8-(4-Methoxyphenyl)pyrazolo[1,5-*a*]-1,3,5-triazines: Selective and Centrally Active Corticotropin-Releasing Factor Receptor-1 (CRF_1) Antagonists

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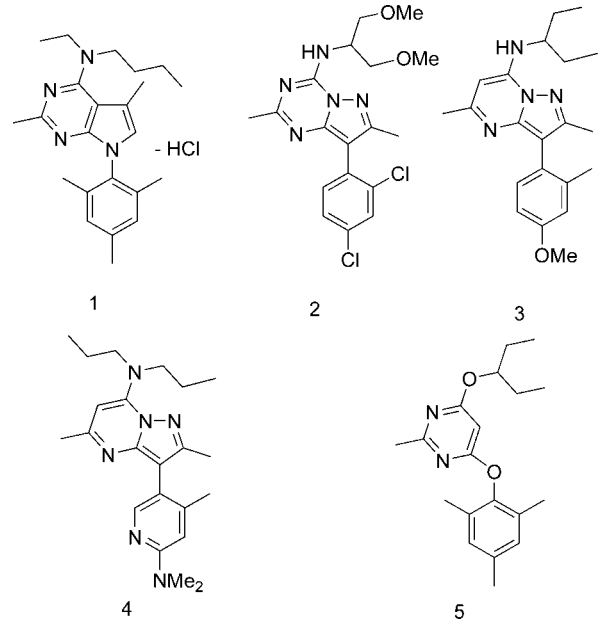
This report describes the syntheses and structure−activity relationships of 8-(4-methoxyphenyl)pyrazolo-[1,5-*a*]-1,3,5-triazine corticotropin releasing factor receptor-1 (CRF_1) receptor antagonists. CRF_1 receptor antagonists may be potential anxiolytic or antidepressant drugs. This research culminated in the discovery of analogue **12-3**, which is a potent, selective CRF_1 antagonist ($hCRF_1$ $IC_{50} = 4.7 \pm 2.0$ nM) with weak affinity for the CRF-binding protein and biogenic amine receptors. This compound also has a good pharmacokinetic profile in dogs. Analogue **12-3** is orally effective in two rat models of anxiety: the defensive withdrawal (situational anxiety) model and the elevated plus maze test. Analogue **12-3** has been advanced to clinical trials.

Corticotropin releasing factor (CRF^a) receptor modulators may be useful in the treatment of stress-related disorders (e.g., anxiety or depression).[1−5] Preclinical studies have documented the essential role of CRF in the regulation of endocrine and autonomic and behavioral responses to stress. Rapid, transient release of CRF mediates several responses to natural stressors in many species; intracerebroventricular (icv) administration of CRF in rodents elicits behavioral and physiological effects identical to those caused by natural stressors.[6] Transgenic mice, which overexpress CRF, demonstrate increased anxiety, whereas mice in which the CRF gene is deleted exhibit a reduced endocrine response to natural stressors.[7,8] Peptidic CRF antagonists (e.g., α-helical CRF_{9-41}, astressin) not only block the effects of exogenous CRF but also block the effects of various natural stressors.[9,10] These data indicate that CRF receptors may be important targets for the treatment of stress-related disorders.

CRF is a 41-amino acid peptide that was first characterized as a pituitary adrenocorticotropic hormone (ACTH) secretagogue[11] and then as a neurotransmitter.[12−14] CRF function is mediated by two G-protein-coupled receptor subtypes, CRF_1 and CRF_2 receptors, which are positively coupled to cyclic adenosine monophosphate (c-AMP) production.[15−22] Splice variants have been identified for each subtype. The CRF_1 receptor is the most abundant subtype found in the rodent and primate pituitary and is involved in the regulation of ACTH secretion from the pituitary.[23−25] The relative roles of the subtypes in neuropsychiatric and feeding disorders in rodents have been reviewed at length.[26] Great progress has been made in defining the structure−activity relationships for peptidic CRF_1- and CRF_2-selective agonists and antagonists (e.g., astressin and anti-sauvagine$_{1-30}$).[9,10,27−29] However, the physical properties of these agents limit their use in a clinical setting, which has prompted a search for nonpeptidic antagonists. To date, only CRF_1-selective nonpeptidic antagonists have been reported. Preclinical reports on CRF_1-selective antagonists **1** (CP-154526),[30,31] **2** (DMP 696),[32] and **3** (DMP 904)[33] (Scheme 1) support the hypothesis that CRF receptors may be good targets for treatment of stress-related disorders. No data on CRF_2-selective nonpeptidic agents have been published.

Scheme 1



Clinical studies have provided equivocal data on the role of CRF in the pathophysiology of depression. A role for CRF in major depression is inferred from the following observations:

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[a] Abbreviations: CRF, corticotropin-releasing factor; ACTH, adrenocorticotropic hormone; c-AMP, cyclic adenosine monophosphate; CSF, cerebrospinal fluid; HEK293, human embryonic kidney-293.

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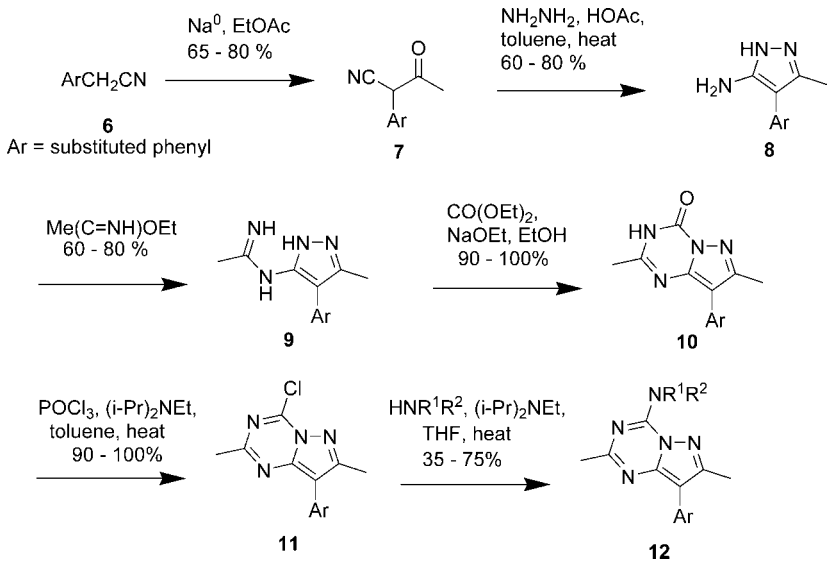
Scheme 2



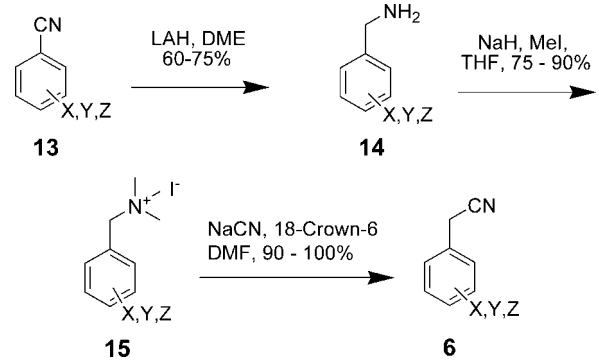
(a) maladaptation to chronic stress and related chronic elevation of corticosteroids may be a major pathway leading to some, but not all, forms of depression;[34] (b) hypercortisolemia in these forms of depression appears to be a direct result of chronic hypersecretion of hypothalamic CRF;[35] (c) the number of CRF-expressing neurons in the hypothalamus of depressed patients is increased relative to that in normal subjects;[36] (d) concentrations of CRF in cerebrospinal fluid (CSF) are increased in some patients with major depression;[37] and (e) successful treatment of depression in these patients is associated with decreased CRF levels in CSF.[37−40] The small molecule CRF_1 receptor antagonist **4** (R121919) had some efficacy in a small open label clinical study in anxiety and depression.[41] However, another CRF_1 antagonist **5** (CP-316311) failed in a double-blind study involving patients suffering from depression unlike the positive control sertraline.[42,43] Additional clinical studies with structurally diverse CRF_1 antagonists may better define the role of CRF in human mental illness.

