Notes

Synthesis and Oral Efficacy of a 4-(Butylethylamino)pyrrolo[2,3-*d*]pyrimidine: A Centrally Active Corticotropin-Releasing Factor$_1$ Receptor Antagonist

Yuhpyng L. Chen,* Robert S. Mansbach, Steven M. Winter, Elizabeth Brooks, Judith Collins, Michael L. Corman, Audrey R. Dunaiskis, W. Stephen Faraci, Randall J. Gallaschun, Anne Schmidt, and David W. Schulz

Medicinal Chemistry and Neuroscience Departments, Central Research Division, Pfizer Inc., Groton, Connecticut 06340

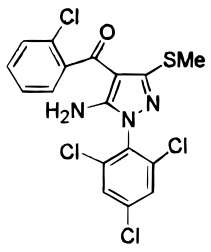
Received December 23, 1996[⊗]

The syntheses of a centrally active nonpeptide CRF_1 receptor antagonist **2**, butylethyl[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (CP-154,526), and its analogs **11**−**14** and [^{3}H]-**2** are reported. The *in vitro* CRF_1 receptor binding affinity in the series **2**, the pharmacokinetic properties of **2** in rats, and the anxiolytic-like effects of orally administered **2** are presented.

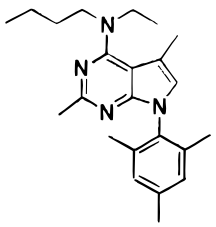
Introduction

Corticotropin-releasing factor (CRF), a 41-amino acid neuropeptide hormone, coordinates the overall response of the body to stress through the release of adrenocorticotropic hormone (ACTH). CRF has been shown to mediate stress-induced changes in the autonomic nervous system, neuroendocrine functions, and behavior.[1] Receptors for CRF are distributed throughout the central and peripheral nervous systems. Receptor subtypes CRF_1 and CRF_2 have been cloned and expressed from mouse, rat, and human.[2−6] Clinical data indicate that patients with depression and posttraumatic stress disorder show significantly elevated concentrations of CRF in cerebrospinal fluid when compared to normal controls.[7] In addition, patients with depression, anxiety, anorexia nervosa, and posttraumatic stress disorder showed blunted ACTH response to intravenous CRF,[8] indicating their CRF receptors may be down-regulated possibly due to chronic hypersecretion of CRF. A CRF antagonist may represent a novel agent for the treatment of anxiety, depression, and stress-related diseases. Several peptide antagonists including α-helical CRF_{9-41}[9] have been discovered and studied extensively. However, a peptide antagonist would not be expected to penetrate the blood−brain barrier, thereby limiting its clinical utility. Intensive research has been focused on seeking nonpeptide CRF receptor antagonists. Several nonpeptide CRF_1 receptor antagonists were published very recently.[10] Herein we describe the discovery of a centrally active nonpeptide CRF_1 receptor antagonist **2**, butylethyl[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]-amine (CP-154,526), and its oral efficacy in an animal model of anxiety.

An initial weak pyrazole lead (**1**)[11] was identified from a high-throughput CRF receptor binding screen. After substantial structural modifications, a structurally distinct pyrazolo[3,4-*d*]pyrimidine series[12] was identified. Replacement of the pyrazolo ring with a pyrrolo ring resulted in a more basic pyrrolo[2,3-*d*]pyrimidine series[13] with increased solubility at pH 2.0. Subsequent research on structure-activity relationships in the pyrrolo[2,3-*d*]pyrimidine series focused on improving potency, brain penetrability, and oral bioavailability, leading to the discovery of compound **2**, which is centrally and orally active. As described in our previous publication,[14] compound **2** has a K_i of 2.7 nM against [^{125}I]Tyr^0-o-CRF (ovine CRF) binding to the CRF_1 receptor and >10 μM against [^{125}I]Tyr^0-sauvagine binding to the CRF_2 receptor. It blocks CRF-induced elevation of ACTH and antagonizes CRF-induced excitation of locus coeruleus neurons. In addition **2** demonstrates efficacy in anxiolytic[14,15] and antidepressant[16] models after intraperitoneal (ip) administration, indicating the therapeutic potential of a CRF_1 receptor antagonist. Herein we report the syntheses of **2** and its derivatives **11**−**14** and [^{3}H]-**2**. The *in vitro* CRF_1 receptor binding affinity of analogs in the series **2**, the pharmacokinetic properties of **2**, and the anxiolytic-like effects of orally administered **2** are presented.



1



2
(CP-154,526)

Chemistry

Synthesis of **2** is illustrated in Scheme 1. Knoevenagel condensation of malononitrile and excess acetone in refluxing toluene or benzene gave **3**[17] in 70% yield after distillation. Bromination of **3** was attempted under various conditions. All reaction conditions tried provided the desired monobromo **4**[18] as a major component contaminated with undesired dibromo derivative **5** and starting material. The best conditions use 1−1.3 equiv

* Corresponding author: phone, 860-441-4208; fax, 860-441-4111; e-mail, yuhpyng_l_chen@groton.pfizer.com.

⊗ Abstract published in *Advance ACS Abstracts,* May 1, 1997.

