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Research Article

Tritiation of SR141716 by metallation–iodination–reduction: tritium-proton nOe study

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Summary

The central cannabinoid receptor antagonist SR141716, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2, 4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, was synthesized from commercially available starting materials. Condensation of an aryl hydrazine with a diketone followed by base promoted isomerization/cyclization of the intermediate anti-imine gave the pyrazole acid which was converted to the title hydrazide via its acid chloride. Facile iodination via metallation followed by *in situ* trapping with an iodine source gave a modest yield of the iodinated SR141716. The iodine was selectively reduced with tritium gas and catalyst while retaining the three aryl chlorine atoms in the structure. The tritiated SR141716 exhibited a tritium-proton nOe both definitively identifying the position of the tritium as well as the sought isomer of the diarylpyrazole. This work provides a direct method for the preparation of preferred iodinated aryl substrates that offer advantages where selectivity and high incorporation in catalytic reduction is sought. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: metallation; iodination; tritiation; tritium-proton nOe; SR141716

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Introduction

Tritium labelling of a compound in the preferred final step of its preparation often involves the synthesis of a derivative that can be reduced back to the parent compound. Halogenation of the target compound on an aromatic ring can preclude a separate, complete synthesis of the halo-analog. However, if the target compound contains chlorine atoms that are to be maintained in the final product, the introduction of the significantly more reactive iodine would be required to achieve good selectivity in the reduction step. The rapid reduction of an aryl iodide offers both greater selectivity and higher specific activities than the more commonly used bromides. Iodine incorporation, however, typically by an iodonium reagent, is often problematic. In this paper, an approach of metallation–iodination followed by selective catalytic reduction is described for the tritiation of the cannabinoid receptor antagonist SR141716.

The hydrochloride of the diarylpyrazole hydrazide SR141716, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)- 4-methyl-1H-pyrazole-3-carboxamide, is the first antagonist for the cannabinoid receptor.[1,2] This ligand has become an important tool in studies leading to a better understanding of the neurochemical system represented by the receptor.[3,4] Essential in pharmacological studies of the receptor is the availability of the corresponding tritiated radioligand. The details of the synthesis of both the unlabelled free base SR141716 and of the tritium-labelled ligand from a metallation–iodination–tritiation sequence are reported. The nuclear Overhauser enhancement (nOe) spectroscopy that was used to unambiguously identify the structure of SR141716 versus its 1,3-isomer is described as well as its extension to a tritium-proton nOe.

Results and discussion

The synthesis of the unlabelled SR141716 is outlined in Figure 1. A preliminary account of this synthesis has been published[5] as have syntheses from other laboratories.[6–8] The synthesis begins with the lithium hexamethyldisilazide promoted condensation[9] of 4-chloropropiophenone **1** with diethyl oxylate **2** to give the readily isolated solid lithium enolate **3** in 35% yield. Use of the stronger base LDA modestly increased the yield to 40%. The further enhancement of the kinetic

