmol) was added portionwise to a magnetically stirred solution of **2** (3.33 g, 7.65 mmol) in DMF (26 ml), cooled with ice. The reaction was heated to 80° C and stirred for 16h. The reaction was quenched with a saturated solution of sodium thiosulphate. The precipitate was filtered and dissolved in a small portion of DCM. The organic phase was washed with water, dried over anhydrous Na2SO4 and concentrate under reduced pressure. The residue was purified by column chromatography on silica gel (Hex/EtOAc 80:20) to give the desired product **3** as a pale yellow solid (79%). 1H NMR (400 MHz, Chloroform-d) δ 7.59 (dd, J = 1.9, 0.7 Hz, 1H), 7.47 – 7.45 (m, 2H), 7.40 (d, J = 4.1 Hz, 1H), 7.07 (d, J = 4.1 Hz, 1H), 4.52 (q, J = 7.1 Hz, 2H), 1.46 (t, J = 7.1 Hz, 3H); 13C NMR (101 MHz, CDCl3) δ 159.3, 145.9, 144.6, 138.4, 133.9, 133.9, 131.0, 130.9, 130.9, 130.8, 128.7, 126.7, 118.2, 112.4, 93.3, 62.5, 14.2. ESMS, calculated m/z $C_{17}H_{10}BrCl_2N_3O_2S$ 470 [M+H]+, found m/z (relative intensity) 469.9 [M+H]+ (29).

*5-(5-Bromothiophen-2-yl)-4-cyano-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carboxylic acid (**4**)*. To a solution of bromo ester 3 (0.10 g, 0.20 mmol) in methanol (4 mL) was added a solution of potassium hydroxide (0.8 g, 1.2 mmol) in methanol (3 mL) dropwise at room temperature. The reaction mixture was stirred for 3 h. After the hydrolysis was complete, the reaction mixture was poured into ice-water and acidified with 2N hydrochloric acid. The precipitate was filtered, washed with water, and dried under vacuum to give the acid 4 (87%) as a white solid. 1H NMR (400 MHz, Methanol-d4) δ 7.82 (d, J = 2.2 Hz, 1H), 7.74 (d, J = 8.5 Hz, 1H), 7.64 (dd, J = 8.5, 2.2 Hz, 1H), 7.36 (d, J = 4.1 Hz, 1H), 7.21 (d, J = 4.1 Hz, 1H); 13C NMR (101 MHz, MeOD) δ 160.7, 146.3, 144.6, 138.2, 134.1, 133.4, 131.3, 131.2, 130.9, 130.3, 128.8, 126.8, 117.6, 112.1, 93.1. ESMS, calculated m/z $C_{15}H_6BrCl_2N_3O_2S$ 442 [M+H]+, found m/z (relative intensity) 441.8 [M+H]+ (26).

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

5-(5-Bromothiophen-2-yl)-4-cyano-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carbonyl chloride. A solution of the acid 4 (0.63 g, 1.39 mmol) and thionyl chloride (306 µl, 4.18 mmol) in toluene (9 mL) was refluxed for 3 h. Solvent was evaporated under reduced pressure and the residue was then re-dissolved in toluene (5 mL) first and then in hexane (5 ml); the crude was concentrated to give the white carboxylic chloride (0.62 g, 98% yield) as a solid.

*5-(5-Bromothiophen-2-yl)-4-cyano-1-(2,4-dichlorophenyl)-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (**5a**)*. A solution in dichloromethane of the carboxylic chloride obtained as described above (2 ml, 0.5 M), was added dropwise to a solution of 1-aminopiperidine (0.13 g, 1.21 mmol) and triethylamine (171 µl, 01.21 mmol) in dichloromethane (2 ml) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 16 h. The reaction was quenched with water and the organic phase was extracted with dichloromethane (3 x 3 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Flash column chromatography on silica gel (n-Hexane/ethyl acetate 5:5) gave carboxamide **5a** as a white solid (36% yield). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.60 (dd, J = 2.0, 0.5 Hz, 1H), 7.49 (dd, J = 8.5, 2.1 Hz, 2H), 7.45 (d, J = 8.8 Hz, 1H), 7.40 (d, J = 4.0 Hz, 1H), 7.05 (d, J = 4.1 Hz, 1H), 2.88 (t, J = 5.4 Hz, 4H), 1.73 (p, J = 5.7 Hz, 4H), 1.43 (dt, J = 10.3, 5.3 Hz, 2H); ^{13}C NMR

(101 MHz, CDCl3) δ 156.1, 148.1, 144.7, 138.4, 134.0, 133.9, 131.0, 130.9, 130.8, 128.8, 126.8, 118.1, 112.4, 92.6, 56.8, 25.3, 23.2. ESMS, calculated *m/z* $C_{20}H_{16}BrCl_2N_5OS$ 524 $[M+H]^+$, found *m/z* (relative intensity) 523.9 $[M+H]^+$ (63). HRMS m/z $[M^+H]^+$ calcd. for $C_{20}H_{17}BrCl_2N_5OS$: 523.9714; found: 523.9703.

