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

Shintaro Tobiishi,[a] Toru Sasada,[a] Yumiko Nojiri,[a] Fumihiko Yamamoto,[a] Takahiro Mukai,[a] Kiichi Ishiwata,[b] and Minoru Maeda*,[a]

[a] *Graduate School of Pharmaceutical Sciences, Kyushu University; 3–1–1 Maidashi, Higashi-ku, Fukuoka 812–8582, Japan: and* [b] *Positron Medical Center, Tokyo Metropolitan Institute of Gerontology; 1–1 Naka-cho, Itabashi-ku, Tokyo 173–0022, Japan.* Received April 11, 2007; accepted May 17, 2007; published online May 21, 2007

Methoxy and fluorine analogs substituted on the terminal carbon of the pentyl chain of *N*-(piperidinyl)-1-(2,4-dichlorophenyl)-4-methyl-5-(4-pentylphenyl)-1*H*-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-1*H*-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 [^{3}H]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 C_3 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 C_3 of the pyrazole ring resulted in a reduction of the CB1 affinity.

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

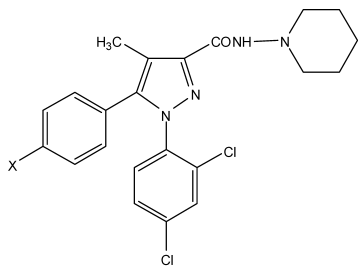
Radiotracers, labeled with a short-lived positron emitter such as ^{11}C or ^{18}F, are being used increasingly as useful probes for diagnosis and for monitoring the course of therapeutic intervention using positron emission tomography (PET).[1] In terms of creating new PET imaging tools, we have been 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,[2,3] 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.[2,3] The most widely studied *N*-(piperidinyl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-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.[4—7] Thus, various types of analogs of SR141716 have been extensively studied as candidate molecules to develop useful PET ligands for nuclear imaging.[8—13] 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 [^{11}C]JHU75528 appears to hold potential as a PET agent for imaging the CB1 receptor in human studies.[14] Recently it has been reported that a SR141716 analog with *p*-pentylphenyl substituted at the C_5 position of the pyrazole ring (O-1302) has high affinity (K_i=2.1 nM for CB1 receptor) with potent antagonistic properties (Fig. 1).[15,16] It appeared that O-1302 may serve as a useful lead structure for the development of high affinity CB1 imaging agents. We have designed and developed a synthetic route to O-1302 analogs with methoxy and fluorine substituents on the terminal carbon of the pentyl chain, for radiolabeling with ^{11}C or ^{18}F of the O-1302 molecule. The *in vitro* binding affinities of these O-1302 derivatives for the CB1 receptor and lipophilicity are also evaluated in this study.

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.[17,18] 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.[16] We pursued an alternative approach, according to a method reported by Dutta *et al.*,[19] in which



