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Pyrazoles with a "click" 4-[*N*-(4-fluorobutyl)-1,2,3-triazole] substituent in position 3 are nanomolar CB_1 receptor ligands

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ABSTRACT

Replacement of the 3-carbonylaminopiperidine substitutent with a "click" 4-[*N*-(4-fluorobutyl)-(1,2,3-triazolyl)] group in Rimonabant-type pyrazoles produced a novel class of nanomolar CB_1 receptor ligands. Molecule **1d** is the most promising lead with a K_i = 23 nM for CB_1, which is very close to that displayed by Rimonabant (SR141716), and fairly good CB_1/CB_2 selectivity (K_i CB_2/K_i CB_1 = 35.5), thus representing a promising candidate for [^{18}F]radiolabeling and PET Imaging studies of the CB_1 receptor.

1. Introduction

Cannabinoid receptors are members of the large family of G-protein coupled receptors (GPCRs) [1]. Two types of cannabinoid receptor have been discovered so far, CB_1 and CB_2 [2], and both of them have been extensively studied. CB_1 receptors are localised predominantly in the brain [2] whereas CB_2 receptors are more abundant in peripheral nervous system (PNS) cells [3], although some studies have shown the presence of CB_1 in the PNS [4] and of CB_2 in the central nervous system, albeit in low density [5]. CB_1 receptors have been associated with a number of disorders, including depression [6], anxiety [7], stress [8], schizophrenia [9], chronic pain [10] and obesity [11]. For this reason, several cannabinoid ligands were developed as drug candidates. Among these ligands, a prominent position is occupied by SR141716 (Rimonabant) [12], which is a pyrazole-core inverse agonist discovered by Sanofi-Synthelabo (now Sanofi-Aventis) in 1994, marketed in Europe as an anti-obesity drug but subsequently withdrawn from the market owing to its side-effects, which included severe depression and suicidal thoughts. Since the relationship between (a) the CB_1 receptors' functional modification, density and distribution, and (b) the onset of a pathological state is still not well understood, the development of radio-ligands suitable for *in vivo* PET functional imaging of CB_1 receptors remains an important area of research in medicine and drug development. To date, a few radiotracers [13] based on the structure of SR141716 (Rimonabant) [12] have been synthesised and tested *in vivo* but most of them afforded unsatisfactory brain imaging results due to their poor ability to cross the blood-brain barrier (BBB). A handful of radiolabelled CB_1 PET ligands [14] have also been submitted to clinical trials in humans [15]. In this paper we describe the synthesis of a conceptually new class of high-affinity CB_1 ligands **1**, bearing a "click" *N*-(4-fluorobutyl)-1,2,3-triazolyl function in position 3 of a pyrazolyl ring, as candidate PET tracers. Furthermore, we synthesised the 4-iodo-1,2,3-triazolyl analogue **10** which might be developed into a theranostic or a multi-modal imaging tool by radioiodination.

2. Results and discussion

2.1. Ligands design

Extensive theoretical and experimental structure-activity relationship studies have been performed on Rimonabant analogues for

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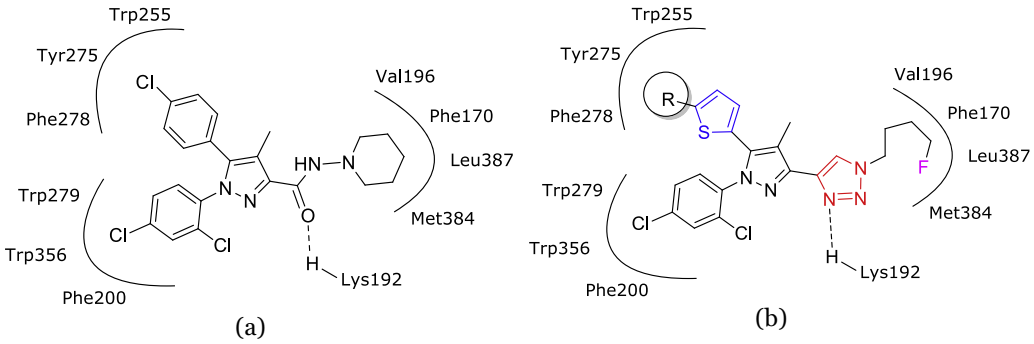


Fig. 1. (a) Rimonabant's pharmacophore. (b) Proposed binding mode of compounds **1** to CB_1.

identifying a general pharmacophore. Hydrophobic interactions between ligands and CB_1 receptor were deemed to be essential. In fact, the two aromatic rings in positions 1 and 5 of the pyrazole ring interact favourably with the residues Trp279/Phe200/Trp356 and Tyr275/Trp255/Phe278 respectively, and likewise the aminopiperidine cyclohexyl with the cavity constituted by Val196/Phe170/Leu387 and Met384 [16] (Fig. 1a). Moreover, the hydrogen bond between the ligand's amidic oxygen and the receptor residue Lys192 plays a crucial role in the binding, favouring the inverse agonism of Rimonabant.

With that in mind, we decided to replace the carbonyl-aminopiperidine residue in position 3 with a 4-(1,2,3-triazolyl) function, since either of the triazolyl sp^2 nitrogen atoms could act as hydrogen bond acceptor with Lys192. The 1,2,3-triazole would carry a *N*-(4-fluorobutyl) group, which should be readily amenable to [^{18}F]radiofluorination and could be accommodated in the lipophilic Val196/Phe170/Leu387/Met384 pocket. Finally, we planned to replace the 4-chlorophenyl group in 5-position with a 5-substituted 2-thiophenyl residue, which was previously shown to be a very advantageous structural modification leading to high-affinity CB_1 ligands, such as NESS125A [17].

2.2. Synthesis of 1,2,3-triazolyl compounds *1*

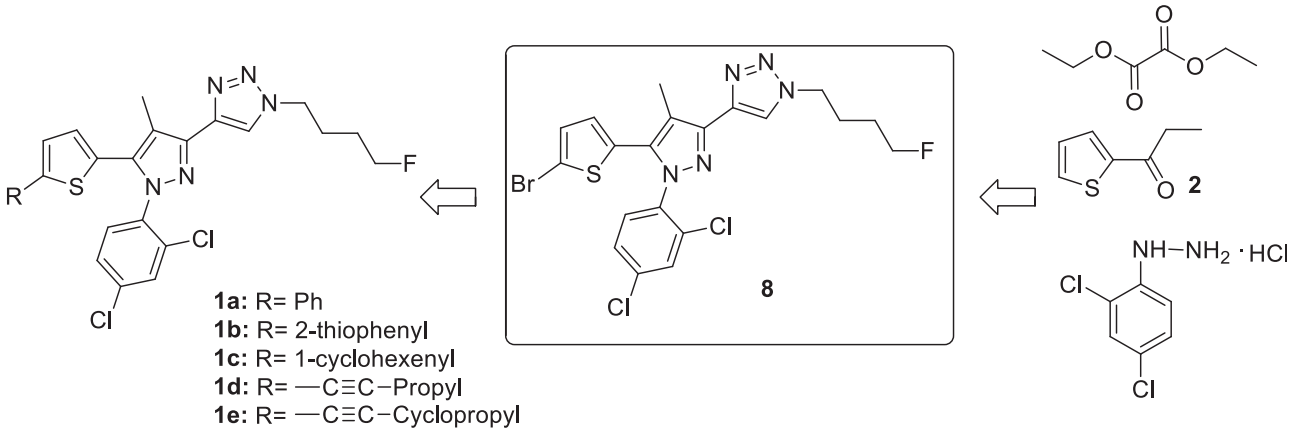
The synthesis of target compounds **1** envisaged the use of a key intermediate **8** (Scheme 1) which was obtained in a few synthetic steps from commercially available reagents such as diethyl oxalate, 1-(thiophen-2-yl) propan-1-one **2**, and 2,4-dichlorophenylhydrazine hydrochloride, and directly converted *via* a palladium-catalysed cross coupling reactions, into the desired target pyrazoles **1a–e**.

