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Research Article

Synthesis of 1-(2,4-dichlorophenyl)-4-cyano-5-(4-[^{11}C]methoxyphenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide ([^{11}C]JHU75528) and 1-(2-bromophenyl)-4-cyano-5-(4-[^{11}C]methoxyphenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide ([^{11}C]JHU75575) as potential radioligands for PET imaging of cerebral cannabinoid receptor

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Summary

Two novel ligands for cerebral cannabinoid receptor (CB1), 1-(2,4-dichlorophenyl)-4-cyano-5-(4-methoxyphenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide (JHU75528) and 1-(2-bromophenyl)-4-cyano-5-(4-methoxyphenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide (JHU75575) have been synthesized. Both JHU75528 and JHU75575 display a combination of higher binding affinity and lower lipophilicity than those of Rimonabant (SR141716), a high affinity CB1 selective antagonist, and AM281, the only available ligand for emission tomography imaging of CB1 in human subjects. Radiolabeled [^{11}C]JHU75528 and [^{11}C]JHU75575 were prepared by reaction of [^{11}C]methyl iodide with nor-methyl precursors. The average radiochemical yield, specific radioactivity, and radiochemical purity of [^{11}C]JHU75528 were 16%, 235 GBq/μmol (6360 mCi/μmol), and 99%, respectively; those of [^{11}C]JHU75575 were 8%, 196 GBq/μmol (5308 mCi/μmol), and 99%, respectively. Both ligands hold promise as PET radioligands for imaging CB1 receptor. Copyright © 2006 John Wiley & Sons, Ltd.

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1. Introduction

The development of the radiotracers for emission tomography imaging of cerebral cannabinoid receptor (CB1) is important for studying the role of CB1 in schizophrenia,[1–4] obesity,[5–8] drug dependence[9,10] and a variety of other disorders[11] as well as for developing cannabinergic medications to treat these conditions.[12–16] The radiotracers studied to date, mainly analogs of Rimonabant and WIN 55,212-2, have exhibited properties that were inadequate for successful quantitative tomography imaging due to low specific binding, high non-specific binding, and insufficient brain uptake of the radiotracers.[17–29]

Low binding potentials of all existing CB1 radioligands are due to either insufficient binding affinity or too high lipophilicity ($\log D > 4$) of these compounds.[18,20,25] Even though many other properties of CNS radioligands are crucially important,[30] it is essential to develop a CB1 radioligand with a combination of high binding affinity and reduced lipophilicity ($\log D < 4$). It was our hypothesis that the structure of one of the best currently available CB1 PET radioligand 1-(2,4-dichlorophenyl)-5-(4-[^{11}C]methoxyphenyl)-4-methyl-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide ([^{11}C]NIDA41020 or [^{11}C]SR149080) (Figure 1),[19,20,31] a methoxy-analog of Rimonabant, could be modified in such a way that the new ligand would exhibit high binding affinity and lower lipophilicity. We attempted to identify a position in the Rimonabant molecule that would tolerate hydrophilic substituents.[20] In our earlier studies we found that introduction of hydrophilic substituents in the 5-phenyl ring of Rimonabant caused a reduction of the binding affinity.[20] Recent studies by other groups demonstrated that non-pyrazole analogs of Rimonabant are tolerant to the attachment of CN-group in the central core ring.[32–34] Because a cyano-group is hydrophilic,[35] we chose to introduce this group in the pyrazole core of NIDA41020 and determine the binding affinities and lipophilicities of the cyano-analogs. We targeted a compound with higher binding affinities and lower lipophilicities than those of AM281 (Figure 1), the only available ligand for emission tomography imaging of CB1 in human subjects.[27] Here we present development of 1-(2,4-dichlorophenyl)-4-cyano-5-(4-methoxyphenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide (JHU75528) and its monobromo-analog (JHU75575) (Figure 2), two CB1 radioligands with high binding affinities and reduced lipophilicities as compared with AM281.

Results and discussion

Chemistry

JHU75528, 1-(2,4-dichlorophenyl)-4-cyano-5-(4-methoxyphenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide **1a**, and JHU75575, 1-(2-bromophenyl)-4-cyano-5-(4-methoxyphenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide **1b**,

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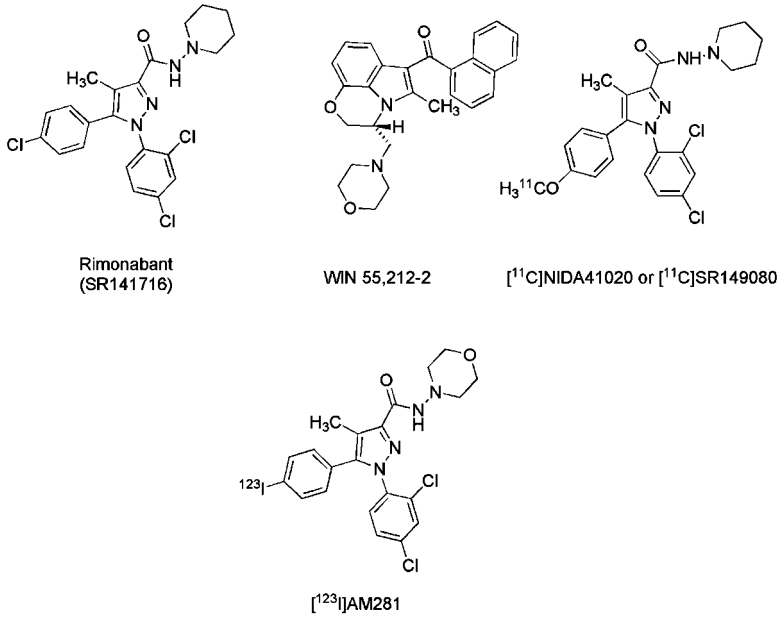