*Tert-Butyl 4-(5-(5-bromothiophen-2-yl)-4-cyano-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carboxamido)piperidine-1-carboxylate (**5b**)*. **5b** (92 mg, 51% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.62 (d, J = 2.2 Hz, 1H), 7.49 (dd, J = 8.5, 2.2 Hz, 1H), 7.44 (d, J = 8.5 Hz, 1H), 7.42 (d, J = 4.0 Hz, 1H), 7.06 (d, J = 4.1 Hz, 1H), 6.62 (d, J = 8.0 Hz, 1H), 4.20 – 4.00 (m, 4H), 2.91 (t, J = 12.8 Hz, 2H), 2.01 (dd, J = 12.9, 4.0 Hz, 2H), 1.45 (s, 9H), 1.39 (dd, J = 11.8, 4.2 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.1, 154.8, 148.6, 145.0, 138.6, 134.1, 134.0, 131.1, 130.9, 128.9, 127.0, 118.2, 112.7, 92.3, 79.8, 77.2, 47.1, 42.7, 32.0, 28.5. ESMS, calculated m/z $C_{25}H_{24}BrCl_2N_5O_3S$ 624 [M+H]+, found m/z (relative intensity) 646.0 [M+Na]+ (54). HRMS m/z [M+H]+ calcd. for $C_{25}H_{25}BrCl_2N_5O_3S$: 624.0239; found: 624.0235.

*5-(5-Bromothiophen-2-yl)-4-cyano-1-(2,4-dichlorophenyl)-N-(1,1-dioxido-thiomorpholino)-1H-pyrazole-3-carboxamide (**5c**)*. **5c** (281 mg, 62% yield) was obtained as a white solid. 1H NMR (400 MHz, Chloroform-d) δ 7.93 (s, 1H), 7.63 (d, J = 2.2 Hz, 1H), 7.50 (dd, J = 8.5, 2.2 Hz, 1H), 7.43 (d, J = 4.1 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.08 (d, J = 4.1 Hz, 1H), 3.58 (t, J = 5.5 Hz, 4H), 3.25 (t, J = 5.3 Hz, 4H); 13C NMR (101 MHz, CDCl3) δ 156.9, 147.0, 145.0, 138.7, 133.9, 133.7, 131.2, 131.0, 130.7, 128.9, 126.5, 118.5, 112.2, 92.4, 52.8, 51.1. ESMS, calculated m/z C19H14BrCl2N5O3S2 574 [M+H]+, found m/z (relative intensity) 573.9 [M+H]+ (57). HRMS m/z M+NH4+ calcd. for C19H15BrCl2N5O3S2: 590.9442; found: 590.9434.

*5-(5-Bromothiophen-2-yl)-4-cyano-1-(2,4-dichlorophenyl)-N-(fluoromethyl)-1H-pyrazole-3-carboxamide (**5d**)*. **5d** (119 mg, 32%) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.62 (dd, J = 2.2, 0.4 Hz, 1H), 7.50 (dd, J = 8.5, 2.2 Hz, 1H), 7.44 (d, J = 0.4 Hz, 1H), 7.43 (d, J = 4.0 Hz, 1H), 7.10 (t, J = 6.4 Hz, 1H), 7.07 (d, J = 4.1 Hz, 1H), 4.60 (dt, J = 47.2, 4.8 Hz, 2H), 3.78 (dq, J = 27.9, 5.2 Hz, 2H); ^{13}C NMR (101 MHz, CDCl3) δ 158.8, 148.2, 144.9, 138.5, 134.0, 133.9, 131.0, 130.9, 130.7, 128.8, 126.8, 118.2, 112.5 , 92.1, 82.43 (d, J = 167.5 Hz), 39.82 (d, J = 19.9 Hz); ^{19}F NMR (376 MHz, Chloroform-d) δ -224.10 (tt, J = 47.3, 27.8 Hz). ESMS, calculated *m/z* $C_{17}H_{10}BrCl_2FN_4OS$ 487 $[M+H]^+$, found *m/z* (relative intensity) 486.9 $[M+H]^+$ (56). HRMS m/z $[M^+H]^+$ calcd. for $C_{17}H_{11}BrCl_2FN_4OS$: 486.9198; found: 486.9188.

General Procedure for the Synthesis of Compounds 6a-c. The general procedure is illustrated below for compound **6a**.

*Ethyl 4-cyano-1-(2,4-dichlorophenyl)-5-(5-(pent-1-yn-1-yl)thiophen-2-yl)-1H-pyrazole-3-carboxylate (**6a**)*. To a solution of the ester **3** (1.00 g, 1.91 mmol) in diisopropyl amine (8 ml) in a sealed vial, magnetically stirred at 40 °C, bis(triphenylphosphine)-palladium(II)dichloride (0.01 g, 0.01 mmol), triphenyl phosphine (0.01 g, 0.02 mmol) and Copper(I) Iodide (0.01 g, 0.02 mmol) were added. The reaction mixture was left stirring for 40 min and then the alkyne 1-pentyne (571 µl, 5.73 mmol) was added in one portion. The mixture was warmed up to 80 °C and stirred for 16 h. The reaction was cooled to room temperature, quenched with a solution of HCl 2N and the organic phase was extracted with EtOAc (3 X 7). The combined organic phases were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The black crude was purified by column chromatography on silica gel (Hex/EtOAc 70:30) to afford the alkyne **6** as a pale yellow solid (88%). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.58 (dd, J = 1.6, 1.0 Hz, 1H), 7.50 (d, J = 4.0 Hz, 1H), 7.46 – 7.44 (m, 2H), 7.06 (d, J = 4.0 Hz, 1H), 4.52 (q, J = 7.1 Hz, 2H), 2.39 (t, J = 7.1 Hz, 2H), 1.61 (h, J = 7.3 Hz, 2H), 1.46 (t, J = 7.1 Hz, 3H), 1.01 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 159.4, 145.9, 145.1, 138.2, 134.2, 134.0, 131.5, 130.9, 130.8, 130.4, 129.4, 128.6, 124.6, 112.7, 98.8, 93.2, 72.6, 62.4, 21.8, 21.7, 14.2, 13.6. ESMS, calculated m/z $C_{22}H_{17}Cl_2N_3O_2S$ 458 [M+H]+, found m/z (relative intensity) 458.0 [M+H]+ (100).