X = Cl: SR141716
X = $(CH_2)_4CH_3$: O-1302

Fig. 1. Structure of SR141716 and O-1302, CB1 Cannabinoid Receptor Antagonist

* To whom correspondence should be addressed. e-mail: maeda@phar.kyushu-u.ac.jp

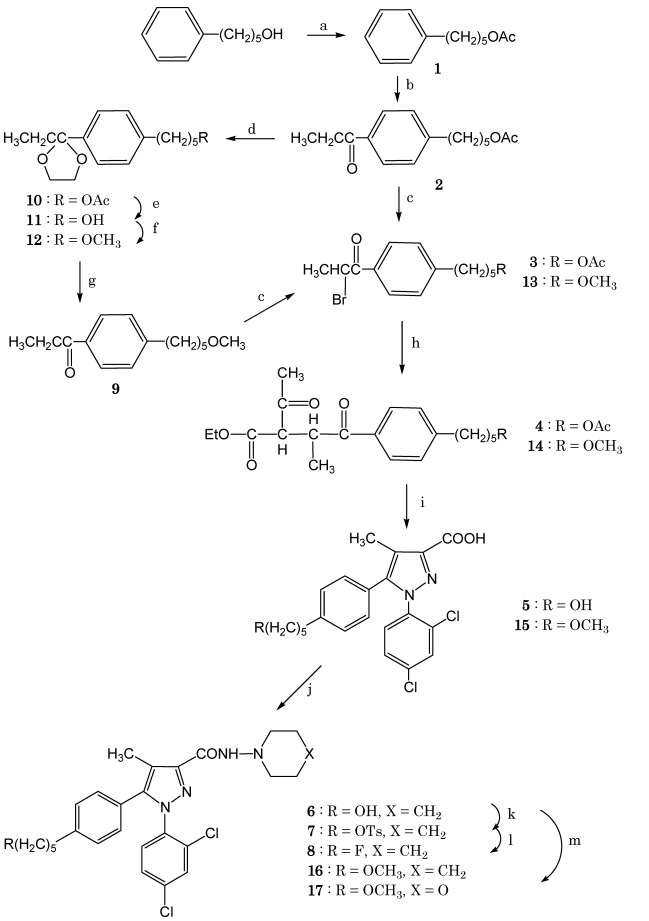
the synthesis was carried out from 4′-(5-acetoxypentyl)-propiophenone (**2**), as outlined in Chart 1, for the preparation of the fluorine analog (**8**) of O-1302. This compound (**2**) was prepared in good yield under Friedel–Crafts acylation with $AlCl_3$ and propionyl chloride after being protected by acetylation of the hydroxy group of 5-phenyl-1-pentanol; the use of *t*-butyldimethylsilyl, *t*-butyldiphenylsilyl or methoxymethyl group 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 acetoacetate (**4**) was achieved by the treatment of ethyl acetoacetate 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-aminopiperidine in the presence of 1-hydroxybenzotriazole (HOBT), *O*-(1,2-dihydro-2-oxo-1-pyridyl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TPTU), and diisopropylethylamine in CH_3CN gave the desired carboxamide (**6**) in 73% yield. The primary alcohol of **6** was tosylated with toluene-*p*-sulfonyl chloride in pyridine and subsequent treatment of the tosyl derivative (**7**) with tetrabutylammonium fluoride in THF afforded the target fluorine-substituted analog (**8**) of O-1302 in 59% yield.

The methoxy-substituted analog bearing an *N*-piperidinyl ring (**16**) or *N*-morpholine ring (**17**) was accessible by a similar reaction sequence described for the fluorine analog (**8**) of O-1302, starting from acetyl pentylpropiophenone (**2**). The acetyl group of **2** was converted to the corresponding *O*-methoxy compound (**9**) *via* the four-step procedure illustrated in Chart 1. This procedure involved ketalization of **2** with ethylene glycol, followed by basic hydrolysis and *O*-methylation with CH_3I, and deprotection of the 1,3-dioxolane group to give the methoxy-propiophenone (**9**). The methoxy-substituted propiophenone (**9**) was then transformed into the corresponding pyrazole carboxylic acid (**15**) *via* the bromoketone (**13**) and acetoacetate intermediate (**14**), analogously to the procedure described above.

The pyrazole carboxylic acid (**15**) was then converted to the acid chloride intermediate by use of oxalyl chloride, followed by coupling with 1-aminopiperidine to give the target *N*-piperidinylamide (**16**) in a reasonable yield. On the other hand, the synthesis of the *N*-morpholinylamide (**17**) was achieved by treatment of the carboxylic acid (**15**) with 4-aminomorpholine in the presence of HOBT, TPTU, and diisopropylethylamine.

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 coupling 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, ^{1}H-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 quantities of the target compounds and also is acceptable for further preparation of experimental radiolabeled material, al-



Reagents: (a) Ac_2O, pyridine; (b) $AlCl_3$, CH_2CH_2COCl; (c) Br_2, AcOH for (**3**) and (**13**); (d) ethylene glycol, pyridinium *p*-toluensulfonate, benzene; (e) NaOH, MeOH; (f) CH_3I, NaH, DMF; (g) HCl, MeOH; (h) NaH, ethyl acetoacetate, THF for (**4**) and (**14**); (i) (1) NaOEt, EtOH, (2) 2,4-dichlorobenzenediazonium chloride, (3) NaOH, EtOH for (**5**) and (**15**); (j) (1) 1-aminopiperidine, HOBT, TPTU, DIPEA, CH_3CN for (**6**), (2) oxalyl chloride, DMF, CH_2Cl_2, Et_3N, 1-aminopiperidine, CH_2Cl_2 for (**16**), (3) HOBT, TPTU, DIPEA, 4-aminomorpholine, CH_3CN for (**17**), and (**18**); (k) TsCl, pyridine; (l) TBAF, THF; (m) MeOTf, 2.6-di-*tert*-butyl-4-methylpyridine, DMF.