Figure 1. Representative cannabinoid ligands and radioligands

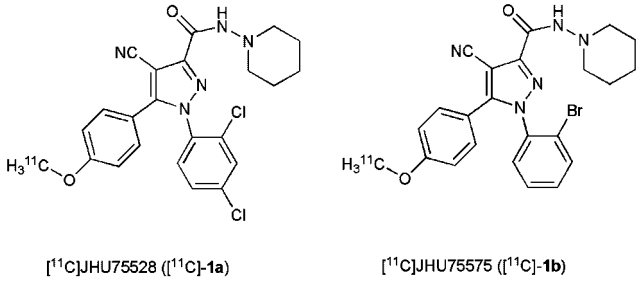

Figure 2. Chemical structures

were synthesized via a conventional method of preparation of 4-cyanopyrazole derivatives[36–39] as shown in Scheme 1. Briefly, 4-methoxybenzoylacetonitrile underwent cycloaddition reaction with chloro[(2,4-dichlorophenyl)hydrazono]ethyl acetate[39] or chloro[(2-bromophenyl)hydrazono]ethyl acetate **7** in the presence of sodium ethoxide to give pyrazole derivatives **4a** or **4b**. The cycloaddition products **4a** and **4b** were saponified with lithium hydroxide to give the carboxylic acids **5a** and **5b**, which were converted to the carbonyl chloride intermediates followed by coupling with 1-aminopiperidine to afford **1a** or **1b** in reasonable yields. Chloro[(2-bromophenyl)hydrazono]ethyl acetate **7** was prepared in two steps (Scheme 2). 2-Bromo aniline was first treated with 24% HCl and $NaNO_2$ to yield

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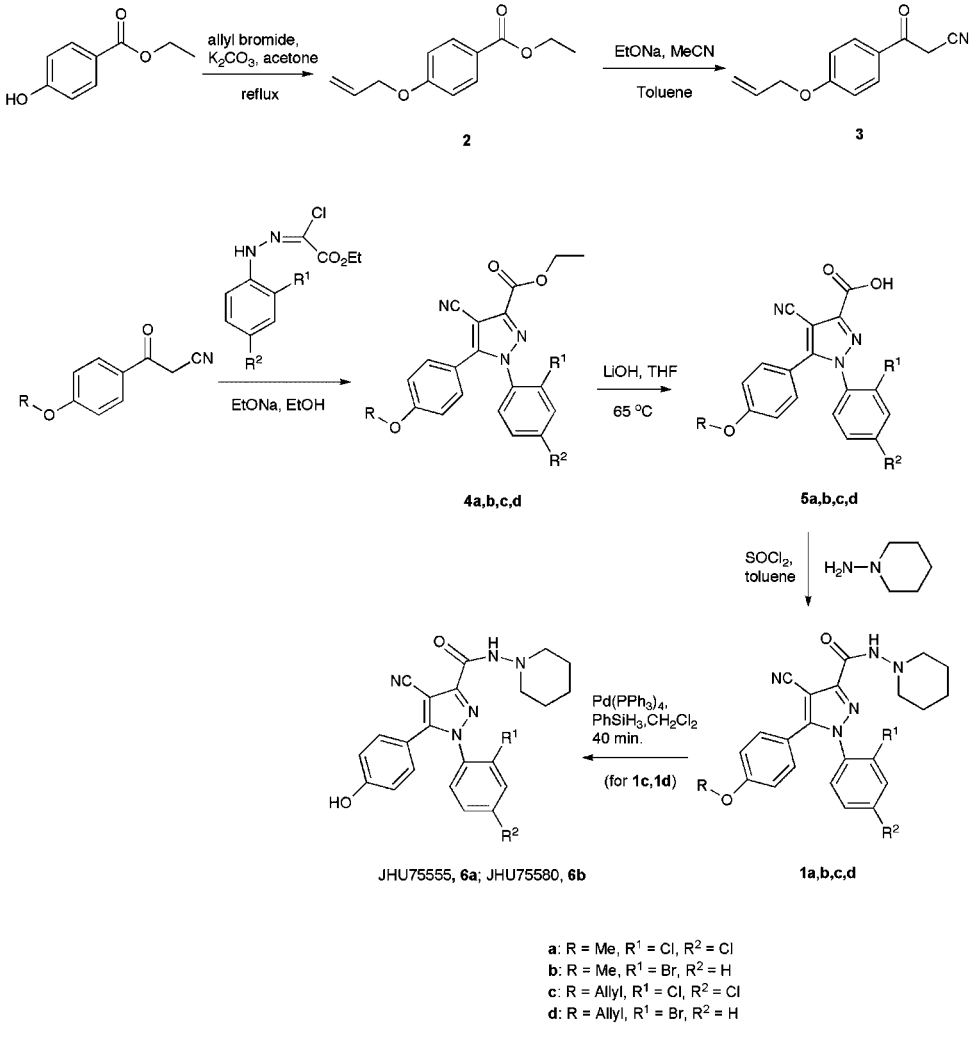
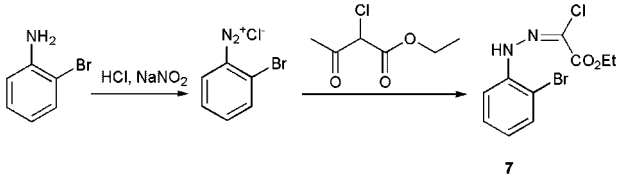