This report describes the syntheses and structure−activity relationships of 8-(4-methoxyphenyl)pyrazolo[1,5-*a*]-1,3,5-triazine CRF_1 receptor antagonists, which may be potential anxiolytic drugs. A preliminary report from this laboratory outlined the structure−activity relationships for substitution on the phenyl ring of 8-phenylpyrazolo[1,5-*a*]-1,3,5-triazines.[44] Additional studies on 8-(4-methoxyphenyl) congeners are reported below. This research has culminated in the discovery of analogues **12-3**, **12-16**, **12-33**, and **12-35**, which are potent, selective CRF_1 antagonists with weak affinity for the CRF-binding protein and biogenic amine receptors. These agents also have good pharmacokinetic profiles in dogs. Analogue **12-3** is effective in two rat models of anxiety: the defensive withdrawal (situational anxiety) model and the elevated plus maze test. These data strengthen the hypothesis that CRF_1 antagonists may be useful for the treatment of anxiety. Analogue **12-3** has been advanced to clinical trials.

Results

Chemistry. Pyrazolo[1,5-*a*]-1,3,5-triazines **12** were synthesized by the general method, which is illustrated in Scheme 2. The phenylacetonitrile precursors were usually prepared by nucleophilic displacement of benzyl halides with cyanide. However, this method sometimes provided low yields, especially when the phenyl ring has electron-donating substituents. In these cases, nucleophilic displacement of benzyltrimethylammonium halides was more efficient (Scheme 3). Alternatively, palladium-mediated coupling of isoxazole **16** with phenylboronic acids followed by basic hydrolysis could be used to generate *β*-ketonitriles **6** (Scheme 4).[45]

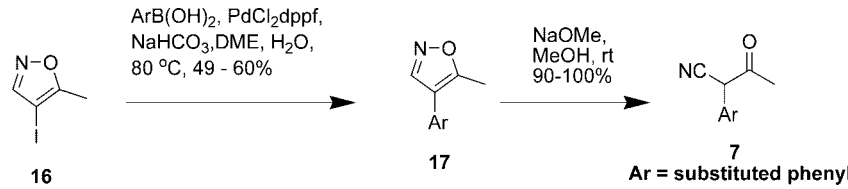
Scheme 3



Analogues with two different ortho substituents on the 8-phenyl group can exist as atropisomers. Compound **12−16** is one such example. Proton NMR spectroscopy reveals the existence of atropisomers for **12−16** (which is a sulfuric acid salt (1:1 molar ratio) crystallized from 2-propanol) due to restricted rotation of the phenyl ring. Two signals are observed in DMSO-d_6 at 500 MHz for the methyl group at the 2-position of the phenyl ring (δ 1.99, 1.98) and for the methoxy group at the 6-position (δ 3.65, 3.64). Chiral HPLC (OD column, 1% 2-propanol-hexanes (v/v)) separates the free bases of the two atropisomers. The melting points for the two atropisomers were similar (60−61 °C for the first isomer; 59−60 °C for the second isomer). Their optical rotations were significantly different ($[\alpha]_D^{25}$ −101° (0.256 g/dL, MeOH) for the first isomer; $[\alpha_D^{25}]$ +42.2° (0.252 g/dL, MeOH) for the second isomer). Variable temperature NMR studies in DMSO-d_6 on the first atropisomer with the low retention time reveal that it is slowly converted to the other atropisomer at 22 °C ($t_{1/2}$ = 2200 h) and rapidly converted at 100 °C ($t_{1/2}$ = 0.5 h). This conversion raises questions about long-term solid state stability of a potential dosage form for this compound.

Pharmacology. Pyrazolo[1,5-*a*]-1,3,5-triazines **12** were progressed through a series of in vitro and in vivo tests to identify

Scheme 4



a preclinical candidate. The compounds were first tested for their binding affinity to rat cortical homogenate CRF receptors.[46] Compounds with high receptor binding affinity ($IC_{50} \leq 10$ nM) were then evaluated in dog plasma ex vivo binding studies to estimate plasma exposure after oral administration (5 mg/kg). Analogues, which displaced ^{125}I-Tyr^0-ovine-CRF ex vivo by more than 30% at 10 h postdose, were submitted to discrete dog pharmacokinetic studies. Select analogues with good discrete dog pharmacokinetic profiles (oral bioavailability of >10% at 5 mg/kg, po) were submitted to secondary pharmacological and behavioral tests. The leading compounds were also tested for their affinity to CRF_1 receptors endogenously expressed in human IMR32 neuroblastoma cells,[30] since rat cortical membranes contain CRF_1 and, to a much lesser extent, CRF_2 sites. Antagonist potency was determined by measuring a compound's effects on c-AMP production in rat cortical homogenates. The potential anxiolytic activity of these agents was assessed in the rat situational anxiety and elevated plus maze models.[47,48]

The rat receptor binding affinity data for compounds **12** are summarized in Table 1. Compounds bearing a methoxy group at the 4-position of the phenyl ring generally have high affinity for rat CRF receptors. Hydrogen, methyl, chloro, and bromo substituents are well tolerated at 2-position. Analogues **12-1**, **12-3**, **12-34**, **12-35**, **12-38**, **12-40** and **12-42** have high affinity for rat cortical CRF receptors. Methyl substitution at the 3-position reduces binding affinity in one case (compare **12-4** with **12-43**). The introduction of a fluorine or methyl group at the 5-position of the phenyl ring and the addition of methyl or methoxy groups at the 6-position are also well tolerated (see analogues **12-34** through **12-42**). Diverse substituted alkyl amino groups (secondary or tertiary) on the bicyclic core at the 6-position contribute to high receptor binding affinity in the 2-methyl-4-methoxyphenyl series and the 2-methyl-4,6-dimethoxyphenyl series, which is consistent with SAR from other bicyclic CRF antagonist series.[1−3] Compounds in the 2-methyl-4,6-dimethoxyphenyl series potentially could form atropisomers, but we could only detect atropisomers in the case of analogue **12-16**. The atropisomers of **12-16** have similar affinity for rat cortical homogenate CRF receptors (rat CRF $IC_{50} = 4.4 \pm 0.6$ nM ($n = 3$) for the first isomer with low retention time and 5.2 ± 1.0 nM ($n = 3$) for the second isomer compared to 1.24 ± 0.8 nM ($n = 8$) for α-helical CRF_{9-41}).

Compounds with high rat receptor binding affinity ($IC_{50} <$ 10 nM) were next submitted to an ex vivo binding assay after oral administration to dogs (5 mg/kg). Table 2 delineates the data only for the leading compounds. Plasma samples were drawn at 2 and 10 h postdose, diluted 10-fold with the receptor binding assay buffer, and submitted to the standard binding assay. Compounds that displaced ^{125}I-Tyr^0-ovine-CRF from rat receptors were prioritized for discrete pharmacokinetic studies based on the magnitude and duration of the binding inhibition. While this assay cannot distinguish parent compound from active metabolite, its high throughput made it a useful screening tool. Several compounds significantly displace the radioligand at 10 h postdose (i.e., >40% inhibition).