Chart 1

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 receptor site were determined by measuring the ability of each ligand to compete with [^{3}H]SR141716 binding in rat cerebellar membrane preparations, and were expressed as K_i values. The results are summarized in Table 1, including the K_i values of the reference pyrazole SR141716 and O-1302 for comparison. In this study the K_i value of SR141716, chosen as a reference ligand, was found to be in agreement with previous literature values.[21] The fluorine and methoxy analogs (**8**, **16**) of O-1302 were found to have nanomolar affinity (K_i value of 0.91 nM for **8**; 0.70 nM for **16**) for the sites labeled by [^{3}H]SR141716, comparable to that of SR141716 and lead compound O-1302, thus confirming the suitability of our initial design of the molecule. The replacement of the piperidine 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-

Table 1. Inhibition of [^{3}H]SR141716 Binding to Rat Cerebellar Membranes and Lipophilicity

Ligand	K_i (nM)[a]	logP (measured)[c]
8	0.91±0.24	2.9
16	0.70±0.26	4.5
17	4.60±2.16	3.6
SR141716	0.57±0.14	3.8
O-1302	2[b]	—

a) The binding assay was performed with [^{3}H]SR141716 and the results are expressed as $K_i=IC_{50}/(1+L/K_d)$. The data correspond to mean values±S.E.M obtained from at least three independent experiments. *b*) Taken from ref. 15. *c*) Lipophilicity (logP) was determined in an octanol/phosphate buffer (pH=7.4) system using the conventional flask-shake technique. The results were presented as mean values ($n=3$) with a maximum range of ±5%.

quired for good blood–brain barrier permeability,[22] although there are some exceptions. Currently, there is no sufficiently general understanding of the relationship between lipophilicity and *in vivo* imaging characteristics for radioligands investigated for neuroimaging of the CB1 receptor. Katoch-Rouse *et al.* found a correlation between the CB1 receptor binding affinities and the calculated lipophilicity values for 1,5-diaryl ligand compounds, and lipophilicity is thought to be an important factor for explaining the inhibitory potency towards the receptor, presumably due to the hydrophobic nature of the binding site region.[23] However, factors other than lipophilicity have also been reported to be key determinants of brain uptake of this class of compounds.[12] The lipophilic properties were determined experimentally as shown in Table 1. The obtained logP values of compounds **8**, **16** and **17** seem to be above the optimal range of lipophilicity for imaging agents targeted at the central nervous system, in which **8** displayed somewhat lower lipophilicity compared to three other compounds. Nevertheless, it is suggested that the high binding affinity and high density of the CB1 receptor in the mammalian brain[24] warrant further investigation *in vivo* using positron-labeled forms of **8** and **16**.

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 [^{3}H]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.[19] All melting points are uncorrected. ^{1}H-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 Na_2SO_4 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 tesque COSMOSIL 5 C18-AR-II (4.6×250 mm) with monitoring of UV absorption (at 254 nm).

Synthesis. (5-Acetoxypentyl)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%). ^{1}H-NMR ($CDCl_3$) 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 $(M+H)^+$.

4′-(5-Acetoxypentyl)propiophenone (2) Anhydrous $AlCl_3$ (3.0 g, 22 mmol) was added with stirring to an ice-cold solution of 5-(acetoxypentyl)benzene (**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 with silica gel chromatography (hexane : EtOAc=10 : 1) to give **2** as a light yellow oil (1.66 g, 88 %). ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, t, J=7.3 Hz), 1.36—1.44 (2H, m), 1.63—1.70 (4H, m), 2.04 (3H, s), 2.68 (2H, t, J=7.6 Hz), 2.98 (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-Acetoxypentyl)-α-bromopropiophenone (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 using a syringe. 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 Na_2CO_3 and brine, dried and evaporated *in vacuo*. The residue was purified by chromatography on silica gel (hexane : EtOAc=15 : 1) to give **3** as a light yellow oil (127 mg, 70 %). ^{1}H-NMR ($CDCl_3$) 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—2.64 (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 $(M+H)^+$.

