Brief Articles

Structure−Activity Relationships of Pyrazole Derivatives as Cannabinoid Receptor Antagonists

Ruoxi Lan,[†] Qian Liu,[†] Pusheng Fan,[†] Sonyuan Lin,[†] Susanthi R. Fernando,[‡] Deirdre McCallion,[‡] Roger Pertwee,[‡] and Alexandros Makriyannis*[,†,§]

Departments of Pharmaceutical Sciences and Molecular and Cell Biology, and Institute of Materials Science, University of Connecticut, Storrs, Connecticut 06269, and Department of Biomedical Sciences, University of Aberdeen, Aberdeen AB9 1AS, Scotland

Received June 15, 1998

As a potent, specific antagonist for the brain cannabinoid receptor (CB1), the biarylpyrazole *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (SR141716A; **1**) was the lead compound for initiating studies designed to examine the structure−activity relationships of related compounds and to search for more selective and potent cannabimimetic ligands. A series of pyrazole derivatives was designed and synthesized to aid in the characterization of the cannabinoid receptor binding sites and also to serve as potentially useful pharmacological probes. Therapeutically, such compounds may have the ability to antagonize harmful side effects of cannabinoids and cannabimimetic agents. Structural requirements for potent and selective brain cannabinoid CB1 receptor antagonistic activity included (a) a *para*-substituted phenyl ring at the 5-position, (b) a carboxamido group at the 3-position, and (c) a 2,4-dichlorophenyl substituent at the 1-position of the pyrazole ring. The most potent compound of this series contained a *p*-iodophenyl group at the 5-position, a piperidinyl carboxamide at the 3-position, and a 2,4-dichlorophenyl group at the 1-position of the pyrazole ring. The iodinated nature of this compound offers additional utility as a γ-enriching SPECT (single photon emission computed tomography) ligand that may be useful in characterizing brain CB1 receptor binding in vivo.

Introduction

Cannabinoids such as (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC), the major psychoactive constituent of marijuana, have long been the focus of study due to their effects on the central nervous system. Early pharmacological testing has shown that cannabinoids possess analgesic, antiemetic, psychotropic, and antiinflammatory properties and has also suggested their potential therapeutic utility for the treatment of asthma and glaucoma.[1−6] However, widespread use of cannabinoids as therapeutic agents has been limited by their psychotropic properties.[4,7]

The effort to develop useful cannabimimetic medications has involved extensive modifications of cannabinoid structures in order to dissect the medicinal properties of these compounds away from their undesirable psychotropic effects. These studies led to several significant discoveries, such as the development of (1*R*,3*R*,4*R*)-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-4-(3-hydroxypropyl)cyclohexan-1-ol (CP-55,940; **2**), a potent, high-affinity agonist, the discovery of cannabinoid receptors in brain (CB1)[8] and spleen (CB2),[9] and the identification of arachidonylethanolamide (anandamide),[10] an endogenous ligand for the cannabinoid receptors. The recently developed cannabinoid receptor antagonists, (4-methoxyphenyl)[6-iodo-1-[2-(4-morpholinyl)ethyl]-1*H*-indol-3-yl]methanone (AM630),[11] SR141716A (**1**),[12−16] and *N*-[(1*S*)-*endo*-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylphenyl)-1-(4-methylbenzyl)-pyrazole-3-carboxamide (SR144528),[17] have provided effective tools to characterize cannabinoid receptors and improve our understanding of the molecular basis for cannabinoid activity.

Compound **1** was reported in 1994 by Sanofi Recherche as a potent selective and orally active antagonist of the brain cannabinoid receptor (CB1).[12−16] As the structure of **1** and congeners developed by Sanofi Recherche differ substantially from that of recognized cannabimimetic compounds, the objective of the present study was to evaluate the structure−activity relationships (SARs) of this class of ligands and to optimize their antagonist activity. From these SARs, we also explored the possibility of developing a highly selective CB1 ligand containing iodine that could serve as an effective SPECT (single photon emission computed tomography) probe for radioimaging of CB1 receptors in vivo. Early reports[18,19] from this laboratory including SAR results of substituted pyrazoles have preceded this publication, which described the synthesis of such analogues and

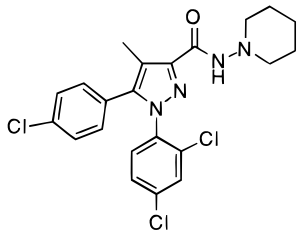
* Direct correspondence to Dr. Alexandros Makriyannis, Department of Pharmaceutical Sciences and Molecular and Cell Biology, U-92, University of Connecticut, Storrs, CT 06269. Tel: (860) 486-2133. Fax: (860) 486-3089. E-mail: Makriyan@uconnvm.uconn.edu.

[†] Departments of Pharmaceutical Sciences and Molecular and Cell Biology, University of Connecticut.

[‡] University of Aberdeen.

[§] Institute of Materials Science, University of Connecticut.

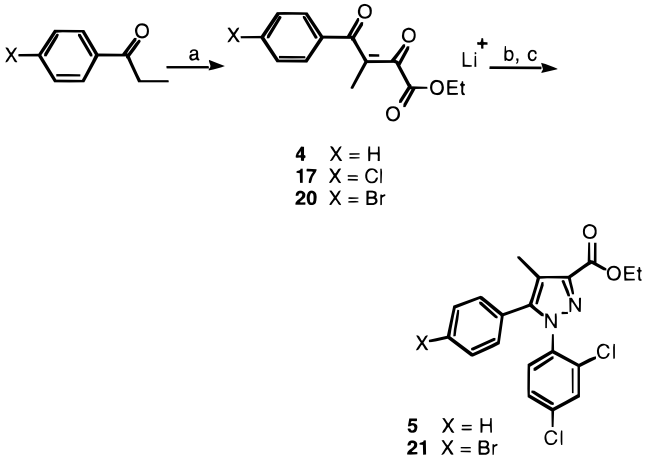
10.1021/jm980363y CCC: $18.00
Published on Web 02/06/1999



1

Figure 1. Structure of SR141716A, a CB1 cannabinoid receptor antagonist.

Scheme 1[a]



[a] Reagents: (a) LiHMDS, ether; EtO_2CCO_2Et; (b) 2,4-dichlorophenylhydrazine hydrochloride, EtOH; (c) AcOH.

their testing as potent selective cannabinoid CB1 receptor antagonists.

Chemistry

Most of the target compounds were synthesized in a manner similar to our published work.[20] The procedures for preparation of these compounds are outlined in Schemes 1−5.

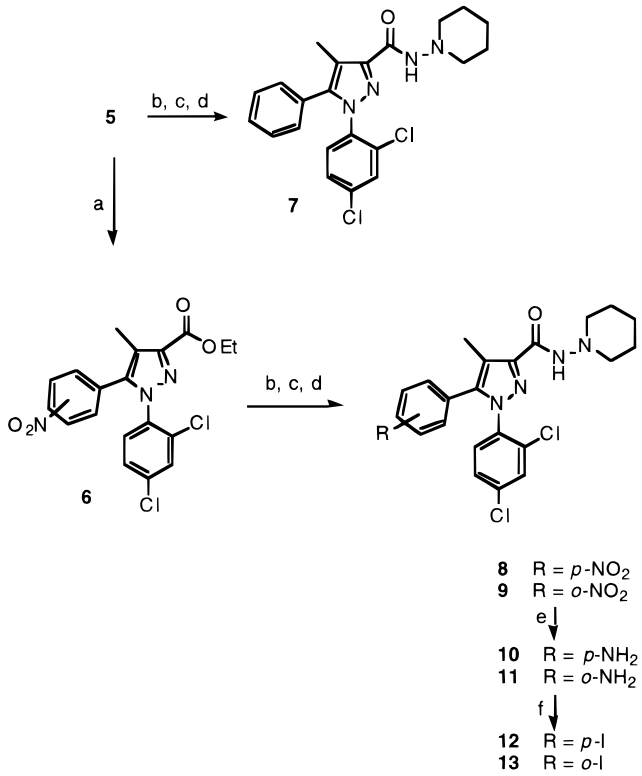
The ketones reacted with diethyl oxalate in the presence of lithium bis(trimethylsilyl)amide to provide the appropriate lithium salts **4**, **14**, **17**, and **20**. These were allowed to react with either 2,4-dichlorophenylhydrazine hydrochloride or 4-chlorophenylhydrazine hydrochloride to yield substituted 1*H*-pyrazole-3-carboxylic acid ethyl esters **5**, **15**, **18**, and **21**, respectively. Nitration of compound **5** by nitronium tetrafluoroborate in acetonitrile[21] gave a mixture of *para*- and *ortho*-nitrated compounds **6** which were used directly for the subsequent reaction without separation. The esters obtained were hydrolyzed to their carboxylic acids and then converted to the corresponding acid chlorides, which reacted with the appropriate primary or secondary amines in the presence of triethylamine to give carboxamides **7**, **8**, **9**, **16**, **19**, and **22**−**31**.