S0022-2623(96)00861-8 CCC: $14.00

Scheme 1

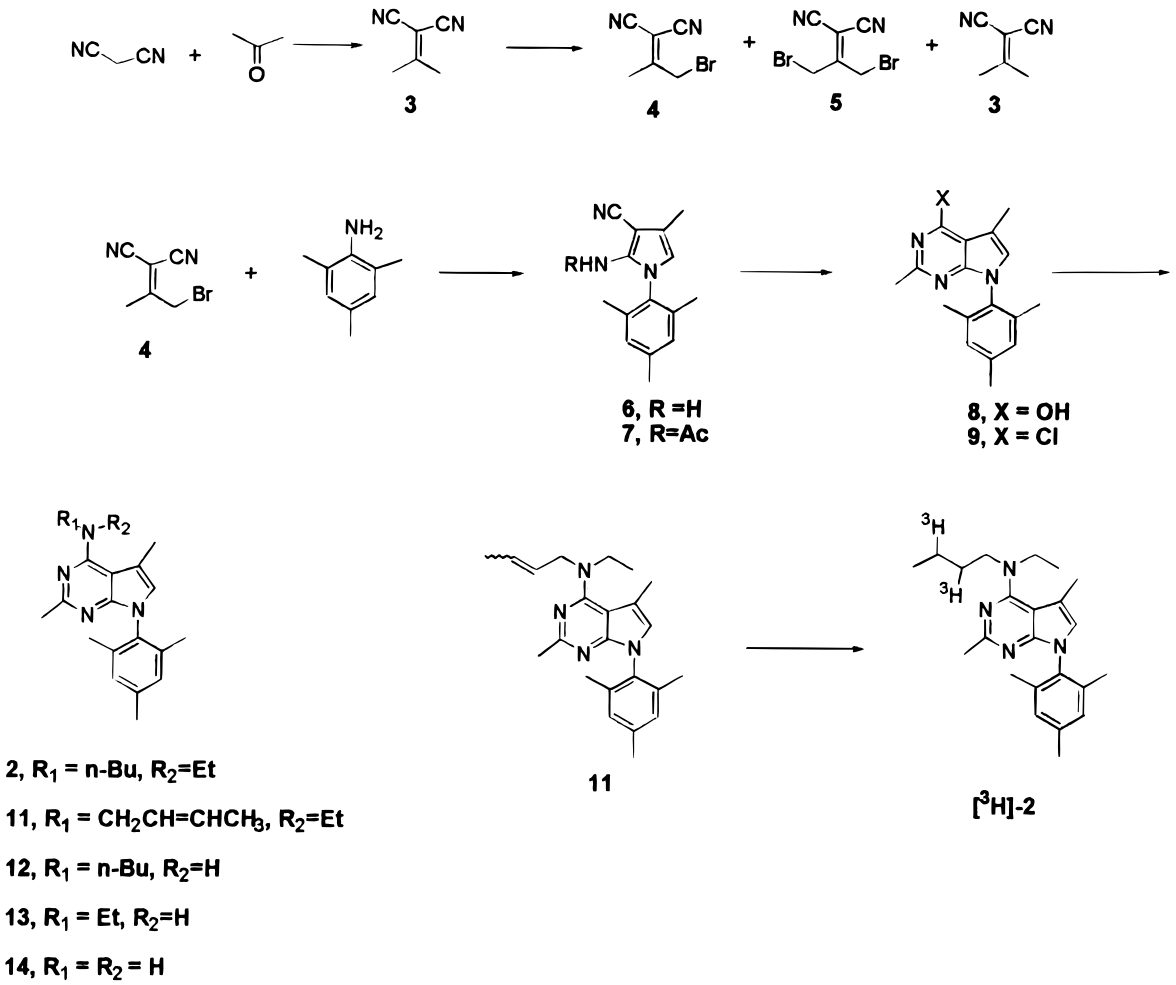


Table 1. Inhibition of Binding of 70 pM [^{125}I]Tyr^0-o-CRF to Rat Brain Membranes

compd	R_1	R_2	pIC_{50} (mean ± SEM)[a]	IC_{50} (nM)
2 (CP-154,526)	*n*-Bu	Et	8.26 ± 0.02	5.5
11	$CH_2-CH=CH-CH_3$	Et	8.12 ± 0.08	7.6
12	*n*-Bu	H	6.94 ± 0.16	110
13	Et	H	6.21 ± 0.08	620
14	H	H	<5	>10000

[a] Geometric means are of at least three experimental determinations.

of *N*-bromosuccinimide in the presence of a catalytic amount of benzoyl peroxide in chloroform. Reaction of **4** (76% pure) with trimethylaniline at room temperature afforded the desired aminopyrrole intermediate **6** as a purple solid that was purified through silica gel column chromatography to give a 48% yield of tan crystals, mp 138−139 °C. Acetylation of aminopyrrole **6** followed by acid hydrolysis and cyclization in 85% phosphoric acid afforded 4-hydroxypyrrolopyrimidine derivative **8** in 81% overall yield. Reaction of **8** with phosphorous oxychloride at reflux gave the corresponding 4-chloro derivative **9** in 79% yield. Coupling **9** with *n*-butylethylamine in dimethyl sulfoxide at 130 °C gave **2** in 88% yield as a tan solid, mp 132−137 °C. The corresponding HCl salt was prepared as white crystals, mp 169−170 °C . The corresponding analogs **11**−**14** were prepared in a similar manner.

[^{2}H]CP-154,526 was synthesized by reduction of the precursor **11** with deuterium in the presence of 10% Pd/C in ethyl acetate at atmospheric pressure. [^{3}H]CP-154,526 was prepared in a similar way with 60 Ci/mmol specific activity.