4′-(5-Acetoxypentyl)-α,α'-dibromopropiophenone was also obtained as a side product: a colorless oily material (55 mg, 24%). ^{1}H-NMR ($CDCl_3$) δ: 1.31—1.37 (2H, m), 1.58—1.64 (4H, m), 1.96 (3H, s), 2.60—2.68 (5H, m), 3.97—4.00 (2H, m), 7.20 (2H, d, J=8.21 Hz), 8.26 (2H, d, J=8.2 Hz); IR (KBr) cm^{-1}: 2935, 1735, 1605; FAB-MS (*m*/*z*): 420 $(M)^+$.

Ethyl [2-Acetyl-4-{4′-(5-acetoxypentyl)phenyl}-3-methyl-4-oxo]butyrate (4) To a suspension of 60% NaH oil (470 mg, 11.7 mmol) in dry THF (10 ml) was added dropwise ethyl acetoacetate (1.1 ml, 8.6 mmol) with stirring 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 silica gel chromatography using hexane : EtOAc=5 : 1 as the eluent to give **4** as a yellow oil (0.998 g, 36%). ^{1}H-NMR ($CDCl_3$) 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}: 2937, 1739, 1716, 1680; FAB-MS (*m*/*z*): 391 $(M+H)^+$.

1-(2,4-Dichlorophenyl)-5-{4′-(5-hydroxypentyl)phenyl}-4-methyl-1*H*-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 $NaNO_2$ (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 extracted with EtOAc. The EtOAc was washed with brine, dried and evaporated *in vacuo*. The crude product was purified by silica gel chromatography (hexane : EtOAc = 2 : 1 + 2% acetic acid → hexane : EtOAc = 1 : 1 + 2% acetic acid) to give **5** (369 mg, 34%) as an orange solid, mp 95—97 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.39—1.45 (2H, m), 1.59—1.66 (4H, m), 2.35 (3H, s), 2.60—2.67 (2H, m), 3.62—3.67 (2H, m), 7.06 (2H, d, J=8.0 Hz), 7.11 (2H, d, J=8.0 Hz), 7.27 (1H, 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-hydroxypentyl)-phenyl}-4-methyl-1*H*-pyrazole-3-carboxamide (6)** *N*,*N*-Diisopropylethylamine (124 μl, 694 μmol) was added to a suspension of **5** (105 mg, 242 μmol), 1-aminopiperidine (34 μl, 314 μmol), 1-hydroxybenzotriazole (HOBT) (63 mg, 465 μmol), and *O*-(1,2-dihydro-2-oxo-1-pyridyl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TPTU) (94 mg, 316 μmol) in dry CH_3CN (3 ml). The reaction mixture was stirred at room temperature for 74 h under argon and then evaporated to dryness. The residue was partitioned between EtOAc and 0.2 M aqueous $NaHSO_3$. The organic layer was washed with saturated aqueous Na_2CO_3 and brine, dried and evaporated to dryness. The crude product was purified by silica gel chromatography using hexane : EtOAc = 1 : 1 + 2% acetic acid as the eluent to give **6** (94 mg, 73%) as a yellow solid, mp 93—95 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.40—1.43 (2H, m), 1.57—1.65 (6H, m), 1.72—1.77 (4H, m), 2.37 (3H, s), 2.59 (2H, t, J=7.7 Hz), 2.86—2.88 (4H, m), 3.62—3.65 (2H, m), 7.01 (2H, d, J=8.0 Hz), 7.11 (2H, d, J=8.0 Hz), 7.25 (1H, s), 7.41 (1H, s), 7.63 (1H, s); IR (KBr) cm^{-1}: 1674; FAB-MS (*m*/*z*): 515 $(M+H)^+$.

***N*-(Piperidin-1-yl)-1-(2,4-dichlorophenyl)-4-methyl-5-{4′-(5-*p*-toluenesulfonyloxypentyl)phenyl}-4-methyl-1*H*-pyrazole-3-carboxamide (7)** *p*-Toluenesulfoyl chloride (300 mg, 1.56 mmol) was added to a solution of **6** (200 mg, 0.38 mmol) in dry pyridine (4 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, and then a solution of saturated aqueous Na_2CO_3 was added. The resulting solution was extracted with EtOAc. The separated organic layers were washed with brine, dried and evaporated *in vacuo*. The residue was purified by silica gel chromatography using hexane : EtOAc = 2 : 1 → hexane : EtOAc = 1 : 1 → hexane : EtOAc = 1 : 2) as the eluent to give **7** as a light yellow solid (145 mg, 57%), mp 88 °C. ^{1}H-NMR ($CDCl_3$) 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=8.2 Hz), 7.06 (2H, d, J=8.2 Hz), 7.25 (1H, s), 7.32 (2H, d, J=8.0 Hz), 7.41 (1H, s), 7.64 (1H, s), 7.77 (2H, d, J=8.0 Hz); IR (KBr) cm^{-1}: 1683; FAB-MS (*m*/*z*): 669 $(M)^+$.