The nitro-substituted compounds **8** and **9** were reduced by hydrazine in ethanol in the presence of catalytic Raney nickel[22] to amines **10** and **11**, which were converted finally to iodides **12** and **13** via the reaction of their diazonium salts with sodium iodide.[23]

Results and Discussion

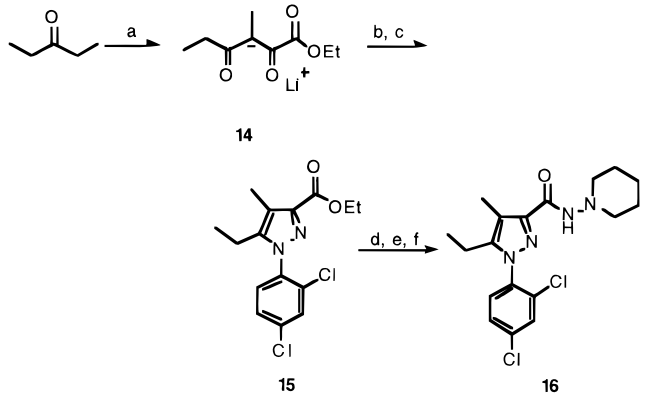
Receptor Binding Studies. Rat forebrain (CB1) and mouse spleen (CB2) membrane preparations were used

Scheme 2[a]



[a] Reagents: (a) BF_4NO_2, acetonitrile; (b) KOH, MeOH; (c) $SOCl_2$, toluene; (d) 1-aminopiperidine, Et_3N, CH_2Cl_2; (e) N_2H_4, Raney Ni, ethanol; (f) $NaNO_2$, HCl; NaI.

Scheme 3[a]

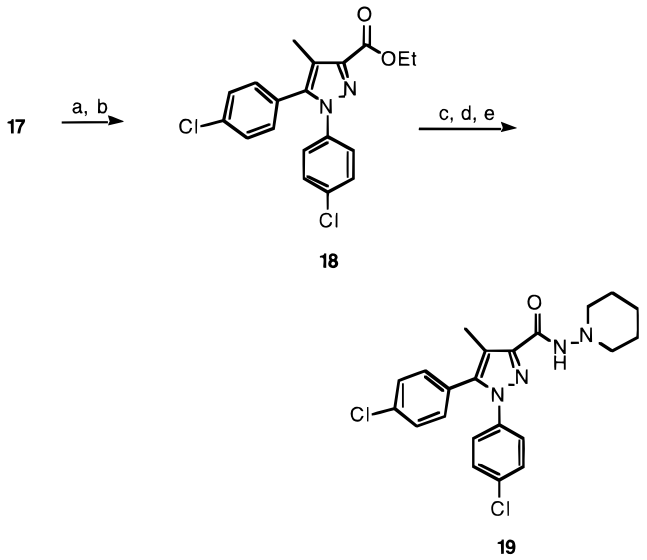


[a] Reagents: (a) LiHMDS, ether; EtO_2CCO_2Et; (b) 2,4-dichlorophenylhydrazine hydrochloride, EtOH; (c) AcOH; (d) KOH, MeOH; (e) $SOCl_2$, toluene; (f) 1-aminopiperidine, Et_3N, CH_2Cl_2.

to assess the affinity of pyrazole derivatives for cannabinoid receptor binding sites. A filtration assay was employed in which specific binding was measured by displacement of [^{3}H]**2** from cannabinoid binding sites by increasing concentrations of the ligands. The biological activities are summarized in Tables 1−3.

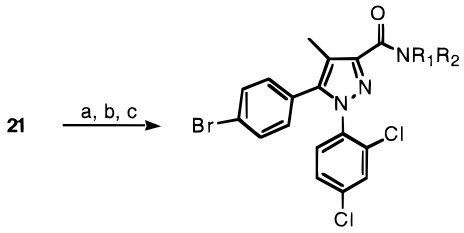
Using **1** as a benchmark, substitution at the 5-position of the pyrazole ring was explored. Generally, all of the analogues tested had selectivity for the CB1 receptor and variably reduced affinity for CB2. In this series, the *p*-iodophenyl analogue (**12**) was found to have the highest affinity (K_i value of 7.5 nM) for CB1 and a high degree of selectivity (306-fold) for the CB1 over the CB2 receptor. *Para*-substituted analogues (**8**, **10**, **12**) generally had higher affinities and selectivities for CB1 than

Scheme 4[a]



[a] Reagents: (a) 4-chlorophenylhydrazine hydrochloride, EtOH; (b) AcOH; (c) KOH, MeOH; (d) $SOCl_2$, toluene; (e) 1-aminopiperidine, Et_3N, CH_2Cl_2.

Scheme 5[a]



22 R_1 = H, R_2 = pyrrolidin-1-yl
23 R_1 = H, R_2 = piperidin-1-yl
24 R_1 = H, R_2 = homopiperidin-1-yl
25 R_1 = H, R_2 = morpholin-4-yl
26 R_1, R_2 = -$(CH_2)_5$-
27 R_1, R_2 = -$(CH_2)_2N(CH_3)(CH_2)_2$-
28 R_1 = H, R_2 = cyclohexyl
29 R_1 = Me, R_2 = cyclohexyl
30 R_1 = H, R_2 = 2-ethanol
31 R_1 = H, R_2 = phenyl

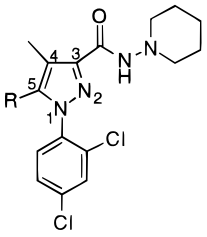
[a] Reagents: (a) KOH, MeOH; (b) $SOCl_2$, toluene; (c) NHR_1R_2, Et_3N, CH_2Cl_2.

the corresponding *ortho*-substituted congeners (**9**, **11**, **13**) and their unsubstituted analogue (**7**). Also, the binding affinity was reduced when the aromatic ring was replaced with an aliphatic ethyl group.

At the 1-position of the pyrazole ring, 2,4-dichlorophenyl substitution was optimal for binding. Other substituents such as 4-chlorophenyl (**19**), 4-nitrophenyl, and 4-aminophenyl led to a decreased affinity.

The SAR of different carboxamides at the 3-position of the pyrazole ring was also investigated. We found that although various *N*-heterocyclic substituted carboxamides had comparable affinities, the piperidino analogue gave optimal selectivity for CB1. *N*,*N*-Disubstitution (**26**, **27**, **29**) resulted in decreased binding. An aromatic carboxamide analogue (**31**) had lower binding affinity, and the *N*-(2-hydroxyethyl) carboxamide (**30**) had very low affinities for both cannabinoid receptors.

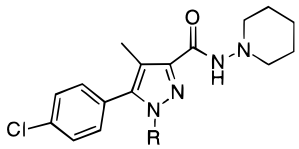
Table 1. Ligands Modified at the 5-Position



ligands	R	K_i (nM)[a] CB1	K_i (nM)[a] CB2	K_i ratio CB1:CB2
7	Ph	123 (112, 135)	217 (165, 284)	1:1.8
8	*p*-NO_2−Ph	57.5 (51.3, 66.4)	252 (192, 331)	1:4.4
9	*o*-NO_2−Ph	255 (233, 279)	691 (599, 797)	1:2.7
10	*p*-NH_2−Ph	81.5 (69.6, 95.4)	958 (777, 1180)	1:12
11	*o*-NH_2−Ph	346 (298, 401)	931 (732, 1180)	1:2.7
12	*p*-I−Ph	7.49 (6.38, 8.78)	2290 (1640, 3190)	1:306
13	*o*-I−Ph	53.8 (49.6, 66.5)	577 (431, 774)	1:11
23	*p*-Br−Ph	16.8 (14.3, 19.7)	1430 (1120, 1820)	1:85
16	Et	183 (165, 203)	744 (612, 905)	1:4.1
1	*p*-Cl−Ph	11.5 (8.45, 13.7)	1640 (1440, 1850)	1:143

[a] Binding affinities (K_i) of ligands for CB1 (rat forebrain) and CB2 (mouse spleen) cannabinoid receptors. The 95% confidence limits are in parentheses.