The synthesis started from **2** (Scheme 2), which was condensed with diethyl oxalate in the presence of sodium ethylate to give, in 85% yield, the 1,3-diketoester **3** as a tautomeric mixture, predominantly containing the alkenylidene structure. Subsequently, tricarbonyl compound **3** and 2,4-dichlorophenylhydrazine were heated in ethanol [18] to afford the pyrazole **4** in rather modest yield (32%). The latter was regioselectively brominated, [19] employing NBS as bromine source, to afford the corresponding bromothiophene **5** in good yield (83%). The following conversion was accomplished through a DIBAL-H hydride reduction, providing the aldehyde **6** which was homologated under Bestmann-Ohira alkynylation conditions [20] to generate the alkyne **7** in a moderate yield (55%). Finally, the key triazole **8** was achieved by means of a copper-catalyzed azide-alkyne cycloaddition protocol [21] in an acceptable 55% yield.

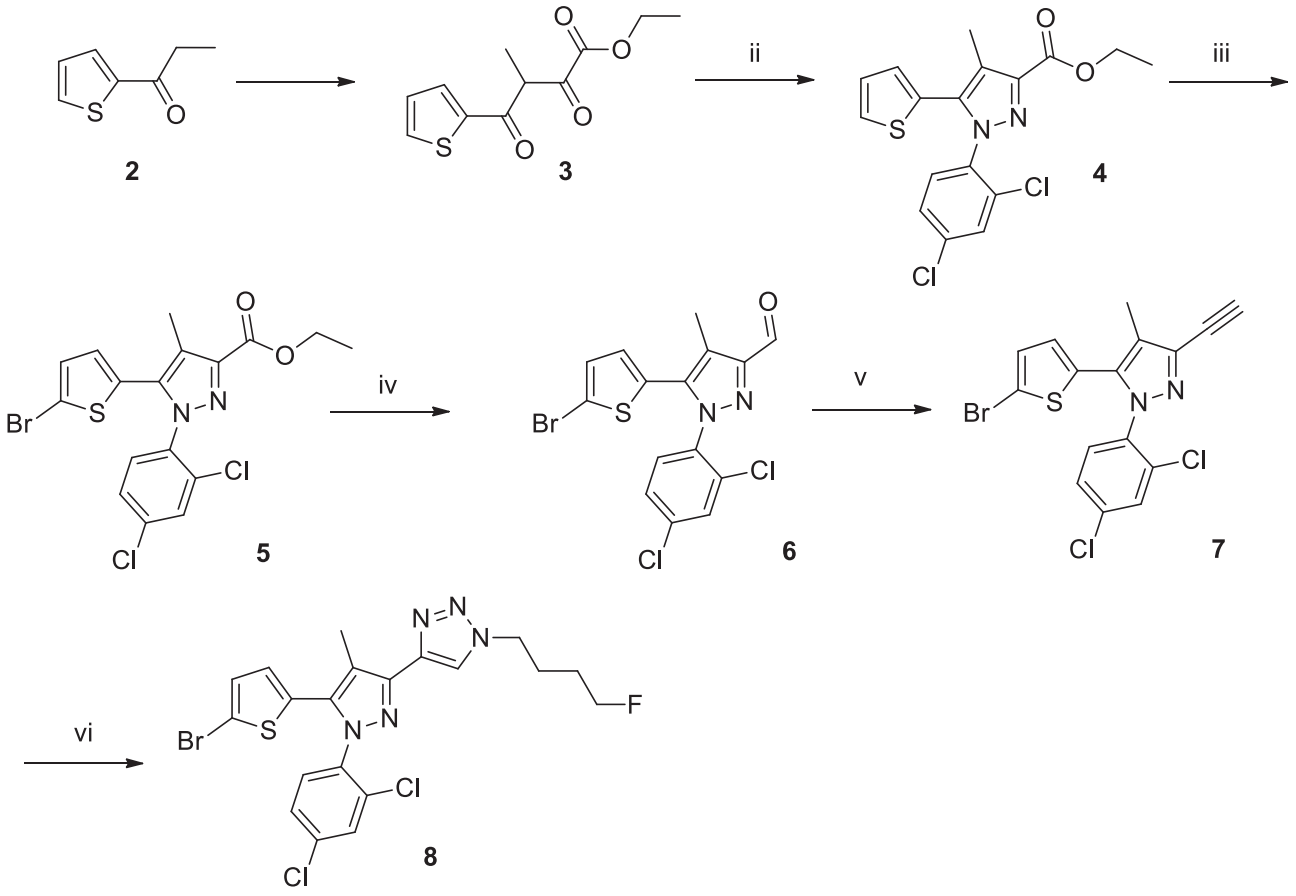
With the intermediate **8** in hand, compounds **1a–c** were obtained by means of a palladium-catalysed Suzuki–Miyaura cross coupling [22] using the respective commercially available boronic acids, while compounds **1d–e** were synthesised employing a copper-palladium catalysed Sonogashira cross coupling [23] using the appropriate alkyne (Scheme 3).

2.3. Synthesis of 5-iodo-1,2,3-triazolyl compound *10*

The synthesis of 4-iodo-1,2,3-triazolyl derivative **10** (Scheme 4) started from the intermediate **7** that was iodinated in a good yield (74%) using 4-iodomorpholine as iodine source. Next, a



Scheme 1. Retro-synthesis of 1,2,3-triazolyl analogues **1a–e**.



Scheme 2. Synthesis of key intermediate **8**. *Reagents and conditions:* (i) diethyl oxalate, EtONa/EtOH, r.t., overnight, (85%); (ii) 2,4-dichlorophenylhydrazine hydrochloride, EtOH, reflux, overnight, (32%); (iii) NBS, CH_3CN, from 0 °C to r.t., overnight, (83%); (iv) DIBAL-H, DCM, -78 °C, 4 h, (55%); (v) dimethyl 1-diazo-2-oxopropylphosphonate, K_2CO_3, MeOH, r.t., overnight, (55%); and (vi) 1-azido-4-fluorobutane, CuI, sodium ascorbate, *tert*-BuOH/H_2O, r.t, overnight, (55%).

copper-catalysed azide-iodoalkyne cycloaddition [24] afforded the desired compound **10** in moderate yield (43%).