*Ethyl 4-cyano-5-(5-(cyclopropylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carboxylate (**6b**)*. **6b** (547 mg, 90% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.57 (dd, J = 1.7, 0.8 Hz, 1H), 7.51 (d, J = 4.0 Hz, 1H), 7.46 – 7.44 (m, 2H), 7.04 (d, J = 4.0 Hz, 1H), 4.51 (q, J = 7.1 Hz, 2H), 1.46 (t, J = 7.1 Hz, 3H), 1.51 – 1.39 (m, 1H), 0.94 – 0.86 (m, 2H), 0.86 – 0.80 (m, 2H); ^{13}C NMR (101 MHz, CDCl3) δ 159.4, 145.9, 145.1, 138.2, 134.2, 134.1, 131.7, 130.9, 130.8, 130.4, 129.4, 128.6, 124.6, 112.7, 101.9, 93.1, 67.7, 62.4, 14.2, 9.0, 0.4. ESMS, calculated m/z $C_{22}H_{15}Cl_2N_3O_2S$ 456 [M+H]+, found m/z (relative intensity) 456.0 [M+H]+ (100).

*Ethyl 4-cyano-5-(5-(cyclopentylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carboxylate (**6c**)*. **6c** (581 mg, 90% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.58 (dd, J = 1.6, 0.9 Hz, 1H), 7.48 (d, J = 4.0 Hz, 1H), 7.45 (d, J = 1.6 Hz, 2H), 7.04 (d, J = 4.0 Hz, 1H), 4.52 (q, J = 7.1 Hz, 2H), 2.82 (p, J = 7.6 Hz, 1H), 2.03 – 1.92 (m, 2H), 1.79 – 1.56 (m, 6H), 1.46 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 159.4, 145.9, 145.1, 138.2, 134.2, 134.0, 131.4, 130.9, 130.7, 130.4, 129.5, 128.6, 124.5, 112.7, 103.0 , 93.1, 72.0, 62.4, 33.5, 30.9, 25.1, 14.2. ESMS, calculated m/z $C_{24}H_{19}Cl_2N_3O_2S$ 484 [M+H]+, found m/z (relative intensity) 484.0 [M+H]+ (100).

General Procedure for the Synthesis of Compounds 7a-c. The general procedure is illustrated below for compound **7a**.

*4-Cyano-1-(2,4-dichlorophenyl)-5-(5-(pent-1-yn-1-yl)thiophen-2-yl)-1H-pyrazole-3-carboxylic acid (**7a**)*. To a solution of bromo ester **6a** (0.60 g, 1.24 mmol) in methanol (20 mL) was added a solution of potassium hydroxide (0.6 g, 1.24 mmol) in methanol (41 mL) dropwise, at room temperature. The reaction mixture was stirred for 3 h. After the hydrolysis was complete, the reaction mixture was poured into ice-water and acidified with 2N hydrochloric acid. The precipitate was filtered, washed with water, and dried under vacuum to give the acid **7a** (84%) as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.60 (dd, J = 1.7, 0.8 Hz, 1H), 7.53 (d, J = 4.0 Hz, 1H), 7.50 – 7.44 (m, 2H), 7.07 (d, J = 4.0 Hz, 1H), 2.39 (t, J = 7.1 Hz, 2H), 1.61 (h, J = 7.3 Hz, 2H), 1.01 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 163.3, 145.6, 144.7, 138.4, 134.0, 133.9, 131.6, 130.8, 130.6, 129.7, 128.7, 124.3, 112.3, 99.0, 93.6 , 72.6, 53.4, 21.7, 13.6. ESMS, calculated m/z $C_{20}H_{13}Cl_2N_3O_2S$ 430 [M+H]+, found m/z (relative intensity) 468.0 [M+K]+ (100).

*4-Cyano-5-(5-(cyclopropylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carboxylic acid (**7b**)*. **7b** (380 mg, 90% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 14.13 (s, 1H), 8.03 (d, J = 2.2 Hz, 1H), 7.92 (d, J = 8.5 Hz, 1H), 7.76 (dd, J = 8.5, 2.3 Hz, 1H), 7.38 (d, J = 4.0 Hz, 1H), 7.27 (d, J = 4.0 Hz, 1H), 1.59 (tt, J = 8.3, 5.0 Hz, 1H), 0.95 – 0.88 (m, 2H), 0.80 – 0.74 (m, 2H); ^{13}C NMR (101 MHz, CDCl3) δ 161.6, 145.1, 144.9, 137.9, 133.6, 133.5, 131.2, 130.4, 130.3, 130.1, 129.0, 128.2, 124.0, 112.1, 101.5,

92.8, 67.2, 50.1, 8.5. ESMS, calculated *m/z* $C_{20}H_{11}Cl_2N_3O_2S$ 428 $[M+H]^+$, found *m/z* (relative intensity) 466.0 $[M+K]^+$ (100).