Table 2. Ligands Modified at the 1-Position

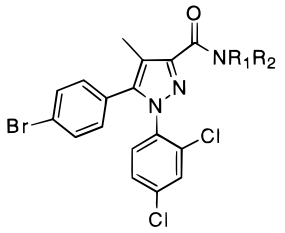


ligands	R	K_i (nM)[a] CB1	K_i (nM)[a] CB2	K_i ratio CB1:CB2
19	4-Cl−Ph	60.4 (49.1, 74.3)	836 (746, 936)	1:14
1	2,4-di-Cl−Ph	11.5 (8.45, 13.7)	1640 (1440, 1850)	1:143

[a] Binding affinities (K_i) of ligands for CB1 (rat forebrain) and CB2 (mouse spleen) cannabinoid receptors. The 95% confidence limits are in parentheses.

Antagonism Studies. A selection of the compounds described in this paper were tested for their ability to decrease 2,3-dihydro-5-methyl-3-[(4-morpholin-yl)methyl]pyrrolo[1,2,3-*de*]-1,4-benzoxazin-6-yl](1-naphthyl)-methanone (WIN55,212-2; **3**)-induced inhibition of electrically evoked contractions of the guinea pig myenteric plexus-longitudinal muscle preparation or mouse vas deferens (Tables 4 and 5). Each of the compounds tested behaved as a competitive cannabinoid receptor antagonist, producing a statistically significant parallel dextral shift in the log concentration−response curve of **3** without any significant reduction in the size of the maximal response to the agonist. Our experiments with the myenteric plexus-longitudinal muscle preparation indicated a good correlation between the K_d values of

Table 3. Ligands Modified at the 3-Position



ligands	R_1	R_2	K_i (nM)[a] CB1	K_i (nM)[a] CB2	K_i ratio CB1:CB2
22	H	pyrrolidinyl	17.1 (15.3, 19.1)	1310 (1140, 1510)	1:76
23	H	piperidinyl	16.8 (14.3, 19.7)	1430 (1120, 1820)	1:85
24	H	homopiperidinyl	7.85 (6.86, 8.97)	215 (174, 266)	1:28
25	H	morpholin-4-yl	53.9 (48.1, 60.5)	2450 (2140, 2810)	1:45
26		$-(CH_2)_5-$	125 (104, 150)	4580 (4020, 5230)	1:37
27		$-(CH_2)_2N(CH_3)(CH_2)_2-$	326 (284, 374)	2340 (1750, 3140)	1:7.2
28	H	cyclohexyl	11.7 (9.21, 14.8)	1010 (851, 1210)	1:87
29	Me	cyclohexyl	76.7 (70.0, 84.0)	1260 (1050, 1510)	1:16
30	H	2-ethanol	1120 (1020, 1240)	1.9e+4[b] 1.7e+4, 2.1e+4)	1:17
31	H	phenyl	31.1 (28.5, 34.7)	6750 (5840, 7810)	1:217

[a] Binding affinities (K_i) of ligands for CB1 (rat forebrain) and CB2 (mouse spleen) cannabinoid receptors. The 95% confidence limits are in parentheses. [b] Read as $1.9 \times 10^{+4}$.

Table 4. Receptor Antagonism in Guinea Pig Small Intestine

ligands	concn of compd (nM)[a]	K_d (nM)	CB1 K_i (nM)
12	100	0.22 (0.06, 0.61)	7.49
23	100	0.32 (0.12, 0.87)	16.8
24	1000	0.38 (0.17, 3.92)	7.85
22	1000	1.18 (0.71, 2.12)	17.1
10	100	2.63 (0.66, 9.57)	81.5
8	1000	2.90 (1.53, 12.1)	57.5
26	1000	4.72 (2.01, 23.0)	125
13	1000	5.87 (3.88, 10.6)	53.8
9	1000	5.96 (3.46, 14.7)	255
7	1000	7.00 (4.26, 16.1)	123
11	100	11.2 (3.46, 43.6)	346
27	1000	12.3 (7.06, 24.1)	326
1	100	0.25 (0.09, 0.66)	11.5

[a] Concentration of compound used to determine K_d value. Dissociation constants (K_d) determined in the myenteric plexus-longitudinal muscle preparation of guinea pig small intestine using the cannabinoid receptor agonist **3**. Linear regression analysis indicates the mean slope of a plot of log K_i for CB1 (x axis) against log K_d (y axis) to be 1.020 with 95% confidence limits of 0.780 and 1.259 ($r^2 = 0.889$). The corresponding r^2 value for a plot of log K_i for CB2 against log K_d is 0.001.

Table 5. Receptor Antagonism in Mouse Vas Deferens

ligands	concn of compd (nM)[a]	K_d (nM)	CB1 K_i (nM)
12	31.6	0.50 (0.31, 0.87)	7.49
23	31.6	2.64 (0.93, 7.44)	16.8
8	316	5.31 (2.46, 10.3)	57.5
24	316	6.80 (1.22, 19.4)	7.85
13	31.6	7.83 (3.17, 33.8)	53.8
9	316	25.4 (14.7, 71.6)	255
7	316	28.0 (16.1, 48.9)	123
10	316	42.6 (23.0, 83.4)	81.5
22	316	47.8 (18.7, 159)	17.1
11	1000	118 (53.9, 283)	125
26	1000	172 (33.8, 581)	346
27	1000	325 (115, 1210)	326
1	31.6	1.98 (0.85, 5.91)	11.5

[a] Concentration of compound used to determine K_d value. Dissociation constants (K_d) determined in the mouse isolated vas deferens using the cannabinoid receptor agonist **3**. Linear regression analysis indicates the mean slope of a plot of log K_i for CB1 (x axis) against log K_d (y axis) to be 1.088 with 95% confidence limits of 0.533 and 1.644 ($r^2 = 0.889$). The corresponding r^2 value for a plot of log K_i for CB2 against log K_d is 0.008.

the compounds investigated and their K_i values for CB1 but not CB2 binding sites (Table 4). This finding is in agreement with previous evidence that **3** and other cannabinoid receptor agonists can act through CB1 receptors to inhibit electrically evoked contractions of

this tissue.[24,25] Interestingly, the K_d value for **1** obtained in the present experiments with the myenteric plexus-longitudinal muscle preparation was significantly lower than that obtained previously using the same preparation and agonist.[24] The cause of this discrepancy remains to be established.

In the vas deferens, there is not a close correlation between K_d values and K_i values for CB1 binding sites (Table 5). Moreover, the K_d values of some compounds (compounds **10**, **11**, **22**, **23**, **26**, **27**, and **1**) were found to be significantly higher in this tissue than in the myenteric plexus-longitudinal muscle preparation. It is tempting to speculate that the vas deferens contains more than one class of receptor with which **3** can interact to cause inhibition of evoked contractions, and indeed, our compounds do not all interact preferentially with the same receptor type. The K_d value for **1** shown in Table 5 is similar to that obtained in previous experiments with the mouse vas deferens using **3** as the agonist.[26]

Compound **12**, incorporating an iodine atom in the 5-*para*-substituted phenyl ring was found to be the most potent and also the most selective antagonist for the cannabinoid CB1 receptor. In this series, compound **12** with about twice the activity of **1** is also an excellent candidate for a SPECT probe. In fact, a recent publication from this laboratory[27] elaborated on its effectiveness in this capacity.