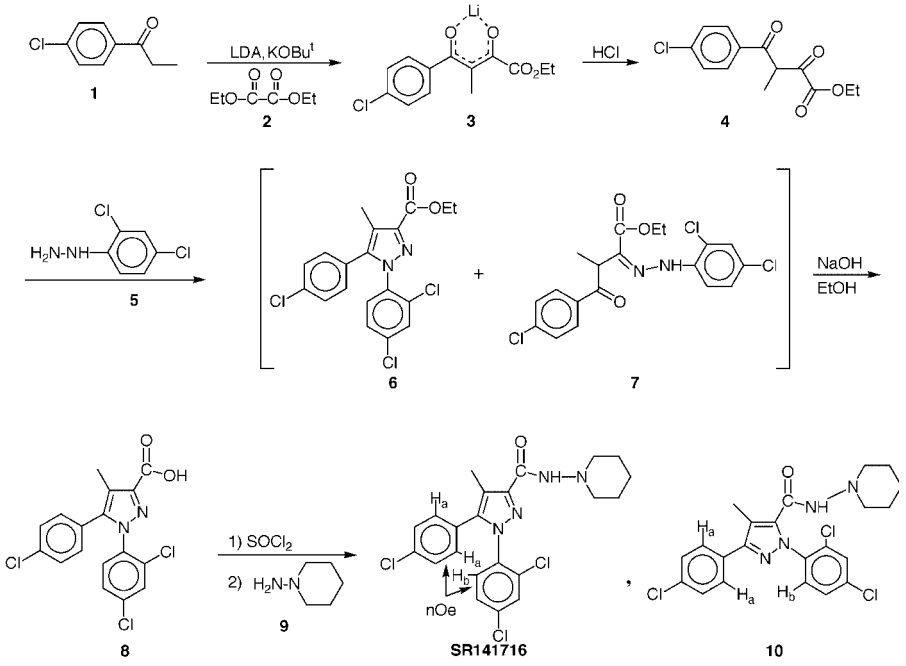

Figure 1.

basicity of LDA by the addition of 10 mol% of potassium *t*-butoxide[10] raised the yield of the salt **3** to 65%. Acidification of **3** gave **4** in 52% overall yield from **1**.

Condensation of **4** with the hydrazine free base **5** in ethanol at reflux[11] gave the pyrazole ester **6** (40%) and the imine **7** (60%) which were readily separated due to solubility differences between the two compounds in the reaction mixture. Further heating of **7** failed to generate more **6**, indicative of a non-reactive *anti* configuration between the anilino nitrogen and the 4-keto group. However, when **7** or the reaction mixture containing **6** and **7** were heated with ethanolic sodium hydroxide,[5] the pyrazole acid **8** was obtained directly in 89% yield. The cyclization of **7** proceeded via base promoted isomerization at the imine double bond to afford a reactive *syn* relationship between the anilino nitrogen and the 4-keto group. *In situ* hydrolysis of the ester **6** then provided the acid **8**.

The acid **8** was converted to the corresponding acid chloride with thionyl chloride[12,13] in 94% yield. Treatment of the acid chloride with 1-aminopiperdine and triethylamine readily afforded SR141716 (free base) in 73% yield after chromatography and recrystallization, for an overall

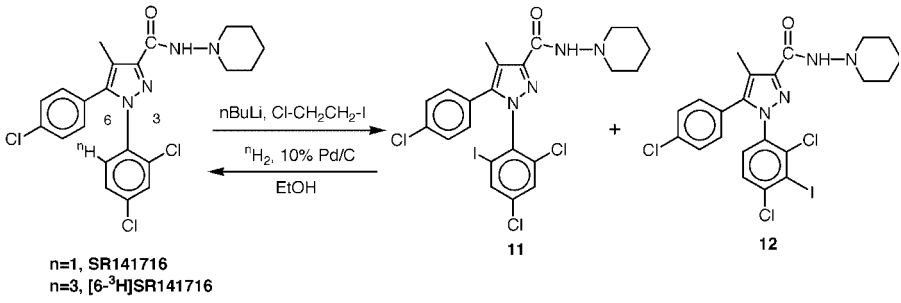

Figure 2.

yield of 32%. A trace amount of the 1,3-isomer **10** was obtained from the mother liquor (see Experimental).

Two isomers are possible from the condensation of the diketone **4** with the hydrazine **5** affording the desired 1,5-diarylpyrazole acid **8** and the isomeric 1,3-diarylpyrazole. The former is usually preferred due to initial attack of the unsubstituted hydrazine nitrogen at the more electrophilic 2-carbonyl of 2,4-diketo esters.[9,14] An nOe spectral determination was used to show the regiochemistry of the product SR141716 and by inference the structure of **8**. The proximity of the two aryl rings in the 1,5-isomer suggests that an nOe interaction would exist between the *ortho* protons H_a and H_b on the respective aryl rings, thus identifying SR141716. No such effect from the similar protons would be anticipated for the 1,3-isomer (**10**). The five aromatic resonances were completely resolved in MeOD allowing a definitive experiment (in contrast to the overlap in $CDCl_3$ or C_6D_6). The NOESY spectrum clearly demonstrated an interaction between the 6-H and the 2′,6′–H_2 of the respective rings and thereby rigorously identified the final product as the 1,5-diarylpyrazole SR141716.