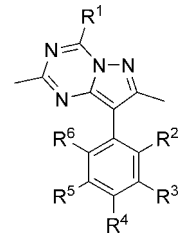
Compounds **12-3**, **12-16**, **12-33**, and **12-35** were profiled in other receptor binding assays to determine their cross-reactivity with other sites. All four compounds have high affinity for human CRF_1 receptors which are endogenously expressed in human IMR32 cells. Analogues **12-3**, **12-16**, **12-33**, and **12-35** have mean IC_{50} values (±standard deviation) equal to 4.7 ± 2.0 nM ($n = 6$), 3.4 ± 1.4 nM ($n = 6$), 15.2 ± 1.6 nM ($n = 3$), and 1.2 ± 0.2 ($n = 5$), respectively, while α-helical CRF_{9-41} has comparable affinity ($IC_{50} = 2.0 \pm 0.5$ nM, $n = 20$). None of the compounds binds human $CRF_{2\alpha}$ receptors ($IC_{50} > 1000$ nM).[26] These compounds have no significant affinity ($IC_{50} >$ 1000 nM) for 30 other receptors or ion channels in a NovaScreen battery of assays (e.g., adrenergic α_{1a}, α_{1b}, α_{2b}, α_{2c}; dopamine D_1, D_2; serotonin, calcium type L or N, or vasopressin V_1). None of these compounds have affinity for the CRF binding protein ($IC_{50} > 1000$ nM).[49,50]

The four leading compounds are silent and selective antagonists of rat CRF_1 receptors. No agonist activity was detected in a CRF-stimulated adenylate cyclase assay, which employed rat brain homogenates. The compounds have no effect on isoproterenol-stimulated or basal adenylate cyclase activity. Compounds **12-3**, **12-16**, **12-33**, **12-35**, and α-helical CRF_{9-41} have rat IC_{50} values equal to 66 ± 10, 110 ± 8, 38 ± 3, 36 ± 5, and 264 ± 85 nM, respectively ($n = 3$ for all compounds). These analogues have no functional agonist or antagonist activity at the cloned human $CRF_{2\alpha}$ receptors expressed in human embryonic kidney-293 (HEK293) cells.[50]

Compounds that have superior magnitude and duration of inhibition in the ex vivo binding assay (>40% inhibition at 10 h postdose) were advanced to discrete dog pharmacokinetic studies. Data for only the leading analogues are presented in Table 3. Compound **12-35** had tolerability issues in the dog and was deprioritized for further studies. The remaining compounds were formulated as salts to facilitate oral dosing. The 2-naphthalenesulfonic acid salt of analogue **12-3** was initially used in animal studies. Later studies would demonstrate that the benzenesulfonic acid salt of **12-3** had better pharmaceutic properties.[51] Three compounds **12-3**, **12-16**, and **12-33** have moderate to low clearances in the dog when compared to the hepatic blood flow (1.86 (L/h)/kg). The compounds also have high volumes of distribution and long elimination half-lives in the dog. Analogues **12-3** and **12-33** have oral bioavailability in the dog (34% and 48%, respectively) which is superior to that for compound **12-16** (4.7%). Analogue **12-16** has the lowest maximal concentration (C_{max}) after oral dosing (0.48 μM normalized to 5 mg/kg). This compound was excluded from further in vivo studies because of its inferior dog pharmacokinetic profile and the atropisomer issue (vide supra). Compound **12-3** was selected for additional studies over analogue **12-33** because of their divergent profiles in dog safety assessment studies.

Compound **12-3** was evaluated in the rat elevated plus maze test (Figure 1).[52] Vehicle-treated animals spent less than 10% of the test time in the open arms of the elevated-plus maze (Figure 1, left panel). The mean percent time in open arms was 8.4 ± 2.3%. The positive control **2** (rat $IC_{50} = 4.2 \pm 0.2$ nM

Table 1. Rat Receptor Binding Affinity Data[a]



compd	R^1	R^2	R^3	R^4	R^5	R^6	rat IC_{50} + SD (nM)	*n*
12-1	$N(CH_2CH_2OMe)_2$	H	H	MeO	H	H	11.52 ± 2.46	3
12-2	$NHCH(Et)_2$	H	H	MeO	H	H	3.80 ± 1.86	3
12-3	$N(CH_2CH_2OMe)_2$	Me	H	MeO	H	H	4.20 ± 0.40	9
12-4	NEt_2	Me	H	MeO	H	H	6.08 ± 1.42	3
12-5	NPr_2	Me	H	MeO	H	H	2.58 ± 0.90	3
12-6	NEtBu	Me	H	MeO	H	H	2.30 ± 0.16	3
12-7	$NPr(CH_2\text{-}c\text{-}C_3H_5)$	Me	H	MeO	H	H	1.96 ± 1.02	3
12-8	$NPr(CH_2CH_2CN)$	Me	H	MeO	H	H	2.16 ± 1.84	3
12-9	$N(c\text{-}C_3H_5)CH_2CH_2CN$	Me	H	MeO	H	H	1.88 ± 0.76	4
12-10	pyrrolidinyl	Me	H	MeO	H	H	80.66 ± 32.40	4
12-11	$NHCH(CH_2OMe)_2$	Me	H	MeO	H	H	9.70 ± 9.68	4
12-12	$NHCH(CH_2OEt)_2$	Me	H	MeO	H	H	19.34 ± 6.18	4
12-13	$NH\text{-}c\text{-}C_4H_7$	Me	H	MeO	H	H	24.64 ± 5.94	4
12-14	$NHCH(Et)_2$	Me	H	MeO	H	MeO	5.00 ± 4.46	4
12-15	(*S*)-NHCH(Me)Et	Me	H	MeO	H	MeO	2.90 ± 0.84	3
12-16	(*R*)-NHCH(Me)Et	Me	H	MeO	H	MeO	5.70 ± 2.10	3
12-17	NHCH(Me)Pr	Me	H	MeO	H	MeO	2.14 ± 0.58	3
12-18	$NH\text{-}c\text{-}C_4H_9$	Me	H	MeO	H	MeO	17.42 ± 3.10	4
12-19	NEt_2	Me	H	MeO	H	MeO	1.20 ± 0.14	4
12-20	NPr_2	Me	H	MeO	H	MeO	3.72 ± 3.64	3
12-21	$N(CH_2CH{=}CH_2)_2$	Me	H	MeO	H	MeO	1.00 ± 0.34	3
12-22	$N(Pr)CH_2\text{-}c\text{-}C_3H_5$	Me	H	MeO	H	MeO	1.48 ± 0.50	5
12-23	N(Me)Pr	Me	H	MeO	H	MeO	6.48 ± 2.26	3
12-24	$N(Me)CH_2CH{=}CH_2$	Me	H	MeO	H	MeO	4.92 ± 1.40	3
12-25	NEtBu	Me	H	MeO	H	MeO	1.72 ± 0.72	3
12-26	$N(Et)CH_2CH_2OMe$	Me	H	MeO	H	MeO	1.78 ± 0.58	4
12-27	$N(CH_2\text{-}c\text{-}C_3H_5)CH_2CH_2OMe$	Me	H	MeO	H	MeO	1.80 ± 0.48	5
12-28	$N(CH_2CH_2OMe)_2$	Me	H	MeO	H	MeO	7.96 ± 0.88	3
12-29	$N(Et)CH_2CH_2CN$	Me	H	MeO	H	MeO	2.48 ± 0.34	3
12-30	2-Et-piperidinyl	Me	H	MeO	H	MeO	3.26 ± 0.82	3
12-31	$N(CH_2CH_2OMe)_2$	Me	H	MeO	H	Me	2.74 ± 0.94	3
12-32	NMeBu	Me	H	MeO	H	Me	4.22 ± 0.72	3
12-33	$N(CH_2CH_2OMe)_2$	Me	H	MeO	Me	H	1.98 ± 0.14	3
12-34	$NHCHEt_2$	Me	H	MeO	F	H	1.90 ± 0.76	3
12-35	$NHCHEt_2$	Cl	H	MeO	F	H	1.00 ± 0.22	5
12-36	NEt_2	Cl	H	MeO	F	H	1.64 ± 0.94	4
12-37	$N(CH_2CH_2OMe)_2$	Cl	H	MeO	F	H	1.18 ± 0.38	3
12-38	$N(CH_2CH_2OMe)_2$	Cl	H	MeO	MeO	H	8.00 ± 3.80	3
12-39	$NHCHEt_2$	Br	H	MeO	MeO	H	6.08 ± 1.48	3
12-40	NEt_2	Br	H	MeO	MeO	H	4.06 ± 0.76	3
12-41	$N(CH_2CH_2OMe)_2$	Br	H	MeO	MeO	H	4.99 ± 1.44	3
12-42	$N(CH_2CH_2OMe)_2$	Me	H	MeO	MeO	H	13.68 ± 2.08	3
12-43	NEt_2	Me	Me	MeO	H	H	38.56 ± 8.22	3
12-44	$NHCHEt_2$	Me	Me	MeO	H	H	11.32 ± 5.92	3
α-helical CRF_{9-41}							1.24 ± 0.8	8