*4-Cyano-5-(5-(cyclopentylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carboxylic acid (**7c**).* **7c** (380 mg, 85% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.60 (dd, J = 1.7, 0.8 Hz, 1H), 7.51 (d, J = 4.0 Hz, 1H), 7.50 – 7.44 (m, 2H), 7.05 (d, J = 4.0 Hz, 1H), 2.82 (p, J = 7.6 Hz, 1H), 2.03 – 1.93 (m, 2H), 1.79 – 1.55 (m, 6H); ^{13}C NMR (101 MHz, CDCl3) δ 163.2, 145.6, 144.8, 138.4, 134.0, 133.9, 131.5, 130.8, 130.8, 130.6, 129.8, 128.7, 124.2, 112.3, 103.2, 93.5, 72.0, 33.5, 30.9, 25.1. ESMS, calculated m/z $C_{22}H_{15}Cl_2N_3O_2S$ 456 [M+H]+, found m/z (relative intensity) 494.0 [M+K]+ (100).

General Procedure for the Synthesis of Compounds 8a-d. The general procedure is illustrated below for compound **8a**.

4-Cyano-1-(2,4-dichlorophenyl)-5-(5-(pent-1-yn-1-yl)thiophen-2-yl)-1H-pyrazole-3-carbonyl chloride. A solution of the acid **6a** (0.15 g, 0.33 mmol) and thionyl chloride (73 µl, 0.99 mmol) in toluene (2.2 mL) was refluxed for 3 h. Solvent was evaporated under reduced pressure and the residue was then re-dissolved in toluene (3 mL) first and then in Hexane (3 ml); the crude was concentrated to give the white carboxylic chloride (98% yield) as a solid

*4-Cyano-1-(2,4-dichlorophenyl)-5-(5-(pent-1-yn-1-yl)thiophen-2-yl)-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (**8a**).* A solution in dichloromethane of the carboxylic chloride obtained as described above (613 µl, 0.7 M), was added dropwise to a solution of 1-aminopiperidine (50 µl, 0.45 mmol) and triethylamine (63 µl, 0.45 mmol) in dichloromethane (0.9 ml) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 16 h. The reaction was quenched with water and the organic phase was extracted with dichloromethane (3 x 3 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Flash column chromatography on silica gel (n-Hexane/ethyl acetate 6:4) gave carboxamide **8a** (48 % yield). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.60 (d, J = 2.1 Hz, 1H), 7.52 (d, J = 4.0 Hz, 1H), 7.48 (dd, J = 8.5, 2.0 Hz, 2H), 7.43 (d, J = 8.4 Hz, 1H), 7.05 (d, J = 4.0 Hz, 1H), 2.89 (t, J = 5.4 Hz, 4H), 2.38 (t, J = 7.1 Hz, 2H), 1.74 (p, J = 5.5 Hz, 4H), 1.60 (h, J = 7.4 Hz, 2H), 1.43 (s, 2H), 1.01 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 156.2, 148.1, 145.2, 138.2, 134.2, 134.0, 131.5, 130.9, 130.8, 130.4, 129.2, 128.7, 124.7, 112.6, 98.7, 92.4, 72.7, 56.8, 25.4, 23.2, 21.8, 21.7, 13.6. ESMS, calculated *m/z* $C_{25}H_{23}Cl_2N_5OS$ 475 $[M+H]^+$, found *m/z* (relative intensity) 475.0 $[M+H]^+$ (100). HRMS m/z $[M^+H]^+$ calcd. for $C_{25}H_{24}Cl_2N_5OS$: 512.1079; found: 512.1066.

*tert-Butyl 4-(4-cyano-1-(2,4-dichlorophenyl)-5-(5-(pent-1-yn-1-yl)thiophen-2-yl)-1H-pyrazole-3-carboxamido)piperidine-1-carboxylate (**8b**).* **8b** (118 mg, 66% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.61 (d, J = 2.2 Hz, 1H), 7.53 (d, J = 3.9 Hz, 1H), 7.48 (dd, J = 8.5, 2.2 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H), 7.05 (d, J = 4.0 Hz, 1H), 6.62 (d, J = 8.1 Hz, 1H), 4.22 – 3.96 (m, 4H), 2.91 (t, J = 12.8 Hz, 2H), 2.38 (t, J = 7.1 Hz, 2H), 2.01 (d, J = 12.5 Hz, 2H), 1.60 (h, J = 7.3 Hz, 2H), 1.45 (s, 9H), 1.39 (dd, J = 12.0, 3.8 Hz, 1H), 1.01 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 158.0, 154.7, 148.5, 145.3, 138.3, 134.1, 131.5, 130.9, 130.8, 130.4, 129.2, 128.7, 124.7, 112.8, 98.8, 92.0, 79.7, 72.6, 46.9, 42.7, 31.9, 28.4, 21.8, 21.7, 13.6. ESMS, calculated *m/z* $C_{30}H_{31}Cl_2N_5O_3S$ 612 $[M+H]^+$, found *m/z* (relative intensity) 634.1 $[M+Na]^+$ (100). HRMS m/z $[M^+H]^+$ calcd. for $C_{30}H_{32}Cl_2N_5O_3S$: 612.1603; found: 612.1607.