To obtain the iodinated analog for reduction, direct iodination of the unlabelled ligand of interest would be preferable to conducting a separate, multistep synthesis from an iodinated fragment, especially if the latter was not readily available. The availability of a site on SR141716 that can be metallated[15–17] by directed *ortho* metallation and followed by trapping with an iodine source made such direct iodination possible. Thus, metallation by *n*-butyllithium at the 6-position of the 2,4-dichlorophenyl ring, directed by the N-linked pyrazole *ortho* directing metallation group, followed by quenching with 1-chloro-2-iodoethane afforded the 6-iodophenyl derivative **11** in 17% yield (Figure 2). Also obtained from this process was the 3-iodophenyl

derivative **12**, in 14% yield, which derived from the combined weak *ortho* metallation directing effects of the two chlorine atoms. The compounds were isolated chromatographically and characterized by ^{1}H NMR (MeOD, only two *meta* coupled doublets for the trihalogenated ring of **11,** δ 7.94 and 7.66, $J = 2.2$ Hz) and MS (DIP, m/z 588 M^+, 84 base). Reduction of **11** under a deuterium atmosphere in the presence of 10% Pd/C (10% w/w) and Et_3N (25°C, Et_2O) afforded deuterium-labelled SR141716 (93% ^{2}H) that was identical (HPLC, ^{1}H NMR, MS) in all other respects with SR141716. No over-reduced product was detected.

Tritiation under similar conditions with carrier-free tritium gas gave [^{3}H]SR141716 as pre-dominantly a single component (HPLC, βRAM). Purification by HPLC afforded >99% pure ligand at a specific activity of 22.4 Ci/mmol as determined by UV quantitation and scintillation counting. While effective, the tritiation in ether proceeded sluggishly and with modest conversion to the radioligand. A change of solvents to ethanol gave faster and more complete reduction in both deuterium and tritium experiments. A specific activity of 23 Ci/mmol was obtained. A proton-decoupled ^{3}H NMR spectrum (MeOD) exhibited one singlet (δ 7.50) for the 6- ^{3}H on the 2,4-dichlorophenyl ring, thus identifying the single site of label incorporation. A proton observed tritium-proton nOe difference spectrum of [6-^{3}H]SR141716 exhibited an enhancement of the 2,6-protons of the 4-chlorophenyl ring and an enhancement of the 5-proton of the 2,4-dihlorophenyl ring upon irradiation of the tritium in a 1:3 ratio, respectively.[†] This demonstrates the position of the tritium atom and its proximity to the 4-chlorophenyl ring with the same rigorousness as for the unlabelled SR141716. In addition to its application to structure determination, tritium-proton nOe can contribute significant understanding to pivotal interactions between proteins and tritiated substrates, in suitable studies. For example, a more complex protein counterpart to this small molecule tritium-proton nOe has been demonstrated in an nOe study of a tritiated substrate's covalent adduct to an enzyme's binding site.[18]

Conclusion

This paper provides the details of a metallation–iodination–tritiation sequence that offers a facile entry to tritiated ligands from numerous

[†]The use of a direct difference nOe experiments for observation of {^{3}H}^{1}H nOe makes determination of percent enhancements difficult. However, the superior spectra obtained without subtraction artifacts makes this method preferable for qualitative detection of nOe interactions.

aryl ligands. The chemistry suggests its wider application to other structures that are amenable to metallation and trapping with the preferred halogen iodine. It is applied here to a cannabinoid receptor ligand of considerable current interest that is proving to be a valuable tool in pharmacological studies of this emerging neurochemical system. Our synthesis of the unlabelled SR141716 from commercially available fragments without the need for purification of intermediates recommend this route for the preparation of this important compound. The demonstration of an intramolecular proton-tritium nOe has implications for the study of the interactions of ligands with receptors.