[a] SD = standard deviation. *n* = number of measurements.

($n = 3$) at a dose of 18 mg/kg significantly increased the percent time spent in open arms ($p = 0.03$). Pretreatment with **12-3** (30 mg/kg) significantly increased percent time in open arms [$F(5,79) = 2.31$, $p = 0.05$, analysis of variance (ANOVA) followed by individual mean comparisons using Fisher's least significant difference test]. The lowest effective dose of **12-3** was 30 mg/kg ($p = 0.02$) (Figure 1, left panel).

Analogue **12-3** was also tested in the rat defensive withdrawal (situational anxiety) model.[52] Vehicle-treated animals showed long latencies to exit the dark chamber and explore the open field (Figure 1, right panel). The mean exit latency was 655 ± 114 s (73% of the total test duration). The positive control **2** at the dose of 10 mg/kg decreased exit latency by 52% relative to vehicle control ($p = 0.04$). Pretreatment with **12-3** decreases exit latency ($H(5) = 11.9$, $p = 0.04$, the Kruskal−Wallis test, followed by individual comparisons using the Mann−Whitney *U* test). The lowest effective dose of 3.0 mg/kg decreased exit latency by 62% relative to vehicle-treated animals ($p = 0.0009$; Figure 1, right panel). Higher doses of **12-3** (10 and 30 mg/kg) decreased exit latency by 73% and 59%, respectively ($p = 0.01$ and $p = 0.03$, respectively).

The differences in the efficacies of compounds **12-3** and **2** between the two behavioral models may be explained by differences in the type and severity of the stressor present in the two tests. Similar observations have been reported for the peptide antagonists α-hel-CRF_{9-41} and astressin.[9,10] The same strain of rats was employed in both tests.

The CRF receptor occupancy of compound **12-3** increases with oral dose. In a separate experiment, rats were dosed at 0.3, 1, 3, 10, and 30 mg/kg (po) using the same vehicle and

Table 2. Dog ex Vivo Binding Data (5 mg/kg, po)[a]

compd	% inhibition ± SD		*n*
	2 h postdose	10 h postdose	
12-16	72 ± 6	83 ± 6	3
12-15	72 ± 9	74 ± 3	3
12-35	97 ± 3	66 ± 7	3
12-14	80 ± 12	56 ± 18	3
12-26	86 ± 5	54 ± 1	3
12-33	84 ± 1	44 ± 7	3[b]
12-18	52 ± 8	46 ± 9	3
12-3	75 ± 3	37 ± 8	3[b]
12-19	56 ± 23	30 ± 17	3
12-34	81 ± 3	25 ± 8	3

[a] The doses are expressed as free base weights. The vehicle is Labrafil unless otherwise noted. SD = standard deviation. *n* = number of measurements. [b] Vehicle = Tween-80/0.5% aqueous methylcellulose, 1:99 (v/v).

Table 3. Dog Discrete Pharmacokinetic Data (5 mg/kg, po)[a]

	12-3[b]	12-16[c]	12-33[d]
dose (iv, mg/kg)	5	1	5
n	4	3	4
CL ((L/h)/kg)	1.20 ± 0.25	0.60 ± 0.10	0.66 ± 0.11
V_{ss} (L/kg)	18.45 ± 3.95	14.80 ± 0.10	23.71 ± 7.69
$t_{1/2}$ (h)	27.3 ± 6.8	33.3 ± 8.6	40.2 ± 9.60
dose (po, mg/kg)	5	20	5
n	4	3	5
C_{max} (μM)	2.38 ± 0.39	1.94 ± 0.30	1.22 ± 0.29
t_{max} (h)	0.5 ± 0.2	4.0 ± 1.0	0.8 ± 0.2
F, po (%)	34.0	4.7	48.0

[a] The vehicle for iv studies was *N*,*N*-dimethylformamide/ethanol/propylene glycol/water, 10:10:55:25. The vehicle for oral studies was 0.5% Methocel−Tween-80 (99:1). Free base weights are reported for the doses. *n* = number of measurements. [b] The 2-naphthalenesulfonic acid salt was used. [c] The sulfuric acid salt was used. [d] The benzenesulfonic acid salt was used.

protocol as those used in the defensive withdrawal test. Rats were sacrificed and their brains were removed, sectioned, and frozen at −80 °C. Subsequent ex vivo binding studies using the published protocol[52] established receptor occupancy relative to vehicle-control animals. The receptor occupancy (±standard error of the mean) was calculated to be 12 ± 13%, 17 ± 8%, 48 ± 12%, 60 ± 8%, and 82 ± 12% for the 0.3, 1, 3, 10, and 30 mg/kg (po). Thus, the minimal effective dose of compound **12-3** (3 mg/kg, po) corresponds to 48 ± 12% receptor occupancy in ex vivo studies.

Compound **12-3** does not cause adverse motor effects at doses that are efficacious in the anxiety models. The compound has no effect in a rat open-field locomotor activity test[47,48] or in a rat rotorod performance test[47,48] up to 30 mg/kg (po, free base weight). The compound was administered by oral gavage in 0.5% methocel−Tween-80 (99:1), and behavior was monitored at 1 h postdose as in the behavioral studies. There was no difference between the vehicle control and compound **12-3** in these two tests, while chlordiazepoxide blocked motor function in these two tests at 30 mg/kg (po) when dosed in the same vehicle. In the rat locomotor activity test, the total distances traveled were 5286 ± 751 and 4986 ± 931 cm for vehicle and compound **12-3** (30 mg/kg free base weight, po), respectively (mean ± SEM, *n* = 8). In a separate experiment in this test, the total distances traveled were 5785 ± 941 and 2249 ± 286 cm for vehicle and chlordiazepoxide (30 mg/kg free base weight, po), respectively (mean ± SEM, *n* = 8). In the rat rotorod test, the times on the rotorod were 153 ± 29 and 158 ± 93 s for vehicle and compound **12-3** (30 mg/kg free base weight, po), respectively (mean ± SEM, *n* = 8). In a separate experiment in this second model, the times on the rotorod were 198 ± 43 and 121 ± 36 s for vehicle and chlordiazepoxide (30 mg/kg free base weight, po), respectively (mean ± SEM, *n* = 8). Compound **12-3** is less likely than chlordiazepoxide to induce motor side effects at 30 mg/kg (po).