Experimental Section

Chemistry. ^{1}H NMR spectra were recorded on either Bruker WP-211SY 200 MHz or Bruker DMX-500 MHz spectrometers. Chemical shifts are reported in ppm (parts per million) relative to tetramethylsilane as the internal standard, and signals are quoted as s (singlet), brs (broad singlet), d (doublet), dd (double doublet), dt (double triplet), t (triplet), q (quartet), or m (multiplet). Mass spectra (electron ionization, 70 eV) were determined on a KRATOS MS-50RFA instrument. GC-MS was measured on a Hewlett-Packard 6890 GC-MS instrument. Melting points were determined on a Thomas-Hoover melting point apparatus. Elemental analyses were performed at Analytical Services Center of Baron Consulting Company. Compounds are indicated by the molecular formula followed by the symbols for the elements (C, H, N) and were found to be within 0.4% of their theoretical values. Flash column chromatography was carried out by using Whatman active silica gel (230−400 mesh), and eluents were distilled before use. Solvents for reactions were dried or purified as required. Reactions were carried out under nitrogen atmosphere unless otherwise noted.

Lithium Salt of Ethyl 2,4-Dioxo-3-methyl-4-phenylbutanoate (4). To a magnetically stirred solution of lithium bis(trimethylsilyl)amide (4.0 mL, 1.0 M solution in hexane, 4.0 mmol) in diethyl ether (20 mL) was added a solution of propiophenone (530 μL, 4.0 mmol) in diethyl ether (5 mL) at −78 °C. After the mixture was stirred at the same temperature for additional 45 min, diethyl oxalate (600 μL, 4.4 mmol) was added to the mixture. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. The precipitate was filtered, washed with diethyl ether, and dried under vacuum to afford the lithium salt **4** (790 mg, 83% yield).

Lithium Salt of Ethyl 2,4-Dioxo-3-methyl-hexanoate (14). Compound **14** was synthesized from 3-pentanone by using the procedure applied to compound **4** as a white solid in 90% yield.

Lithium Salt of Ethyl 2,4-Dioxo-3-methyl-4-(4-chlorophenyl)butanoate (17). Compound **17** was synthesized from 4′-chloropropiophenone according to the procedure applied to compound **4** and obtained as a light yellow solid in 85% yield.

Lithium Salt of Ethyl 2,4-Dioxo-3-methyl-4-(4-bromophenyl)butanoate (20). Compound **20** was synthesized from 4′-bromopropiophenone according to the procedure applied to compound **4** and obtained as a light yellow solid in 79% yield.

1-(2,4-Dichlorophenyl)-4-methyl-5-phenyl-1*H*-pyrazole-3-carboxylic Acid, Ethyl Ester (5). To a magnetically stirred solution of lithium salt **4** (700 mg, 2.9 mmol) in 10 mL of ethanol was added 2,4-dichlorophenylhydrazine hydrochloride (701 mg, 3.2 mmol) at room temperature. The resulting mixture was stirred at room temperature for 20 h. The precipitate was filtered, washed with ethanol and diethyl ether, and then dried under vacuum to give a light yellow solid (853 mg). This solid was dissolved in acetic acid (7 mL) and heated under reflux for 24 h. The reaction mixture was poured into cold water (30 mL) and extracted with ethyl acetate (3 × 20 mL). The combined extracts were washed with water, saturated aqueous sodium bicarbonate, and brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Purification by flash column chromatography on silica gel with petroleum ether/ethyl acetate (9:1) gave the ester **5** (776 mg, 71% yield) as a white solid: mp 80−82 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.37−7.11 (m, 8H, ArH), 4.46 (q, J = 7.1 Hz, 2H, OCH_2), 2.35 (s, 3H, CH_3), 1.43 (t, J = 7.1 Hz, 3H, CH_3); MS *m/e* 374 (M^+), 329 ($M^+ - C_2H_5O$), 301 ($M^+ - C_3H_5O_2$). Anal. ($C_{19}H_{16}Cl_2N_2O_2$) C, H, N.

1-(2,4-Dichlorophenyl)-5-ethyl-4-methyl-1*H*-pyrazole-3-carboxylic Acid, Ethyl Ester (15). To a magnetically stirred solution of lithium salt **14** (1.00 g, 5.37 mmol) in 20 mL of ethanol was added 2,4-dichlorophenylhydrazine hydrochloride (1.29 g, 5.9 mmol) at room temperature. The resulting mixture was stirred at room temperature for 20 h and then evaporated under reduced pressure to give a brown oil (1.98 g). The above crude product was dissolved in acetic acid (15 mL) and heated to reflux for 24 h. The reaction mixture was poured into cold water (30 mL) and then extracted with ethyl acetate (3 × 30 mL). The combined extracts were washed with water, saturated aqueous sodium bicarbonate, and brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Purification by flash column chromatography on silica gel with petroleum ether/ethyl acetate (9:1) gave the ester **15** (454 mg, 26% yield) as a white solid: mp 102−104 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (s, 1H, ArH), 7.39−7.34 (m, 2H, ArH), 4.41 (q, J = 7.0 Hz, 2H, OCH_2), 2.47 (brs, 2H, CH_2), 2.32 (s, 3H, CH_3), 1.43 (t, J = 7.1 Hz, 3H, OCH_2CH_3), 0.99 (t, J = 7.4 Hz, 3H, CH_2CH_3); GC-MS *m/e* 326 (M^+), 311 ($M^+ - CH_3$), 297 ($M^+ - C_2H_5$), 291 ($M^+ - Cl$).

1-(4-Chlorophenyl)-5-(4-chlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic Acid, Ethyl Ester (18). Compound **18** was synthesized from lithium salt **17** and 4-chlorophenylhydrazine hydrochloride according to the procedure applied to **5** and was obtained in 43% yield as a white solid: mp 118−121 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, J = 8.3 Hz, 2H, ArH), 7.28 (d, J = 8.6 Hz, 2H, ArH), 7.18 (d, J = 8.7 Hz, 2H, ArH), 7.08 (d, J = 8.2 Hz, 2H, ArH), 4.46 (q, J = 7.1 Hz, 2H, OCH_2), 2.30 (s, 3H, CH_3), 1.43 (t, J = 7.1 Hz, 3H, CH_3); GC-MS *m/e* 374 (M^+), 345 ($M^+ - C_2H_5$), 301 ($M^+ - C_3H_5O_2$).

5-(4-Bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic Acid, Ethyl Ester (21). Compound **21** was synthesized from lithium salt **20** and 2,4-dichlorophenylhydrazine hydrochloride according to the procedure applied to **5** and was obtained in 59% yield as a white solid: mp 143−144 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.48−7.27 (m, 5H, ArH), 7.01 (dd, J = 1.8 and 6.6 Hz, 2H, ArH), 4.46 (q, J = 7.1 Hz, 2H, OCH_2), 2.33 (s, 3H, CH_3), 1.43 (t, J = 7.1 Hz, 3H, CH_3); MS *m/e* 452 (M^+), 407 ($M^+ - C_2H_5O$), 379 ($M^+ - C_3H_5O_2$). Anal. ($C_{19}H_{15}BrCl_2N_2O_2$) C, H, N.

1-(2,4-Dichlorophenyl)-4-methyl-5-nitrophenyl-1*H*-pyrazole-3-carboxylic Acid, Ethyl Ester (6). To a magnetically stirred solution of compound **5** (890 mg, 2.37 mmol) in 50 mL of acetonitrile was added nitronium tetrafluoroborate (365 mg, 2.61 mmol) at room temperature for 8 h. After removal of acetonitrile, the residue was dissolved in dichloromethane, washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Flash column chromatography on silica gel with toluene and ethyl acetate (95:1) gave a mixture of *para*- and *ortho*-nitration product **6** (920 mg, 92% yield) as

a white solid: ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.22−7.19 (m, 7H, ArH), 4.47 (q, J = 7.1 Hz, 2H, OCH_2), 2.38 and 2.39 (s, 3H, CH_3), 1.44 (t, J = 7.1 Hz, 3H, CH_3); MS *m/e* 419 (M^+), 373 ($M^+ - C_2H_6O$), 346 ($M^+ - C_3H_5O_2$). Anal. ($C_{19}H_{15}Cl_2N_3O_4$) C, H, N.