***N*-(Piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-{4′-(5-fluoropentyl)-phenyl}-4-methyl-1*H*-pyrazole-3-carboxamide (8)** Under argon, **7** (46 mg, 75.4 mmol) was added dropwise to solution of *n*-Bu_4NF (400 μl; 1 M solution in THF). The mixture was heated under reflux for 15 min and evaporated to dryness. The residue was purified by chromatography on silica gel (hexane : EtOAc = 1 : 2) to give **8** (23 mg, 59%) as a yellow gum. ^{1}H-NMR ($CDCl_3$) δ: 1.36—1.42 (2H, m), 1.55—1.68 (6H, m), 1.70—1.78 (4H, m), 2.37 (3H, s), 2.59 (2H, t, J=7.7 Hz), 2.87 (4H, m), 4.43 (2H, dt, J=47, 5.9 Hz), 7.01 (2H, d, J=8.2 Hz), 7.10 (2H, d, J=8.2 Hz), 7.26 (1H, s), 7.42 (1H, s), 7.64 (1H, s); IR (KBr) cm^{-1}: 1681; ESI-HR-MS (*m*/*z*): 517.1937. Calcd for $C_{27}H_{32}{}^{35}Cl_2FN_4O$ (M+H): 517.1932.

4′-(5-Hydroxypentyl)propiophenone Ethylene Acetal (11) Ethylene glycol (35 ml, 630 mmol) and pyridinium *p*-toluensulfonate (130 mg, 0.52 mmol) were added to a solution of ketone (**2**) (3.00 g, 11.4 mmol) in benzene (130 ml) and the mixture was refluxed for 27 h by water separation with a Dean–Stark trap. After cooling to room temperature, the solvent was concentrated and ether was added. The mixture was washed with saturated aqueous $NaHCO_3$ and brine, then dried and evaporated *in vacuo*. The crude product was chromatographed on silica gel using hexane : EtOAc = 10 : 1 as the eluent to give ketal (**10**) (2.77 g, 93%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 0.84 (3H, t, J=7.5 Hz), 1.36 (2H, m), 1.59—1.64 (4H, m), 2.00 (2H, q, J=7.0 Hz), 2.57 (2H, t, J=7.8 Hz), 3.31 (3H, s), 3.75 (2H, t, J=6.2 Hz), 3.97 (2H, t, J=6.5 Hz), 4.02 (2H, t, J=6.7 Hz), 7.10 (2H, d, J=8.0 Hz), 7.31 (2H, d, J=8.0 Hz); IR (KBr) cm^{-1}: 1739.

To a solution of the above (**10**) (2.77 g, 9.04 mmol) in methanol (8 ml) was added a solution of NaOH in water (2 ml). The mixture was stirred at room temperature for 5 min and diluted by the addition of water (2 ml). The mixture was evaporated *in vacuo* and the residue was extracted with ether. The ether was washed with water and brine, dried and evaporated *in vacuo* to leave hydroxyl acetal (**11**) (2.17 g, 91%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=7.5 Hz), 1.41 (2H, m), 1.56—1.67 (4H, m), 1.90 (2H, q, J=7.5 Hz), 2.61 (2H, t, J=7.5 Hz), 3.64 (2H, t, J=6.5 Hz), 3.78 (2H, t, J=6.3 Hz), 4.00 (2H, t, J=6.5 Hz), 7.13 (2H, d, J=7.8 Hz), 7.34 (2H, d, J=8.0 Hz); IR (KBr) cm^{-1}: 3363.

