Methoxy- and Fluorine-Substituted Analogs of O-1302: Synthesis and in Vitro Binding Affinity for the CB1 Cannabinoid Receptor

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Methoxy and fluorine analogs substituted on the terminal carbon of N-(piperidinyl)-1-(2,4-dichlorophenyl)-4-methyl-5-(4-pentylphenyl)-1H-pyrazole-3-carboxamide (O-1302) were synthesized in a multi-step process from 5-phenyl-1-pentanol, which was based on the 1,5-diarylpyrazole core template of N-(piperidinyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR141716) through condensation of the respective amine with pyrazole carboxylic acid, in order to develop tracers for medical imaging. Their potency for inhibiting the binding of the CB1 antagonist [3H]SR141716 was evaluated with the aim of developing positron emission tomography (PET) ligands for the cerebral cannabinoid CB1 receptor. These analogs bearing a piperidinyl carboxamide at the C3 of the pyrazole ring exhibited affinities comparable to those of the CB1 reference antagonist SR141716, which warrants further investigation using the radiolabeled form for biological imaging studies. A morpholine ring substituted at the C3 of the pyrazole ring resulted in a reduction of the CB1 affinity.

Key words: cannabinoid CB1 receptor; 1,5-diarylpyrazoles; O-1302; binding

Radiotracers, labeled with a short-lived positron emitter such as 11C or 18F, are being used increasingly as useful probes for diagnosis and for monitoring the course of therapeutic intervention using positron emission tomography (PET). In terms of creating new PET imaging tools, we have been interested in the progress of characterizing cerebral cannabinoid ligands and exploring the possibility of using them as tracers.

The cannabinoid CB1 receptor is the most abundant cannabinoid receptor subtype found in the central nervous system, and is responsible for inhibitory modulation of synaptic transmission by presynaptic actions on a range of transmitters. The brain CB1 receptor has been recognized as an interesting therapeutic target for the treatment of several psychotropic and neurodegenerative disorders, although the role of the CB1 receptor in the cause and treatment of these disorders is not fully understood.

Diarylpyrazoles are well known ligands for the central cannabinoid receptor, although cannabinoid ligands are currently classified into several different structural groups. The most widely studied N-(piperidinyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (SR141716), a highly potent and selective CB1 receptor antagonist/agonist, has served as a unique pharmacological and biochemical tool for the characterization of the CB1 receptor. Thus, various types of analogs of SR141716 have been extensively studied as candidate molecules to develop useful PET ligands for nuclear imaging. However, the PET imaging of the cerebral CB1 receptor in vivo is still problematic, due to high nonspecific binding and insufficient brain uptake of the radiotracers, although a very recent publication shows that [11C]H75528 appears to hold potential as a PET agent for imaging the CB1 receptor in human studies. Recently it has been reported that a SR141716 analog with p-pentylphenyl substituted at the C3 position of the pyrazole ring (O-1302) has high affinity (Kd=2.1 nm for CB1 receptor) with potent antagonistic properties (Fig. 1).

Results and Discussion

Currently, the general route to construct a pyrazole skeleton includes procedures based on the cyclocondensation of diketone ester enolates with appropriate hydrazine compounds. In the first stage of this approach, our attempts to perform Claisen condensation of 4'-pentylpropiophenone or 4'-chloropropiophenone as a model with diethyl oxalate in the presence of a strong base such as lithium bis(trimethylsilyl)amide for the preparation of the corresponding diketo ester were not successful. While our study was in progress, Alekseeva et al. reported the synthesis of 5-substituted pyrazole derivatives of O-1302, using a Suzuki-type coupling with an alkene. We pursued an alternative approach, according to a method reported by Dutta et al., in which...
the synthesis was carried out from 4′-(5-acetoxypentyl)-
propiophenone (2), as outlined in Chart 1, for the preparation
of the fluorine analog (8) of O-1302. This compound (2) was
prepared in good yield under Friedel–Crafts acylation with
AlCl₃ and propionyl chloride after being protected by acetyla-
tion of the hydroxy group of 5-phenyl-1-pentanol; the use of t-butyldimethylsilyle, t-butyldiphenylsilyle or me-
thoxyethylmethyl as the protecting group of the hydroxy
group gave either poor yield or was mostly unreacted under
conditions for Friedel–Crafts acylation. The synthesis of ace-