Biological Results

Table 1 lists the *in vitro* CRF_1 binding affinity in rat cortex against radioligand [^{125}I]Tyr^0-o-CRF by compounds **2** and **11**−**14**. As shown, both 4-disubstituted amino analogs **2** and **11** showed potent CRF_1 binding affinity. The monosubstituted amino analogs **12** and **13** had reduced potency, and the amino analog **14** is inactive. Compound **2** was selected for anxiolytic-like activity studies after oral administration in rats.

Previously we reported the activity of **2** in fear-potentiated startle after ip administration.[13] In this report we present efficacy data after oral administration. The paradigm for the fear-potentiated startle experiments is similar to that described previously. In the training session, rats received light paired with electric shock. In this way, the light acquired conditioned aversive properties. Three days later, the acoustic startle session was performed in the presence and absence of light without electric shock. At the oral dose of 17.8 mg/kg, compound **2** significantly attenuated the enhancement of startle amplitude induced by the light-

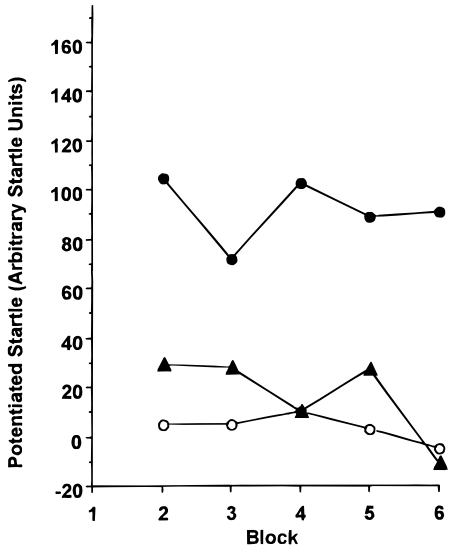


Figure 1. Effect of compound **2** (17.8 mg/kg, po, 60 min prior to testing) on potentiated startle in rats. Animals were exposed to 108 dB[A] acoustic stimuli, some of which were presented in darkness and others in the presence of a conditioned stimulus (electric light, 25 W) formerly paired with brief electric shock. Data are expressed as the absolute difference in startle amplitude between light-paired and light-unpaired startle trials, over 5 blocks of 10 trials each. The initial block consisted of light-unpaired trials only and was presented to eliminate the rapid phase of startle habituation. Controls (no shock history/vehicle) received previous exposure to light without the accompanying shock. Compound **2** significantly reduced fear-potentiated startle without affecting baseline startle magnitude ($n = 12$): (○) no shock history/vehicle, (●) vehicle, and (▲) compound **2**.

conditioned stimulus to that of vehicle controls as shown in Figure 1.

Pharmacokinetics in Rat. The pharmacokinetics of **2** were determined in male Sprague−Dawley rats following an intravenous (iv) dose of 5 mg/kg ($n = 4$) and an oral (po) dose of 10 mg/kg ($n = 4$). Following iv administration, drug concentrations declined over time in a biphasic manner (Figure 1). The pharmacokinetics of **2** were characterized by a high plasma clearance (CL 82 mL/min/kg) and a large volume of distribution (V_{dss} 6.7 L/kg), resulting in an elimination half-life of 1.5 h. Following oral administration in DMSO/emulphor/water (5:5:90, v/v/v) the mean maximal plasma concentration (C_{max}) of drug was 367 ng/mL and occurred at 0.5−1 h following dose administration (Figure 2). Oral bioavailability of **2** at this dose was estimated to be 37%. Assuming that blood concentrations are similar to those measured in plasma, CL exceeds hepatic blood flow. An oral bioavailability of 37% suggests that hepatic extraction of **2** was ≤63% when administered orally at 10 mg/kg in DMSO/emulphor/water (5:5:90, v/v/v).

Conclusions

After substantial structural modifications of the initial pyrazole lead **1** aimed at improving potency, pharmacokinetics, and physicochemical properties, we have discovered a centrally and orally active CRF_1 antagonist (**2**). Compound **2** not only shows potent CRF_1 binding affinity but also demonstrates oral efficacy in animals. An orally bioavailable CRF_1 receptor antagonist, such as **2**, may be a therapeutic alternative to current antidepressant and anxiolytic drugs. Details of structure−activity relationships will be reported later.