4′-(5-Methoxypentyl)propiophenone (9) Under argon, to a solution of hydroxyl acetal (**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 *O*-methylated product (**12**) as a pale yellow oil (1.99 g, 87%). ^{1}H-NMR ($CDCl_3$) δ: 0.87 (3H, t, J=7.5 Hz), 1.40 (2H, m), 1.59—1.64 (4H, m), 1.90 (2H, q, J=7.5 Hz), 2.60 (2H, t, J=7.6 Hz), 3.31 (3H, s), 3.36 (2H, t, J=6.5 Hz), 3.77 (2H, t, J=4.8 Hz), 3.99 (2H, t, J=4.9 Hz), 7.13 (2H, d, J=7.8 Hz), 7.33 (2H, d, J=8.2 Hz); IR (KBr) cm^{-1}: 3363.

To a solution of the *O*-methylated product (**12**) (1.98 g, 7.11 mmol) in methanol (13 ml) was added hydrochloric acid (3 M; 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 **9** (1.44 g, 87%) as colerless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, t, J=7.1 Hz), 1.37 (2H, m), 1.54—1.66 (4H, m), 2.65 (2H, t, J=7.7 Hz), 2.95 (2H, q, J=7.7 Hz), 3.30 (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 $(M+H)^+$.

4′-(5-Methoxypentyl)-α-bromopropiophenone (13) Compound (**13**) was obtained from **9** according to the procedure described for **3** and was isolated as a pale yellow oil in 64% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (2H, m), 1.55—1.66 (4H, m), 1.87 (3H, d, J=6.7 Hz), 2.66 (2H, t, J=7.7 Hz), 3.30 (3H, s), 3.34 (2H, t, J=6.4 Hz), 5.25 (1H, quintet, J=6.6 Hz), 7.26 (2H, d, J=8.2 Hz), 7.92 (2H, d, J=8.2 Hz); IR (KBr) cm^{-1}: 1685; FAB-MS (*m*/*z*): 313 $(M+H)^+$. 4′-(5-Acetoxypentyl)-α-bromopropiophenone (**3**) was also obtained in 27% yield as a side product.

Ethyl [2-Acetyl-4-{4′-(5-methoxypentyl)phenyl}-3-methyl-4-oxo]butyrate (14) Compound (**14**) was obtained from **13** according to the procedure described for **4** and was isolated as a pale yellow oil in 37% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.16 (3H, d, J=6.9 Hz), 1.31 (3H, t, J=7.1 Hz), 1.39 (2H, m), 1.55—1.65 (4H, m), 2.27 (3H, s), 2.65 (2H, t, J=7.7 Hz), 3.30 (3H, s), 3.34 (2H, t, J=6.5 Hz), 4.05—4.15 (2H, m), 4.18—4.27 (2H, m), 7.25 (2H, d, J=8.2 Hz), 7.88 (2H, d, J=8.0 Hz); IR (KBr) cm^{-1}: 1743, 1716, 1679; FAB-MS (*m*/*z*): 363 $(M+H)^+$.

1-(2,4-Dichlorophenyl)-5-{4′-(5-methoxypentyl)phenyl}-4-methyl-1*H*-pyrazole-3-carboxylic Acid (15) Compound (**15**) was obtained from **14** according to the procedure described for **5** and isolated as a brown oil in 44% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (2H, m), 1.55—1.62 (4H, m), 2.34 (3H, s), 2.57 (2H, t, J=8.0 Hz), 3.30 (3H, s), 3.34 (2H, t, J=6.5 Hz), 7.00 (2H, d, J=8.0 Hz), 7.11 (2H, d, J=8.2 Hz), 7.25 (2H, d, J=3.5 Hz), 7.40 (1H, s); IR (KBr) cm^{-1}: 1716; ESI-MS (*m*/*z*): 445 $(M-H)^+$.