carboxylic acid (4) was achieved by the treatment of ethyl aceto-
cetate with the bromo ketone intermediate (3), prepared by bromination of 2. The sodium salt of 4 was allowed to react
with a solution of 2,4-dichlorobenzenediazonium chloride, followed by base hydrolysis to give the 5′-hydroxy pyrazole
carboxylic acid (5). Acid-amine coupling of 5 with 1-ami-
nopiperidine in the presence of 1-hydroxybenzotriazole
(HOBt), O-(1,2-di-hydro-2-oxo-1-pyridyl)-N,N,N',N'-tetra-
methyluronium tetrafluoroborate (TPTU), and diisopropyl-


toacetate (4) gave either poor yield or was mostly unreacted under
conditions for Friedel–Crafts acylation. The synthesis of ace-

toacetate intermediate provided access to usable quantities
of the target compounds and also is acceptable for further
preparation of experimental radiolabeled material, al-
though the overall yield is low and the long synthetic steps
required are undesirable.

The affinities of 8, 16 and 17 for binding to the CB1 recep-
tor site were determined by measuring the ability of each
ligand to compete with [³H]SR141716 binding in rat cerebel-
lar membrane preparations, and were expressed as Kᵢ values.
The results are summarized in Table 1, including the Kᵢ values of the reference pyrazole SR141716 and O-1302 for
comparison. In this study the Kᵢ value of SR141716, chosen
as a reference ligand, was found to be in agreement with pre-
vious literature values.²¹ The fluorine and methoxy analogs
(8, 16) of O-1302 were found to have nanomolar affinity (Kᵢ
value of 0.91 nM for 8; 0.70 nM for 16) for the sites labeled by
[³H]SR141716, comparable to that of SR141716 and lead
compound O-1302, thus confirming the suitability of our ini-
tial design of the molecule. The replacement of the piperi-
dine ring of 16 by the more polar morpholine ring resulted in
less than seven-fold affinity, in agreement with the findings
in other studies to the effect that the affinity is quite sensitive
to carboxamide group modifications.⁹,¹⁰,¹⁸

It is well understood that an optimally high lipophilicity (a
partition coefficient of logP of about 2 of a compound) is re-

Chart 1

Reagents: (a) Ac₂O, pyridine; (b) AlCl₃, CH₂CH₂COCl; (c) Br₂, AcOH for (4); (d) ethylene glycol, pyridinium
p-toluenesulfonate, benzene; (e) NaOH, MeOH; (f) CH₂CN; (g) MeOTf, 2.6-di-t-butyl-4-methylpyridine, DMF.
(h) NaH, ethyl acetoacetate, THF for (4); (i) HOBT, TPTU, DIPEA, CH₂Cl₂ for (6); (k) TsCl, pyridine; (l)
TBAF, THF; (m) MeOTf, 2,6-di-tert-butyl-4-methylpyridine, DMF.

Access to the target compound (17) was also envisaged by
use of O-methylation of the hydroxyl pyrazole carboxamide
(18). The O-methylation of 18, which was prepared by cou-
pling of 5 with 4-amino-morpholine, which occurred with
the use of MeOTf in the presence of 2,6-di-tert-butyl-4-
methylpyridine,²⁰ but resulting in isolation of the desired
compound (17) in only 16% yield.

The IR, ¹H-NMR and MS spectra for all intermediates and
final target compounds were consistent with the assigned
structures. Moreover, the purity of the final compounds was
checked by HPLC analysis. Thus, the synthetic route via the
acetoacetate intermediate provided access to useable quan-
tities of the target compounds and also is acceptable for fur-
ther preparation of experimental radiolabeled material, al-

Vol. 55, No. 8

1214
In summary, the present study describes the synthesis of the methoxy and fluorine analogs of O-1302 in a multi-step reaction, and their potency for inhibiting the binding of CB1 receptor antagonist [1H]SR141716 was also evaluated in vitro. These analogs displayed desirably high binding affinity at the CB1 receptor and one of these, the fluorine analog of O-1302 (8), appears to possess a somewhat lower lipophilicity value compared to that of 16, 17 and SR141716. Studies using positron-labeled analogs are proceeding to assess the suitability for the imaging of the CB1 receptor in vivo.