***N*-(Piperidin-1-yl)-1-(2,4-dichlorophenyl)-4-methyl-5-phenyl-1*H*-pyrazole-3-carboxamide (7).** To a magnetically stirred solution of ester **5** (520 mg, 1.38 mmol) in methanol (7 mL) was added a solution of potassium hydroxide (155 mg, 2.76 mmol) in methanol (5 mL). The mixture was heated under reflux for 3 h. The cooling reaction mixture was then poured into water (10 mL) and acidified with 10% hydrochloric acid. The precipitate was filtered, washed with water, and dried under vacuum to yield the corresponding acid (430 mg, 90% yield) as a solid.

A solution of the crude acid (430 mg) and thionyl chloride (270 μL, 4.14 mmol) in toluene (10 mL) was refluxed for 3 h. Solvent was evaporated under reduced pressure, and the residue was then redissolved in toluene (20 mL) and evaporated to yield the crude carboxylic chloride (450 mg, 100% yield) as a solid. A solution of the above carboxylic chloride (450 mg, 1.24 mmol) in dichloromethane (5 mL) was added dropwise to a solution of 1-aminopiperidine (215 μL, 1.92 mmol) and triethylamine (270 μL, 1.92 mmol) in dichloromethane (5 mL) at 0 °C. After stirring at room temperature for 3 h, the reaction mixture was added with brine and extracted with dichloromethane (3 × 15 mL). The combined extracts were washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Flash column chromatography on silica gel with petroleum ether/ethyl acetate (2:1) gave carboxamide **7** (540 mg, 98% yield) as a white solid: mp 186−187 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.64 (s, 1H, NH), 7.42−7.27 (m, 6H, ArH), 7.14−7.11 (m, 2H, ArH), 2.87 (t, J = 5.2 Hz, 4H, NCH_2), 2.38 (s, 3H, CH_3), 1.79−1.66 (m, 4H, CH_2), 1.46−1.42 (m, 2H, CH_2); MS *m/e* 428 (M^+), 329 ($M^+ - C_5H_{11}N_2$). Anal. ($C_{22}H_{22}Cl_2N_4O$) C, H, N.

***N*-(Piperidin-1-yl)-1-(2,4-dichlorophenyl)-4-methyl-5-nitrophenyl-1*H*-pyrazole-3-carboxamide (8 and 9).** Compounds **8** and **9** were synthesized from *p*- and *o*-nitrate esters **6** according to the procedure described for compound **7**. Purification of the crude product by flash column chromatography on silica gel with petroleum ether/ethyl acetate (3:2 to 1:1) gave the *p*-nitro compound **8** and *o*-nitro compound **9** in a total yield of 98%. Compound **8**: mp 142−143 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (d, J = 8.3 Hz, 2H, ArH), 7.66 (s, 1H, NH), 7.43−7.32 (m, 3H, ArH), 7.32 (d, J = 8.2 Hz, 2H, ArH), 2.87 (t, J = 5.0 Hz, 4H, NCH_2), 2.41 (s, 3H, CH_3), 1.77−1.72 (m, 4H, CH_2), 1.44 (bs, 2H, CH_2); MS *m/e* 473 (M^+), 374 ($M^+ - C_5H_{11}N_2$). Anal. ($C_{22}H_{21}Cl_2N_5O_3$) C, H, N. Compound **9**: mp 139−140 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.19 (d, J = 7.7 Hz, 1H, ArH), 8.01 (s, 1H, ArH), 7.64 (s, 1H, NH), 7.54 (t, J = 7.8 Hz, 1H, ArH), 7.74 (d, J = 7.4 Hz, 1H, ArH), 7.42−7.34 (m, 3H, ArH), 2.87 (t, J = 5.1 Hz, 4H, NCH_2), 2.41 (s, 3H, CH_3), 1.79−1.74 (m, 4H, CH_2), 1.44 (bs, 2H, CH_2); MS *m/e* 473 (M^+), 374 ($M^+ - C_5H_{11}N_2$). Anal. ($C_{22}H_{21}Cl_2N_5O_3$) C, H, N.

***N*-(Piperidin-1-yl)-5-(4-aminophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (10).** Hydrazine (61 μL, 1.9 mmol) was added to a solution of **8** (300 mg, 0.63 mmol) in ethanol (20 mL) at room temperature. The mixture was warmed to 30−40 °C and Raney nickel (10 mg, 50% slurry in water) was added. After no more nitrogen was generated, another portion of Raney nickel (5.0 mg) was added and the reaction mixture was warmed to 70 °C and stirred for 3 h. The catalyst was removed by filtration, and the crude product was purified by flash column chromatography on silica gel with petroleum ether/ethyl acetate (1:1) to afford *p*-amine **10** (263 mg, 94% yield) as a white solid: mp 211−212 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.65 (s, 1H, NH), 7.42 (dd, J = 0.9 and 1.7 Hz, 1H, ArH), 7.27−7.26 (m, 2H, ArH), 6.88 (dd, J = 2.0 and 6.5 Hz, 2H, ArH), 6.59 (dd, J = 2.0 and 6.6 Hz, 2H, ArH), 3.74 (brs, 2H, NH_2), 2.86 (t, J = 5.2 Hz, 4H, NCH_2), 2.35 (s, 3H, CH_3), 1.76−1.69 (m, 4H, CH_2), 1.45−1.41 (m, 2H, CH_2); MS *m/e* 443 (M^+), 344 ($M^+ - C_5H_{11}N_2$). Anal. ($C_{22}H_{23}Cl_2N_5O$) C, H, N.

***N*-(Piperidin-1-yl)-5-(2-aminophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (11).** Compound **11** was synthesized from **9** according to the procedure applied to **10** and obtained in 89% yield as a white solid: mp 256−258 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.67 (s, 1H, NH), 7.42 (dd, J = 0.7 and 1.6 Hz, 1H, ArH), 7.24−7.22 (m, 2H, ArH), 7.05 (t, J = 7.7 Hz, 1H, ArH), 6.63−6.58 (m, 1H, ArH), 1.77−1.68 (m, 4H, CH_2), 1.44−1.41 (m, 2H, CH_2); MS *m/e* 443 (M^+), 344 ($M^+ - C_5H_{11}N_2$). Anal. ($C_{22}H_{23}Cl_2N_5O$) C, H, N.

***N*-(Piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (12).** To a magnetically stirred solution of **10** (44 mg, 0.1 mmol) in concentrated hydrochloric acid (0.5 mL) and ice (1 g) was added dropwise a 1.0 M solution of sodium nitrite (100 μL, 0.1 mmol) at 0 °C until iodine-starch paper turned blue. After being stirred at the same temperature for additional 10 min, this cold solution was transferred into a solution of potassium iodide (166 mg, 1.0 mmol) in water (0.5 mL) at room temperature, and stirring was continued for 4 h. The reaction mixture was extracted with dichloromethane (3 × 10 mL). The combined dichloromethane solution was washed with brine, dried over anhydrous sodium sulfate, filtered, and evaporated. Purification by flash column chromatography on silica gel with petroleum ether/ethyl acetate (2:1) gave the *p*-iodide **12** (22 mg, 39% yield) as a white solid: mp 195−196 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.58 (d, J = 8.2 Hz, 1H, ArH), 7.56 (s, 1H, NH), 7.37 (s, 1H, ArH), 7.38−7.18 (m, 3H, ArH), 6.78 (d, J = 8.2 Hz, 2H, ArH), 2.79 (t, J = 5.0 Hz, 4H, NCH_2), 2.29 (s, 3H, CH_3), 1.71−1.63 (m, 4H, CH_2), 1.38−1.36 (m, 2H, CH_2); MS *m/e* 554 (M^+), 455 ($M^+ - C_6H_{11}N_2O$); HRMS *m/e* calcd, 554.0137; found, 554.0121. Anal. ($C_{22}H_{21}Cl_2IN_4O$) C, H, N.

***N*-(Piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-(2-iodophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (13).** Compound **13** was obtained from **11** by the method described for compound **12** and was isolated as a white solid in 29% yield: mp 107−109 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.61−7.55 (m, 2H, ArH and NH), 7.43 (s, 1H, ArH), 7.37−7.36 (m, 1H, ArH), 7.24−7.18 (m, 2H, ArH), 6.99−6.96 (m, 2H, ArH), 2.79 (t, J = 5.2 Hz, 4H, NCH_2), 2.30 (s, 3H, CH_3), 1.74−1.60 (m, 4H, CH_2), 1.41−1.36 (m, 2H, CH_2); MS *m/e* 554 (M^+), 455 ($M^+ - C_6H_{11}N_2O$); HRMS *m/e* calcd, 554.0137; found, 554.0147. Anal. ($C_{22}H_{21}Cl_2IN_4O$) C, H, N.