Analogue **12-3** was evaluated in a rat pharmacokinetic study. The single dose intravenous (iv) pharmacokinetics of compound **12-3** in male (*n* = 3) and female (*n* = 3) Sprague−Dawley CD rats was investigated following administration of a 1 mg/kg iv bolus dose. The single dose oral (po) pharmacokinetics in male rats (*n* = 3) was investigated at a dose level of 5 mg/kg. Compound **12-3** was formulated in 20% ethanol in physiological saline (apparent pH 3.4) for iv administration and in 0.5% aqueous methylcellulose containing 1% Tween-80 for oral administration. Following the iv bolus administration of **12-3**, plasma concentrations exhibited a biexponential decline with time. The apparent volume of distribution at steady-state (V_{ss}) was high with a mean value of 14.6 ± 3.9 and 12.2 ± 2.3 L/kg for the male and female rats, respectively, suggesting extensive extravascular tissue distribution (standard deviations are reported). Analogue **12-3** was cleared from the systemic circulation in rats with mean values of systemic clearance (CL) of 1.2 ± 0.2 (L/h)/kg in males and 0.87 ± 0.28 (L/h)/kg in females (hepatic blood flow = 3.3 (L/h)/kg). The mean elimination half-life was 9.7 ± 2.8 h in males and 11.9 ± 2.9 h in females. Overall, the iv pharmacokinetics were similar between the male and female rats. Following oral administration of 5 mg/kg in male rats, **12-3** appeared to be rapidly absorbed as indicated by a mean T_{max} of 0.5 h. The mean C_{max} (observed peak plasma concentrations) of **12-3** after the oral dose was 0.851 μM in male rats. The apparent bioavailability (*F*) in the male rats was 51% relative to the 1 mg/kg iv dose.

Compound **12-3** (BMS 561388)[51,53] was advanced to clinical studies in humans on the basis of its favorable preclinical profile. It is a potent and selective antagonist of CRF_1 receptors. It is rapidly absorbed in both rats and dogs after oral administration. Furthermore, this analogue did not cause toxicity in dogs unlike some closely related analogues. The details of the safety assessment studies and clinical trials will be presented in separate publications.

Experimental Section

Analytical data were recorded for the compounds described below using the following general procedures. Proton NMR spectra were recorded on a Varian VXR or Unity 300 FT-NMR instruments (300 MHz); chemical shifts were recorded in ppm (δ) from an internal tetramethysilane standard in deuterochloroform or deuterodimethyl sulfoxide as specified below. Mass spectra (MS) or high resolution mass spectra (HRMS) were recorded on a Finnegan MAT 8230 spectrometer or a Hewlett-Packard 5988A model spectrometer (using chemi-ionization (CI) with NH_3 as the carrier gas, electrospray (ESI), atmospheric pressure chemi-ionization (APCI) or gas chromatography (GC)). Melting points were recorded on a MelTemp 3.0 heating block apparatus and are uncorrected. Boiling points are uncorrected. All pH determinations during workup were made with indicator paper.

Reagents were purchased from commercial sources and, where necessary, purified prior to use according to previously described general procedures.[54] Chromatography was performed on silica gel using the solvent systems indicated below. For mixed solvent systems, the volume ratios are given. Otherwise, parts and percentages are by weight.

The purity of final compounds was assessed by two analytical HPLC methods or combustion analysis and was found to be greater than or equal to 95% for all cases. Analytical HPLC analyses were performed on a Rainin HPLC machine (dual SD-200 pumps) using a C18 column (Dynamax 60A, 83-201C, 250 mm × 4.6 mm, 100 Å pore size, flow rate = 1 mL/min, solvent A = 0.1% TFA-H_2 O, solvent B = 0.1%

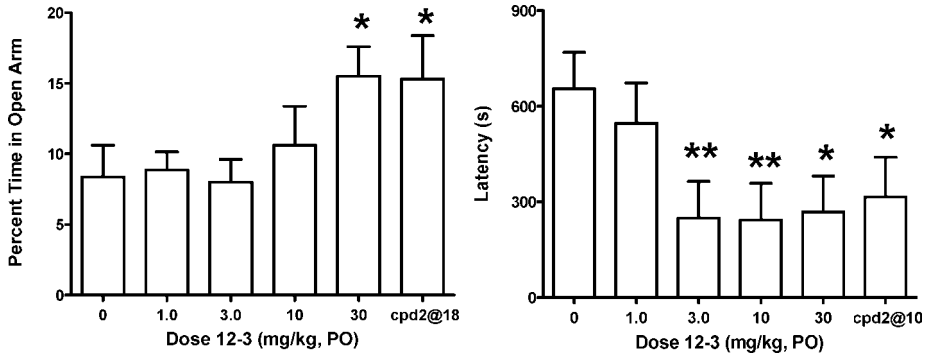


Figure 1. Anxiolytic-like effects of **12-3** in the elevated-plus maze (left) and defensive withdrawal tests (right) in rats. Abscissae indicate the dose (mg/kg). cpd2@18 is compound **2** at 18 mg/kg, and cpd2@10 is compound **2** at 10 mg/kg. Left panel ordinate displays the mean (±SEM) percent time in open arms for $n = 13-15$ animals per dose. Right panel ordinate displays the mean (±SEM) latency to exit the dark chamber (in seconds) for $n = 12$ animals per dose. Maximum latency is 900 s. Analogue **12-3** and compound **2** were administered in 0.25% Methocel, po, 60 min prior to testing: (*) $p < 0.05$; (**) $p < 0.01$.

TFA−CH_3CN, gradient of 15−95% B over 15 min) (method A). Analytical HPLC analyses were also performed on a Shimadzu HPLC machine (model LC-10AT) using a C18 column (Zorbax SB, 700 mm × 4.6 mm, 100 Å pore size, flow rate = 2.5 mL/min, solvent A = 0.1% TFA−H_2 O, solvent B = 0.1% TFA−MeOH, gradient of 5−95% B over 15 min) (method B). Combustion analyses were performed by Quantitative Technologies, Whitehouse, NJ.

Commonly used abbreviations are AIBN (azobis(isobutyro)nitrile), DMF (*N*,*N*-dimethylformamide), EtOH (ethanol), MeOH (methanol), EtOAc (ethyl acetate), HOAc (acetic acid), DME (1,2-diethoxyethane), and THF (tetrahydrofuran).

4-(Bis(2-methoxyethyl)amino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)[1,5-*a*]pyrazolo-1,3,5-triazine, Benzenesulfonic Acid Salt (12-3). Step A: 4-Methoxy-2-methylbenzyl Cyanide. A mixture of 3,4-dimethylanisole (24.5 g, 0.18 mol), *N*-bromosuccinimide (32 g (0.18 mol), and AIBN (1 g, 0.006 mol) in carbon tetrachloride (350 mL) was stirred at reflux temperature for 2 h. The reaction mixture was cooled to ambient temperature and filtered. Solvent was removed in vacuo to provide a yellow oil (30 g), which was used immediately without further purification. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.21 (d, 1H, $J = 8$), 6.70 (m, 2H), 4.5 (s, 2H), 3.80 (s, 3H), 2.4 (s, 3H).