*4-Cyano-1-(2,4-dichlorophenyl)-N-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-5-(5-(pent-1-yn-1-yl)thiophen-2-yl)-1H-pyrazole-3-carboxamide (**8c**).* **8c** (98 mg, 46% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.97 (s, 1H), 7.61 (d, J = 2.2 Hz, 1H), 7.52 (d, J = 4.0 Hz, 1H), 7.49 (dd, J = 8.5, 2.2 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.05 (d, J = 4.0 Hz, 1H), 3.64 – 3.52 (m, 4H), 3.24 (t, J = 5.3 Hz, 4H), 2.38 (t, J = 7.1 Hz, 2H), 1.60 (h, J = 7.3 Hz, 2H), 1.00 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 156.9, 147.0, 145.5, 138.5, 134.0, 131.6, 131.0, 130.7, 130.6, 129.6, 128.8, 124.3, 112.4, 99.0, 92.3, 72.6, 52.8, 51.1, 21.8, 21.7, 13.6. ESMS, calculated *m/z* $C_{24}H_{21}Cl_2N_5O_3S_2$ 562 $[M+H]^+$, found *m/z* (relative intensity) 584.0 $[M+Na]^+$ (100). HRMS m/z $[M^+H]^+$ calcd. for $C_{24}H_{22}Cl_2N_5O_3S_2$: 562.0541; found: 562.0531.

*4-Cyano-1-(2,4-dichlorophenyl)-N-(2-fluoroethyl)-5-(5-(pent-1-yn-1-yl)thiophen-2-yl)-1H-pyrazole-3-carboxamide (**8d**).* **8d** (20 mg, 33% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.61 (d, J = 2.2 Hz, 1H), 7.53 (d, J = 4.0 Hz, 1H), 7.48 (dd, J = 8.5, 2.2 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H), 7.12 – 7.07 (m, 1H), 7.05 (d, J = 4.0 Hz, 1H), 4.60 (dt, J = 47.3, 4.8 Hz, 2H), 3.78 (dq, J = 28.0, 5.2, 4.8 Hz, 2H), 2.38 (t, J = 7.1 Hz, 2H), 1.61 (h, J = 7.3 Hz, 2H), 1.01 (t, J = 7.4 Hz, 3H); ^{13}C NMR (101 MHz, CDCl3) δ 158.9, 148.1, 145.3, 138.3, 134.1, 134.0, 131.5, 130.9, 130.8, 130.4, 129.3, 128.7, 124.7, 112.8, 98.8, 92.0, 82.43 (d, J = 167.5 Hz), 72.6, 39.79 (d, J = 20.0 Hz), 21.8, 21.7, 13.6; ^{19}F NMR (376 MHz, Chloroform-d) δ -224.06 (tt, J = 47.3, 27.8 Hz). ESMS, calculated *m/z* $C_{22}H_{17}Cl_2FN_4OS$ 475 $[M+H]^+$, found *m/z* (relative intensity) 475.0 $[M+H]^+$ (100). HRMS m/z $[M^+H]^+$ calcd. for $C_{22}H_{18}Cl_2FN_4OS$: 475.0562; found: 475.0551.

General Procedure for the Synthesis of Compounds 8e-h. The general procedure is illustrated below for compound **8e**.

4-Cyano-5-(5-(cyclopropylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carbonyl chloride. A solution of the acid **6b** (0.12 g, 0.27 mmol) and thionyl chloride (59 µl, 0.80 mmol) in toluene (1.8 mL) was refluxed for 3 h. Solvent was evaporated under reduced pressure and the residue was then re-dissolved in toluene (3 mL) first and then in Hexane (3 ml); the crude was concentrated to give the white carboxylic chloride (97% yield) as a solid.

*4-Cyano-5-(5-(cyclopropylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (**8e**).* A solution in dichloromethane of the carboxylic chloride obtained as described above (515 µl, 0.5 M), was added dropwise to a solution of 1-aminopiperidine (35 µl, 0.31 mmol) and triethylamine (44 µl, 0.31 mmol) in dichloromethane (0.6 ml) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 16 h. The reaction was quenched with water and the organic phase was extracted with dichloromethane (3 x 3 mL). The combined extracts were washed with brine, dried over anhydrous Na2SO4, filtered, and evaporated under reduced pressure. Flash column chromatography on silica gel (n-Hexane/ethyl acetate 5:5) gave carboxamide 8e as a white solid (50% yield). 1H NMR (400 MHz, Chloroform-d) δ 7.60 (d, J = 2.2 Hz, 1H), 7.53 (d, J = 4.0 Hz, 1H), 7.50 (s, 1H), 7.48 (dd, J = 8.5, 2.2 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.03 (d, J = 4.0 Hz, 1H), 2.94 – 2.84 (m, 4H), 1.75 (p, J = 5.6 Hz, 4H), 1.49 – 1.39 (m, 3H), 0.94 – 0.77 (m, 4H); 13C NMR (101 MHz, CDCl3) δ 156.2, 148.1, 145.2, 138.2, 134.2, 134.1, 131.7, 130.9, 130.4, 129.2, 128.7, 124.7, 112.6, 101.8, 92.4, 67.7, 56.8, 25.4, 23.2, 9.0, 0.4. ESMS,

calculated m/z C25H21Cl2N5OS 510 [M+H]+, found m/z (relative intensity) 510.0 [M+H]+ (100). HRMS m/z [M+H]+ calcd. for C25H22Cl2N5OS: 510.0922; found: 510.0906.