Experimental

In general, chemical reagents and solvents were of commercial quality and were used without further purification unless otherwise noted. SR141716 was prepared according to that described in previous literature. All melting points are uncorrected. 1H-NMR spectra were obtained on a JOEL GX-270 spectrometer (270 MHz) or Varian Inova 400 (400 MHz), and the chemical shifts are reported in parts per million downfield from tetramethylsilane. Infrared (IR) spectra were recorded with a Shimadzu FTIR-8400 spectrometer. Mass spectra were obtained with a JOEL JMS DX-300 (FAB-MS), or an Applied Biosystems Mariner System 5299 spectrometer (ESI-MS). Column chromatography was performed on Kieselgel 60 (70–230 mesh, Merck), the progress of the reaction was monitored by TLC on Silica gel 60F 254 plates (Merck), and spots were visualized with UV light. In the synthetic procedures, organic extracts were routinely dried over anhydrous Na2SO4, and evaporated with a rotary evaporator under reduced pressure. All reactions involving air- or moisture-sensitive compounds were performed under an argon atmosphere. HPLC was done using a HITACHI L-2000 series HPLC system fitted with a nacalai texes COSMOSIL 5 C18-AR-II (4.6×250 mm) with monitoring of UV absorption (at 254 nm).

Synthesis. (5-Acetoxyethyl)benzene (1) Acetic anhydride (2.25 ml, 24 mmol) was added dropwise to a stirred and ice-cold solution of 5-phenyl-1-pentanol (1.0 g, 6 mmol) in pyridine (50 ml) and stirring was continued at room temperature for 3 h. EtOAc (10 ml) was then slowly added to the mixture; the resulting solution was washed with water and brine, and then dried and evaporated in vacuo. The residue was purified by chromatography on a silica gel column (hexane : EtOAc = 10 : 1) to give 1 as a colorless oil (1.2 g, 97%). 1H-NMR (CDCl3) δ: 1.36—1.43 (2H, m), 1.62—1.65 (4H, m), 2.03 (3H, s), 2.62 (2H, t, J = 7.7 Hz), 4.05 (2H, t, J = 6.7 Hz), 7.16—7.19 (3H, m), 7.25—7.34 (2H, m); IR (KBr) cm⁻¹: 2935, 1739, FAB-MS (m/z): 207 (M+H)⁺.

(5-Acetoxyethyl)propio phenone (2) Anhydrous AcCl (3.0 g, 22 mmol) was added to a stirred and ice-cold solution of 5-acetoxyethylbenzene (1) (1.5 g, 7.3 mmol) and propionyl chloride (1.0 ml, 11 mmol). The mixture was allowed to stir at room temperature for 30 min under argon, poured into ice-cold water and then extracted with ether. The ether was successively washed with 1 M HCl, 1 M NaOH, brine, dried and evaporated in vacuo. The crude product was purified by silica gel chromatography (hexane: EtOAc = 10 : 1) to give 2 as a yellow oil (1.66 g, 88%). 1H-NMR (CDCl3) δ: 1.23 (2H, t, J = 7.3 Hz), 1.36—1.44 (4H, m), 1.82 (2H, t, J = 7.7 Hz), 2.68 (2H, t, J = 7.1 Hz), 2.93 (2H, q, J = 7.1 Hz), 4.05 (2H, t, J = 6.7 Hz), 7.25 (2H, d, J = 8.6 Hz), 7.88 (2H, d, J = 8.4 Hz); IR (KBr) cm⁻¹: 2937, 1739, 1683, 1608, FAB-MS (m/z): 263 (M+H)⁺.

4’-(5-Acetoxyethyl)-2’-bromobiphenylone (3) Under argon, to a stirred solution of 2 (140 mg, 0.53 mmol) in glacial acetic acid (0.5 ml) was added dropwise bromine (42 µl, 0.79 mmol) at 0 °C under a stirring. The mixture was kept at room temperature for 3 h with stirring, was then diluted by the addition of water, and extracted with ether. The ether was washed with water, saturated aqueous Na2CO3 and brine, dried and evaporated in vacuo. The residue was purified by chromatography on a silica gel column (hexane : EtOAc = 10 : 1) to give 3 as a yellow oil (127 mg, 70%). 1H-NMR (CDCl3) δ: 1.30—1.37 (3H, m), 1.52—1.65 (4H, m), 1.82 (2H, d, J = 6.7 Hz), 1.95 (3H, m), 2.60—2.62 (2H, m), 3.94—4.00 (2H, m), 5.20 (1H, q, J = 6.7 Hz), 7.21 (2H, d, J = 8.2 Hz), 7.88 (2H, d, J = 8.2 Hz); IR (KBr) cm⁻¹: 2935, 1735, 1606; FAB-MS (m/z): 341 (M+H)⁺.