Experimental

NMR spectra were obtained on a Bruker AMX-500 spectrometer operating at 500.13 MHz for ^{1}H or on a Bruker AM-250 spectrometer operating at 250.13 MHz for ^{1}H. Proton spectra were referenced to internal tetramethylsilane at 0.0 ppm and tritium spectra were referenced to internal tetramethylsilane by the relationship $\delta_{\text{tritium}} = f_{\text{tritium}}\text{-}(f_{\text{TMS}}{}^{*}\gamma_{T}/\gamma_{H}) \times 10^{6}$ where f_{tritium} and f_{TMS} are the observational frequencies of the tritium of interest and tetramethylsilane, respectively, and γ_{T}/γ_{H} is the ratio of the gyromagnetic ratios of ^{3}H and ^{1}H or 1.06663975. Two-dimensional (2D) NOESY spectroscopy at 500 MHz was obtained on a sample of SR141716 that was dissolved in d4-methanol and then degassed using several freeze–pump–thaw cycles and sealed under vacuum. NOESY spectra were acquired with a 800 ms mixing time as a $2K \times 1K$ data matrix and then zero-filled to $2K \times 2K$ prior to apodization with a phase shifted squared sine function and 2D Fourier transformation. $\{^{3}H\}^{1}H$ nOe difference spectra were obtained on a 10 mCi sample of tritium-labelled SR141716 that was dissolved in d4-methanol and placed in a Teflon NMR tube insert that had been cut to a length of 5″ and placed in an 8″ NMR tube. The sample was then degassed using several freeze–pump–thaw cycles and sealed under vacuum. Difference spectra were obtained by a proton observation direct difference. In this method, a transient is obtained after low power ($\sim$10 mW) continuous wave irradiation at the tritium resonance frequency during the relaxation delay of 2 s. The irradiation frequency is gated off during acquisition of the proton signal during which time the irradiation frequency is shifted off resonance by 5000 Hz and the next transient is obtained with the detector phase shifted by 180°. This results

in the accumulation of a difference spectrum after each even number of transients.

Mass spectra were obtained on a model HP 5989A mass spectrometer or a VG ZAB-E high resolution, double focusing mass spectrometer.

*Ethyl 2,4-dioxo-3-methyl-4-(4-chlorophenyl)-butanoate (**4**)*

Under anhydrous conditions, potassium t-butoxide (70 mg, 0.59 mmol) was cooled to −78°C under dry argon and treated with anhydrous ethyl ether (20 ml) and stirred for 10 min. Commercial LDA in heptane/THF/ethyl benzene (2.77 ml, 5.93 mmol) was added by syringe and the mixture stirred for 15 min. 4-Chloropropiophenone (1.00 g, 5.93 mmol) in ether (7 ml) was added drop-wise over 30 min with stirring. After a further 30 min, diethyl oxylate (0.81 ml, 5.93 mmol) was added in one portion. The cold bath was removed and the reaction stirred 2.5 h after reaching ambient temperature. After standing 3 days, the precipitated yellow solid was filtered to afford 1.06 g (65%) of the lithium enolate of the title compound. On larger scale (723 mmol), a second crop of lithium enolate was also obtained for a total yield of 54%. The solid was sequentially partitioned between 1 N HCl and CH_2Cl_2 (3 times) affording 0.84 g (52% overall) of the title compound as an orange oil after drying the organic layers (Na_2SO_4), filtering and evaporation of the volatiles in vacuo. ^{1}H NMR ($CDCl_3$), δ 7.93 (d, 2H, 8.8 Hz, ArH_2), 7.50 (d, 2H, 8.8 Hz, ArH_2'), 5.00 (q, 1H, 7.1 Hz, HCMe), 4.28 (q, 2H, 7.2 Hz, OCH_2), 1.45 (d, 3H, 7.1 Hz, $HCCH_3$), 1.30 (*t*, 3H, 7.1 Hz).