The yellow oil was dissolved in DMF (75 mL). The solution was added dropwise to a mechanically stirred solution of sodium cyanide (12.3 g, 0.35 mol) in ethanol (500 mL) and water (250 mL) at 90 °C. The mixture was stirred at this temperature for 18 h. The mixture was diluted with water (1000 mL) and extracted three times with EtOAc (200 mL). The combined organic layers were dried over $MgSO_4$ and filtered. Solvent was removed in vacuo to give a light-brown oil. Column chromatography (EtOAc/hexane, 1:9) provided the desired product as a yellow oil (6.5 g, 22% overall yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.25 (d, 1H, $J = 8$), 6.75 (m, 2H), 3.8 (s, 3H), 3.6 (s, 2H), 2.30 (s, 3H). APCI-MS: 162 (M + H).

Step B: 1-Cyano-1-(2-methyl-4-methoxyphenyl)propan-2-one. The above nitrile (6.5 g, 40.4 mmol) was dissolved in EtOAc (200 mL). Sodium pellets (1.2 g, 52.2 mmol) were added portionwise over 15 min. The reaction mixture was heated to reflux temperature and stirred for 3 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature, and the creme colored suspension was filtered. The precipitate was dissolved in a minimum amount of water. A concentrated HCl solution was added dropwise with stirring until pH 5 (test paper) was attained. The mixture was extracted three times with EtOAc (100 mL). The combined organic layers were dried over $MgSO_4$ and filtered. Solvent was removed in vacuo to provide a yellow oil (4.5 g, 55% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.30 (dd, 1H, $J = 8, 1$), 6.80 (m, 2H), 4.75 (s, 1H), 3.8 (s, 3H), 2.3 (s, 3H), 2.2 (s, 3H). APCI-MS: 203 (M + H).

Step C: 5-Amino-4-(2-methyl-4-methoxyphenyl)-3-methylpyrazole. A mixture of 1-cyano-1-(2-methyl-4-methoxyphenyl)-propan-2-one (4.5 g, 22.2 mmol), hydrazine hydrate (2.25 mL, 44.4 mmol), glacial acetic acid (4.3 mL, 75 mmol), and toluene (57 mL) was stirred in an apparatus fitted with a Dean−Stark trap at reflux temperature for 18 h under a nitrogen atmosphere. The reaction mixture was cooled to ambient temperature, and solvent was removed in vacuo. The residue was dissolved in 4 N HCl, and the resulting solution was extracted with ether three times. A concentrated sodium hydroxide solution was added to the aqueous layer until pH 11 (test paper) was attained. The resulting semisolution was extracted three times with ethyl acetate (100 mL). The combined organic layers were dried over $MgSO_4$ and filtered. Solvent was removed in vacuo to give a yellow solid (4.0 g, 83% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.10 (d, 1H, $J = 8$), 6.85 (d, 1H, $J = 1$), 6.80 (dd, 1H, $J = 8,1$), 3.85 (s, 3H), 2.20 (s, 3H), 2.18 (m, 2H), 2.05 (s, 3H). APCI-MS (NH_3): 218 (M^+ + H).

Step D: 5-Acetamidino-4-(2,5-dimethyl-4-methoxyphenyl)-3-methylpyrazole, Acetic Acid Salt. A mixture of glacial acetic acid (1.04 mL, 18.4 mmol), 5-amino-4-(2-methyl-4-methoxyphenyl)-3-methylpyrazole (4.0 g, 18.4 mol), ethyl acetamidate (2.37 g, 27.2 mol), and acetonitrile (80 mL) was stirred at room temperature for 20 h. Solvent was removed in vacuo to about one-third of the original volume. The resulting suspension was filtered, and the collected solid was washed with copious amounts of ether. The white solid was dried in vacuo (4.2 g, 76% yield). ^{1}H NMR (DMSO-d_6,300 MHz): δ 6.95 (d, 1H, $J = 8$), 6.8 (d, 1H, $J = 1$), 6.75 (dd, 1H, $J = 8, 1$), 3.75 (s, 3H), 2.10 (s, 3H), 2.0 (s, 3H), 1.9 (s, 3H). APCI-MS (NH_3): 259 (M^+ + H, free base).

Step E: 2,7-Dimethyl-8-(2-methyl-4-methoxyphenyl)[1,5-*a*]-pyrazolo[1,3,5]triazin-4(3*H*)-one. Sodium pellets (4.0 g, 0.175 mol) were added portionwise to ethanol (100 mL) with vigorous stirring. After all the sodium reacted, the reaction mixture was cooled to room temperature. 5-Acetamidino-4-(2-methyl-4-methoxyphenyl)-3-methylpyrazole, acetic acid salt (1.65 g, 5.48 mmol), and diethyl carbonate (8.6 mL, 430 mmol) were added sequentially. The resulting reaction mixture was heated to reflux temperature and stirred for 69 h. The mixture was cooled to room temperature. Solvent was removed in vacuo. The residue was dissolved in water, and a concentrated HCl solution was added slowly until pH 7 (test paper) was attained. The aqueous mix was extracted with ethyl acetate (100 mL) three times; the combined organic layers were dried over $MgSO_4$ and filtered. Solvent was removed in vacuo to give a white solid (1.33 g, 85% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.10 (d, 1H, $J = 8$), 6.83 (m, 2H), 3.85 (s, 3H), 2.50 (s, 3H), 2.30 (s, 3H), 2.15 (s, 3H). CI-MS (NH_3): 285 (M^+ + H).

Step F: 4-Chloro-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)[1,5-*a*]pyrazolotriazine. A mixture of 2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)[1,5-*a*]pyrazolo-1,3,5-triazin-4-one (8.2 g, 28.9 mmol), *N*,*N*-diethylaniline (18.5 mL, 116 mmol), phosphorus

oxychloride (10.8 mL, 116 mmol), and toluene (200 mL) was stirred at reflux temperature for 42 h. The reaction mixture was concentrated in vacuo to give an oil. Flash column chromatography (ethyl acetate.hexanes, 1:9) gave a yellow oil after removal of solvent in vacuo (5.1 g, 58% yield). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.10 (d, 1H, J = 7), 6.90 (d, 1H, J = 1), 6.85 (dd, 1H, J = 7,1), 3.85 (s, 3H), 2.65 (s, 3H), 2.45 (s, 3H), 2.15 (s, 3H). CI-MS: 303, 305 (M^+ + H).