Ethyl [2-Acryl-4’-(5-acetoxyethyl)phenyl]-3-methyl-4-oxo-butyrate (4) To a suspension of 60% NaH oil (470 mg, 11.7 mmol) in dry THF (40 ml) was added dropwise ethyl acetoacetate (1.1 ml, 11 mmol) at -78 °C under argon. After stirring at room temperature for 0.5 h, bromoketone (3) (2.38 g, 6.97 mmol) was added dropwise. The mixture was kept at room temperature for 30 min and then refluxed for 2 h. After cooling, excess NaH was decomposed by the addition of water and the THF was removed in vacuo. The residue was extracted with ether. The ether was washed with water and brine, dried and evaporated in vacuo. The residue was purified by column chromatography on silica gel (hexane : EtOAc = 10 : 1) to give 4 as a yellow oil (0.999 g, 36%). 1H-NMR (CDCl3) δ: 1.13—1.19 (4H, m), 1.32 (2H, t, J = 7.0 Hz), 1.38—1.46 (2H, m), 1.62—1.69 (6H, m), 2.04 (3H, s), 2.29 (2H, s), 2.39 (1H, s), 2.68 (2H, t, J = 7.6 Hz), 4.14—4.28 (4H, m), 7.27 (2H, d, J = 8.6 Hz), 7.90 (2H, d, J = 8.0 Hz); IR (KBr) cm⁻¹: 2935, 1735, 1605; FAB-MS (m/z): 420 (M⁺).

1-(2,4-Dichlorophenyl)-5-[4’-(5-hydroxyethyl)phenyl]-4-methyl-1H-pyrazole-3-carboxylic Acid (5) To a solution of sodium ethoxide (1.5 ml, 21 wt % in absolute ethanol) in absolute ethanol (3.5 ml) was added a solution of 4 (990 mg, 2.53 mmol) in absolute ethanol (3 ml). After stirring for 30 min at room temperature under argon, the mixture was cooled in an ice bath. To this solution with stirring was added a solution of 2,4-dichlorobenzenediazonium chloride prepared from 2,4-dichloroaniline (820 mg, 5.07 mmol) in 35% HCl (1.3 ml) and diazotized at 0 °C with the slow addition (0.5 h) of a solution of NaNO2 (350 mg, 5.07 mmol) in water (20 ml). After stirring under argon for 5 h at 0 °C, the reaction mixture was diluted with water (20 ml) and was allowed to stand in the refrigerator (4 °C) for 16 h. The ethanol was evaporated in vacuo and the residue was redissolved in EtOAc, which was washed with water and brine. The EtOAc was removed in vacuo and the residue was redissolved in ethanol (20 ml), followed by the addition of a solution of NaOH (338 mg, 8.42 mmol) in water (1.5 ml). The
mixture was refluxed for 9 h. The EtOH was evaporated in vacuo after further addition of water. The residue was again washed with brine, dried and evaporated in vacuo. The crude product was purified by silica gel chromatography (hexane:EtOAc= 2:1) as the eluent to give 5 (369 mg, 34%) as an orange solid, mp 95—97°C. 1H-NMR (CDCl3) δ: 1.39—1.45 (2H, m), 1.59—1.66 (4H, m), 2.35 (3H, s), 2.60—2.67 (2H, m), 3.60—3.67 (2H, m), 7.06 (2H, d, J = 8.0 Hz), 7.11 (2H, d, J = 8.0 Hz). (7H, s), 7.38—7.42 (1H, m), 7.48—7.52 (1H, m); IR (KBr) cm⁻¹: 1705; ESI-MS (m/z): 433 M⁺.