2.4. Biological tests

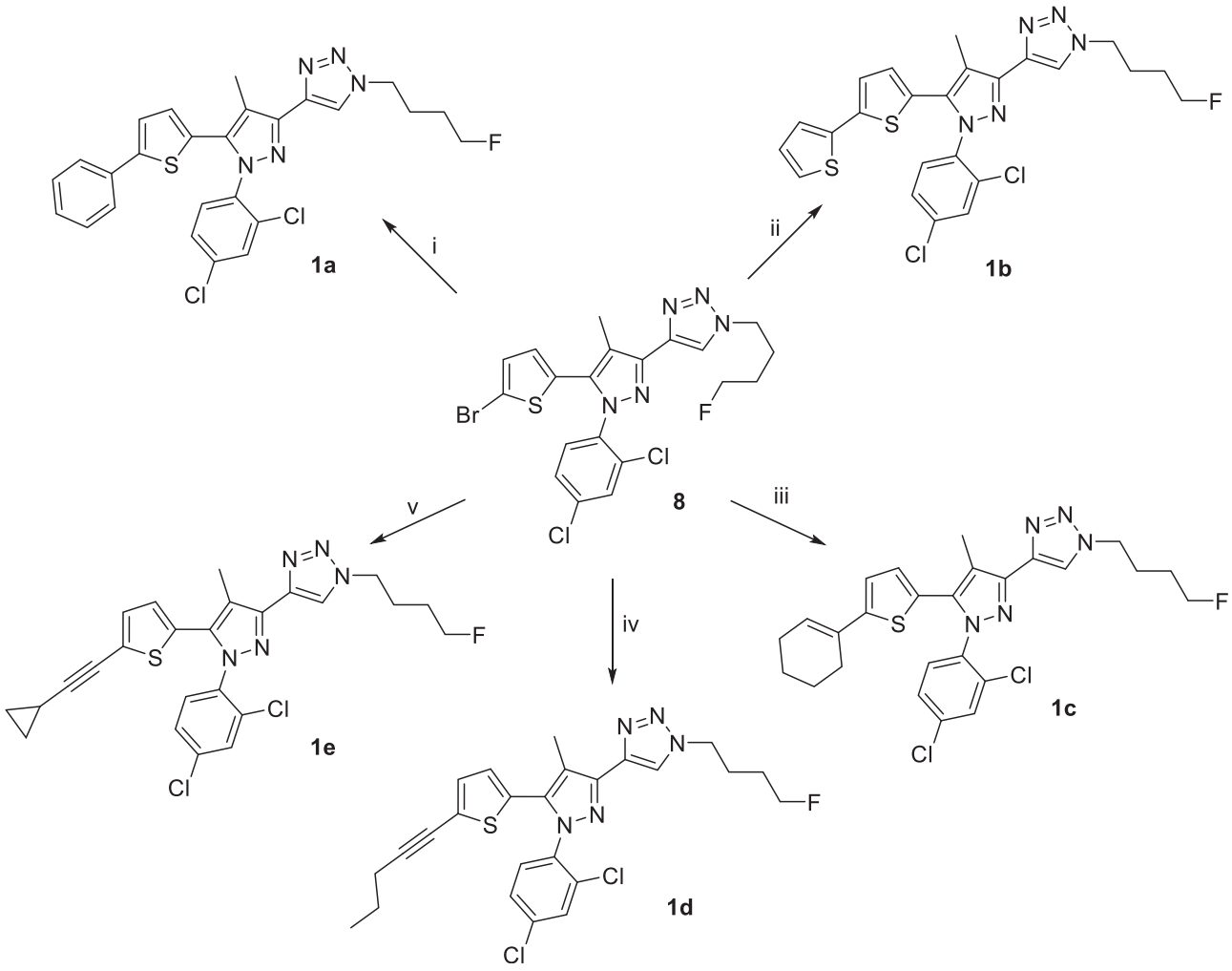
We next performed [^{3}H]CP55940 displacement binding assays with membranes obtained from hCB_1 and hCB_2 CHO cells using methods we have described previously [25]. Results are summarised in Table 1 (affinity to CB_1 and CB_2 are expressed as K_i values).

Compound **1d** stands out for its high CB_1 affinity, which was comparable to that displayed by Rimonabant (SR141716). Moreover, **1d** showed fairly good CB_1/CB_2 selectivity (K_i CB_2/K_i CB_1 = 35.5). All the other compounds showed K_i CB_1 one order of magnitude higher than that of **1d**, and low to moderate CB_1/CB_2 selectivity. Compound **1d** is therefore the most promising candidate for further development, including its possible use as a PET tracer for imaging the CB_1 receptor *in vivo*.

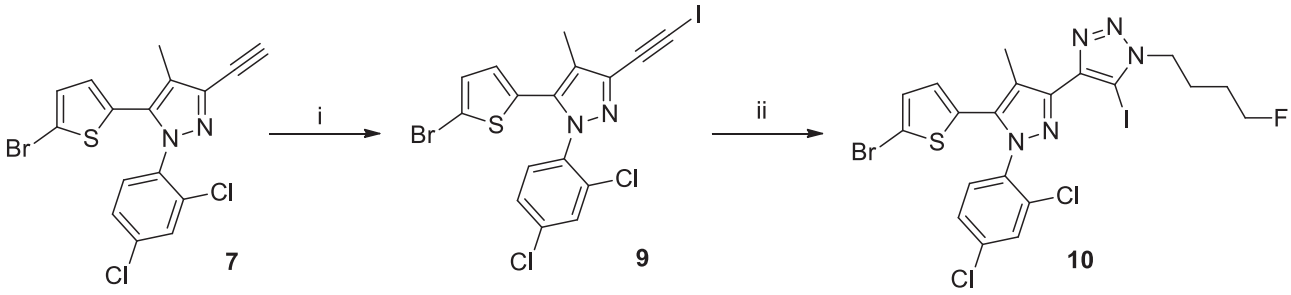
Table 1

Compound	hCB_1 CHO cells		hCB_2 CHO cells	
	K_i[a,d] (95% CL[b])	% Maximum displacement (95% CL[b])	K_i[a,d] (95% CL[b])	% Maximum displacement (95% CL[b])
1a	**200** (94.9–420)	76.5 (66.3–86.7)	**2.36×10^3** ($641–8.70 \times 10^3$)	73.3 (47.3–99.2)
1b	**353** ($103–1.20 \times 10^3$)	85.1 (66.5–103)	**1.70×10^3** ($953–3.02 \times 10^3$)	97.5 (82.6–112)
1c	**119** (40.2–353)[f]	80.0 (65.9–94.1)	**471** ($95.1–2.33 \times 10^3$)	55.6 (41.8–69.5)
1d	**23.4** (6.80–80.0)	75.6 (62.0–89.2)	**830** ($281–2.45 \times 10^3$)	74.5 (55.0–94.0)
1e	**164** (32.5–825)	58.8 (43.2–74.5)	n.a.[c]	n.a.[c]
8	**312** (113–862)	100 (81.0–120)	**1.02×10^3** ($603–1.72 \times 10^3$)	92.0 (79.7–104)
10	**422** (235–757)	71.8 (65.6–77.9)	n.a.[c]	n.a.[c]
SR141716	**18.7** (11.1–31.4)[e]	90.2 (85.0–95.3)	**1.40×10^3** ($500–3.70 \times 10^3$)[g]	92.4 (70.4–114)

[a] nM.
[b] CL, confidence limits.
[c] Plateau could not be reached.
[d] $n = 4$, unless otherwise indicated.
[e] $n = 14$.
[f] $n = 12$.
[g] $n = 2$.



Scheme 3. Synthesis of analogues **1a–e**. *Reagents and conditions*: (i) phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, r.t., overnight, (35%); (ii) 2-thienylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, r.t., overnight, (35%); (iii) 1-cyclohexen-1-yl-boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, r.t., overnight, (75%); (iv) 1-pentyne, $Pd(PPh_3)_4$, DIPEA, CuI, 80 °C, 20 h, (20%); and (v) cyclopropylacetylene, $Pd(PPh_3)_4$, DIPEA, CuI, 80 °C, 20 h, (25%).



Scheme 4. Synthesis of iodinated analogue **10**. *Reagents and conditions*: (i) 4-iodomorpholine, CuI, THF, r.t. 1 h, (74%); and (ii) 1-azido-4-fluorobutane, CuI, TEA, THF, r.t, 72 h, (43%).