*5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid (**8**)*

The diketone **4** (36.0 g, 134 mmol) dissolved in 500 ml of absolute ethanol was treated with solid 2,4-dichlorophenylhydrazine free-base (23.7 g, 134 mmol) in one portion with mechanical stirring under dry nitrogen. The reaction was heated at reflux for 2.5 h. An aliquot that was evaporated in vacuo, dissolved in CH_2Cl_2 and analyzed by TLC (SiO_2 F254, EtOAc:hex 1:2, UV detection) showed the diketone to be essentially consumed and two higher Rf products to be present. The ambient temperature reaction was treated with solid NaOH (8.0 g, 200 mmol) and heated under nitrogen at reflux for 2 h. TLC (SiO_2, ace:CH_2Cl_2:HOAc, 1:1: trace, UV detection) of an aliquot quenched in aqueous HCl and extracted with ether showed the intermediate imine

and pyrazole ester to be consumed and a lower Rf product to be formed. Water was added (~1000 ml) and the ethanol removed by rotary evaporation in vacuo. The residual basic solution was extracted with ether (2 ×) to remove neutrals. The aqueous phase was acidified to pH 1 with HCl and extracted with ether (3 ×). The combined organic layers of the acid extract was dried (Na_2SO_4) and evaporated in vacuo and high vacuum dried to afford 45.9 g (89%) of the title acid as a beige solid. This acid was suitable for use in the next reaction. ^{1}H NMR (MeOD) δ 7.58 (d, 1H, J = 2.2 Hz, 3-H), 7.53 (d, 1H, J = 8.5 Hz, 6-H), 7.44 (dd, 1H, J = 2.2 Hz, 8.5 Hz, 5-H), 7.37 (d, 2H, J = 8.5 Hz, 3′, 5′-H_2), 7.21 (d, 2H, J = 8.5 Hz, 2′, 6′-H_2), 2.30 (s, 3H, CH_3).

When the reaction is run in more concentrated solution (10 ml ethanol per gram of diketone), the intermediate anti imine precipitates (60%) leaving the pyrazole ester (40%), derived from the syn-imine, in solution, thus enabling their easy separation. In situ processing of the mixture often requires further dilution with ethanol to maintain fluidity in the subsequent base-promoted step of the reaction.

5-(4-Chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxylic acid chloride

The unpurified acid **8** (43.7 g, 114 mmol) dissolved in dry toluene (2500 ml) was treated with $SOCl_2$ (16.7 ml, 229 mmol) under N_2 and heated at reflux for 3–6 h. Rotary evaporation in vacuo and high vacuum drying afforded 44.7 g (94%) of the acid chloride as a golden foam. The completeness of the reaction can be determined by the observation of SR141716, and the absence of the precursor acid **8**, after treatment of a high vacuum dried reaction aliquot residue with triethylamine and 1-aminopiperidine, as in the next step, and analyzing by TLC. HRMS: calculated for $C_{17}H_{10}N_2OCl_4$ 397.9547, found 397.9549.

N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, SR141716

The acid chloride (44.7 g, 107 mmol) dissolved in dry CH_2Cl_2 (2000 ml) was treated with 1-aminopiperidine (17.4 ml, 161 mmol) followed by triethylamine (46.0 ml, 321 mmol) under N_2 with stirring at ambient temperature. After 2 h, TLC (SiO_2, EtOAc:hex, 4:1, phosphomolybdic acid, Ce^{+4}) on a worked up aliquot showed the reaction to be complete.