N-(Piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-[4'- (5-hydroxy-pentyl)phenyl]-4-methyl-1H-pyrazole-3-carboxamide (7) as a yellow solid, mp 97—99°C. 1H-NMR (CDCl3) δ: 1.24—1.43 (2H, m), 1.53—1.68 (6H, m), 1.73—1.76 (4H, m), 2.36 (3H, s), 2.43 (3H, s), 2.53 (2H, t, J = 7.7 Hz), 3.40 (1H, t, J = 6.3 Hz), 7.10 (2H, d, J = 8.0 Hz); IR (KBr) cm⁻¹: 1720; FAB-MS (m/z): 476 (M + H)⁻.

N-(Piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-[4'- (5-fluorooxy-pentyl)phenyl]-4-methyl-1H-pyrazole-3-carboxamide (8) as a yellow solid, mp 80—81°C. 1H-NMR (CDCl3) δ: 1.24—1.43 (2H, m), 1.53—1.68 (6H, m), 1.73—1.76 (4H, m), 2.36 (3H, s), 2.43 (3H, s), 2.53 (2H, t, J = 7.7 Hz), 4.01 (2H, t, J = 6.3 Hz), 7.00 (2H, d, J = 8.0 Hz), 7.06 (2H, d, J = 8.0 Hz), 7.25 (2H, d, J = 8.0 Hz); IR (KBr) cm⁻¹: 1720; ESI-MS (m/z): 476 (M + H)⁻.

4'-(5-Hydroxy-pentyl)phenyl-4-methyl-1H-pyrazole-3-carboxamide (11) (21.7 g, 82.0 mmol) in dry DMF (18 ml) was added NaH (1.64 g, 41.0 mmol) at room temperature. After cooling to 0°C, methyl iodide (2.55 ml, 40.9 mmol) was added to the mixture with stirring. The solution was kept at 0°C for 1 h, poured onto ice and extracted with ether. The ether was washed with brine, dried and evaporated to dryness. The crude product was purified by silica gel chromatography using hexane:EtOAc= 5:1 as the eluent to give the 2,2-dimethylbutyl (12) as a pale yellow oil (1.99 g, 87%). 1H-NMR (CDCl3) δ: 0.87 (3H, t, J = 7.5 Hz), 1.40 (2H, m), 1.59—1.64 (4H, m), 1.90 (2H, q, J = 6.8 Hz), 2.30—2.35 (2H, m), 2.70 (2H, d, J = 8.0 Hz), 3.30 (2H, t, J = 7.3 Hz), 3.70 (2H, t, J = 4.8 Hz); IR (KBr) cm⁻¹: 1710; ESI-MS (m/z): 235 M⁺.

4'-(5-Hydroxy-pentyl)phenyl-4-methyl-1H-pyrazole-3-carboxamide (17) (0.98 g, 7.11 mmol) in methanol (13 ml) was added hydrochloric acid (3 ml; 5.0 ml) at room temperature with stirring. The mixture was kept at room temperature for 4 h, then diluted with water and the methanol was removed in vacuo. The residue was extracted with ether and the ether was washed with brine, dried and evaporated to dryness. The crude product was purified by silica gel chromatography using hexane:EtOAc= 5:1 as the eluent to give the 2,2-dimethylbutyl (12) as a pale yellow oil (1.94 g, 87%) as colorless oil. 1H-NMR (CDCl3) δ: 1.19 (3H, t, J = 7.1 Hz), 1.73 (2H, m), 1.54—1.61 (4H, m), 2.65 (2H, t, J = 7.7 Hz), 3.00 (3H, s), 3.34 (2H, t, J = 6.3 Hz), 7.30 (2H, d, J = 6.7 Hz), 7.86 (2H, d, J = 8.0 Hz); IR (KBr) cm⁻¹: 1670; FAB-MS (m/z): 235 (M⁺).