3. Conclusions

In Rimonabant-type pyrazoles, replacement of the 3-carbonylaminopiperidine substitutent with a "click" [4-(1,2,3-triazolyl)] group carrying a *N*-(4-fluorobutyl) function produced a novel class of nanomolar CB_1 receptor ligands displaying nanomolar affinity for the CB_1 receptor. This may be explained by the capacity of the 1,2,3-triazole ring to mimic Rimonabant's 3-carboxamide residue and behave as hydrogen-bond acceptor with Lys192 of the CB_1 receptor. Molecule **1d** is a particularly promising candidate for [^{18}F]radiolabeling and PET Imaging studies of the CB_1 receptor, as it displayed a K_i = 23 nM for the CB_1, in the same range as that displayed by Rimonabant (SR141716), and fairly good CB_1/CB_2 selectivity (K_i CB_1 approx.36-fold lower than K_i CB_2).

4. Experimental

4.1. General information

^{1}H (400.13 MHz), ^{13}C (100.58 MHz) and ^{19}F (376.45 MHz) NMR spectra were recorded on a Bruker ADVANCE III spectrometer. ^{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 with the solvent resonance as the internal standard ($CDCl_3$, δ = 77.0). ^{19}F NMR spectra were recorded with complete proton decoupling. The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet-doublet, dt = doublet-triplet, q = quartet. All chemical shifts (δ) are expressed in parts per million and coupling constant (J) are given in Hertz. LC–MS experiments were performed on an Agilent Technologies 1200 Series HPLC system equipped with a DAD and a 6120 MS detector composed by a ESI ionisation source and a Single Quadrupole mass selective detector using an Analytical C18 RP column (Phenomenex Luna, C18, 250 mm × 4.60 mm, 5 μ, 100 Å). HPLC purifications were performed on the Agilent 1200 system using a semi preparative C18 RP column (Phenomenex Luna, 250 mm × 10.00 mm, 5 μ, 100 Å). All reactions were carried out in oven- or flame-dried glassware under nitrogen atmosphere, unless stated otherwise. All commercially available reagents were used as received. Reactions were magnetically stirred and monitored by TLC on silica gel (60 F254 pre-coated glass plates, 0.25 mm thickness). Visualisation was accomplished by irradiation with a UV lamp and/or staining with a ceric ammonium molibdate or $KMnO_4$ solution. Flash chromatography was performed on silica gel (60 Å, particle size 0.040–0.062 mm). Yields refer to chromatographically and spectroscopically pure compounds, unless stated otherwise. Abbreviations used: DCM for dichloromethane, EtOAc for ethyl acetate, Et_2O for diethyl ether, NBS for *N*-bromosuccinimide, DIBAL-H for diisobutylaluminium hydride, DME for dimethoxyethane, DIPEA *N*,*N*-Diisopropylethylamine, THF for tetrahydrofuran, MeOH for methanol and TEA for triethylamine. [^{3}H]CP55940 displacement binding assays with membranes obtained from hCB_1 and hCB_2 CHO cells using methods were performed as described previously [25].

4.2. Ethyl-3-methyl-2,4-dioxo-4-(thiophen-2-yl)butanoate (**3**)

Under a nitrogen atmosphere, sodium (0.86 g, 37.50 mmol) was added in small portions to dry ethanol (25 mL) and stirred at room temperature until all the sodium was dissolved. Diethyl oxalate (7.6 mL, 56.30 mmol) was then added, followed by dropwise addition of a solution of commercially available 1-(thiophen-2-yl) propan-1-one **2** (2.63 g, 18.65 mmol) in dry ethanol (26 mL). The mixture was stirred at room temperature for 18 h, than slowly poured into a mixture of ice and aqueous 1 N HCl. The resulting mixture was extracted with Et_2O, the organic layers were dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 8:2) to give compound **3** (3.8 g, 85%) as a white solid. R*f* 0.57 (Hexane/EtOAc 8:2); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.24 (t, 3H, J = 7.1 Hz), 1.45 (d, 3H, J = 7.0 Hz), 4.25 (q, 2H, J = 7.1 Hz), 4.70 (q, 1H, J = 7.0 Hz), 7.37 (t, 1H, J = 8.4 Hz), 7.37 (d, 1H, J = 8.4 Hz), 7.51 (d, 1H, J = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 13.4, 14.0, 52.6, 63.1, 128.6, 133.5, 135.3, 142.4, 160.4, 187.5, 190.1; MS (ESI), calculated m/z $C_{11}H_{12}O_4S$: 241.0 $[M+H]^+$, 263.0 $[M+Na]^+$, found m/z (relative intensity): 241.0 $[M+H]^+$ (100), 263.0 $[M+Na]^+$ (40).

4.3. Ethyl 5-(thiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylate (**4**)

The α,γ-diketoester **3** (4.63 g, 20.51 mmol) was dissolved in absolute EtOH (36 mL) and 2,4-dichlorophenylhydrazine hydrochloride (4.38 g, 20.51 mmol) was added in one portion, then the mixture was refluxed overnight. The solvent was removed under reduced pressured and the crude product was purified by flash chromatography (Hexane/EtOAc 8:2). A final recrystallization (Hexane/EtOAc 7:3) gave compound **4** (2.01 g, 32%) as a white solid. R*f* 0.30 (Hexane/EtOAc 8:2); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.45 (t, 3H, J = 7.1 Hz), 2.46 (s, 3H), 4.47 (q, 2H, J = 7.1 Hz), 6.92 (dd, 1H, J = 1.2, 3.6 Hz), 7.02 (dd, 1H, J = 3.6, 5.1 Hz), 7.33 (dd, 1H, J = 2.2, 8.5 Hz), 7.38 (dd, 1H, J = 1.2, 5.1 Hz), 7.40 (d, 1H, J = 8.5 Hz), 7.46 (d, 1H, J = 2.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 9.9, 14.5, 61.0, 120.0, 127.2, 127.7, 127.8, 128.6, 128.9, 130.0, 131.0, 133.9, 136.0, 136.3, 137.8, 142.9, 162.7; MS (ESI), calculated m/z $C_{17}H_{14}{}^{35}Cl_2N_2O_2S$: 381.0 $[M+H]^+$, 383.0 $[M+2+H]^+$, found m/z (relative intensity): 381.0 $[M+H]^+$ (100), 383.0 $[M+2+H]^+$ (70).

4.4. Ethyl 5-(5-bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylate (**5**)

Compound **4** (2.21 g, 1.31 mmol) was dissolved in acetonitrile (4.5 mL) and the solution was cooled to 0 °C. NBS (0.39 g, 2.23 mmol) was added in small portions, than the mixture was stirred overnight at r.t. A saturated solution of $Na_2S_2O_3$ (5 mL) was added and the solvent was removed under reduced pressure. The resulting mixture was extracted with EtOAc, the organic layers were washed with water, brine, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 8:2) to give compound **5** (510 mg, 83%) as a pale yellow solid. R*f* 0.38 (Hexane/EtOAc 8:2); ^1H NMR (400 MHz, $CDCl_3$) δ: 1.40 (t, 3H, J = 7.1 Hz), 2.41 (s, 3H), 4.43 (q, 2H, J = 7.1 Hz), 6.63 (d, 1H, J = 3.9 Hz), 6.94 (d, 1H, J = 3.9 Hz), 7.33 (d, 1H, J = 2.0 Hz), 7.35 (d, 1H, J = 0.4 Hz), 7.45 (dd, 1H, J = 0.4, 2.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 10.1, 14.6, 61.2, 115.1, 120.5, 128.0, 129.4, 130.3, 130.3, 131.0, 133.9, 134.1, 135.8, 136.7, 137.0, 142.8, 162.6; MS (ESI), calculated m/z $C_{17}H_{13}{}^{78}Br^{35}Cl_2N_2O_2S$: 458.9 $[M+H]^+$, 460.9 $[M+2+H]^+$, found m/z (relative intensity): 458.9 $[M+H]^+$ (65), 460.9 $[M+2+H]^+$ (100).