*tert-Butyl 4-(4-cyano-5-(5-(cyclopropylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carboxamido)piperidine-1-carboxylate (**8f**).* **8f** (95 mg, 54% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.61 (d, J = 2.2 Hz, 1H), 7.53 (d, J = 4.0 Hz, 1H), 7.48 (dd, J = 8.5, 2.2 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H), 7.03 (d, J = 4.0 Hz, 1H), 6.61 (d, J = 8.1 Hz, 1H), 4.20 – 3.98 (m, 4H), 2.91 (t, J = 12.8 Hz, 2H), 2.01 (d, J = 14.4 Hz, 2H), 1.45 (s, 9H), 1.42 (dd, J = 8.7, 3.5 Hz, 2H), 0.93 – 0.78 (m, 4H); ^{13}C NMR (101 MHz, CDCl3) δ 158.2, 154.8, 148.6, 145.4, 138.4, 134.3, 134.2, 131.8, 131.0, 130.9, 130.5, 129.3, 128.8, 124.8, 112.9, 102.0, 92.1, 79.8, 67.8, 47.1, 42.8, 32.0, 28.5, 9.1, 0.6. ESMS, calculated *m/z* $C_{30}H_{29}Cl_2N_5O_3S$ 610 $[M+H]^+$, found *m/z* (relative intensity) 632.1 $[M+Na]^+$ (100). HRMS m/z $[M^+H]^+$ calcd. for $C_{30}H_{30}Cl_2N_5O_3S$: 610.1446; found: 610.1444.

*4-Cyano-5-(5-(cyclopropylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-N-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-1H-pyrazole-3-carboxamide (**8g**).* **8g** (71 mg, 40% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.92 (s, 1H), 7.62 (d, J = 2.2 Hz, 1H), 7.54 (d, J = 4.0 Hz, 1H), 7.49 (dd, J = 8.5, 2.2 Hz, 1H), 7.41 (d, J = 8.5 Hz, 1H), 7.04 (d, J = 4.0 Hz, 1H), 3.64 – 3.53 (m, 4H), 3.29 – 3.20 (m, 4H), 1.45 (tt, J = 8.1, 5.0 Hz, 1H), 0.95 – 0.78 (m, 4H); ^{13}C NMR (101 MHz, CDCl3) δ 156.9, 147.0, 145.5, 138.5, 134.0, 133.9, 131.8, 131.0, 130.7, 130.6, 129.5, 128.8, 124.3, 112.4, 102.1, 92.2, 67.6, 52.9, 51.1, 9.0, 0.5. ESMS, calculated *m/z* $C_{24}H_{19}Cl_2N_5O_3S_2$ 560 $[M+H]^+$, found *m/z* (relative intensity) 582.1 $[M+Na]^+$ (100). HRMS m/z $[M^+H]^+$ calcd. for $C_{24}H_{20}Cl_2N_5O_3S_2$: 560.0385; found: 560.0370.

*4-Cyano-5-(5-(cyclopropylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-N-(2-fluoroethyl)-1H-pyrazole-3-carboxamide (**8h**).* **8h** (58mg, 28% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.61 (d, J = 2.1 Hz, 1H), 7.54 (d, J = 4.0 Hz, 1H), 7.48 (dd, J = 8.5, 2.2 Hz, 1H), 7.43 (d, J = 8.8 Hz, 1H), 7.09 (t, J = 6.1 Hz, 1H), 7.03 (dd, J = 4.0, 0.4 Hz, 1H), 4.60 (dt, J = 47.3, 4.7 Hz, 2H), 3.77 (dq, J = 27.7, 4.9 Hz, 2H), 1.45 (tt, J = 8.2, 5.1 Hz, 1H), 0.94 – 0.79 (m, 4H); ^{13}C NMR (101 MHz, CDCl3) δ 158.9, 148.1, 145.3, 138.3, 134.1, 134.0, 131.7, 130.9, 130.8, 130.4, 129.3, 128.7, 124.7, 112.8, 101.8, 92.0, 82.44 (d, J = 167.5 Hz), 67.7, 39.79 (d, J = 19.9 Hz), 9.0, 0.44; ^{19}F NMR (376 MHz, Chloroform-d) δ -224.06 (tt, J = 47.2, 27.8 Hz). ESMS, calculated m/z $C_{22}H_{15}Cl_2FN_4OS$ 472.0 [M+H]+, found m/z (relative intensity) 474 [M+H]+ (100). HRMS m/z [M+H]+ calcd. for $C_{22}H_{16}Cl_2FN_4OS$: 473.0406; found: 473.0394.

General Procedure for the Synthesis of Compounds 8i-l. The general procedure is illustrated below for compound **8i**.

4-Cyano-5-(5-(cyclopentylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carbonyl chloride. A solution of the acid **6c** (0.10 g, 0.21 mmol) and thionyl chloride (46 µl, 0.62 mmol) in toluene (2.2 mL) was refluxed for 3 h. Solvent was evaporated under reduced pressure and the residue was then re-dissolved in toluene (3 mL) first and then in Hexane (3 ml); the crude was concentrated to give the white carboxylic chloride (96% yield) as a solid