4'-(5-Hydroxy-pentyl)phenyl-4-methyl-1H-pyrazole-3-carboxamide (17) (0.98 g, 7.11 mmol) in methanol (13 ml) was added hydrochloric acid (3 ml; 5.0 ml) at room temperature with stirring. The mixture was kept at room temperature for 4 h, then diluted with water and the methanol was removed in vacuo. The residue was extracted with ether and the ether was washed with brine, dried and evaporated to dryness. The crude product was purified by silica gel chromatography using hexane:EtOAc= 5:1 as the eluent to give the 2,2-dimethylbutyl (12) as a pale yellow oil (1.94 g, 87%) as colorless oil. 1H-NMR (CDCl3) δ: 1.19 (3H, t, J = 7.1 Hz), 1.73 (2H, m), 1.54—1.61 (4H, m), 2.65 (2H, t, J = 7.7 Hz), 3.00 (3H, s), 3.34 (2H, t, J = 6.3 Hz), 7.30 (2H, d, J = 6.7 Hz), 7.86 (2H, d, J = 8.0 Hz); IR (KBr) cm⁻¹: 1670; FAB-MS (m/z): 235 (M⁺).

1H-NMR (CDCl3) δ: 1.19 (3H, t, J = 7.1 Hz), 1.73 (2H, m), 1.54—1.61 (4H, m), 2.65 (2H, t, J = 7.7 Hz), 3.00 (3H, s), 3.34 (2H, t, J = 6.3 Hz), 7.30 (2H, d, J = 6.7 Hz), 7.86 (2H, d, J = 8.0 Hz); IR (KBr) cm⁻¹: 1670; FAB-MS (m/z): 363 (M⁺).

N-(Piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4'-(5-fluorooxy-pentyl)phenyl)-4-methyl-1H-pyrazole-3-carboxamide (16) under argon, to a solution of hydroxyl acetel (11) (2.17 g, 8.20 mmol) in dry DMF (18 ml) was added NaH (1.64 g, 41.0 mmol) at room temperature. After cooling to 0°C, methyl iodide (2.55 ml, 40.9 mmol) was added to the mixture with stirring. The solution was kept at 0°C for 1 h, poured onto ice and extracted with ether. The ether was washed with brine, dried and evaporated to dryness. The crude product was purified by silica gel chromatography using hexane:EtOAc= 5:1 as the eluent to give the 2,2-dimethylbutyl (12) as a pale yellow oil (1.94 g, 87%) as colorless oil. 1H-NMR (CDCl3) δ: 1.19 (3H, t, J = 7.1 Hz), 1.73 (2H, m), 1.54—1.61 (4H, m), 2.65 (2H, t, J = 7.7 Hz), 3.00 (3H, s), 3.34 (2H, t, J = 6.3 Hz), 7.30 (2H, d, J = 6.7 Hz), 7.86 (2H, d, J = 8.0 Hz); IR (KBr) cm⁻¹: 1670; ESI-HR-MS (m/z): 529.2162.}

CaCl₂,H₂F₂,Cl₂O₄ (M⁺): 517.1937.

C₁₁H₁₂F₂N₂O₂ (M⁺): 253.1139.

C₁₁H₁₂F₂N₂O₂ (M⁺): 253.1139.

C₁₁H₁₂F₂N₂O₂ (M⁺): 253.1139.

CaCl₂,H₂F₂,Cl₂O₄ (M⁺): 517.1937.

C₁₁H₁₂F₂N₂O₂ (M⁺): 253.1139.
the procedure described for 6 and was isolated as a pale yellow gum in 67% yield. 1H-NMR (DMSO-d6): δ: 1.22—1.27 (2H, m), 1.45—1.55 (4H, m), 2.20 (3H, s), 2.53 (2H, t, J=7.8 Hz), 2.83 (4H, t, J=4.8 Hz), 3.18 (3H, s), 3.26 (2H, t, J=6.5 Hz), 3.63 (4H, t, J=5.1 Hz), 7.08 (2H, d, J=8.0 Hz), 7.17 (2H, d, J=8.0 Hz), 7.54 (1H, dd, J=2.2, 8.4 Hz), 7.69—7.73 (2H, m), 9.25 (1H, s); IR (KBr) cm⁻¹: 1683; ES-HR-MS (m/z): 531.1933. Calcd for C27H33Cl2N2O3 (M+H): 531.1924.