4.5. 5-(5-Bromothiophen-2-yl)-3-(2,4-dichlorophenyl)-5-methylcyclopenta-1,4-dienecarbaldehyde (**6**)

Ester **5** (0.2 g, 0.43 mmol) was dissolved in anhydrous dichloromethane (2 mL) and the mixture was cooled to −78 °C. DIBAL-H (0.5 mL) was added drop wise over 45 min and the mixture was stirred for 4 h at −78 °C. MeOH (0.5 mL) was added and the solvent was removed under reduced pressure. The resulting mixture was extracted with EtOAc, the organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 5:5) to give aldehyde **6** (0.45 g, 55%) as a white solid. R*f* 0.50 (Hexane/EtOAc 5:5); ^1H NMR (400 MHz, $CDCl_3$) δ: 2.36 (s, 3H), 6.58 (d, 1H, J = 3.9 Hz), 6.90 (d, 1H, J = 3.9 Hz), 7.29 (d, 1H, J = 0.5 Hz), 7.30 (d, 1H, J = 2.0 Hz), 7.46 (dd, 1H, J = 0.5, 2.0 Hz), 10.0 (s, 1H); MS (ESI), calculated m/z $C_{15}H_9{}^{78}Br^{35}Cl_2N_2OS$: 414.9 $[M+H]^+$, 416.9 $[M+2+H]^+$, found m/z (relative intensity): 414.9 $[M+H]^+$ (55), 416.9 $[M+2+H]^+$ (100).

4.6. 2-Bromo-5-(5-(2,4-dichlorophenyl)-3-ethynyl-2-methylcyclopenta-1,3-dienyl) thiophene (**7**)

K_2CO_3 (0.11 g, 0.86 mmol) and dimethyl 1-diazo-2-oxopropylphosphonate (0.09 g, 0.52 mmol) were added to an ice cold solution of aldehyde **6** (0.45 g, 1.09 mmol) in MeOH (0.5 mL). After 5 min the ice bath was removed and the reaction was allowed to warm to r.t. and stirred for additional 12 h. Rochelle salt (2 mL) and Et_2O (2 mL) were added. The organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash

chromatography (Hexane/EtOAc 7:3) to give alkyne **7** (0.15 g, 55%) as a white solid. R*f* 0.82 (Hexane/EtOAc 7:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.28 (s, 3H), 3.31 (s, 1H), 6.62 (d, 1H, J = 3.9 Hz), 6.96 (d, 1H, J = 3.9 Hz), 7.36 (d, 2H, J = 1.3 Hz), 7.50 (t, 1H, J = 1.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 9.4, 75.0, 81.4, 114.5, 120.1, 127.9, 128.4, 130.1, 130.2, 130.7, 130.9, 133.7, 135.2, 135.8, 136.3, 136.4; MS (ESI), calculated m/z $C_{16}H_9{}^{78}Br^{35}Cl_2N_2S$: 410.9 $[M+H]^+$, 412.9 $[M+2+H]^+$, 432.9 $[M+Na]^+$, 434.9 $[M+2+Na]^+$, found *m/z* (relative intensity): 410.9 $[M+H]^+$ (35), 412.9 $[M+2+H]^+$ (70), 432.9 $[M+Na]^+$ (55), 434.9 $[M+2+Na]^+$ (100).

4.7. 1-Azido-4-fluorobutane

NaN_3 (0.25 g, 3.96 mmol) was added to a stirred solution of 1-bromo-4-fluorobutane (0.5 g, 2.61 mmol) in 40 mL of water/acetone (1:4). The resulting suspension was stirred at r.t. for 24 h. The mixture was extracted with DCM, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. 1-Azido-4-fluorobutane was obtained (300 mg, 76%) as a yellow oil and was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.71–1.79 (m, 1H), 1.79–1.87 (m, 1H), 1.89–1.99 (m, 2H), 3.39 (t, 2H, J = 6.5 Hz), 4.42 (dt, 2H, $J_{H\text{-}F}$ = 47.3 Hz, $J_{H\text{-}H}$ = 5.7 Hz).

4.8. 4-(5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1-(4-fluorobenzyl)-1H-1,2,3-triazole (**8**)

Sodium ascorbate (0.08 g, 0.38 mmol) and copper sulphate (0.02 g, 0.07 mmol) were added to a solution of alkyne **7** (0.80 g, 1.94 mmol) and 1-azido-4-fluorobutane (0.34 g, 2.11 mmol) in *tert*-butanol/water (50 mL, 4:1). The mixture was stirred at r.t. for 24 h. A saturated aqueous solution of ammonium chloride (20 mL) was added and the aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 6:4) to give triazole **8** (0.12 g, 55%) as a white solid. R*f* 0.24 (Hexane/EtOAc 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.60–1.68 (m, 1H), 1.69–1.76 (m, 1H), 1.99–2.09 (m, 2H), 2.47 (s, 3H), 4.41 (dt, 2H, $J_{H\text{-}F}$ = 47.2 Hz, $J_{H\text{-}H}$ = 5.7 Hz), 4.41 (t, 2H, J = 7.0 Hz), 6.60 (d, 1H, J = 3.9 Hz), 6.89 (d, 1H, J = 3.9 Hz), 7.25–7.32 (m, 2H), 7.42 (d, 1H, J = 2.1 Hz), 7.88 (s, 1H); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: −219.3 (s, 1F); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 10.3, 26.7 (d, $J_{C\text{-}F}$ = 4.1 Hz), 26.7 (d, $J_{C\text{-}F}$ = 20.1 Hz), 50.0, 83.3 (d, $J_{C\text{-}F}$ = 165.5 Hz), 114.5, 121.3, 128.1, 128.9, 130.3, 130.4, 131.2, 134.1, 136.2, 136.3, 140.2, 142.4, 144.1, 147.0, 150.5; MS (ESI), calculated m/z $C_{20}H_{17}{}^{79}Br^{35}Cl_2FN_5S$: 528.0 $[M+H]^+$, 530.0 $[M+2+H]^+$, 550.0 $[M+Na]^+$, 552.0 $[M+2+Na]^+$, found *m/z* (relative intensity): 528.0 $[M+H]^+$ (30), 530.0 $[M+2+H]^+$ (65), 550.0 $[M+Na]^+$ (52), 552.0 $[M+2+Na]^+$ (100); HRMS calcd. for $C_{20}H_{18}BrCl_2FN_5S$: 527.9822 and 529.9799, found: 527.9813 and 529.9787.

4.9. Suzuki–Miyaura cross coupling: general procedure

A mixture of alkyne **8** (0.18 mmol), $Pd(PPh_3)_4$ (0.09 mmol), the appropriate boronic acid (0.28 mmol) and aqueous Na_2CO_3 (0.23 mmol) in DME (2 mL), was heated to reflux and stirred overnight. The reaction was cooled down to r.t., poured into water, extracted with DCM, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 6:4) to give the desired compound **1a–c**.