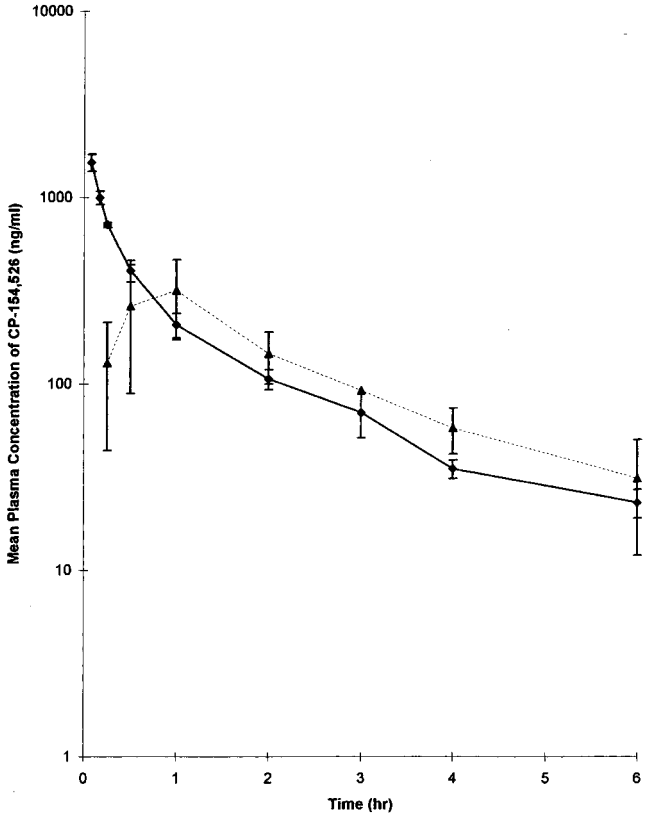


Figure 2. Mean plasma concentrations of compound **2** in Sprague−Dawley rats administered a bolus intravenous dose at 5 mg/kg (◆, solid line) or an oral dose at 10 mg/kg (▲, dotted line). Data represent mean ± SD from four animals.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. High-field ^{1}H NMR spectra were recorded on a Varian XL-300, Bruker AM 250, or Bruker AM 300 instrument. Elemental analyses were carried out by Mr. J. W. Greene, Analytical Department, Pfizer Inc., or by Schwarzkopf Microanalytical, Woodside, NY.

Isopropylidenemalononitrile (3). A mixture of malononitrile (90 g, 1.362 mol), acetone (400 mL), sodium acetate (25 g, 0.3 mol), acetic acid (18 mL), and benzene (400 mL) was heated at reflux using a Dean−Stark trap for 4 h. The reaction mixture was concentrated to dryness. The reaction was quenched with water and the residue extracted with ethyl acetate. The organic layer was separated, dried, and concentrated to give a light yellow oil, which was distilled at 70 °C/1 mmHg to give 101.34 g (70.1%) of the title compound: ^{1}H NMR ($CDCl_3$) δ 2.24 (s, 6H).

2-(2-Bromo-1-methylethylidene)malononitrile (4). A mixture of isopropylidenemalononitrile (30.000 g, 282.68 mmol), *N*-bromosuccinimide (65.410 g, 367.48 mmol), and benzoyl peroxide (0.600 g, 2.48 mmol) in 90 mL of chloroform was heated at reflux for 15 h. The reaction mixture was allowed to cool and filtered. The collected solid was washed with chloroform. The filtrate was concentrated to dryness to give a brown oil which showed a mixture of **4**, **5**, and **3** with a ratio of 67%, 15%, and 18%, respectively. The product was fractionated: bp 95−98 °C at 0.6 mmHg, to give 40.21 g (77%) of light oil which showed a mixture of **4**, **5**, and **3** with a ratio of 76%, 12%, and 12%, respectively, according to the H NMR spectrum. The material was used directly for the next reaction step: ^{1}H NMR ($CDCl_3$) δ 4.38 (s, 0.12 × 4H), 4.10 (s, 0.76 × 2H), 2.40 (s, 0.76 × 3H), 2.24 (s, 0.12 × 6H).

2-Amino-4-methyl-1-(2,4,6-trimethylphenyl)-1*H*-pyrrole-3-carbonitrile (6). A mixture of 2-(2-bromo-1-methylethylidene)malononitrile (76% pure, 20.000 g, 91.24 mmol) and 2,4,6-trimethylaniline (12.330 g, 91.24 mmol) in 40 mL of 2-propanol was stirred at room temperature for 15 h. The reaction mixture was concentrated to dryness and diluted with

chloroform and water. The chloroform layer was neutralized with dilute sodium hydroxide, washed with brine, separated, dried, and concentrated to give 33.000 g of a dark brown oily solid. The solid was purified through silica gel column chromatography using chloroform as eluent to give 9.35 g (47.5%) of **6** as an orange-yellow solid. The solid was recrystallized from ethanol to give beige crystals: mp 138−139 °C; ^{1}H NMR ($CDCl_3$) δ 6.95 (s, 2H), 5.8 (s, 1H), 3.75 (brs, 2H), 2.35 (s, 3H), 2.15 (s, 3H), 2.0 (s, 6H). Anal. Calcd for $C_{15}H_{17}N_3$: C, 75.28; H, 7.16; N, 17.56. Found: C, 74.85; H, 6.92; N, 17.59.

***N*-[3-Cyano-4-methyl-1-(2,4,6-trimethylphenyl)-1*H*-pyrrol-2-yl]acetamide (7).** A mixture of **6** (3.200 g, 12.54 mmol) and acetic anhydride (1.410 g, 13.82 mmol) in 3 mL of acetic acid was refluxed for 45 min. The reaction mixture was concentrated to dryness. The residue was treated with ice water, neutralized, and extracted with ethyl acetate. The organic layer was washed with brine, dried, and concentrated to give 3.71 g of **7** as a brown foam. The brown foam was used directly for the next reaction step. A small portion of the crude material was purified through column chromatography to give a pink glassy foam: ^{1}H NMR ($CDCl_3$) δ 6.9 (s, 2H), 6.8 (brs, 1H, NH), 6.2 (s, 1H), 2.32 (s, 3H), 2.2 (s, 3H), 1.95 (s, 6H). Anal. Calcd. for $C_{17}H_{19}N_3O$: C, 72.57; H, 6.81; N, 14.93. Found: C, 72.79; H, 6.64; N, 14.98.