Scheme 1. Synthesis of precursors and target compounds



Scheme 2. Synthesis of Chloro[(2-bromophenyl)hydrazono]ethyl-acetate

arenediazonium salt. Then the diazonium salt was converted to chloro[(2-bromophenyl)hydrazono]ethyl acetate by reacting with ethyl 2-chloro-acetoacetate.[39]

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Attempts to generate nor-methyl precursors **6a** (JHU75555) and **6b** (JHU75580) for radiosynthesis either via demethylation or by a direct synthesis were not successful. Therefore, preparation of precursors required a protective group approach. Among the several protective groups including *t*-butyl carbamate (Boc), triisopropylsilyl (TIPS), and *t*-butyl, we found that allyl group was the best protective group for this reaction scheme. The intermediate for this synthesis, 3-(4-(allyloxy)phenyl)-3-oxopropanenitrile **3**, was obtained by treating ethyl 4-hydroxybenzoate with allyl bromide, and then condensing with acetonitrile in the presence of sodium ethoxide. In the final step, **6a** (JHU75555) and **6b** (JHU75580) were prepared via Pd-catalyzed removal of allylic protective group of **1c** and **1d** in the presence of phenyltrihydrosilane.[40]

Binding affinity and lipophilicity

In the inhibition binding assay experiments, JHU75528 and JHU75575 displayed substantially higher binding affinities than those of AM281 and Rimonabant (Table 1).

The conventional criterion for selection of CNS receptor radiotracer, the value of B_{max}/K_i, has to be 10 or greater.[41] Assuming that the B_{max} of CB1 receptors in the mammalian brain reaches values above 1 pmol/mg tissue (10^{-6} M),[14] the B_{max}/K_i ratios of JHU75528 and JHU75575 are fairly high (see Table 1).

Table 1. Inhibition binding affinity ([^{3}H]CP55940, recombinant hCB1) and lipophilicity of Rimonabant (SR141716), AM281 and JHU75528

Compound	Binding affinity, K_i, (nM)	B_{max}/K_i[a]	Calculated log $D_{7.4}$[b]	Experimental log $D_{7.4}$	Target-to-non-target ratio/time
Rimonabant	35.4 46.2	—	6.0	4.6 ± 0.8(4)[c]	—
AM281	422 525	2.4 1.9	4.9	—	2.0 (mice)/2 h[26][f] 0.22 (human)/[27][g]
JHU75528	11 ± 7 (3)[d]	91	4.3	3.6 ± 0.3 (4)[c] 3.3 ± 0.02 (4)[e]	3.4 (mice)/2 h[42][h] 2.5(baboon)/1.5 h[42][i]
JHU75575	4.7	213	3.8	3.4 ± 0.1(8)[e]	—

[a] $B_{max} = 10^{-6}$ M[14] and K_i unit is M.
[b] Calculated by ACD/logD software (Advanced Chemistry Development Inc., Toronto).
[c] The octanol/phosphate buffer (pH = 7.4) partition coefficient of non-labeled JHU75528 was measured in four assays (mean ± S.D.) using conventional flask-shake technique.
[d] Mean ± S.D. (number of independent assays).
[e] The partition coefficient between octanol and phosphate buffer (pH = 7.4) was measured using radioactivity-counting procedure[43].
[f] Cerebellum/brainstem.
[g] Estimated value of the binding potential (BP) in the lentiform nuclei with white matter as the reference region.
[h] Striatum/brainstem.
[i] Putamen/pons.

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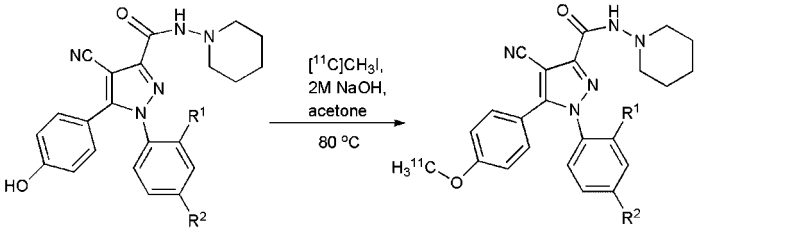
Lipophilicities of JHU75528, JHU75575, AM281, and Rimonabant were calculated and determined experimentally (Table 1). Unlike the previously studied PET radioligands for imaging CB1, the lipophilicities of JHU75528 and JHU75575 are lower than those of AM281 and Rimonabant and they fall in the range that is conventionally optimal for BBB permeability.

These *in vitro* studies demonstrate that both JHU75528 and JHU75575 are CB1 ligands with a combination of high binding affinity and relatively low lipophilicity. This combination of the ligand properties and the high density of CB1 receptors in mammalian brain[14] suggest that radiolabeled [^{11}C]JHU75528 and [^{11}C]JHU75575 could label CB1 receptors *in vivo* with high specific binding and low non-specific binding. Imaging properties of [^{11}C]JHU75528 have been recently evaluated in rodents and baboon.[42] This radioligand holds promise as the first PET radiotracer for quantitative PET imaging of CB1 receptor in human subjects.