***N*-(Piperidin-1-yl)-1-(2,4-dichlorophenyl)-5-ethyl-4-methyl-1*H*-pyrazole-3-carboxamide (16).** Compound **16** was obtained from ester **15** according to the procedure described for **7** and was isolated as a white solid in 88% yield: mp 151−153 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58−7.55 (m, 2H, ArH and NH), 7.41−7.40 (m, 1H, ArH), 7.32 (d, J = 8.4 Hz, 1H, ArH), 2.83 (t, J = 4.6 Hz, 4H, NCH_2), 2.46−2.45 (m, 2H, CH_2), 2.35 (s, 3H, CH_3), 1.74−1.72 (m, 4H, CH_2), 1.41 (brs, 2H, CH_2), 0.97 (t, J = 7.4 Hz, 3H, CH_3); GC-MS *m/e* 380 (M^+), 365 ($M^+ - CH_3$), 281 ($M^+ - C_5H_{11}N_2$). Anal. ($C_{18}H_{22}Cl_2N_4O$) C, H, N.

***N*-(Piperidin-1-yl)-1-(4-chlorophenyl)-5-(4-chlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (19).** Compound **19** was obtained from ester **18** according to the procedure described for **7** and was isolated as a white solid in 82% yield: mp 169−170 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (s, 1H, NH), 7.35 (d, J = 8.0 Hz, 2H, ArH), 7.30 (d, J = 8.3 Hz, 2H, ArH), 7.15 (d, J = 8.2 Hz, 2H, ArH), 7.07 (d, J = 8.1 Hz, 2H, ArH), 2.88 (t, J = 5.1 Hz, 4H, NCH_2), 2.34 (s, 3H, CH_3), 1.78−1.71 (m, 4H, CH_2), 1.46 (brs, 2H, CH_2); GC-MS *m/e* 428 (M^+), 329 ($M^+ - C_5H_{11}N_2$), 301 ($M^+ - C_6H_{11}N_2O$). Anal. ($C_{22}H_{22}Cl_2N_4O$) C, H, N.

***N*-(Pyrrolidin-1-yl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (22).** Compound **22** was obtained from 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic chloride (44 mg, 0.1 mmol), 1-aminopyrrolidine hydrochloride (25 mg, 0.2 mmol), and triethylamine (55 μL, 0.4 mmol) according to the procedure described for **7** and was isolated as a white solid in 90% yield: mp 197−198 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (s, 1H, NH), 7.45 (d, J = 8.1 Hz, 2H, ArH), 7.43 (s, 1H, ArH), 7.31−7.27 (m, 2H, ArH), 6.99 (d, J = 8.2 Hz, 2H, ArH), 3.02 (brs, 4H,

NCH_2), 2.37 (s, 3H, CH_3), 1.91 (brs, 4H, CH_2); MS *m/e* 492 (M^+), 408 ($M^+ - C_4H_8N_2$). Anal. ($C_{21}H_{19}BrCl_2N_4O$) C, H, N.

***N*-(Piperidin-1-yl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (23).** Compound **23** was obtained from ester **21** according to the procedure described for **7** and was isolated as a white solid in 96% yield: mp 106−108 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.63 (s, 1H, NH), 7.45 (dd, J = 1.8 and 8.4 Hz, 2H, ArH), 7.47−7.42 (m, 1H, ArH), 7.30−7.27 (m, 2H, ArH), 6.99 (dd, J = 1.9 and 8.5 Hz, 2H, ArH), 2.86 (t, J = 5.2 Hz, 4H, NCH_2), 2.36 (s, 3H, CH_3), 1.81−1.70 (m, 4H, CH_2), 1.45−1.41 (m, 2H, CH_2); MS *m/e* 506 (M^+), 407 ($M^+ - C_5H_{11}N_2$); HRMS *m/e* calcd, 506.0276; found, 506.0268. Anal. ($C_{22}H_{21}BrCl_2N_4O$) C, H, N.

***N*-(Homopiperidin-1-yl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (24).** Compound **24** was obtained from 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic chloride (44 mg, 0.1 mmol) and 1-aminohomopiperidine (24 μL, 0.2 mmol) according to the procedure described for **7** and was isolated as a white solid in 88% yield: mp 127−129 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.07 (s, 1H, NH), 7.45 (d, J = 8.2 Hz, 2H, ArH), 7.42 (s, 1H, ArH), 7.30−7.29 (m, 2H, ArH), 6.98 (d, J = 8.3 Hz, 2H, ArH), 3.15 (t, J = 5.3 Hz, 4H, NCH_2), 2.37 (s, 3H, CH_3), 1.74−1.72 (m, 4H, CH_2), 1.66−1.63 (m, 4H, CH_2); MS *m/e* 520 (M^+), 407 ($M^+ - C_6H_{13}N_2$). Anal. ($C_{23}H_{23}BrCl_2N_4O$) C, H, N.

***N*-(Morpholin-4-yl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (25).** Compound **25** was synthesized from 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic chloride (44 mg, 0.1 mmol) and 4-aminomorpholine (20 μL, 0.2 mmol) according to the procedure described for **7** and was isolated as a white solid in 96% yield: mp 247−249 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (s, 1H, NH), 7.45 (d, J = 8.4 Hz, 2H, ArH),7.42 (d, J = 1.9 Hz, 1H, ArH), 7.31−7.27 (m, 2H, ArH), 6.98 (d, J = 8.4 Hz, 2H, ArH), 3.84 (t, J = 4.4 Hz, 4H, NCH_2), 2.94 (t, J = 4.2 Hz, 4H, OCH_2), 2.35 (s, 3H, CH_3); MS *m/e* 508 (M^+), 407 ($M^+ - C_4H_9N_2O$), 379 ($M^+ - C_5H_9N_2O_2$). Anal. ($C_{21}H_{19}BrCl_2N_4O_2$) C, H, N.

5-(4-Bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-(1-piperidylcarboxamide) (26). Compound **26** was obtained from 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic chloride (44 mg, 0.1 mmol) and piperidine (20 μL, 0.2 mmol) according to the procedure described for **7** and was isolated as a white solid in 96% yield: mp 142−144 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (d, J = 8.2 Hz, 2H, ArH), 7.44 (s, 1H, ArH), 7.26−7.24 (m, 1H, ArH), 7.17 (d, J = 8.3 Hz, 1H, ArH), 7.00 (d, J = 7.9 Hz, 2H, ArH), 3.75−3.67 (m, 4H, NCH_2), 2.18 (s, 3H, CH_3), 1.68 (brs, 4H, CH_2), 1.63−1.61 (m, 2H, CH_2); GC-MS *m/e* 491 (M^+), 407 ($M^+ - C_5H_{10}N$), 379 ($M^+ - C_6H_{10}NO$). Anal. ($C_{22}H_{20}BrCl_2N_3O$) C, H, N.

5-(4-Bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-(1-(4-methyl)piperazylcarboxamide) (27). Compound **27** was obtained from 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic chloride (44.0 mg, 0.1 mmol) and 1-methylpiperazine (11 μL, 0.2 mmol) according to the procedure described for **7** and was isolated as a semisolid in 98% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (d, J = 8.4 Hz, 2H, ArH), 7.45 (s, 1H, ArH), 7.25 (d, J = 8.5 Hz, 1H, ArH), 7.16 (d, J = 8.3 Hz, 1H, ArH), 7.00 (d, J = 8.2 Hz, 2H, ArH), 3.85 (t, J = 4.9 Hz, 4H, NCH_2), 2.51−2.44 (m, 4H, CH_2), 2.33 (s, 3H, CH_3), 2.20 (s, 3H, CH_3); GC-MS *m/e* 506 (M^+), 407 ($M^+ - C_5H_{11}N_2$). Anal. ($C_{22}H_{21}BrCl_2N_4O$) C, H, N.