4.10. 4-(5-(2,2′-bithiophen-5-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1-(4-fluorobutyl)-1H-1,2,3-triazole (**1a**)

Starting from **8** and phenylboronic acid, compound **1a** (46 mg, 35%) was obtained as yellow oil. R*f* 0.36 (Hexane/EtOAc 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.67–1.90 (m, 2H), 2.07–2.22 (m, 2H), 2.63 (s, 3H), 4.52 (dt, 2H, $J_{H\text{-}F}$ = 47.2 Hz, $J_{H\text{-}H}$ = 5.7 Hz), 4.52 (t, 2H, J = 6.9 Hz), 6.88 (d, 1H, J = 3.8 Hz), 7.22 (d, 1H, J = 3.8 Hz), 7.30–7.46 (m, 6H), 7.51 (d, 1H, J = 2.2 Hz), 7.56 (d, 1H, J = 2.2 Hz), 7.98 (s, 1H); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: −219.3 (s, 1F); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 10.3, 26.5 (d, $J_{C\text{-}F}$ = 4.1 Hz), 27.3 (d, $J_{C\text{-}F}$ = 20.1 Hz), 49.8, 83.2 (d, $J_{C\text{-}F}$ = 165.7 Hz), 121.2, 123.1, 125.8, 127.9, 128.0, 128.3, 129.0, 129.2, 129.7, 130.2, 130.4, 131.1, 133.6, 134.0, 135.9, 136.5, 136.8, 137.3, 142.5, 143.9, 146.2; MS (ESI), calculated *m/z* $C_{26}H_{22}{}^{35}Cl_2FN_5S$: 526.1 $[M+H]^+$, 528.1 $[M+2+H]^+$, found *m/z* (relative intensity): 526.1 $[M+H]^+$ (100), 528.1 $[M+2+H]^+$ (70); HRMS calcd. for $C_{26}H_{23}Cl_2FN_5S$: 526.1030, found: 526.1026.

4.11. 4-(1-(2,4-Dichlorophenyl)-4-methyl-5-(5-phenylthiophen-2-yl)-1H-pyrazol-3-yl)-1-(4-fluorobutyl)-1H-1,2,3-triazole (**1b**)

Starting from **8** and 2-thienylboronic acid, compound **1b** (48 mg, 35%) was obtained as yellow oil. R*f* 0.31 (Hexane/EtOAc 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.71–1.79 (m, 1H), 1.79–1.86 (m, 1H), 2.10–2.20 (m, 2H), 2.62 (s, 3H), 4.52 (dt, 2H, $J_{H\text{-}F}$ = 47.2 Hz, $J_{H\text{-}H}$ = 5.7 Hz), 4.52 (t, 2H, J = 7.0 Hz), 6.81 (d, 1H, J = 3.8 Hz), 7.04 (dd, 1H, J = 3.6, 5.1 Hz,) 7.08 (d, 1H, J = 3.8 Hz), 7.17 (dd, 1H J = 1.1, 3.6 Hz), 7.26 (dd, 1H, J = 1.1, 5.1 Hz), 7.38 (dd, 1H, J = 2.2, 8.4), 7.45 (d, 1H, J = 8.4 Hz), 7.52 (d, 1H, J = 2.2 Hz), 8.00 (s, 1H); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: −219.3 (s, 1F); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 10.2, 26.5 (d, $J_{C\text{-}F}$ = 4.2 Hz), 27.3 (d, $J_{C\text{-}F}$ = 20.1 Hz), 49.8, 83.2 (d, $J_{C\text{-}F}$ = 165.7 Hz), 115.5, 120.7, 123.6, 124.2, 125.0, 127.9 (2C), 129.0, 130.2, 131.1, 134.0, 136.0, 136.3, 136.5, 137.1, 139.3, 142.4, 143.9, 146.1; MS (ESI), calculated *m/z* $C_{24}H_{20}{}^{35}Cl_2FN_5S_2$: 532.1 $[M+H]^+$, 534.1 $[M+2+H]^+$, found *m/z* (relative intensity): 532.1 $[M+H]^+$ (100), 534.1 $[M+2+H]^+$ (75); HRMS calcd. for $C_{24}H_{21}Cl_2FN_5S_2$: 532.0594, found: 532.0588.

4.12. 4-(5-(5-Cyclohexenylthiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1-(4-fluorobutyl)-1H-1,2,3-triazole (**1c**)

Starting from **8** and 1-cyclohexen-1-yl-boronic acid, compound **1c** (15 mg, 75%) was obtained as yellow oil. R*f* 0.36 (Hexane/EtOAc 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.58–1.71 (m, 4H), 1.71–1.84 (m, 4H), 2.16–2.23 (m, 2H), 2.34–2.40 (m, 2H), 2.58 (s, 3H), 4.51 (t, 2H, J = 7.0 Hz), 4.51 (dt, 2H, $J_{H\text{-}F}$ = 47.2 Hz, $J_{H\text{-}H}$ = 5.7 Hz), 6.15–6.18 (m, 1H), 6.73 (d, 1H, J = 3.8 Hz), 6.82 (d, 1H, J = 3.8 Hz), 7.34 (dd, 1H, J = 2.2, 8.5 Hz), 7.40 (d, 1H, J = 8.5 Hz), 7.50 (d, 1H, J = 2.2 Hz), 7.98 (s,1H); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: −219.3 (s, 1F); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 10.2, 22.0, 22.6, 25.6, 26.5 (d, $J_{C\text{-}F}$ = 4.0 Hz), 27.4 (d, $J_{C\text{-}F}$ = 22.0 Hz), 29.7, 49.8, 83.2 (d, $J_{C\text{-}F}$ = 165.7 Hz), 115.2, 121.1, 125.2, 126.3, 127.8, 128.1, 128.5, 130.1, 130.3, 130.7, 131.1, 134.0, 135.7, 136.6, 142.0, 143.8, 149.0; MS (ESI), calculated m/z $C_{26}H_{26}{}^{35}Cl_2FN_5S$: 530.1 $[M+H]^+$, 532.1 [M+2+H], found *m/z* (relative intensity): 530.1 $[M+H]^+$ (100), 532.1 [M+2+H] (70); HRMS calcd. for $C_{26}H_{27}Cl_2FN_5S$: 530.1333, found: 530.1343.

4.13. Sonogashira reaction: general procedure

A mixture of alkyne **8** (0.09 mmol), $Pd(PPh_3)_4$ (0.003 mmol), DIPEA (1 mL) and the appropriate alkyne (0.19 mmol of either 1-pentyne or cyclopropylacetylene) was stirred at 40 °C for 20 min. CuI (0.006 mmol) was added and the reaction was stirred at 80 °C overnight. The mixture was cooled down to r.t., diluted with EtOAc (1 mL), dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified first by HPLC (Semi-preparative C18 Luna column, Eluent: A: H_2O, Eluent B: CH_3CN; in isocratic condition Eluent A: 20% and Eluent B: 80%, 5 mL/min) to give the desired compound **1d–e**.

4.14. 4-(1-(2,4-Dichlophenyl)-4-methyl-5-(5-(pent-1-ynyl)thiophen-2-yl)-1H-pyrazol-3-yl)-1-(4-fluorobutyl)-1H-1,2,3-triazole (**1d**)

Starting from **8** and 1-pentyne, compound **1d** (50 mg, 20%) was obtained as a yellow oil after an HPLC purification (Semi-preparative C18 Luna column, Eluent A: H_2O, Eluent B: CH_3CN; in isocratic condition Eluent A: 20% and Eluent B: 80%, 5 mL/min, retention time: 8.9 min). R*f* 0.24 (Hexane/EtOAc 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.02 (t, 3H, J = 7.4 Hz), 1.52–1.66 (m, 2H), 1.68–1.75 (m, 1H), 1.75–1.85 (m, 1H), 2.04–2.19 (m, 2H), 2.39 (t, 2H, J = 7.1 Hz), 2.55 (s, 3H), 4.48 (t, 2H, J = 7.0 Hz), 4.49 (dt, 2H, $J_{H\text{-}F}$ = 47.2 Hz, $J_{H\text{-}H}$ = 5.7 Hz), 6.77 (d, 1H, J = 3.8 Hz), 6.99 (d, 1H, J = 3.8 Hz), 7.33 (dd, 1H, J = 2.1, 8.4 Hz), 7.38 (d, 1H, J = 8.4 Hz), 7.48 (d, 1H, J = 2.1 Hz), 7.96 (s, 1H); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: −219.3 (s, 1F); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 10.3, 13.7, 21.8, 22.1, 26.5 (d, $J_{C\text{-}F}$ = 4.2 Hz), 27.3 (d, $J_{C\text{-}F}$ = 20.1 Hz), 29.8, 50.0, 73.3, 83.3 (d, $J_{C\text{-}F}$ = 165.7 Hz), 96.6, 115.6, 116.5, 121.3, 126.4, 128.1, 130.4, 131.1, 131.2, 134.1, 136.1, 136.3, 137.0, 142.4, 144.0; MS (ESI), calculated m/z $C_{25}H_{24}{}^{35}Cl_2FN_5S$: 516.1 $[M+H]^+$, 518.1 [M+2+H], found *m/z* (relative intensity): 516.1 $[M+H]^+$ (100), 518.1 [M+2+H] (75); HRMS calcd. for $C_{25}H_{25}Cl_2FN_5S$: 516.1186, found: 516.1176.