Radiochemistry

Radiomethylation of **6** with [^{11}C]iodomethane at 80°C in acetone in the presence of 2 M NaOH proved straightforward (Scheme 3). The average non-decay corrected radiochemical yields based on trapped radio-activities of [^{11}C]CH_3I for [^{11}C]**1a** and [^{11}C]**1b** were 16 ± 5% ($n = 12$) and 8.2 ± 1% ($n = 4$), respectively, which are comparable to C-11 methylations reported on other members of the phenolic series.[44, 45] The non-optimized time of synthesis for [^{11}C]**1a** and [^{11}C]**1b**, including HPLC purification and formulation as a sterile solution in saline containing 10% ethanol was approximately 28 min.

Radiochemical purity and specific activity were determined by analytical HPLC. Specific activity was calculated by relating the area of the UV absorbance peak of carrier ligand in an aliquot of known radioactivity to the area of a standard sample of ligand. The final radioligands were obtained with radiochemical purity greater than 99% and specific activities 235 ± 108 GBq/



Scheme 3. Radio-synthesis

DOI: 10.1002/jlcr

μmol (6360 ± 2914 mCi/μmol) for [^{11}C]**1a** in 11 production runs, and 196 ± 15 GBq/μmol (5308 ± 419 mCi/μmol) for [^{11}C]**1b** in 4 runs at the end-of-synthesis.

Materials and methods

General

DMF was prepared by sequential distillation under reduced pressure from CaH_2 and BaO. All other chemicals and solvents were reagent grade, and were used as received from Aldrich. ^{1}H NMR spectra were obtained with a Vivian 400 MHz spectrometer. Chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane in $CDCl_3$. High resolution mass spectrometry was performed at the University of Notre Dame Mass Spectrometry Facility. Elemental analysis was done by Galbraith Laboratories Inc. (Knoxville, TN). Flash chromatography purification was performed using E. Merck 7729 (< 230 mesh) silica gel. [^{11}C]iodomethane was prepared with the PETtrace MeI MicroLab (GE, Milwaukee, WI) using a PETtrace cyclotron. HPLC analyses and purification for [^{11}C]JHU75528 and [^{11}C]JHU75575 were performed with two model 590EF HPLC pumps (Waters, Milford, MA), an in-line model 441 fixed wavelength (254 nm, Waters) ultraviolet (UV) detector, and a flow-through 2-in NaI(Tl) crystal scintillation detector (model 276; Ortec, Oak Ridge, TN). All HPLC chromatograms were recorded by a Varian Galaxie system. Semi-preparative (10 mm × 250 mm) and analytical (4.6 mm × 100 mm) 10 μm C-18 Luna columns (Phenomenex Torrance, CA) were used for purification and quality control, respectively.

*Chloro[(2-bromophenyl)hydrazono]ethyl acetate (**7**)*

A mixture of 2-bromo-aniline (7.8 g, 45 mmol) in 75 ml 24% HCl, and 200 ml water was stirred for 2 h at room temperature. A solution of sodium nitrite (3.17 g, 46 mmol) in 21 ml water was added dropwise to the reaction mixture, which was cooled with ice, for 30 min. The resulting solution was treated with a solution of sodium acetate (3.51 g, 43 mmol) and ethyl 2-chloro-acetoacetate (6.21 ml, 45 mmol) in 450 ml EtOH, then cooled with ice. The temperature was allowed to slowly increase for 2 h. The precipitate was filtered, washed with water and dried to give desired product 12.5 g, (91%). ^{1}H NMR ($CDCl_3$, δ) 1.41 (t, J = 6.8 Hz, 3H), 4.40 (q, J = 6.8 Hz, 2H), 6.91 (m, 1H), 7.33 (m, 1H), 7.51 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H), 7.62 (dd, J_1 = 8.4 Hz, J_2 = 1.6 Hz, 1H), 8.86 (b, 1H, NH). FAB-HRMS: calculated for $C_{10}H_{10}BrClN_2O_2$: 303.9614; found: 303.9638.

*Ethyl 4-(allyloxy)benzoate (**2**)*

Ethyl 4-hydroxybenzoate (2.32 g, 14 mmol), allyl bromide (1.69 ml, 20 mmol), and potassium carbonate (2.84 g, 20 mmol) in acetone (50 ml) were heated at

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reflux for 3 h. The reaction solution was cooled and filtered. The filtrate was evaporated to dryness to yield colorless oil 2.90 g, 100%. ^{1}H NMR ($CDCl_3$, δ) 1.37 (t, J = 7.2 Hz, 3H), 4.34 (dd, J_1 = 8.4 Hz, J_2 = 7.2 Hz, 2H), 4.58(m, 2H), 5.31 (m, 1H), 5.42 (m, 1H), 6.04 (m, 1H), 6.92 (dd, J_1 = 7.2 Hz, J_2 = 1.6 Hz, 2H), 7.99 (dd, J_1 = 6.8 Hz, J_2 = 1.6 Hz, 2H). FAB-HRMS: calculated for $C_{12}H_{14}O_3$: 206.2378; found: 207.1022 $(M+H)^+$.