***N*-Cyclohexyl-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (28).** Compound **28** was obtained from 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic chloride (44 mg, 0.1 mmol) and cyclohexylamine (23 μL, 0.2 mmol) according to the procedure described for **7** and was isolated as a semisolid in 86% yield: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (d, J = 8.2 Hz, 2H, ArH), 7.43 (s, 1H, NH), 7.31−7.28 (m, 2H, ArH), 6.99 (d, J = 8.3 Hz, 2H, ArH), 6.81 (d, J = 8.0 Hz, 1H, ArH), 3.96−3.94 (m, 1H, CH), 2.37 (s, 3H, CH_3), 2.01 (dm, J = 10.3 Hz, 2H, CH_2), 1.75 (dm, J = 12.2 Hz, CH_2), 1.64 (dm, J = 12.7 Hz, 1H, C*H*H), 1.42−1.37 (m, 2H, CH_2), 1.29−1.17 (m, 3H, CH_2); GC-MS *m/e* 505 (M^+), 407 ($M^+ - C_6H_{12}N$). Anal. ($C_{23}H_{22}BrCl_2N_3O$) C, H, N.

***N*-Cyclohexyl-*N*-methyl-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (29).** Compound **29** was obtained from 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic chloride (44 mg, 0.1 mmol) and *N*-methylcyclohexylamine (26 μL, 0.2 mmol) according to the procedure described for **7** and was isolated as a semisolid in 64% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (s, 1H, ArH), 7.45 (d, J = 7.6 Hz, 2H, ArH), 7.26−7.12 (m, 2H, ArH), 7.01 (t, J = 7.2 Hz, 2H, ArH), 4.58 and 4.07−4.03 (brs and m, 1H, CH), 3.03 (d, J = 25.9 Hz, 3H, CH_3), 2.17 (s, 3H, CH_3), 1.82−1.68 (m, 4H, CH_2), 1.63−1.57 (m, 4H, CH_2), 1.26−1.06 (m, 2H, CH_2); GC-MS *m/e* 521 ($M^+ + 2$), 407 ($M^+ - C_7H_{14}N$). Anal. ($C_{24}H_{24}BrCl_2N_3O$) C, H, N.

***N*-(2-Hydroxyethyl)-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (30).** Compound **30** was obtained from 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic chloride (100 mg, 0.23 mmol) and ethanolamine (27 μL, 0.45 mmol) according to the procedure described for **7** and was isolated as a white solid in 23% yield: mp 89−90 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (d, J = 8.2 Hz, 2H, ArH), 7.43 (s, 1H, NH), 7.34−7.26 (m, 3H, ArH), 6.99 (d, J = 8.1 Hz, 2H, ArH), 3.84−3.83 (m, 2H, NCH_2), 3.60 (t, J = 4.9 Hz, 2H, OCH_2), 2.92 (brs, 1H, OH), 2.37 (s, 3H, CH_3); MS *m/e* 467 (M^+), 425 ($M^+ - C_2H_5O$), 407 ($M^+ - C_2H_6NO$). Anal. ($C_{19}H_{16}BrCl_2N_3O_2$) C, H, N.

***N*-Phenyl-5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (31).** Compound **31** was obtained from 5-(4-bromophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxylic chloride (44 mg, 0.1 mmol) and aniline (18 μL, 0.2 mmol) according to the procedure described for **7** and was isolated as a white solid in 65% yield: mp 149−150 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.76 (s, 1H, NH), 7.68 (d, J = 7.8 Hz, 2H, ArH), 7.47 (d, J = 8.6 Hz, 2H, ArH), 7.46 (s, 1H, ArH), 7.37−7.30 (m, 4H, ArH), 7.12 (t, J = 7.2 Hz, 1H, ArH), 7.02 (d, J = 8.0 Hz, 1H, ArH), 2.43 (s, 3H, CH_3); MS *m/e* 501 ($(M + 2)^+$), 499 (M^+), 409 ($(M + 2)^+ - C_6H_6N$); 407 ($M^+ - C_6H_6N$). Anal. ($C_{23}H_{16}BrCl_2N_3O$) C, H, N.

Receptor Binding Assay. For CB1 receptor binding studies, rat forebrain membranes were prepared following earlier procedure.[28,29] For CB2 receptor binding studies, membranes were prepared from frozen mouse spleen according to Dodd.[30] Silanized centrifuge tubes were used throughout to minimize receptor loss due to the adherent properties of the CB2 containing macrophages.[28] The binding of the novel probes to the cannabinoid receptors was assessed as previously described.[28,31] Briefly, approximately 50 μg of rat forebrain or mouse spleen membranes were incubated in silanized 96-well microtiter plate with TME containing 0.1% essentially fatty acid free bovine serum albumin (BSA), 0.8 nM [^{3}H] **2**, and various concentrations of the synthesized cannabinoid ligands in a final volume of 200 μL. The assays were incubated for 1 h at 30 °C and then immediately filtered on Unifilter GF/B filterplates using a Packard Filtermate 196 harvester, followed by four washes with ice cold wash buffer containing 0.5% BSA. Radioactivity was detected by adding MicroScint 20 scintillation cocktail directly to the dried filterplates which were counted using a Packard instruments TopCount Microplate Scintillation Counter. Nonspecific binding was assessed using 100 nM **2**. Data collected from three independent experiments performed with duplicate determinations were normalized between 100% and 0% specific binding for [^{3}H] **2**. The normalized data were then analyzed using a four-parameter nonlinear logistic equation to yield IC_{50} values. The IC_{50} values from three independent experiments were combined and converted to K_i values using the assumptions of Cheng and Prusoff.[32]

Pharmacological Studies in Vitro. The in vitro pharmacology of all compounds was investigated using the myenteric plexus-longitudinal muscle preparation of guinea pig small intestine and the isolated mouse vas deferens assays. For both these preparations, the measured response was

3-induced inhibition of electrically evoked contractions.[28,29] All drugs were mixed with two parts of Tween 80 by weight and dispersed in a 0.9% aqueous solution of NaCl (saline). The size of the maximal responses to **3** (E_{max} values) and their 95% confidence limits were calculated by nonlinear regression analysis using GraphPAD InPlot (GraphPAD Software, San Diego). The K_d values of antagonists were calculated using the equation $(x - 1) = B/K_d$, where x (the "dose ratio") is the concentration of **3** that produces a particular degree of inhibition in the presence of the antagonist at a concentration, B, divided by the concentration of **3** that produces an identical degree of inhibition in the presence of Tween 80.[28,29,33,34] The dose ratios and their 95% confidence limits have been determined by symmetrical (2 + 2) dose parallel line assay,[24] using responses to pairs of **3** concentrations located on the steepest part of each log concentration−response curve. In none of these assays did pairs of log concentration−response curves show significant deviation from parallelism ($P > 0.05$).

Acknowledgment. This work was supported by NIDA Grants DA9158, DA-3801, DA-152, and DA-7215 from the National Institute on Drug Abuse and a Wellcome Trust Grant 039538. We thank Dr. L. S. Melvin and Pfizer, Inc., for providing us with CP-55,-940 and Packard Instruments Inc. for providing the Filtermate 196 and Top-Count.