The reaction solution was washed with water (3 ×), aqueous $NaHCO_3$ (3 ×), and 1 N HCl (3 ×) then dried over Na_2SO_4 and rotary evaporated to afford 52.4 g (105%) of a foam. The crude product was dissolved in EtOAc and diluted with three volumes of hexane. The resulting precipitate of the title compound (13.3 g) was filtered and the mother liquor was loaded on a silica gel column (2500 g) and eluted with 25% EtOAc–hexane (14 l) followed by 50% EtOAc–hexane (22 l). The product containing fractions were combined, rotary evaporated and the residue (36 g) recrystallized from in methanol/water. Filtration afforded 17.7 g (36%) of 98% pure (HPLC: Reverse Phase C-18 Waters Radial Nova Pak; 10 µml 8 mm × 10 cm, CH_3CN–H_2O, 75:25 UV, 280 nm) title compound. A second crop of 7.45 g (14%) of 95% pure SR141716 was similarly obtained from the mother liquor residue. The above 13.3 g of precipitate was recrystallized from methanol/water to afford 11.5 g (23%) of 98% pure SR141716. Analytically pure product was obtained from subsequent recrystallization from ether/hexane; m.p. 157–159°C. ^{1}H NMR (MeOD), δ 7.56 (d, 1H, J = 2.2 Hz, 3-H), 7.53 (d, 1H, J = 8.5 Hz, 6-H), 7.45 (dd, 1H, J = 2.3, 8.5 Hz, 5-H), 7.36 (d, 2H, J = 8.3 Hz, 3′,5′-H_2), 7.19 (d, 2H, J = 8.3 Hz, 2′,6′-H_2), 2.82 (br, 4H, CH_2-N-CH_2), 2.30 (s, 3H, CH_3), 1.73 (p, 4H, J = 5.7 Hz, 2 CH_2), 1.44 (br, 2H, CH_2). HRMS: calculated for $C_{22}H_{21}N_4OCl_3$ 462.0783, found 462.0781. CHNCl within 0.06%. NOESY (MeOD), interaction between 6-H and 2′,6′-H_2.

Recrystallization, from ether, of the mother liquor residue from the above second crop afforded 645 mg of the isomeric N-(piperidin-1-yl)-3-(4-chlorophenyl) - 1-(2, 4 - dichlorophenyl) -4-methyl-1H-pyrazole-5-carboxamide (**10**). ^{1}H NMR (MeOD), δ 7.61 (d, 2H, J = 8.5 Hz, 3′,5′-H_2), 7.51 (d, 1H, J = 2.2 Hz, 3-H), 7.48 (d, 1H, J = 8.5 Hz, 6H), 7.42 (d, 2H, J = 8.5 Hz, 2′,6′-H_2), 7.38 (dd, 1H, J = 2.2, 8.5 Hz, 5-H), 2.76 (br, 4H, CH_2-N-CH_2), 2.40 (s, 3H, CH_3), 1.70 (p, 4H, J = 5.5 Hz, 2CH_2), 1.61 (br, 2H, CH_2), MS, m/z; 462 (M^+), 344 (M-84-35 + 1), 99 (base).

*N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichloro-6-iodophenyl)-4-methyl-1H-pyrazole-3-carboxamide (**11**) and N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichloro-3-iodophenyl)-4-methyl-1H-pyrazole-3-carboxamide (**12**)*

N - (Piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3- carboxamide (SR141716) (300 mg, 0.65 mmol) and a few crystals of 1,10-phenanthroline indicator were dissolved in 12 ml dry

ether (distilled from Na/benzophenone) in a 50 ml flask fitted with a septum, a gas inlet and a Teflon stir bar under a dry argon atmosphere. The flask was cooled to −30°C and treated with n-BuLi (0.416 ml, 1.56 M, 0.65 mmol) with stirring until a dark red end-point. This was followed by a further addition of n-BuLi (1.25 ml, 1.95 mmol, 3 eq). After stirring for 2.5 h at −30°C, the reaction was cooled to −78°C and 1-chloro-2-iodoethane (0.94 ml, 10.4 mmol) was added. The reaction was stirred at −78°C for 0.5 h and −30°C to −18°C for 0.75 h after which the reaction was allowed to warm slowly over the next 0.5 h to ambient temperature. The opaque brown mixture was quenched with MeOH (10 ml) and the volatiles evaporated in vacuo. The residue was partitioned between water (50 ml) and ether (3 × 50 ml). The combined organics were dried (Na_2SO_4) and evaporated *in vacuo* to yield 0.41 g of a yellow resin which was chromatographed on a Merck size B prepacked silica gel column eluting with methyl t-butylether: toluene (1:9) to afford 64 mg (17%) of **11** and 53 mg (14%) of **12**. **11**: ^{1}H NMR (250 MHz, MeOD), δ 7.94 (d, 1H, J = 2.2 Hz, 5-H), 7.66 (d, 1H, J = 2.2 Hz, 3-H), 7.39 (d, 1H, J = 8.5 Hz, 3′,5′-H_2), 7.30 (d, 1H, J = 8.5 Hz, 2′,6′-H_2), 2.84 (br m, 4H, N–$(CH_2)_2$), 2.31 (s, 3H, CH_3), 1.73 (br p, 4H, J = 5.5 Hz, N–$(CH_2$-C$\underline{H}_2)_2$), 1.45 (br m, 2H, N-CH_2-CH_2-C$\underline{H}_2$); MS (DIP) (*m*/*z* 588 M^+, 84 base).