***N*-(Piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-{4′-(5-methoxypentyl)-phenyl}-4-methyl-1*H*-pyrazole-3-carboxamide (16)** Under argon, one drop of dry DMF was added to a solution of **15** (61 mg, 0.13 mmol) in dry dichloromethane (2 ml) and to this solution was added dropwise a solution of oxalyl chloride (0.12 ml, 0.24 mmol; 2.0 M solution in dichloromethane). The mixture was kept at room temperature for 3 h with stirring, and concentrated *in vacuo*. The residue was then redissolved in dry dichloromethane (2 ml). To this solution was added dry triethylamine (32 μl, 0.23 mmol) followed by 1-aminopiperidine (21 μl, 0.22 mmol). After stirring at room temperature for 16 h, the reaction mixture was quenched by the addition of water (1 ml) and extracted with EtOAc. The EtOAc was then washed with water, dried and evaporated to dryness. The crude product was purified by silica gel chromatography using hexane : EtOAc = 2 : 1 as the eluent to give **16** (59 mg, 86%) as a pale yellow solid, mp 97—99 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.31—1.40 (2H, m), 1.40—1.47 (3H, br), 1.53—1.61 (4H, m), 1.72—1.81 (4H, br), 2.34 (3H, s), 2.57 (2H, t, J=7.8 Hz), 2.85–3.00 (3H, br), 3.30 (3H, s), 3.33 (2H, t, J=6.5 Hz), 6.98 (2H, d, J=8.2 Hz), 7.08 (2H, d, J=8.2 Hz), 7.23 (2H, d, J=1.3 Hz), 7.40 (1H, dd, J=0.56, 1.88 Hz); IR (KBr) cm^{-1}: 1670; ESI-HR-MS (*m*/*z*): 529.2162. Calcd for $C_{28}H_{35}{}^{35}Cl_2N_4O_2$ (M+H): 529.2132.

***N*-(Morphorin-1-yl)-1-(2,4-dichlorophenyl)-5-{4′-(5-methoxypentyl)-phenyl}-4-methyl-1*H*-pyrazole-3-carboxamide (17)** (a) Compound (**17**) was obtained from **15** (39 mg, 87 μmol), 4-aminomorpholine (11 μl, 114 μmol), 1-hydroxybenzotriazole (23 mg, 170 μmol), TPTU (34 mg, 114 μmol) and *N*,*N*-diisopropylethylamine (45 μl, 252 μmol) according to

the procedure described for **6** and was isolated as a pale yellow gum in 67% yield. ^{1}H-NMR (DMSO-d_6) 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; ESI-HR-MS (m/z): 531.1933. Calcd for $C_{27}H_{33}{}^{35}Cl_2N_4O_3$ (M+H): 531.1924.

(b) *N*-(Morphorin-1-yl)-1-(2,4-dichlorophenyl)-5-{4′-(5-hydroxypentyl)-phenyl}-4-methyl-1*H*-pyrazole-3-carboxamide (**18**) was prepared from **5** (71 mg, 163 μmol), 4-aminomorpholine (20 μl, 207 μmol), 1-hydroxybenzotriazole (43 mg, 318 μmol), TPTU (62 mg, 209 μmol), and *N*,*N*-diisopropylamine (84 μl, 694 μmol) according to the procedure described for **6** and was isolated as an orange gum in 57% yield. ^{1}H-NMR ($CDCl_3$) 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 $NaHCO_3$ 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→1 : 2 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 procedures[21] 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 $MgCl_2$, pH 7.4) and centrifuged at 45500×***g*** for 20 min at 4 °C. The pellets were washed by resuspension and centrifugation as above. The resulting 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.[25]

The binding of [^{3}H]SR141716 (1.63 TBq/mmol, Amersham Biosciences, 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 $MgCl_2$, 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 [^{3}H]SR141716 were made in buffer C consisting of 20 mM Hepes–NaOH, 1 mM $MgCl_2$, and 5.0 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 [^{3}H]SR141716 (=1 nM) 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 $MgCl_2$, 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 (ACSII, Amersham Bioscience, U.S.A.). All assays were performed in triplicate. The IC_{50} values were obtained from the concentration–response curves. Non-specific binding was estimated in the presence of 1 μM SR141716. The K_i values were calculated by the Cheng–Prusoff equation[26] and the K_d values were determined in direct binding assays with [^{3}H]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 0.05 M 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