References

(1) Segal, M. Cannabinoids and Analgesia. In *Cannabinoids as Therapeutic Agents*; Mechoulam, R., Ed.; CRC Press: Boca Raton, FL, 1986; p 105−120.
(2) Pertwee, R. G. Pharmacology of Cannabinoid CB1 and CB2 Receptors. *Pharmacol. Ther.* **1997**, *74*, 129−180.
(3) Pertwee, R. G. The Central Neuropharmacology of Psychotropic Cannabinoids. *Pharmacol. Ther.* **1988**, *36*, 189−261.
(4) Hollister, L. E. Health Aspects of Cannabis. *Pharmacol. Rev.* **1986**, *38*, 1−20.
(5) Dewey, W. L. Cannabinoid Pharmacology. *Pharmacol. Rev.* **1986**, *38*, 151−178.
(6) Razdan, R. K.; Howes, J. F. Drugs Related to Tetrahydrocannabinol. *Med. Res. Rev.* **1983**, *3*, 119−146.
(7) Makriyannis, A.; Rapaka, R. S. The Molecular Basis of Cannabinoid Activity. *Life Sci.* **1990**, *47*, 2173−2184.
(8) Devane, W. A.; Dysarz, F. A., III; Johnson, M. R.; Melvin, L. S.; Howlett, A. C. Determination and Characterization of a Cannabinoid Receptor in Rat Brain. *Mol. Pharmacol.* **1988**, *34*, 605−613.
(9) Munro, S.; Thomas, K. L.; Abu-Shaar, M. Molecular Characterization of a Peripheral Receptor for Cannabinoids. *Nature (London)* **1993**, *365*, 61−65.
(10) Devane, W. A.; Hanus, L.; Breuer, A.; Pertwee, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. Isolation and Structure of a Brain Constituent that Binds to the Cannabinoid Receptor. *Science* **1992**, *258*, 1946−1949.
(11) Pertwee, R. G.; Griffin, G.; Fernando, S.; Li, X.; Hill, W. A. G.; Makriyannis, A. AM630, A Competitive Cannabinoid Receptor Antagonist. *Life Sci.* **1995**, *56*, 1949−1955.
(12) Barth, F.; Casellas, P.; Congy, C.; Martinez, S.; Rinaldi, M.; Anne-Archard, G. Pyrazole Derivatives, Method of Preparing them and Pharmaceutical Compositions in which they Are Present. US Patent 5,624,941, April 29, 1997.
(13) Rinaldi-Carmona, M.; Barth, F.; Héaulme, M.; Shire, D.; Calandra, B.; Congy, C.; Martinez, S.; Maruani, J.; Néliat, G.; Caput, D.; Ferrara, P.; Soubrié, P.; Breliere, J. C.; Le Fur, G. SR141716A, A Potent and Selective Antagonist of the Brain Cannabinoid Receptor. *FEBS Lett.* **1994**, *350*, 240−244.
(14) Rinaldi-Carmona, M.; Barth, F.; Héaulme, M.; Alonso, R.; Shire, D.; Congy, C.; Soubrié, P.; Breliere, J. C.; Le Fur, G. Biochemical and Pharmacological Characterization of SR141716A, The First Potent and Selective Brain Cannabinoid Receptor Antagonist. *Life Sci.* **1995**, *56*, 1941−1947.
(15) Shire, D.; Calandra, B.; Delpech, M.; Dumont, X.; Kaghad, M.; Le Fur, G.; Caput, D.; Ferrara, P. Structural Features of the Central Cannabinoid CB1 Receptor Involved in the Binding of the Specific CB1 Antagonist SR 141716A. *J. Biol. Chem.* **1996**, *271*, 6941−6946.
(16) Rinaldi-Carmona, M.; Pialot, F.; Congy, C.; Redon, E.; Barth, F.; Bachy, A.; Breliere, J. C.; Soubrié, P.; Le Fur, G. Characterization and Distribution of Binding Sites for [3H]-SR 141716A, a Selective Brain (CB1) Cannabinoid Receptor Antagonist, in Rodent Brain. *Life Sci.* **1996**, *58*, 1239−1247.
(17) Rinaldi-Carmona, M.; Barth, F.; Millan, J.; Derocq, J. M.; Casellas, P.; Congy, C.; Oustric, D.; Sarran, M.; Bouaboula, M.; Calandra, B.; Portier, M.; Shire, D.; Breliere, J. C.; Le Fur, G. SR144528, The First Potent and Selective Antagonist of the CB2 Cannabinoid Receptor. *J. Pharmacol. Exp. Ther.* **1998**, *284*, 644−650.
(18) Lan, R.; Gatley, S. J.; Gifford, A. N.; Volkow, N. D.; Hill, W. A. G.; Makriyannis, A. Iodine-123 Labeled AM251: A Radioligand which Binds *In Vivo* to Mouse Brain Cannabinoid Receptors. *1996 Symposium on Cannabis and the Cannabinoids*, 1996, p76.
(19) Fernando, S. R.; Stevenson, L. A.; Hill, W. A. G.; Lan, R.; Makriyannis, A.; Pertwee, R. Structure−Activity Relationships of Some Novel Analogues of SR141716A. 1996 *Symposium on Cannabis and the Cannabinoids*, 1996, p22.
(20) Lan, R.; Gatley, S. J.; Makriyannis, A. Preparation of Iodine-123 Labeled AM251: A Potential SPECT Radioligand for the Brain Cannabinoid CB1 Receptor. *J. Label. Compds. Radiopharma.* **1996**, *38*, 875−881.
(21) Olah, G. A.; Malhotra, R.; Narang, S. C. *Nitration: Methods and Mechanisms*; VCH Publishers: New York, 1989.
(22) Balcom, D.; Furst, A. Reductions with Hydrazine Hydrate Catalyzed by Raney Nickel. I. Aromatic Nitro Compounds to Amines. *J. Am. Chem. Soc.* **1953**, *75*, 4334.
(23) Clive, D. L. J.; Angoh, A. G.; Bennett, S. M. Radical Spirocyclization: Synthesis of an Appropriately Oxygenated Spiro Compound Related to the Antitumor Antibiotic Fredericamycin A. *J. Org. Chem.* **1987**, *52*, 1339−1342.
(24) Colquhoun, D. *Lectures on Biostatistics*; Oxford University Press: Oxford, 1971.
(25) Pertwee, R. G.; Fernando, S. R.; Nash, J. E.; Coutts, A. A. Further Evidence for the Presence of Cannabinoid CB1 Receptors in Guinea-Pig Small Intestine. *Br. J. Pharmacol.* **1996**, *118*, 2199−2205.
(26) Pertwee, R. G.; Griffin, G.; Lainton, J. A.; Huffman, J. W. Pharmacological Characterization of Three Novel Cannabinoid Receptor Agonists in the Mouse Isolated *Vas Deferens*. *Eur. J. Pharmacol.* **1995**, *284*, 241−247.
(27) 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. Imaging the Brain Marijuana Receptor: Development of a Radioligand that Binds to Cannabinoid CB1 Receptors *In Vivo*. *J. Neurochem.* **1998**, *70*, 417−423.
(28) Drake, D. J.; Jensen, R. S.; Busch-Petersen, J.; Kawakami, J. K.; Fernandez-Garcia, M. C.; Fan, P.; Makriyannis, A.; Tius, M. A. Classical/Nonclassical Hydrid Cannabinoids: Southern Aliphatic Chain-Functionalized C-6β Methyl, Ethyl, and Propyl Analogues. *J. Med. Chem.* **1998**, *41*, 3596−3608.
(29) Abadji, V.; Lin, S.; Taha, G.; Griffin, G.; Stevenson, L. A.; Pertwee, R. G.; Makriyannis, A. (R)-Methanandamide: A Chiral Novel Anandamide Possessing Higher Potency and Metabolic Stability. *J. Med. Chem.* **1994**, *37*, 1889−1893.
(30) Dodd, P. R.; Hardy, J. A.; Oakley, A. E.; Edwardson, J. A.; Perry, E. K.; Delaunoy, J. P. A Rapid Method for Preparing Synaptosomes: Comparison, with Alternative Procedures. *Brain Res.* **1981**, *226*, 107−118.
(31) Charalambous, A.; Guo, Y.; Houston, D. B.; Howlett, A. C.; Compton, D. R.; Martin, B. R.; Makriyannis, A. 5′-Azido-Δ^8-THC: A Novel Photoaffinity Label for the Cannabinoid Receptor. *J. Med. Chem.* **1992**, *35*, 3076−3079.
(32) Cheng, Y.; Prusoff, W. H. Relationship between the Inhibition Constant (K_i) and the Concentration of Inhibitor which Causes 50% (IC_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.
(33) Coutts, A. A.; Pertwee, R. G. Inhibition by Cannabinoid Receptor Agonists of Acetylcholine Release from the Guinea-Pig Myenteric Plexus. *Br. J. Pharmacol.* **1997**, *121*, 1557−1566.
(34) Tallarida, R. J.; Cowan, A.; Adler, M. W. pA2 and Receptor Differentiation: A Statistical Analysis of Competitive Antagonism. *Life Sci.* **1979**, *25*, 637−654.

JM980363Y