2,5-Dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ol (8). A suspension of the crude compound of **7** (9.820 g, 34.90 mmol) in 17 mL of 85% phosphoric acid was immersed in an oil bath preheated to 130 °C for 30 min. The reaction mixture was cooled, poured onto ice water and extracted with chloroform. The organic layer was washed with brine, dried, filtered, and concentrated to give 7.97 g (81.1%) of **8** as a brown solid. The solid was used directly for the next reaction step. The solid was recrystallized from a mixture of ethanol and water to give brown crystals: ^{1}H NMR ($CDCl_3$) δ 6.95 (s, 2H), 6.42 (s, 1H), 2.45 (s, 3H), 2.41 (s, 3H), 2.32 (s, 3H), 1.92 (s, 6H). Anal. Calcd for $C_{17}H_{19}N_3O$: C, 72.57; H, 6.81; N, 14.93. Found: C, 72.24; H, 7.01; N, 14.89.

4-Chloro-2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (9). A mixture of 2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ol (7.800 g, 27.72 mmol) and $POCl_3$ (10 mL) was heated at reflux for 2.5 h and cooled. The reaction mixture was poured into ice−water and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous sodium sulfate, and concentrated to dryness to give **9** as a tan solid which was purified via silica gel column chromatography using chloroform as eluent to give 6.600 g (79.4%) of **9** as an off-white solid: mp 108−110 °C; ^{1}H NMR ($CDCl_3$) δ 7.00 (s, 2H), 6.78 (s, 1H), 2.65 (s, 3H), 2.50 (s, 3H), 2.35 (s, 3H), 1.90 (s, 6H). Anal. Calcd for $C_{17}H_{18}ClN_3$: C, 68.11; H, 6.05; H, 14.02. Found: C, 67.85; H, 5.90; N, 13.75.

Butyl[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]ethylamine (2). A mixture of **9** (6.600 g, 22.01 mmol) and *N*-butylethylamine (11.130 g, 110.05 mmol) in 27 mL of DMSO was heated at 130 °C for 3 h. The reaction was quenched with water and the mixture extracted with ethyl acetate. The organic layer was washed with brine, dried, filtered, and concentrated to give 9.1 g of brown oil. The oil was purified via silica gel column chromatography using 2% methanol in chloroform as eluent to give 7.040 g (88%) of **2** as an oil, which crystallized out as a tan solid upon standing: mp 132−137 °C; ^{1}H NMR ($CDCl_3$) δ 6.95 (s, 2H), 6.55 (s, 1H), 3.66 (q, 2H), 3.58 (t, 2H), 2.46 (s, 3H), 2.40 (s, 3H), 2.30 (s, 3H), 1.92 (s, 6H), 1.60−1.70 (m, 2H), 1.35 (m, 2H), 1.23 (t, 3H), 0.90 (t, 3H).

The corresponding HCl salt was prepared by adding 1.5 equiv of 1 M HCl in methanol followed by evaporation of solvent to give a white solid. The solid was recrystallized from ethyl acetate: mp 169−170 C; ^{1}H NMR ($CDCl_3$) δ 14.45 (brs, 1H), 7.09 (s, 2H), 6.78 (s, 1H), 3.80−3.94 (m, 4H), 2.85 (s, 3H), 2.40 (s, 3H), 2.30 (s, 3H), 1.90 (s, 6H), 1.70 (m, 2H), 1.40 (m, 2H), 1.32 (t, 3H), 0.92 (t, 3H); IR (KBr) 2922, 2868, 1609, 1489 cm^{-1}. Anal. Calcd for $C_{23}H_{32}N_4$·HCl: C, 68.89; H, 8.30; N, 13.97. Found: C, 69.23; H, 8.34; N, 14.04.

***N*-(2-Butenyl)ethylamine (10).** To a −78 °C solution of crotyl bromide (3.000 g, 22.22 mmol) in 15 mL of dry methylene chloride was added ethylamine (30 mL). The reaction mixture was allowed to warm to room temperature for 2 h. Excess ethylamine was evaporated to give 3.2 g of a yellow oil. The oil was fractionated to give 1.32 g (60%) of **10** as a clear liquid: bp 110−120 °C; ^{1}H NMR ($CDCl_3$) δ 5.4−5.6 (m, 2H), 3.1−3.3 (m, 2H), 2.6 (m, 2H), 1.6−1.7 (m, 3H), 1.0−1.2 (m, 3H).

But-2-enyl[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]ethylamine (11). A mixture of **9** (0.500 g, 1.67 mmol) and *N*-(2-butenyl)ethylamine (1.320 g, 13.36 mmol) in 5 mL of DMSO was heated at 130 °C for 2 h. The reaction was quenched with water and the mixture extracted with ethyl acetate. The organic layer was washed with brine, dried, filtered, and concentrated to give 0.850 g of brown oil. The oil was purified via silica gel column chromatography using 1% methanol in chloroform as eluent to give 0.540 g (90%) of a mixture of *E* and *Z* isomers **11** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 6.95 (s, 2H), 6.58 (s, 1H), 5.5−5.8 (m, 2H), 4.25 (d, 0.2 × 2H), 4.15 (d, 0.8 × 2H), 3.6 (m, 2H), 2.5 (s, 3H), 2.4 (s, 3H), 2.3 (s, 3H), 1.9 (s, 6H), 1.7 (d, 3H), 1.2 (t, 3H).