12: ^{1}H NMR (250 MHz, MeOD), δ 7.60 (d, 1H, J = 8.6 Hz, 6-H), 7.54 (d, 1H, J = 8.3 Hz, 5-H), 7.38 (d, 2H, J = 8.5 Hz, 3′,5′-H_2), 7.20 (d, 2H, J = 8.5 H,z, 2′,6′–H_2), 2.82 (t, 4H, J = 5.2 Hz, N–$(CH_2)_2$), 2.30 (s, 3H, CH_3), 1.73 (br p, 4H, J = 5.4 Hz, N-$(CH_2$-C$\underline{H}_2)_2$), 1.44 (br m, 2H, N-CH_2-CH_2-C$\underline{H}_2$).

N-(Piperidin-1-yl)-5-(4-chlorophenyl)-1-([6-^{3}H]-2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide, tritium-labelled SR141716

N-(Piperidin - 1-yl)-5 -(4-chlorophenyl)-1-(2,4-dichloro-6-iodophenyl)-4-methyl-1H-pyrazole-3-carboxamide (10 mg, 0.017 mmol) in 1 ml absolute EtOH was shaken with 10% (w/w) Pd/C (4 mg) and filtered, evaporated under a stream of nitrogen and high vacuum dried. The residue was redissolved in absolute EtOH (0.8 ml) and transferred to a 2 ml tritiation flask containing 10% (w/w) Pd/C (4 mg) and then treated with anhydrous Et_3N (from basic Al_2O_3) (7 µl, 0.0507 mmol) and stirred under carrier-free tritium gas (4.9 Ci) for 3.5 h. The catalyst was removed by filtration through celite and diluted with 25 ml absolute EtOH for temporary storage. Evaporation of EtOH (3 ×) to remove

exchangeable tritium afforded 317 mCi as a single component by TLC-radioscan (SiO_2, 10% MeOH/$CHCl_3$). The product was eluted from a Waters RP-C18 25 mm × 10 cm, 10 cm column with 75% CH_3CN/H_2O monitoring by UV at 280 nm. The radioactivity containing fractions were identified by non-eluted TLC-radioscan and analyzed by HPLC in the above system. The product was identified by HPLC retention time versus an unlabelled standard. Fractions were combined affording 139 mCi at a specific activity of 23 Ci/mmol determined by counting and UV determination of yield. ^{1}H NMR (MeOD), δ 7.56 (s, 3-H), 7.53 (d, J = 7.0 Hz, residual 6-H), 7.45 (d, J = 7.0 Hz, 5-H), 7.36 (d, J = 8.0 Hz, 3′,5′-H_2), 7.19 (d, J = 8.0 Hz, 2′,6′-H_2), 2.82 (br s, CH_2-N-CH_2), 2.33 (s, CH_3), 1.74 (br s, 2 CH_2), 1.47 (br, 2H, CH_2). ^{3}H NMR (MeOD) 7.51 (d, J = 7.4 Hz). Difference nOe (MeOD), interaction between 6-^{3}H and 5-H, and 6-^{3}H and 2′,6′-H_2.

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