Step G: 4-(Bis(2-methoxyethyl)amino)-2,7-dimethyl-8-(2,5-dimethyl-4-methoxyphenyl)[1,5-*a*]pyrazolo-1,3,5-triazine (the Free Base of 12-3). Sodium hydride (60% in oil, 1.32 g, 33 mmol) was washed with hexanes, and the solvent was decanted. The solid was immediately taken up in THF (50 mL). Bis(2-methoxyethyl)-amine (4.55 g, 33 mmol) was added dropwise with vigorous stirring. Gas evolution ensued. The reaction mixture was stirred for 30 min. A mixture of 4-chloro-2,7-dimethyl-8-(2,5-dimethyl-4-methoxyphenyl)[1,5-*a*]pyrazolo-1,3,5-triazine (5.1 g, 17 mmol), and tetrahydrofuran (50 mL) was added dropwise, and stirring was continued at ambient temperature for 18 h. The reaction mixture was poured carefully onto water (250 mL) and extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and filtered. Solvent was removed in vacuo to give an oil. The product became a white solid after prolonged standing (4.2 g, 32% yield): mp 57.3 °C (DSC); HPLC log P = 4.76. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.10 (d, 1H, J = 8), 6.85 (d, 1H, J = 3), 6.78 (dd, 1H, J = 8,3), 4.45−4.25 (br s, 4H), 3.82 (s, 3H), 3.76 (t, 4H, J = 5), 3.38 (s, 6H), 2.39 (s, 3H), 2.24 (s, 3H), 2.17 (s, 3H). ^{13}C NMR ($CDCl_3$, 75.44 MHz): δ 162.2, 159.0, 153.2, 149.4, 149.0, 139.5, 132.2, 123.5, 115.7, 111.2, 106.8, 71.9, 58.9 (2C), 55.2 (2C), 50.8 (2C), 25.7, 20.6, 13.3. IR (neat, KBr, cm^{-1}): 2926 (m), 2893 (m), 2830 (m), 1616 (sh), 1604 (s), 1563 (s), 1534 (s), 1465 (s), 1442 (s), 1404 (m), 1373 (m), 1356 (m), 1310 (m), 1292 (m), 1278 (m), 1241 (m), 1204 (m), 1186 (m), 1162 (m), 1116 (m), 1047 (m), 1013 (m), 1002 (m). CI-HRMS (NH_3) calcd for $C_{21}H_{29}N_5O_3$: 400.2349. Found: 400.2336 (M^+ + H). Anal. ($C_{21}H_{29}N_5O_3$) C, H, N.

Step H: 4-(Bis(2-methoxyethyl)amino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)[1,5-*a*]pyrazolo-1,3,5-triazine, Benzenesulfonic Acid Salt (12-3). A mixture of 4-(bis(2-methoxyethyl)-amino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)[1,5-*a*]pyrazolo-1,3,5-triazine (1.5 g, 3.7 mmol), benzenesulfonic acid (0.5 g, 3.7 mmol), ether (50 mL), and ethanol (10 mL) was stirred at ambient temperature for 1 h. Solvent was removed in vacuo; the residue was triturated with copious amounts of ether. Filtration and drying in vacuo afforded a white crystalline solid (1.2 g, 82% yield) which is a hemihydrate: mp 97.5 °C (DSC). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.60 (m, 2H), 7.31 (m, 3H), 7.10 (d, 1H, J = 9), 6.92 (d, 1H, J = 3), 6.84 (dd, 1H, J = 9,3), 4.30 (br s, 4H), 3.79 (s, 3H), 3.69 (t, 4H, J = 5), 3.28 (s, 6H), 2.35 (s, 3H), 2.19 (s, 3H), 2.09 (s, 3H). Anal. ($C_{21}H_{29}N_5O_3 \cdot C_{10}H_8O_3S \cdot 0.5H_2O$) C, H, N, S.

4-(Bis(2-methoxyethyl)amino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)[1,5-*a*]pyrazolo-1,3,5-triazine, 2-Naphthalenesulfonic Acid Salt (2-Naphthalenesulfonic Acid Salt Analogue of 12-3). A mixture of 4-(bis(2-methoxyethyl)amino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)[1,5-*a*]-pyrazolo-1,3,5-triazine (132 mg, 0.23mmol), 2-naphthalenesulfonic acid (36.1 mg, 0.23 mmol), and ether (5 mL) was stirred at ambient temperature for 1 h. Solvent was removed in vacuo; the residue was triturated with copious amounts of ether. Filtration and drying in vacuo afforded a white crystalline solid (100 mg, 77% yield): mp 129.5 °C (differential scan calorimetry). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 8.14 (s, 1H), 8.00−7.84 (m, 4H), 7.70 (d, 1H, J = 8), 7.55−7.50 (m, 2H), 7.10 (d, 1H, J = 8), 6.93 (d, 1H, J = 3), 6.84 (dd, 1H, J = 8,3), 4.45−4.25 (br s, 4H), 3.79 (s, 3H), 3.71 (t, 4H, J = 5), 3.28 (s, 6H), 2.36 (s, 3H), 2.19 (s, 3H), 2.09 (s, 3H). IR (neat, KBr, cm^{-1}): 3440 (br, m), 3056 (m), 2926 (m), 2836 (m), 2552 (m), 1660 (s), 1606 (s), 1568 (s), 1504 (m), 1454 (s), 1374 (m), 1358 (m), 1344 (m), 1316 (s), 1244 (s), 1202 (m), 1166 (s), 1118 (s), 1134 (s), 1118 (s), 1090 (s), 1016 (s). CI-HRMS (NH_3) calcd for $C_{21}H_{29}N_5O_3$: 400.2349. Found: 400.2336 (M^+ + H). Anal. ($C_{21}H_{29}N_5O_3 \cdot C_{10}H_8O_3S$) C, H, N, S.

The following compounds were prepared by methods analogous to those for compound **12-3**, by converting the appropriate phenylacetonitrile precursor to the chloropyrazolotriazine intermediate, followed by nucleophilic displacement with an amine.

12-1: oil. NMR ($CDCl_3$, 300 MHz): δ7.58 (d, 2H, J = 9), 6.99 (d, 2H, J = 9), 4.32 (s, 4H), 3.84 (s, 3H), 3.76 (t, 4H, J = 6), 3.37 (s, 6H), 2.47 (s, 3H), 2.43 (s, 3H). CI-MS calcd for $C_{20}H_{28}N_5O_3$: 386. Found: 386 (M + H). Anal.: HPLC.

12-2: oil. NMR ($CDCl_3$, 300 MHz): δ 7.57 (d, 2H, J = 9), 7.00 (d, 2H, J = 9), 6.16 (d, 1H, J = 9), 4.20 (m, 1H), 3.84 (s, 3H), 2.53 (s, 3H), 2.51 (s, 3H), 1.76 (m, 2H), 1.62 (m, 2H), 0.99 (t, 6H, J = 7). CI-MS calcd for $C_{19}H_{26}N_5O$: 340. Found: 340 (M + H). Anal.: HPLC.

12-4: oil. NMR ($CDCl_3$, 300 MHz): δ 7.10 (d, 1H, J = 8), 6.85 (d, 1H, J = 1), 6.75 (dd, 1H, J = 8,1), 4.20−4.00 (br m, 4H), 3.85 (s, 3H, 2.40 (s, 3H), 2.30 (s, 3H), 2.20 (s, 3H), 1.35 (t, 6H, J = 7). CI-HRMS calcd for $C_{19}H_{26}N_5O$: 340.2137. Found: 340.2122 (M + H). Anal.: HPLC.

12-5: oil. NMR ($CDCl_3$, 300 MHz): δ 7.03 (d, 1H, J = 8), 6.86 (d, 1H, J = 1), 6.76 (dd, 1H, J = 8, 1), 4.94 (m, 4H), 3.75 (s, 3H), 2.31 (s, 3H), 2.18 (s, 3H), 2.11 (s, 3H), 1.70 (m, 4H), 0.91 (t, 6H, J = 7). CI-HRMS calcd for $C_{21}H_{30}N_5O$: 368.2450. Found: 368.2454 (M + H). Anal.: HPLC.

12-6: oil. NMR ($CDCl_3$, 300 MHz): δ 7.12 (d, 1H, J = 8), 6.85 (d, 1H, J = 1), 6.78 (dd, 1H, J = 8, 1), 4.24 (m, 4H), 3.75 (s, 3H), 2.39 (s, 3H), 2.25 (s, 3H), 2.18 (s, 3H), 1.75 (m, 2H), 1.4 (m, 5H), 1.00 (t, 3H, J = 7). CI-HRMS calcd for $C_{21}H_{30}N_5O$: 368.2451. Found: 368.2473 (M + H). Anal.: HPLC.