The corresponding HCl salt was prepared to give white crystals after recrystallization from ethyl acetate: mp 157−159 °C; ^{1}H NMR ($CDCl_3$) δ 7.00 (s, 2H), 6.76 (s, 1H), 5.8−6.0 (m, 1H), 5.4−5.6 (m, 1H), 4.4−4.6 (m, 2H), 3.9−4.1 (m, 2H), 2.86 (s, 3H), 2.43 (s, 3H), 2.35 (s, 3H), 1.89 (s, 6H), 1.75 (m, 3H), 1.32 (m, 3H); IR (KBr) 2917, 1599, 1490 cm^{-1}. Anal. Calcd for $C_{23}H_{30}N_4$·HCl: C, 69.24; H, 7.83; N, 14.04. Found: C, 68.97; H, 7.74; N, 13.97.

The following compounds **12**−**14** were prepared from compound **9** and an appropriate amine by the same procedure as described for **2**. Compounds **13** and **14** were heated in a high-pressure reactor to 110 °C at 150 and 250 psi, respectively.

Butyl[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (12). A 99% yield of brown solid was obtained. The brown solid was recrystallized from petroleum ether. A 40% yield of white crystals was collected from the first crop, and a 55% yield of crude product was obtained from the concentration of the filtrate: mp 114−116 °C; ^{1}H NMR ($CDCl_3$) δ 6.93 (s, 2H), 6.39 (s, 1H), 4.90 (t, 1H), 3.57 (q, 2H), 2.45 (s, 3H), 2.44 (s, 3H), 2.30 (s, 3H), 1.90 (s, 6H), 1.62 (m, 2H), 1.41 (m, 2H), 0.98 (t, 3H); IR (KBr) 3648, 2918, 2858, 1591, 1577 cm^{-1}. Anal. Calcd for $C_{21}H_{28}N_4$: C, 74.96; H, 8.39; N, 16.65. Found: C, 75.04; H, 8.70; N, 16.50.

Ethyl[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (13). A 88% yield of light brown solid was obtained. The light brown solid was recrystallized from petroleum ether. A 44% yield of tan crystals was collected from the first crop: mp 153−156 °C; ^{1}H NMR ($CDCl_3$) δ 6.93 (s, 2H), 6.40 (s, 1H), 4.90 (t, 1H), 3.62 (q, 2H), 2.46 (s, 3H), 2.44 (s, 3H), 2.30 (s, 3H), 1.90 (s, 6H), 1.30 (t, 3H); IR (KBr) 3670, 3458, 2917, 1592, 1578 cm^{-1}. Anal. Calcd for $C_{19}H_{24}N_4$: C, 73.99; H, 7.84; N, 18.17. Found: C, 74.29; H, 7.95; N, 17.84.

2,5-Dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ylamine (14). A 63% yield of golden solid was obtained after silica gel column chromatography using 5% MeOH in chloroform as eluent: mp 208−210 °C; ^{1}H NMR ($CDCl_3$) δ 6.95 (s, 2H), 6.51 (s, 1H), 5.12 (s, 2H), 2.46 (s, 6H), 2.32 (s, 3H), 1.91 (s, 6H); IR (KBr) 3517, 3405, 2916, 1609, 1592 cm^{-1}. Anal. Calcd for $C_{17}H_{20}N_4$: C, 72.83; H, 7.19; N, 19.98. Found: C, 72.47; H, 6.94; N, 19.58.

[^{2}H]Butyl[2,5-dimethyl-7-(2,4,6-trimethylphenyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl]ethylamine. A mixture of **11** (50 mg, 0.138 mmol) and 50 mg of 10% Pd/C in 10 mL of ethyl acetate was hydrogenated using deuterium gas under atomospheric pressure for 2 h. The mixture was filtered through Celite and concentrated to give 50 mg (98%) of a clear oil. The oil was purified via silica gel column chromatography using 1% methanol in chloroform as eluent to give 38 mg (75%) of [^{2}H]-**2** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 6.96 (s, 2H), 6.56 (s, 1H), 3.67 (q, 2H), 3.59 (d, 2H), 2.47 (s, 3H), 2.42 (s, 3H), 2.33 (s, 3H), 1.92 (s, 6H), 1.60−1.70 (m, 1H), 1.35 (m, 1H), 1.23 (t, 3H), 0.90 (t, 3H).

The above procedure was used for the synthesis of [^{3}H]-**2**, and the radiolabeled synthesis was performed by Chemsyn Science Laboratory.

Biological Methods. Receptor Binding Studies. Rats were sacrificed and their brains removed. The cortex was dissected and homogenized in 50 mM Tris HCl buffer (pH 7.4) using a Brinkman Polytron Model PT3000 (setting 15 000 rpm, 15 s). The homogenate was centrifuged for 10 min at 40000*g*. The pellet was resuspended in fresh ice-cold 50 mM Tris HCl buffer using the Polytron and recentrifuged for 10 min at 40000*g*. The final pellet was resuspended in 20 mM piperazine-*N*,*N*′-bis[2-ethanesulfonic acid], 10 mM $MgCl_2$, 2 mM EGTA, 0.015% bacitracin, and aprotinin (100 kallikrein units/mL) (pH 7.0). Incubations were initiated by the addition of tissue homogenate (100 μL) to tubes containing [^{125}I]o-CRF (70 pM) and buffer with 0.04% bovine serum albumin and either vehicle or compound in a final volume of 200 μL. Nonspecific binding was defined as the radioactivity remaining in the presence of 1 μM rat/human CRF. After a 120 min incubation at room temperature, assay samples were centrifuged at 1000*g* for 10 min. The supernatant was discarded, and 100 μL of ice-cold assay buffer (same as tissue buffer described above plus 0.04% bovine serum albumin) was added to each assay sample. After another centrifugation, assay samples were filtered onto LKB Betaplate filtermats, presoaked in 0.2% polyethylenimine, using a Skatron cell harvester and ice-cold 50 mM Tris buffer (pH 7.2). Radioactivity was quantified by liquid scintillation counting (Betaplate, LKB/Wallac).