*3-(4-(allyloxy)phenyl)-3-oxopropanenitrile (**3**)*

A mixture of ethyl (4-allyloxy) benzoate **2** (2.06 g, 10 mmol), sodium ethoxide (0.748 g, 11 mmol), and acetonitrile (0.65 ml, 12 mmol) in anhydrous toluene (5 ml) was stirred at 110°C for 20 h. The reaction was cooled and diluted with 30 ml water to dissolve solid. The aqueous layer was washed with Et_2O (2 × 30 ml), then was acidified with 1 N HCl to pH 7. The solution was extracted with CH_2Cl_2 (3 × 30 ml). The organic phase was washed with brine and dried over Na_2SO_4. The solvent was then removed to afford the desired product 555 mg, 27.6%. ^{1}H NMR ($CDCl_3$, δ) 4.02 (s, 2H), 4.63(m, 2H), 5.35 (m, 1H), 5.46 (m, 1H), 6.05 (m, 1H), 6.99 (dd, J_1 = 6.8 Hz, J_2 = 2.0 Hz, 2H), 7.99 (dd, J_1 = 7.2 Hz, J_2 = 2.0 Hz, 2H). FAB-HRMS: calculated for $C_{12}H_{11}NO_2$: 201.2212; found: 202.0854$(M+H)^+$.

*Ethyl 1-(2,4-dichlorophenyl)-4-cyano-5-(4-methoxyphenyl)-1H-pyrazole-3-carboxylate (**4a**)*

A mixture of chloro[(2,4-dichlorophenyl)hydrazono]ethyl acetate[39] (1.63 g, 5.5 mmol), 4-methoxybezoylacetonitrile (0.97 g, 5.5 mol) , 60 ml EtOH, and sodium ethoxide, which was prepared by dissolving 0.14 g sodium in 12.5 ml EtOH, was heated to reflux for 18 h. After cooling to room temperature, the solvent was removed by reduced pressure. EtOAc (75 ml) was added and the precipitate was filtered. The organic solution was washed with water and brine, dried over Na_2SO_4, and evaporated with a rotary evaporator. The crude product was purified by flash chromatography 10:90 EtOAc/ CH_2Cl_2 to afford the desired product 440 mg, 19%. ^{1}H NMR ($CDCl_3$, δ) 1.46 (t, J = 6.0 Hz, 3H), 3.81 (s, 3H), 4.52 (dd, J_1 = 14.4 Hz, J_2 = 2.8 Hz, 2H), 6.89 (dd, J_1 = 6.8 Hz, J_2 = 2.0 Hz, 2H), 7.26 (dd, J_1 = 7.2 Hz, J_2 = 2.4 Hz, 2H), 7.37 (m, 1H), 7.42 (s,1H), 7.46 (m, 1H). FAB-HRMS: calculated for $C_{20}H_{15}Cl_2N_3O_3$: 415.0490; found: 416.0569 $(M+H)^+$.

*Ethyl 1-(2-bromophenyl)-4-cyano-5-(4-methoxyphenyl)-1H-pyrazole-3-carboxylate (**4b**)*

Compound **4b** was prepared from chloro[(2-bromophenyl)hydrazono]ethyl acetate **7** with the same procedure described for **4a**, to afford the desired product 15%. ^{1}H NMR ($CDCl_3$, δ) 1.47 (t, J = 7.2 Hz, 3H), 3.80 (s, 3H), 4.52

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(q, J = 1.2 Hz, 2H), 6.86 (d, J = 8.8 Hz), 7.28 (d, J = 8.8 Hz), 7.36 (m, 1H), 7.44 (m, 2H), 7.62 (m, 1H). FAB-HRMS: calculated for $C_{20}H_{16}BrN_3O_3$: 425.0375; found: 425.0359.

*Ethyl 5-(4-(allyloxy)phenyl)-1-(2,4-dichlorophenyl)-4-cyano-1H-pyrazole-3-carboxylate (**4c**)*

Compound **4c** was prepared from 3-(4-(allyloxy)phenyl)-3-oxopropanenitrile **3** and chloro[(2,4-dichlorophenyl)hydrazono]ethyl acetate[39] with the same procedure described for **4a**, to afford the desired product 34%. ^{1}H NMR ($CDCl_3$, δ) 1.46 (t, J = 6.8 Hz, 3H), 4.53(m, 4H), 5.31 (m, 1H), 5.40 (m, 1H), 6.00 (m, 1H), 6.90 (dd, J_1 = 6.8 Hz, J_2 = 2.0 Hz, 2H), 7.25 (dd, J_1 = 7.2 Hz, J_2 = 2.4 Hz, 2H), 7.37 (dd, J_1 = 8.8 Hz, J_2 = 2.4 Hz, 1H), 7.42 (d, J = 8.4 Hz 1H), 7.46 (d, J = 2.4 Hz, 1H). FAB-HRMS: calculated for $C_{22}H_{17}Cl_2N_3O_3$: 441.0647; found: 442.0742 $(M+H)^+$.

*Ethyl 5-(4-(allyloxy)phenyl)-1-(2-bromophenyl)-4-cyano-1H-pyrazole-3-carboxylate (**4d**)*

Compound **4d** was prepared from 3-(4-(allyloxy)phenyl)-3-oxopropanenitrile **3** and chloro[(2-bromophenyl)hydrazono]ethyl acetate **7** with the same procedure described for **4a**, to afford the desired product 21%. ^{1}H NMR ($CDCl_3$, δ) 1.47 (t, J = 6.8 Hz, 3H), 4.53(m, 4H), 5.30 (m, 1H), 5.39 (m, 1H), 6.01 (m, 1H), 6.86 (d, J = 8.8 Hz, 2H), 7.27 (d, J = 8.8 Hz, 2H), 7.34 (m, 1H), 7.42 (m, 2H), 7.46 (dd, J_1 = 8.4 Hz, J_2 = 1.2 Hz, 1H). FAB-HRMS: calculated for $C_{22}H_{18}BrN_3O_3$: 451.0532; found: 451.0514.