1) Gatley S. J., Volkow N. D., Wang G.-J., Fowler J. S., Logan J., Ding Y.-S., Gerasimov M., *Curr. Pharm. Des.*, **11**, 3203—3220 (2005).
2) Pacher P., Batkai S., Kunos G., *Pharmacol. Rev.*, **58**, 389—462 (2006).
3) Padgett L. W., *Life Sci.*, **77**, 1767—1798 (2005).
4) Rinaldi-Carmona M., Barth F., Heaulme M., Shire D., Calandra B., Congy C., Martinez S., Maruani J., Neliat G., Caput D., Ferrara T., Soubrie P., Breliere J. C., LeFur G., *FEBS Lett.*, **350**, 240—244 (1994).
5) Carai M. A. M., Colombo G., Gessa G. L., *Life Sci.*, **77**, 2339—2350 (2005).
6) Shim J.-Y., Welsh W. J., Cartier E., Edwards J. L., Howlett A. C., *J. Med. Chem.*, **45**, 1447—1459 (2002).
7) Reggio P. H., *Curr. Pharm. Des.*, **9**, 1607—1633 (2003).
8) Gatley S. J., Lan R., Volkow N. D., Pappas N., King P., Wong C. T., Gifford A. N., Pyatt B., Dewey S. L., Makriyannis A., *J. Neurochem.*, **70**, 417—423 (1998).
9) Mathews W. B., Scheffel U., Finley P., Ravert H. T., Frank R. A., Rinaldi-Carmona M., Barth F., Dannals R. F., *Nucl. Med. Biol.*, **27**, 757—762 (2000).
10) Mathews W. B., Scheffel U., Rauseo P. A., Ravert H. T., Frank R. A., Ellames G. J., Herbert J. M., Barth F., Rinaldi-Carmona M., Dannals R. F., *Nucl. Med. Biol.*, **29**, 671—677 (2002).
11) Kumar J. S. D., Prabhakarman J., Arango V., Parsey R. V., Underwood M. D., Simpson N. R., Kassir S. A., Majo V. J., Van Heertum R. L., Mann J. J., *Bioorg. Med. Chem. Lett.*, **14**, 2393—2396 (2004).
12) Li Z., Gifford A., Liu Q., Thotapally R., Ding Y.-S., Makriyannis A., Gatley S. J., *Nucl. Med. Biol.*, **32**, 361—366 (2005).
13) Gielow P., Klinge P., Knapp W. H., Berding G., *Appl. Radiat. Isot.*, **64**, 763—768 (2006).
14) Hori A. G., Fan H., Kuwabara H., Hilton J., Ravert H. T., Holt D. P., Alexander M., Kumar A., Rahmim A., Scheffel U., Wong D. F., Dannals R. F., *J. Nucl. Med.*, **47**, 1689—1696 (2006).
15) Willy J. L., Jefferson R. G., Grier M. C., Mahadevan A., Razdan R. K., Martin B. R., *J. Pharmacol. Exp. Ther.*, **296**, 1013—1022 (2001).
16) Alekseeva O. O., Mahadevan A., Wiley J. L., Martin B. R., Razdan R. K., *Tetrahedron Lett.*, **46**, 2159—2161 (2005).
17) Seltzman H. H., Carroll F. I., Burgess J. P., Wyrick C. D., Burch D. F., *J. Chem. Soc., Chem. Commun.*, **1995**, 1549—1550 (1995).
18) Lan R., Liu Q., Fan P., Lin S., Fernando S. R., McCallion D., Pertwee R., Makriyannis A., *J. Med. Chem.*, **42**, 769—776 (1999).
19) Dutta A. K., Sard H., Ryan W., Razdan R. K., Compton D. C., Martin B. R., *Med. Chem. Res.*, **5**, 54—62 (1994).
20) Marshall J. A., Xie S., *J. Org. Chem.*, **60**, 7230—7237 (1995).
21) Petitet F., Marhin L., Doble A., *Neuro Report*, **7**, 789—792 (1996).
22) Dischino D. D., Welch M. J., Kilbourn M. R., Raichle M. E., *J. Nucl. Med.*, **24**, 1030—1038 (1983).
23) Katoch-Rouse R., Pavlova O. A., Caulder T., Hoffman A. F., Mukhin A. G., Horti A. G., *J. Med. Chem.*, **46**, 642—645 (2003).
24) Howlett A. C., Barth F., Bonner T. I., Cabral G., Casellas P., Devane W. A., Felder C. C., Herkenham M., Mackie K., Martin B. R., Mechoulam R., Pertwee R. G., *Pharmacol. Rev.*, **54**, 161—202 (2002).
25) Bradford M. M. A., *Anal. Biochem.*, **72**, 248—254 (1976).
26) Cheng Y., Prusoff W. H., *Biochem. Pharmacol.*, **22**, 3099—3108 (1973).