Fear-Potentiated Startle. One week after delivery, rats were exposed to a startle response session consisting of twenty 120 dB[A], 40 ms acoustic startle stimuli, interspersed by 15 s of 68 dB[A] background noise. The results of this initial baseline matching test were used to assign rats to experimental groups with approximately equal startle reactivity. One day later, subjects were exposed to a classical conditioning procedure in which illumination of an incandescent light (25 W a.c.) was paired with presentation of a scrambled foot shock (1.2 mA). The shock occurred during the final 500 ms of the 3.2 s light presentation. Twenty such pairings were presented, 2 min apart, in darkened startle chambers. One group of animals, "no shock", was exposed to the light presentations without the accompanying shocks. On the test day, conducted 3 days later, rats were exposed to a startle session in which some of the 108 dB stimuli were preceded by a 3.2 s light presentation and others were not. An initial block of 11 "noise-alone" startle stimuli were presented to eliminate the rapid phase of startle habituation. These trials were not included in the data analysis. Compound **2** was administered po 60 min prior to the acoustic startle session. For each subject, the difference between light-paired and -unpaired startle responses was calculated. This difference score represents the magnitude of potentiated startle. Also analyzed was the effect of drug on baseline (unpaired) startle, as a measure of nonspecificity.

Pharmacokinetic Studies in Rat. Male Sprague−Dawley rats (300−350 g) were surgically prepared with indwelling jugular vein canulae for the intravenous administration of **2** and the sampling of blood. Surgery was performed under pentobarbital anesthesia (65 mg/kg, ip) 1 day before dosing. Two groups of rats ($n = 4$) received either a bolus iv (5 mg/kg) or po (10 mg/kg) dose of **2**. For iv dosing, the compound was prepared in dimethyl sulfoxide/emulphor/saline (5:5:90, v/v/v) and administered as a 5 mg/mL solution at 1 mL/kg. For oral dosing the compound was administered as a 2 mg/mL solution in dimethyl sulfoxide/emulphor/water (5:5:90, v/v/v) at 5 mL/kg. The dosing solutions were prepared immediately prior to administration. Serial blood samples (0.5 mL) were collected from the indwelling cannula at 0.08, 0.17, 0.25, 0.50, 1, 2, 3, 4, 6, and 24 h following iv dose administration into heparinized syringes. Sampling time points following the oral dose were 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h. The blood was immediately centrifuged and the plasma transferred to clean Eppendorf tubes and stored at −20 °C prior to analysis.

The concentration of **2** in plasma samples was determined by HPLC using a Zorbax RX-C8 5 μm (15 cm × 4.6 mm id) analytical column. The flow rate of the mobile phase, acetonitrile:0.05 M phosphate buffer, pH 4.7 (70/30, v/v), was 1.5 mL/min. The column effluent was monitored by UV absorption at 300 nm, and the limit of detection was 5 ng/mL. The dynamic range of the assay used for these samples was 5−500 ng/mL. Plasma samples with concentrations of drug above 500 ng/mL were diluted appropriately with control plasma and reanalyzed.

Pharmacokinetic parameters were calculated using the program PK_PARAM. The concentration of drug at time $t = 0$ following iv dose administration was determined by regression of the data using data points from the distribution phase of the apparent biexponential elimination. Area under the plasma concentration−time curves (AUC(0 − t_{last}) and AUC(0 − inf)) were calculated by the linear trapezoidal method. Clearance (CL) was calculated as dose/AUC(0 − inf), and the apparent volume of distribution at steady state was calculated as CL × mean residence time (MRT), where MRT equaled area under the momment curve (AUMC)/AUC. The terminal elimination rate constant (K_{el}) was determined by unweighted linear regression. The oral bioavailability (F) of **2** was estimated by comparing the AUCs following oral and iv administration and normalizing for dose.

Acknowledgment. We are grateful to Dr. Jon Bordner for the X-ray structure analyses and Chemsyn Science Laboratories, Lenexa, KS, for conducting the radiolabeled synthesis.

References

(1) (a) Vale, W.; Spiess, J.; Rivier, C.; Rivier, J. Characterization of a 41 residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta endorphin. *Science* **1981**, *213*, 1394−1397. (b) Owens, M. J.; Nemeroff, C. B. Physiology and pharmacology of corticotropin-releasing factor. *Pharmacol. Rev.* **1991**, *43*, 425−473. (c) Black, P. H. Psychoneuroimmunology: brain and immunity. *Sci. Am. Sci. Med.* **1996**, 16−25.

(2) Chen, R.; Lewis, K. A.; Perrin, M. H.; Vale, W. W. Expression cloning of a human corticotropin-releasing-factor receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8967−8971.