*1-(2,4-dichlorophenyl)-4-cyano-5-(4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid (**5a**)*

A mixture of ethyl 1-(2,4-dichlorophenyl)-4-cyano-5-(4-methoxyphenyl)-1*H*-pyrazole-3-carboxylate **4a** (0.51 g, 1.22 mmol) in 35 ml THF and LiOH (35 mg, 1.5 mmol) in 3 ml water was heated to 65°C for 2 h. After cooling to room temperature, 25 ml of cold water and 2.5 ml of HCl (5%) were added. The solution was extracted with dichloromethane (3 × 50 ml). The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed to afford the desired product 432 mg, 91%. ^{1}H NMR ($CDCl_3$, δ) 3.82 (s, 3H), 6.90 (dd, J_1 = 7.2 Hz, J_2 = 2.0 Hz, 2H), 7.26 (dd, J_1 = 7.2 Hz, J_2 = 2.4 Hz, 2H), 7.39 (d, J = 2.0 Hz, 1H), 7.41 (s, 1H), 7.48 (d, J = 2.0 Hz, 1H). FAB-HRMS: calculated for $C_{19}H_{11}Cl_2N_3O_3$: 387.0177; found: 388.0238 $(M+H)^+$.

*1-(2-bromophenyl)-4-cyano-5-(4-methoxyphenyl)-1H-pyrazole-3-carboxylic acid (**5b**)*

Compound **5b** was prepared from **4b** with the same procedure described for **5a**, to afford the desired product 96%. ^{1}H NMR (CD_3OD, δ) 3.79 (s, 3H), 6.93

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(d, J = 8.8 Hz, 2H), 7.32 (d, J = 8.8 Hz, 2H), 7.49 (m, 2H), 7.63 (m, 1H), 7.71 (m, 1H). FAB-HRMS: calculated for $C_{18}H_{12}BrN_3O_3$: 397.0062; found: 397.0063.

*5-(4-(allyloxy)phenyl)-1-(2,4-dichlorophenyl)-4-cyano-1H-pyrazole-3-carboxylic acid (***5c***)*

Compound **5c** was prepared from **4c** with the same procedure described for **5a**, to afford the desired product 97%. ^{1}H NMR ($CDCl_3$, δ) 4.54 (d, J = 4.8 Hz, 2H), 5.31 (m, 1H), 5.41 (m, 1H), 6.03 (m, 1H), 6.90 (dd, J_1 = 6.8 Hz, J_2 = 2.0 Hz, 2H), 7.24 (dd, J_1 = 7.2 Hz, J_2 = 2.4 Hz, 2H), 7.40 (m, 2H), 7.48 (d, J = 2.4 Hz, 1H). FAB-HRMS: calculated for $C_{20}H_{13}Cl_2N_3O_3$: 413.0334; found: 414.0391 $(M+H)^+$.

*5-(4-(allyloxy)phenyl)-1-(2-bromophenyl)-4-cyano-1H-pyrazole-3-carboxylic acid (***5d***)*

Compound **5d** was prepared from **4d** with the same procedure described for **5a**, to afford the desired product 66%. ^{1}H NMR ($CDCl_3$, δ) 4.51 (d, J = 4.8 Hz, 2H), 5.30 (dd, J_1 = 10.8 Hz, J_2 = 1.6 Hz, 1H), 5.39 (dd, J_1 = 17.2 Hz, J_2 = 1.6 Hz, 1H), 6.03 (m, 1H), 6.86 (d, J = 8.4 Hz, 2H), 7.27 (d, J = 8.4 Hz, 2H), 7.36 (m, 1H), 7.44 (m, 2H), 7.61 (d, J = 8.0 Hz, 1H). FAB-HRMS: calculated for $C_{20}H_{14}BrN_3O_3$: 423.0219; found: 423.0212.

*1-(2,4-dichlorophenyl)-4-cyano-5-(4-methoxyphenyl)-N-(piperidin-1-yl)-1H-pyrazole-3- Carboxamide (***1a***)*

A mixture of a solution of 1-(2,4-dichlorophenyl)-4-cyano-5-(4-methoxyphenyl)-1*H*-pyrazole-3-carboxylic acid **5a** (0.19 g, 0.5 mmol) in toluene (5 ml) and thionyl chloride (0.1 ml, 1.33 mol) was refluxed for 2 h. The solvent was removed under reduced pressure. Extra toluene (5 ml) was added and evaporated under vacuum. The crude acid chloride in CH_2Cl_2 (5 mL) was cooled to 0°C, and Et_3N (0.1 ml, 0.71 mmol) with 1-amino piperidine (79 μL, 0.71 mmol) added. The solution was stirred at room temperature for 20 h. The reaction mixture was diluted with CH_2Cl_2, and washed the organic phase washed with brine, dried over Na_2SO_4. The solvent was removed and the crude product was purified by flash chromatography 1:3 EtOAc/hexanes to afford the desire product 87 mg, 36.7%. ^{1}H NMR ($CDCl_3$, δ) 1.43 (b, 2H), 1.74 (m, 4H), 2.89 (b, 4H), 3.81 (s, 3H), 6.87 (dd, J_1 = 6.8 Hz, J_2 = 2.4 Hz, 2H), 7.23 (dd, J_1 = 6.8 Hz, J_2 = 2.4 Hz, 2H), 7.41 (d, J = 2.0 Hz, 1H), 7.43 (d, J = 7.6 Hz, 1H), 7.48 (d, J = 2.0 Hz, 1H), 7.57 (b, 1H). FAB-HRMS: calculated for $C_{23}H_{21}N_5O_2Cl_2$: 469.1072; found: 470.1125 $(M+H)^+$. Analytical ($C_{23}H_{21}N_5O_2Cl_2$•0.55EtOAc) C, H, N.