(b) N-(Morpholin-1-yl)-l-1-(2-A-dichlorophenyl)-5-[(5-hydroxypentyl)phenyl]-4-methyl-1H-pyrazole-3-carboxamide (18) was prepared from 5 (71 mg, 163 μmol), 4-ammonomorpholine (20 μl, 207 μmol), 1-hydroxybenzotriazole (43 mg, 318 μmol), and N,N-diisopropylamine (84 μl, 964 μmol) according to the procedure described for 6 and was isolated as an orange gum in 57% yield. 1H-NMR (CDCl3) δ: 1.37—1.42 (2H, m), 1.55—1.65 (4H, m), 2.37 (3H, s), 2.59 (2H, t, J=8.2 Hz), 2.96 (3H, s), 3.64 (2H, t, J=6.7 Hz), 3.86 (4H, m), 7.01 (2H, d, J=8.0 Hz), 7.11 (2H, d, J=7.8 Hz), 7.26 (2H, br), 7.43 (1H, d, J=1.8 Hz), 7.73 (1H, br); IR (KBr) cm⁻¹: 3446; FAB-MS (m/z): 517 (M+H)+. This material was used for the O-methylation.

To a solution of 18 (11 mg, 20 μmol) in dry DMF (500 μl) was added 2,6-di-tert-butyl-4-methylpyridine (15 μl, 110 μmol) in one portion. The solution was cooled to 0°C, followed by the addition of methyl triflate (3 μl, 27 μmol). After being stirred at room temperature for 15 h, the mixture was poured into a saturated NaHCO3 solution and extracted with EtOAc. The extracts were washed with brine, dried and evaporated to dryness, and chromatographed on silica gel. Elution with hexane : EtOAc (1:1—2:1) gave 17 (2 mg, 17%) as a pale yellow gum, which was identical to the sample obtained from 15.

Radioligand Binding Assay The preparations for the rat cerebellar membranes and the binding assay were carried out according to the reported procedures25 but with slight modifications. Male Sprague-Dawley rats (200—300 g; Kyudo Co., Ltd., Japan) were sacrificed by decapitation and their cerebella were rapidly removed. The tissues were subjected to homogenization (1 : 100 w/v) in ice-cold buffer A (20 mM Hepes, 1 mM MgCl2, pH 7.4). After a 20 min at 4°C. The pellets were resuspended (1 : 120 w/v) in buffer A and stored at −80°C until use. Protein determination was performed by means of the Bradford protein assay using BSA as the standard.26

The binding of [1H]SR141716 (1.63 Tg/mol, Amershams Biociences, U.S.A.) to rat cerebellar membranes was carried out in a final volume of 500 μl of buffer B (20 mM Hepes–NaOH buffer, 1 mM MgCl2, pH 7.4). Stock solutions of the tested compounds were dissolved in DMSO or EtOH and the concentration of DMSO or EtOH in the different assays never exceeded 1% (v/v). Dilutions of the tested compounds and [1H]SR141716 were made in buffer C consisting of 20 mM Hepes–NaOH, 1 mM MgCl2, and 50 mg/ml of fatty acid free BSA. A membrane suspension (50 μl; 15 μg protein) was added to an incubation medium for 90 min at 22°C with [1H]SR141716 (1 μM) and with or without the unlabelled ligand. The reaction was terminated by vacuum filtration through Whatman GF/B filters pretreated with ice-cold buffer D (20 mM Hepes–NaOH, 1 mM MgCl2, BSA 0.5 mg/ml, pH 7.4). The reaction tubes were rinsed five times with 1 ml of aliquots of ice-cold buffer D, and the filters were also washed five times with 5 ml aliquots of the same buffer D. The filter-bound radioactivity was measured using a liquid scintillation counter (LCS-3500, Aloka, Japan) with 10 ml of scintillation fluid (ACSIII, Amershams Biocience, U.S.A.). All assays were performed in triplicate. The IC50 values were obtained from the concentration–response curves. Non-specific binding was estimated in the presence of 1 μM SR141716. The Kd values were calculated by the Cheng–Prusoff equation26 and the Kc values were determined in direct binding assays with [1H]SR141716.

Partition Coefficients The logP value was measured using the standard shake flask method. The sample was shaken well with a mixture of a 1:octanol (2.5 ml) and 0.05μ phosphate buffer (2.5 ml, pH 7.4) for 20 min at 25°C, after which aliquots of both phases were taken for analysis by HPLC quantitation. The reported logP value represents the mean of three experiments.

References
20) Howlett A. C., Barth F., Bonner T. I., Cabral G., Casellas P., Devane W ., Felder C. C., Herkenham M., Mackie K., Martin B. R., Me-