4.15. 4-(5-(5-(2-cyclopropylethynyl)thiophen-2-yl]-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl)-1-(4-fluorobutyl)-1H-1,2,3-triazole (**1e**)

Starting from **8** and cyclopropylacetylene, compound **1e** (40 mg, 25%) was obtained as a yellow oil after an HPLC purification (Semi-preparative C18 Luna column, Eluent: A: H_2O, Eluent B: CH_3CN; in isocratic condition Eluent A: 20% and Eluent B: 80%, 5 mL/min, Retention time: 12.0 min). R*f* 0.24 (Hexane/EtOAc 6:4); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.77–0.86 (m, 2H), 0.87–0.94 (m, 2H), 1.41–1.54 (m, 1H), 1.70–1.77 (m, 1H), 1.77–1.85 (m, 1H), 2.08–2.19 (m, 2H), 2.56 (s, 3H), 4.51 (t, 2H, J = 7.0 Hz), 4.51 (dt, 2H, $J_{H\text{-}F}$ = 47.2 Hz, $J_{H\text{-}H}$ = 5.7 Hz), 6.69 (d, 1H, J = 3.9 Hz), 6.98 (d, 1H, J = 3.9 Hz), 7.33–7.41 (m, 2H), 7.51 (d, 1H, J = 2.0 Hz), 7.98 (s, 1H); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: −219.3 (s, 1F); ^{13}C NMR (100 MHz, $CDCl_3$) δ: −1.47, 8.50, 8.68 (2C), 26.5 (d, $J_{C\text{-}F}$ = 4.1 Hz), 27.0 (d, $J_{C\text{-}F}$ = 19.2 Hz), 46.1, 49.8, 68.0, 83.2 (d, $J_{C\text{-}F}$ = 165.5 Hz), 90.2, 115.6, 118.7, 121.1, 126.1, 126.7, 127.9, 128.5, 129.5, 130.1, 131.2, 133.9, 135.9, 137.6, 143.4; MS (ESI), calculated m/z $C_{25}H_{22}{}^{35}Cl_2FN_5S$: 514.1 $[M+H]^+$, 516.1 [M+2+H], found *m/z* (relative intensity): 514.1 $[M+H]^+$ (100), 516.1 [M+2+H] (75); HRMS calcd. for $C_{25}H_{23}Cl_2FN_5S$: 514.1030, found: 514.1020.

4.16. 4-Iodomorpholine

To a solution of iodine (4 g, 31.5 mmol) in MeOH (63 mL) morpholine (2.75 mL, 31.5 mmol) was added drop wise and the mixture was stirred for 1 h. The precipitate formed was collected by filtration, dried under vacuum and used crude, without further purification. R*f* 0.32 (DCM/MeOH 95:5); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.89–3.00 (m, 2H), 3.69–3.77 (m, 2H).

4.17. 5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-3-(iodoethynyl)-4-methyl-1H-pyrazole (**9**)

CuI (20.0 mg, 0.07 mmol) and 4-iodomorpholine (340 mg, 1.56 mmol) were added to a solution of alkyne **7** (590 mg, 1.43 mmol) in THF (4 mL). The reaction mixture was stirred at room temperature for 1 h. The mixture was filtered on a neutral alumina pad and the solvent was evaporated under reduced pressure. The iodinated derivative **9** was obtained (570 mg, 74%) as a yellow oil and was used without further purification. R*f* 0.84 (Hexane/EtOAc 7:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 2.16 (s, 3H), 6.51 (d, 1H, J = 3.9 Hz), 6.83 (d, 1H, J = 3.9 Hz), 7.25 (m, 2H), 7.38 (m, 1H); MS (ESI), calculated *m/z* $C_{16}H_8{}^{79}Br^{35}Cl_2IN_2S$: 536.8 $[M+H]^+$, 538.8 $[M+2+H]^+$, 558.8 $[M+Na]^+$, 560.8 $[M+2+Na]^+$, found *m/z* (relative intensity): 536.8 $[M+H]^+$ (20), 538.8 $[M+2+H]^+$ (50), 558.8 $[M+Na]^+$ (70), 560.8 $[M+2+Na]^+$ (100).

4.18. 4-[5-(5-Bromothiophen-2-yl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazol-3-yl]-1-(4-fluorobutyl)-5-iodo-1H-1,2,3-triazole (**10**)

1-Azido-4-fluorobutane (400 mg, 0.37 mmol,) CuI (40 mg, 0.19 mmol) and TEA (10 mL, 0.74 mmol) were added to a solution of propargyl iodide **9** (200 mg, 0.37 mmol) in THF (5 mL). The reaction was stirred at r.t. for 72 h, then quenched with a 10% aqueous solution of NH_4OH (10 mL) and the solvent was removed under reduced pressure. The residue was diluted with Et_2O, washed with water, dried over $MgSO_4$, filtered and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (Hexane/EtOAc 4:1) to give the triazole **10** (100 mg, 42%) as a white solid. R*f* 0.52 (Hexane/EtOAc 4:1); ^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.70–1.79 (m, 1H), 1.79–1.87 (m, 1H), 2.05–2.19 (m, 2H), 2.41 (s, 3H), 4.50 (dt, 2H, $J_{H\text{-}H}$ = 5.8 Hz, $J_{C\text{-}F}$ = 47.2 Hz), 4.54 (t, 2H, J = 7.1 Hz), 6.66 (d, 1H, J = 3.9 Hz), 6.96 (d, 1H, J = 3.9 Hz), 7.30–7.38 (m, 2H), 7.50 (d, 1H, J = 1.9 Hz); ^{19}F NMR (376.45 MHz, $CDCl_3$) δ: −219.3 (s, 1F); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 10.1, 26.0 (d, $J_{C\text{-}F}$ = 4.4 Hz), 27.3 (d, $J_{C\text{-}F}$ = 20.3 Hz), 50.3, 78.0, 83.1 (d, $J_{C\text{-}F}$ = 165.8 Hz), 114.4, 116.9, 127.9, 128.7, 130.1, 130.3, 130.9, 131.2, 133.9, 135.9, 136.0, 136.2, 143.1, 145.1; MS (ESI), calculated *m/z* $C_{20}H_{16}{}^{79}Br^{35}Cl_2FIN_5S$: 653.9 $[M+H]^+$, 655.9 $[M+2+H]^+$, found *m/z* (relative intensity): 653.9 $[M+H]^+$ (75), 655.9 $[M+2+H]^+$ (100); HRMS calcd. for $C_{20}H_{17}BrCl_2FIN_5S$: 653.8788 and 655.8765, found: 653.8784 and 655.8757.