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*1-(2-Bromophenyl)-4-cyano-5-(4-methoxyphenyl)-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (**1b**)*

Compound **1b** was prepared from **5b** with the same procedure described for **1a**, to afford the desired product 26%. ^{1}H NMR ($CDCl_3$, δ) 1.43 (b, 2H), 1.75 (m, 4H), 2.85 (b, 4H), 3.79 (s, 3H, CH_3), 6.84 (d, J = 8.4 Hz), 7.25 (d, J = 8.8 Hz), 7.43 (m, 3H), 7.60 (b, 1H), 7.66 (d, J = 7.6 Hz, 1H). FAB-HRMS: calculated for $C_{23}H_{22}BrN_5O_2$: 479.0597; found: 480.1028 $(M+H)^+$. Analytical ($C_{23}H_{22}BrN_5O_2 \cdot 0.4EtOAc \cdot 0.3CH_2Cl_2$) C, H, N.

*5-(4-(allyloxy)phenyl)-1-(2,4-dichlorophenyl)-4-cyano-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (**1c**)*

Compound **1c** was prepared from **5c** with the same procedure described for **1a**, to afford the desired product 41%. ^{1}H NMR ($CDCl_3$, δ) 1.44 (b, 2H), 1.75 (m, 4H), 2.91 (b, 4H), 4.53 (d, J = 4.6 Hz, 2H), 5.31 (d, J = 9.2 Hz, 1H), 5.40 (d, J = 18.8, Hz, 1H), 6.02 (m, 1H), 6.88 (dd, J_1 = 11.2 Hz, J_2 = 2.4 Hz, 2H), 7.22 (dd, J_1 = 7.2 Hz, J_2 = 1.6 Hz, 2H), 7.38 (m, 2H), 7.49 (d, J = 2.0 Hz, 1H), 7.55 (b, 1H). FAB-HRMS: calculated for $C_{25}H_{23}Cl_2N_5O_2$: 495.1229; found: 496.1294 $(M+H)^+$.

*5-(4-(allyloxy)phenyl)-1-(2-bromophenyl)-4-cyano-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (**1d**)*

Compound **1d** was prepared from **5d** with the same procedure described for **1a**, to afford the desired product 50%. ^{1}H NMR ($CDCl_3$, δ) 1.44 (b, 2H), 1.76 (m, 4H), 2.90 (b, 4H), 4.51 (m, 2H), 5.30 (dd, J_1 = 10.4 Hz, J_2 = 1.2 Hz, 1H), 5.39 (dd, J_1 = 10.4 Hz, J_2 = 1.2 Hz, 1H), 6.01 (m, 1H), 6.86 (m, 2H), 7.26 (m, 2H), 7.39 (m, 3H), 7.59 (s, 1H), 7.67 (d, = 7.6 Hz, 1H). FAB-HRMS: calculated for $C_{25}H_{23}BrN_5O_2$: 491.1004; found: 491.1082.

*1-(2,4-dichlorophenyl)-4-cyano-5-(4-hydroxyphenyl)-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (**6a**)[40]*

A mixture of 5-(4-(allyloxy)phenyl)-1-(2,4-dichlorophenyl)-4-cyano-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide **1c** (324 mg, 0.65 mmol), $Pd(PPh_3)_4$ (15 mg, 0.013 mmol), and $PhSiH_3$(140 mg, 1.3 mmol) in 20 ml CH_2Cl_2 was stirred at room temperature for 1 h. The reaction was monitored by TLC (1:1 EtOAc/hexanes). After the reaction was complete, the solvent was removed under reduced pressure. EtOAc (50 ml) was added to dissolve the residue. The organic layer was washed with saturated $NaHCO_3$, brine, dried over Na_2SO_4 and evaporated to an oil. The residue was dissolved and purified by flash chromatograph 1:1 EtOAc/CH_2Cl_2, to yield 287 mg, 96%. ^{1}H NMR ($CDCl_3$, δ) 1.42 (b, 2H), 1.73 (m, 4H), 2.87 (b, 4H), 6.87 (dd, J_1 = 6.4 Hz, J_2 = 1.6 Hz, 2H), 7.16 (dd, J_1 = 6.4 Hz, J_2 = 1.6 Hz, 2H), 7.37 (m, 2H), 7.49 (d, J =

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2.0 Hz, 1H), 7.59 (b, 1H). FAB-HRMS: calculated for $C_{22}H_{19}Cl_2N_5O_2$: 455.0916; found: 456.1013 $(M+H)^+$.

*1-(2-bromophenyl)-4-cyano-5-(4-hydroxyphenyl)-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide (***6b***)*

Compound **6b** was prepared from **1d** with the same procedure described for **6a**, to afford the desired product 75%. ^{1}H NMR ($CDCl_3$, δ) 1.37 (b, 2H), 1.66 (m, 4H), 2.82 (b, 4H), 6.89 (d, J = 8.8 Hz, 2H), 7.16 (d, J = 8.4 Hz, 2H), 7.38 (m, 3H), 7.64 (d, J = 7.6 Hz, 1H), 7.68 (s, 1H), 8.23 (b, 1H). FAB-HRMS: calculated for $C_{22}H_{20}BrN_5O_2$: 465.0800; found: 466.0855 $(M+H)^+$.