*4-Cyano-5-(5-(cyclopentylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (**8i**).* A solution in dichloromethane of the carboxylic chloride obtained as described above (578 µl, 0.4 M), was added dropwise to a solution of 1-aminopiperidine (30 µl, 0.27 mmol) and triethylamine (38 µl, 0.27 mmol) in dichloromethane (0.5 ml) at 0 °C. The reaction mixture was allowed to reach room temperature and stirred for 16 h. The reaction was quenched with water and the organic phase was extracted with dichloromethane (3 x 3 mL). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. Flash column chromatography on silica gel (n-Hexane/ethyl acetate 6:4) gave carboxamide **8i** as a white solid (43% yield). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.60 (d, J = 2.2 Hz, 1H), 7.50 (d, J = 4.0 Hz, 2H), 7.48 (dd, J = 8.4, 2.2 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H), 7.03 (d, J = 4.0 Hz, 1H), 2.94 – 2.86 (m, 4H), 2.81 (p, J = 7.6 Hz, 1H), 2.04 – 1.90 (m, 2H), 1.81 – 1.54 (m, 10H), 1.49 – 1.24 (m, 2H); ^{13}C NMR (101 MHz, CDCl3) δ 156.2, 148.1, 145.2, 138.2, 134.2, 134.0, 131.5, 130.9, 130.4, 129.3, 128.7, 124.6, 112.6, 102.9, 92.4, 72.1, 56.8, 33.6, 31.0, 25.4, 25.1, 23.2. ESMS, calculated *m/z* $C_{27}H_{25}Cl_2N_5OS$ 538 $[M+H]^+$, found *m/z* (relative intensity) 538.1 $[M+H]^+$ (17). HRMS m/z $[M^+H]^+$ calcd. for $C_{27}H_{26}Cl_2N_5OS$: 538.1235; found: 538.1220.

*tert-Butyl 4-(4-cyano-5-(5-(cyclopentylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-1H-pyrazole-3-carboxamido)piperidine-1-carboxylate (**8j**).* **8j** (75 mg, 48% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.61 (d, J = 2.1 Hz, 1H), 7.51 (d, J = 4.0 Hz, 1H), 7.48 (dd, J = 8.5, 2.2 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.04 (d, J = 4.0 Hz, 1H), 6.62 (d, J = 8.1 Hz, 1H), 4.21 – 3.99 (m, 4H), 2.91 (t, J = 12.7 Hz, 2H), 2.81 (p, J = 7.6 Hz, 1H), 2.05 – 1.93 (m, 4H), 1.81 – 1.56 (m, 6H), 1.45 (s, 9H), 1.39 (dd, J = 12.6, 3.5 Hz, 1H); ^{13}C NMR (101 MHz, CDCl3) δ 158.0, 154.7, 148.5, 145.3, 138.2, 134.2, 134.0, 131.5, 130.9, 130.8, 130.4, 129.4, 128.7, 124.6, 112.9, 102.9, 92.0, 79.7, 72.0, 46.9, 42.8, 33.6, 31.9, 31.0, 28.4, 25.1. ESMS, calculated *m/z* $C_{32}H_{33}Cl_2N_5O_3S$ 638 $[M+H]^+$, found *m/z* (relative intensity) 660.1 $[M+Na]^+$ (70). HRMS m/z $[M^+H]^+$ calcd. for $C_{32}H_{34}Cl_2N_5O_3S$: 638.1759; found: 638.1750.

*4-Cyano-5-(5-(cyclopentylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-N-(1,1-dioxidotetrahydro-2H-thiopyran-4-yl)-1H-pyrazole-3-carboxamide (**8k**).* **8k** (50 mg, 39% yield) was obtained as a white solid. 1H NMR (400 MHz, Chloroform-d) δ 7.99 (s, 1H), 7.61 (d, J = 4.0 Hz, 1H), 7.53 – 7.40 (m, 3H), 7.03 (d, J = 4.1 Hz, 1H), 3.59 (d, J = 10.6 Hz, 4H), 3.24 (s, 4H), 2.81 (p, J = 7.4 Hz, 1H), 1.97 (s, 2H), 1.81 – 1.50 (m, 6H); 13C NMR (101 MHz, CDCl3) δ 157.0, 147.0, 145.5, 138.4, 134.0, 133.9, 131.5, 131.0, 130.7, 130.6, 129.7, 128.8, 124.3, 112.4, 103.2, 92.3, 72.0, 52.8, 51.1, 33.6, 31.0, 25.1. ESMS, calculated m/z C26H23Cl2N5O3S2 588 [M+H]+, found m/z (relative intensity) 610 [M+Na]+ (40). HRMS m/z [M+H]+ calcd. for C26H24Cl2N5O3S2: 588.0698; found: 588.0685.

*4-Cyano-5-(5-(cyclopentylethynyl)thiophen-2-yl)-1-(2,4-dichlorophenyl)-N-(2-fluoroethyl)-1H-pyrazole-3-carboxamide (**8l**).* **8l** (15mg, 31% yield) was obtained as a white solid. ^{1}H NMR (400 MHz, Chloroform-d) δ 7.61 (d, J = 2.2 Hz, 1H), 7.51 (d, J = 4.0 Hz, 1H), 7.49 (dd, J = 8.5, 2.2 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.09 (t, J = 6.1 Hz, 1H), 7.04 (d, J = 4.0 Hz, 1H), 4.60 (dt, J = 47.2, 4.7 Hz, 2H), 3.78 (dq, J = 27.5, 5.1, 4.6 Hz, 2H), 2.81 (p, J = 7.5 Hz, 1H), 2.03 – 1.89 (m, 2H), 1.81 – 1.56 (m, 6H); ^{13}C NMR (101 MHz, CDCl3) δ 158.9, 148.1, 145.4, 138.2, 134.2, 134.0, 131.4, 130.9, 130.8, 130.4, 129.4, 128.7, 124.6, 112.8, 102.9, 92.0, 82.44 (d, J = 167.5 Hz), 72.0, 39.79 (d, J = 19.9 Hz), 33.6, 31.0, 25.1; ^{19}F NMR (376 MHz, Chloroform-d) δ -224.06 (tt, J = 47.2, 27.8 Hz). ESMS, calculated m/z $C_{24}H_{19}Cl_2FN_4OS$ 501 [M+H]+, found m/z

(relative intensity) 501.0 [M+H]+ (100). HRMS m/z [M+H]+ calcd. for $C_{24}H_{20}Cl_2FN_4OS$: 501.0719; found: 501.0706.