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References

[1] (a) R.G. Pertwee, A.C. Howlett, M.E. Abood, S.P.H. Alexander, V. Di Marzo, M.R. Elphick, P.J. Greasley, H.S. Hansen, G. Kunos, K. Mackie, R. Mechoulam, R.A. Ross, Pharmacol. Rev. 62 (2010) 588–631;
(b) S. Munro, K.L. Thomas, M. Abu-Shaar, Nature 365 (1993) 61–65.
[2] M. Herkenham, A.B. Lynn, M.D. Little, M.R. Johnson, L.S. Melvin, B.R. de Costa, K.C. Rice, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 1932–1936.
[3] G. Griffin, S.R. Fernando, R.A. Ross, N.G. McKay, M.L.J. Ashford, D. Shire, J.W. Huffman, S. Yu, J.A.H. Lainton, R.G. Pertwee, Eur. J. Pharmacol. 339 (1997) 53–61.
[4] R.G. Pertwee, Life Sci. 65 (1999) 597–605.
[5] J.C. Ashton, D. Friberg, C.L. Darlington, P.F. Smith, Neurosci. Lett. 396 (2006) 113–116.
[6] J. Horder, M. Browning, M. Di Simplicio, P.J. Cowen, C.J. Harmer, J. Psychopharmacol. 26 (2012) 125–132.
[7] G. Kunos, D. Osei-Hyiaman, S. Bátkai, K.A. Sharkey, A. Makriyannis, Trends Pharmacol. Sci. 30 (2009) 1–7.
[8] E. Kirilly, X. Gonda, G. Bagdy, Acta Physiol. 205 (2012) 41–60.
[9] B.-C. Ho, T.H. Wassink, S. Ziebell, N.C. Andreasen, Schizophr. Res. 128 (2011) 66–75.
[10] B. Costa, A.E. Trovato, M. Colleoni, G. Giagnoni, E. Zarini, T. Croci, Pain 116 (2005) 52–61.
[11] P. Gazzerro, M.G. Caruso, M. Notarnicola, G. Misciagna, V. Guerra, C. Laezza, M. Bifulco, Int. J. Obes. 31 (2006) 908–912.
[12] M. Rinaldi-Carmona, F. Barth, M. Héaulme, D. Shire, B. Calandra, C. Congy, S. Martinez, J. Maruani, G. Néliat, D. Caput, P. Ferrara, P. Soubrié, J.C. Brelière, G. Le Fur, FEBS Lett. 350 (1994) 240–244.
[13] (a) S.J. Gatley, A.N. Gifford, N.D. Volkow, R. Lan, A. Makriyannis, Eur. J. Pharmacol. 307 (1996) 331–338;
(b) S.J. Gatley, R. Lan, N.D. Volkow, N. Pappas, P. King, C.T. Wong, A.N. Gifford, B. Pyatt, S.L. Dewey, A. Makriyannis, J. Neurochem. 70 (1998) 417–423.
[14] (a) H.D. Burns, K.V. Laere, S. Sanabria-Bohórquez, T.G. Hamill, G. Bormans, W. Eng, R. Gibson, C. Ryan, B. Connolly, S. Patel, S. Krause, A. Vanko, A. Van Hecken, P. Dupont, I. De Lepeleire, P. Rothenberg, S.A. Stoch, J. Cote, W.K. Hagmann, J.P. Jewell, L.S. Lin,

P. Liu, M.T. Goulet, K. Gottesdiener, J.A. Wagner, J. de Hoon, L. Mortelmans, T.M. Fong, R.J. Hargreaves, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 9800–9805;
(b) F. Yasuno, A.K. Brown, S.S. Zoghbi, J.H. Krushinski, E. Chernet, J. Tauscher, J.M. Schaus, L.A. Phebus, A.K. Chesterfield, C.C. Felder, R.L. Gladding, J. Hong, C. Halldin, V.W. Pike, R.B. Innis, Neuropsychopharmacology 33 (2008) 259–269;
(c) G.E. Terry, J. Hirvonen, J.-S. Liow, S.S. Zoghbi, R. Gladding, J.T. Tauscher, J.M. Schaus, L. Phebus, C.C. Felder, C.L. Morse, S.R. Donohue, V.W. Pike, C. Halldin, R.B. Innis, J. Nucl. Med. 51 (2010) 112–120;
(d) S.R. Donohue, V.W. Pike, S.J. Finnema, P. Truong, J. Andersson, B. Gulyás, C. Halldin, J. Med. Chem. 51 (2008) 5608–5616.

[15] Retrieved from: http://clinicaltrials.gov/ (27.06.12).

[16] J.H.M. Lange, C.G. Kruse, Drug Discov. Today 10 (2005) 693–702.

[17] (a) P. Lazzari, A. Pau, S. Tambaro, B. Asproni, S. Ruiu, G. Pinna, A. Mastinu, M.M. Curzu, R. Reali, M.E.H. Bottazzi, G.A. Pinna, G. Murineddu, Cent. Nerv. Syst. Agents Med. Chem. 12 (2012) 254–276;
(b) S. Frau, S. Dall'angelo, G.L. Baillie, R.A. Ross, M. Pira, C.-C. Tseng, P. Lazzari, M. Zanda, J. Fluor. Chem. 152 (2013) 166–172, and references therein.

[18] B.K. Srivastava, R. Soni, J.Z. Patel, A. Joharapurkar, N. Sadhwani, S. Kshirsagar, B. Mishra, V. Takale, S. Gupta, P. Pandya, P. Kapadnis, M. Solanki, H. Patel, P. Mitra, M.R. Jain, P.R. Patel, Bioorg. Med. Chem. Lett. 19 (2009) 2546–2550.

[19] (a) C.-L. Tai, M.-S. Hung, V.D. Pawar, S.-L. Tseng, J.-S. Song, W.-P. Hsieh, H.-H. Chiu, H.-C. Wu, M.-T. Hsieh, C.-W. Kuo, C.-C. Hsieh, J.-P. Tsao, Y.-S. Chao, K.-S. Shia, Org. Biomol. Chem. 6 (2008) 447–450;
(b) S.-L. Tseng, M.-S. Hung, C.-P. Chang, J.-S. Song, C.-L. Tai, H.-H. Chiu, W.-P. Hsieh, Y. Lin, W.-L. Chung, C.-W. Kuo, C.-H. Wu, C.-M. Chu, Y.-S. Tung, Y.-S. Chao, K.-S. Shia, J. Med. Chem. 51 (2008) 5397–5412.

[20] (a) S. Ohira, Synth. Commun. 19 (1989) 561–564;
(b) S. Müller, B. Liepold, G.J. Roth, H.J. Bestmann, Synlett (1996) 521–522.

[21] F. Himo, T. Lovell, R. Hilgraf, V.V. Rostovtsev, L. Noodleman, K.B. Sharpless, V.V. Fokin, J. Am. Chem. Soc. 127 (2005) 210–216.

[22] N. Miyaura, T. Ishiyama, H. Sasaki, M. Ishikawa, M. Saton, A. Suzuki, J. Am. Chem. Soc. 111 (1989) 314–321.

[23] K. Sonogashira, Y.N. Tohda, Tetrahedron Lett. 16 (1975) 4467–4470.

[24] J.E. Hein, J.C. Tripp, L.B.H. Krasnova, K.B. Sharpless, V.V. Fokin, Angew. Chem. Int. Ed. 48 (2009) 8018–8021.

[25] (a) M.G. Cascio, L.A. Gauson, L.A. Stevenson, R.A. Ross, R.G. Pertwee, Br. J. Pharmacol. 159 (2010) 129–141;
(b) D. Bolognini, B. Costa, S. Maione, F. Comelli, P. Marini, V. Di Marzo, D. Parolaro, R.A. Ross, L.A. Gauson, M.G. Cascio, R.G. Pertwee, Br. J. Pharmacol. 160 (2010) 677–687.