1-(2,4-dichlorophenyl)-4-cyano-[^{11}C]5-(4-methoxyphenyl)-N-(piperidin-1-yl)-1H-pyrazole-3- carboxamide ([^{11}C]**1a***)*

To a 1 ml V-vial was added 1-(2,4-dichlorophenyl)-4-cyano-5-(4-hydroxyphenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide **6a** (1 mg). The precursor was dissolved in 0.2 ml of acetone. Five microliters of 2 M sodium hydroxide was added, and the vial was capped with a septum seal. [^{11}C]methyl iodide, carried by a stream of helium, was trapped in the above solution cooled in a dry ice/acetonitrile bath. The reaction was heated in an 80°C water bath for 5 min, then quenched with 0.2 ml of HPLC mobile phase consisting of 70% acetonitrile/0.1 M ammonium formate. The crude reaction product was purified by reverse-phase HPLC at 10 ml/min. The radioproduct (t_R = 4.9 min) that was separated from the precursor (t_R = 2.8 min) was remotely collected. After concentration to dryness under reduced pressure and heat (80°C), the radiotracer was reconstituted in ethanol (1 ml) and sterile 0.9% saline (9.0 ml) and passed through a 0.2 μM sterile filter (Millex-FG, Millipore, Carrigtwohill, Co. Cork, Ireland) into a sterile, pyrogen-free multi-dose vial. The average non-decay corrected radiochemical yield for [^{11}C]**1a** was 16 ± 5% (n = 12). An aliquot (0.1 ml) was assayed for radioactivity, and checked by analytical HPLC using the mobile phase described above at 3 ml/min. A single radioactive peak (t_R = 2.9 min) corresponding to **1a** was observed. The specific radioactivity at the end-of-synthesis was calculated by relating radioactivity to the mass associated with the UV absorbance peak of carrier. An average specific radioactivity at end-of-synthesis (n = 11) of 235 ± 108 GBq/μmol (6360 ± 2914 mCi/μmol) was obtained.

1-(2-bromophenyl)-4-cyano-[^{11}C]5-(4-methoxyphenyl)-N-(piperidin-1-yl)-1H-pyrazole-3- carboxamide ([^{11}C]**1b***)*

Radioligand [^{11}C]**1b** was prepared from **6b** with the same procedure described for [^{11}C]**1a**. The final radioligand was obtained with average non-decay corrected radiochemical yield 8.2 ± 1% (n = 4), radiochemical purity greater

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than 99%, and specific activity 196 ± 15 GBq/μmol (5308 ± 419 mCi/μmol) in 4 runs at the end-of-synthesis.

Inhibition binding assay

The *in vitro* inhibition binding assays of JHU75528, JHU75575, AM281 and Rimonabant were performed commercially by NovaScreen (Hanover, MD) under condition similar to that previously published.[46] In brief, membranes from HEK-293 cells expressing the human recombinant cannabinoid receptor CB1 were incubated with [^{3}H]CP55,940 ($K_d = 0.6$ nM) at a concentration of 0.5 nM in 50 mM *Tris*-HCl buffer with 5 mM $MgCl_2$, 5 mg/ml BSA and 2.5 mM EDTA at pH 7.4 for 90 min at 30°C. The binding reaction was terminated by rapid vacuum filtration of the assay contents onto presoaked (0.5% PEI) Whatman GF/C filters. Radioactivity trapped onto the filters was assessed using liquid scintillation counting. Non-specific binding was defined as that remaining in the presence of 1 μM HU-210. The assays were done in duplicate at multiple concentrations of the test compounds. Binding assay results were analyzed using a 1 site competition models and IC_{50} curves were generated based on a sigmoidal dose response with variable slope. Inhibition binding affinity values (K_i) were calculated using Cheng-Prusoff equation[47].

Determination of partition coefficients of [^{11}C]JHU75528 and [^{11}C]JHU75575

700 μCi of the respective radioligand was added to a separatory funnel containing 1-octanol (20 ml) and 0.02 M phosphate buffer (pH 7.4, 20 ml) pre-saturated each other. The mixture was shaken mechanically for 3 min and the layers were separated. The aqueous layer was discarded and 1-octanol (15 ml) was transferred to a second separatory funnel containing phosphate buffer (pH 7.4, 15 ml) and the mixture shaken. 1-Octanol (2 ml each) was pipetted into four test tubes containing phosphate buffer (2 ml each). The test tubes were stopped, vortexed for 10 min and centrifuged (5 min, 1000 g). The 1-octanol and aqueous phases (1 ml each) were transferred into counting tubes. The amount of radioactivity in each tube was measured by a γ counter and corrected for decay. The partition coefficient calculated as follows: P = counts in 1-octanol/counts in phosphate buffer.

Conclusion

In summary, [^{11}C]JHU75528 and [^{11}C]JHU75575, two high affinity CB1 radiotracers and analogs of the selective CB1 antagonist Rimonabant, have been developed. The experimental lipophilicities of [^{11}C]JHU75528 and [^{11}C]JHU75575 are in the optimal range for BBB permeation and substantially lower than those of all previously described PET tracers for CB1 receptor imaging. The *in vitro* results with [^{11}C]JHU75528 and [^{11}C]JHU75575 suggest

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that these radioligands are promising potential PET tracers for imaging CB1 receptor with high specific and low non-specific binding. This conclusion is supported by our recent *in vivo* studies with [^{11}C]JHU75528[42]. Animal imaging studies with [^{11}C]JHU75575 are warranted.

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