Determination of the partition coefficients

Measurement of chromatographic capacity factor (k') by C-18 HPLC method was performed using the equation $k' = (t_r-t_0)/t_0$ [17,18], 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 1ml/min. A solution of urea in a methanol – water solvent (85:15) was used for measurement of the column dead time (t_0 = 2,582±0,005, n=3). Seven compounds having known LogP values, tabulated and in the range from 1.10 to 5.70 (Benzyl alcohol, LogP 1.10; Benzene, LogP 2.10; Toluene, LogP 2.70; Naphtalene, LogP 3.60; Biphenyl, LogP 4; Phenanthrene, LogP 4.50; Triphenylamine, LogP 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 ºC. Capacity factors (k') were calculated. The Logk' value for each of seven compounds was plotted against its relative lipophilicity value reported in literature, based on the established linear relationship (LogP = 2.60 Logk' + 3.58, correlation coefficient = 0.92). The capacity factor of each compound was determined (the value was obtained on average of three experiments) and the relative LogP value was obtained by extrapolation.

Equilibrium Binding Assays

Binding assays were performed with the CB_1 receptor agonist, [^{3}H]CP55940 (0.7 nM), 1 mg ml-1 bovine serum albumin (BSA) and 50 mM Tris buffer containing 0.1mM EDTA and 0.5mM $MgCl_2$ (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 (5µg). 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.

[^{35}S]GTPγS Binding Assay

Mouse brain membranes (5µg protein) were preincubated for 30 minutes at 30°C with adenosine deaminase (0.5 U ml^{-1}). The membranes were then incubated with the agonist with vehicle or modulator for 60 minutes at 30 °C in assay buffer (50 mM Tris-HCl; 50 mM Tris-Base; 5 mM $MgCl_2$; 1 mM EDTA; 100 mM NaCl; 1 mM DTT; 0.1% BSA) in the presence of 0.1 nM [^{35}S]GTPγS and 30 µM GDP, in a final volume of 500 µl. Binding was initiated by the addition of [^{35}S]GTPγS. Nonspecific binding was measured in the presence of 30 µM GTPγS. The reaction was terminated by rapid vacuum filtration (50 mM Tris-HCl; 50 mM Tris-Base; 0.1% BSA) using a 24-well sampling manifold (cell harvester; Brandel, Gaitherburg, MD) and GF/B filters (Whatman, Maidstone, UK) that had been soaked in buffer (50 mM Tris-HCl; 50 mM Tris-Base; 0.1% BSA) for at least 24 hours. Each reaction tube was washed six times with a 1.2 ml aliquot of ice-cold wash buffer. The filters were oven-dried for at least 60 minutes and then placed in 5 ml of scintillation fluid (Ultima Gold XR, Packard). Radioactivity was quantified by liquid scintillation spectrometry.

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Electronic Supplementary Information (ESI) available: copies of ^{1}H, ^{13}C, ^{19}F NMR spectra of all the new compounds, ligand displacement and functional assays curves. See DOI: 10.1039/b000000x/

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Graphical abstract

4-Cyano-5-(2-Thiophenyl)-Pyrazoles are High Affinity CB_1 Receptor Ligands

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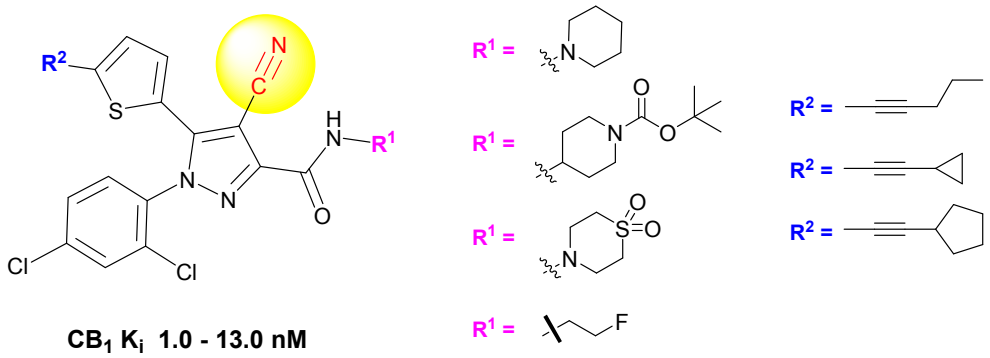

CB_1 K_i 1.0 - 13.0 nM

Introduction of a cyano group on position 4 of 5-thiophenyl-pyrazoles afforded high affinity cannabinoid ligands displaying low nanomolar K_i values and moderate selectivity for the CB1 receptor. Relatively low LogP values (< 4) and high TPSA (>90) suggest that some of this compounds might be peripherally restricted.