(3) Perrin, M. H.; Donaldson, C. J.; Chen, R.; Lewis, K. A.; Vale, W. W. Cloning and functional expression of a rat brain corticotropin releasing factor (CRF) receptor. *Endocrinology* **1993**, *133*, 3058−3061.

(4) Perrin, M.; Donaldson, C.; Chen, R.; Blount, A.; Berggren, T.; Bilezikjian, L.; Sawchenko, P.; Vale, W. Identification of a second corticotropin-releasing factor receptor gene and characterization of a cDNA expressed in heart. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2969−2973.

(5) Liaw, C. W.; Lovenberg, T. W.; Barry, G.; Oltersdorf, T.; Grigoriadis, D. E.; De Souza, E. B. Cloning and characterization of the human corticotropin-releasing factor-2 receptor complementary deoxyribonucleic acid. *Endocrinology* **1996**, *137*, 72−77.

(6) Chalmers, D. T.; Lovenberg, T. W.; Grigoriadis, D. E.; Behan, D. P.; De Souza, E. B. Corticotrophin-releasing factor receptors: from molecular biology to drug design. *Trends Pharmacol. Sci.* **1996**, *17*, 166−172.

(7) (a) Nemeroff, C. B.; Widerlov, E.; Bissette, G.; Wallens, H.,; Karlsson, I.; Eklund, K.; Kilts, C. D.; Loosen, P. T.; Vale, W. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* **1984**, *226*, 1342−1344. (b) Darnell, A.; Bremner, J. D.; Licinio, J.; Krystal, J.; Nemeroff, C. B.; Owens, M.; Erdos, J.; Charnev, D. S. CSF levels of corticotropin releasing factor in chronic post-traumatic stress disorder. *Soc. Neurosci. Abstr.* **1994**, *20*, 17.

(8) (a) Holsboer, F.; Von Bardeleben, U.; Gerken, A.; Stalla, G. K.; Muller, O. A. Blunted corticotropin and normal cortisol response to human corticotropin-releasing-factor in depression. *N. Engl. J. Med.* **1984**, *311*, 1127. (b) Taylor, A. L.; Fishman, L. M. Corticotropin-releasing hormone. *N. Engl. J. Med.* **1988**, *319*, 213−222.

(9) Rivier, J.; Rivier, C.; Vale, W. Synthetic competitive antagonists of corticotropin-releasing factor: effect on ACTH secretion in the rat. *Science* **1984**, *224*, 889−891.

(10) (a) Chen, Y. L. Pyridine, pyrimidine, purinones, pyrrolopyrimidinones and pyrrolopyridinones as corticotropin releasing factor antagonists. International Publication Number WO 9533750-A1. The disclosed pyrimidine series in this patent was later published by another group: Chen, C.; Dagnino, R.; De Souza, E. B.; Grigoriasdis, D. E.; Huang, C. Q.; Kim, K.; Liu, Z.; Moran, T.; Webb, T. R.; Whitten, J. P.; Xie, Y. F.; McCarthy, J. R. Design and synthesis of a series of non-peptide high-affinity human corticotropin-releasing factor$_1$ receptor antagonists. *J. Med. Chem.* **1996**, *39*, 4358−4360. (b) Whitten, J. P.; Xie, Y. F.; Erickson, P. E.; Webb, T. R.; De Souza, E. B.; Grigoriadis, D. E.; McCarthy, J. R. Rapid microscale synthesis, a new method

for lead optimization using robotics and solution phase chemistry: application to the synthesis and optimization of corticotropin-releasing factor$_1$ receptor antagonists. *J. Med. Chem.* **1996**, *39*, 4354−4357.
(11) Faraci, W. S.; Welch, W. M., Jr. Pyrazoles and pyrazolopyrimidines having corticotropin-releasing factor antagonist activity. International Publication Number WO 9413643-A1.
(12) Chen, Y. L. Pyrazolopyrimidines as CRF antagonists. International Publication Number WO 9413677-A1.
(13) Chen, Y. L. Pyrrolopyrimidines as CRF antagonists.International Publication Number WO 9413676-A1.
(14) Schulz, W. D.; Mansbach, R. S.; Sprouse, J.; Braselton, J. P.; Collins, J.; Corman, M.; Dunaiskis, A.; Faraci, S.; Schmidt, A.; Seeger, T.; Seymour, P.; Tingley, F. D., III; Winston, E. N.; Chen, Y. L.; Heym, J. CP-154,526: a potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10477−10482.
(15) Lundkvist, J.; Chai, Z.; Teheranian, R.; Hasanvan, H.; Bartfai, T.; Jenck, F.; Widmer, U.; Moreau, J. A non peptide corticotropin releasing factor receptor antagonist attenuates fever and exhibits anxiolytic-like activity. *Eur. J. Pharamcol.* **1996**, *309*, 195−200.
(16) Mansbach, R. S.; Winston, E. N.; Chen, Y. L. Antidepressant-like effects of CP-154,526, a selective CRF$_1$ receptor antagonist. *Eur. J. Pharmacol.* **1997**, *323*, 21−26.
(17) Prout, F. S. Amino acid catalysis of the Knoevenagel reaction. *J. Org. Chem.* **1953**, *18*, 928−933.
(18) Gewald, K. 2-Amino-thiophene aus α-oxo-mercaptanen und methyleneaktiven nitrilen. *Chem. Ber.* **1965**, *98*, 3571−3577.

JM960861B