12-7: oil. NMR ($CDCl_3$, 300 MHz): δ 7.11 (d, 1H, J = 9), 6.84 (d, 1H, J = 3), 6.78 (dd, 1H, J = 9, 3), 4.10 (m, 2H), 3.93 (m, 2H), 3.82 (s, 3H), 2.39 (s, 3H), 2.25 (s, 3H), 2.18 (s, 3H), 1.80 (m, 2H), 1.25 (m, 1H), 0.97 (t, 3H, J = 7), 0.56 (m, 2H), 0.37 (m, 2H). CI-HRMS calcd for $C_{22}H_{30}N_5O$: 379.5. Found: 380.2 (M + H). Anal.: HPLC.

12-8: oil. NMR ($CDCl_3$, 300 MHz): δ 7.10 (d, 1H, J = 8), 6.85 (d, 1H, J = 1), 6.80 (dd, 1H, J = 8,1), 4.15 (m, 4H), 3.85 (s, 3H), 3.00 (t, 2H, J = 7), 2.45 (s, 3H), 2.30 (s, 3H), 2.20 (s, 3H), 1.85 (m, 2H), 1.00 (t, 3H, J = 7). CI-HRMS calcd for $C_{21}H_{27}N_6O$: 379.2246. Found: 379.2248 (M + H). Anal.: HPLC.

12-9: solid; mp 129 °C. NMR ($CDCl_3$, 300 MHz): δ 7.10 (d, 1H, J = 8), 6.90 (d, 1H, J = 1), 6.80 (dd, 1H, J = 8,1), 4.45 (m, 2H), 3.85 (s, 3H), 3.35 (m, 1H), 2.85 (t, 2H, J = 7), 2.50 (s, 3H), 2.30 (s, 3H), 2.20 (s, 3H), 1.05 (m, 2H), 0.70 (m, 2H). CI-HRMS calcd for $C_{21}H_{25}N_6O$: 377.2090. Found: 377.2092 (M + H). Anal.: HPLC.

12-10: oil. NMR ($CDCl_3$, 300 MHz): δ 7.11 (d, 1H, J = 9), 6.84 (d, 1H, J = 3), 6.78 (dd, 1H, J = 9,3), 4.10 (m, 2H), 3.82 (s, 3H), 2.40 (s, 3H), 2.25 (s, 3H), 2.18 (s, 3H), 2.03 (m, 4H), 1.20 (m, 2H). CI-MS calcd for $C_{18}H_{24}N_5O$: 338. Found: 338 (M + H). Anal.: HPLC.

12-11: solid, mp 45−46 °C. NMR ($CDCl_3$, 300 MHz): δ 7.10 (d, 1H, J = 8), 6.90 (d, 1H, J = 1), 6.80 (dd, 1H, J = 8,1), 6.70 (br d, 1H, J = 8), 4.65 (m, 1H), 3.85 (s, 3H), 3.63 (m, 4H), 3.45 (s, 6H), 2.50 (s, 3H), 2.30 (s, 3H), 2.15 (s, 3H). CI-HRMS calcd for $C_{20}H_{28}N_5O3$: 386.2192. Found: 386.2181 (M + H). Anal.: HPLC.

12-12: oil. NMR ($CDCl_3$, 300 MHz): δ 7.10 (d, 1H, J = 8), 6.85 (d, 1H, J = 1), 6.75 (dd, 1H, J = 8,1), 4.60 (m, 1H), 3.85 (s, 3H), 3.72 (m, 4H), 3.60 (q, 4H, J = 7), 2.50 (s, 3H), 2.30 (s, 3H), 2.20 (s, 3H), 1.25 (t, 6H, J = 7). CI-HRMS calcd for $C_{22}H_{32}N_5O_3$: 413.2427. Found: 413.2416 (M + H). Anal.: HPLC.

12-13: solid; mp 94.5−96 °C. NMR ($CDCl_3$, 300 MHz): δ 7.11 (d, 1H, J = 8), 6.85 (d, 1H, J = 3), 6.78 (dd, 1H, J = 8,3), 6.53 (d, 1H, J = 8), 4.77 (m, 1H), 3.82 (s, 3H), 2.51 (m, 2H), 2.48 (s, 3H), 2.30 (s, 3H), 2.17 (s, 3H), 2.10 (m, 2H), 1.95 (m, 2H). CI-MS calcd for $C_{19}H_{24}N_5O$: 366 $(M + H)^+$. Anal.: HPLC.

12-14: solid; mp 145−146 °C. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d,1H, J = 3), 6.39 (d, 1H, J = 3), 6.16 (d, 1H, J = 10), 4.18 (m, 1H), 3.83 (s, 3H), 3.72 (s, 3H), 2.45 (s, 3H), 2.23 (s, 3H), 2.12 (s, 3H), 1.63 (m, 4H), 1.00 (t, 6H, J = 8). CI-HRMS calcd for

$C_{21}H_{30}N_5O_2$: 368.2450. Found: 368.2454 (M + H). Anal. ($C_{21}H_{29}N_5O_2$) C, H, N.

12-15: solid. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d,1H, $J = 2$), 6.39 (d, 1H, $J = 2$), 6.21 (d, 1H, $J = 9$), 4.30 (m, 1H), 3.83 (s, 3H), 3.71 (d, 3H, $J = 2$), 2.46 (s, 3H), 2.22 (s, 3H), 2.11 (d, 3H, $J = 3$), 1.69 (m, 2H), 1.33 (t, 3H, $J = 7$), 1.01 (q, 3H, $J = 7$). CI-HRMS calcd for $C_{20}H_{28}N_5O_2$: 370.2243. Found: 370.2244 (M + H). Anal.: HPLC.

12-16, free base: solid; mp 111−112 °C. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d,1H, $J = 2$), 6.39 (d, 1H, $J = 2$), 6.22 (d, 1H, $J = 9$), 4.29 (m, 1H), 3.83 (s, 3H), 3.71 (s, 3H), 2.46 (s, 3H), 2.27 (s, 3H), 2.11 (d, 3H, $J = 3$), 1.68 (m, 2H), 1.33 (t, 3H, $J = 7$), 1.01 (q, 3H, $J = 7$). CI-HRMS calcd for $C_{20}H_{28}N_5O_2$: 370.2243. Found: 370.2242 (M + H). Anal.: HPLC.

12-16: solid; mp 202−203 °C. NMR (DMSO-d_6, 300 MHz): δ 6.57 (d, 1H, $J = 2$), 6.53 (d, 1H, $J = 2$), 4.33 (m, 1H), 3.82 (s, 3H), 3.68 (s, 3H), 2.44 (s, 3H), 2.16 (s, 3H), 2.03. 2.01 (2s, 3H), 1.73 (m, 2H), 1.3 (d, 3H, $J = 3$), 0.90 (t, 3H, $J = 7$). Anal. ($C_{20}H_{25}N_5O_2 \cdot H_2SO_4$) C, H, N.

12-17: oil. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d,1H, $J = 2$), 6.39 (d, 1H, $J = 2$), 6.20 (d, 1H, $J = 9$), 4.39 (m, 1H), 3.83 (s, 3H), 3.71 (d, 3H, J=2), 2.46 (s, 3H), 2.22 (s, 3H), 2.11 (d, 3H, $J = 2$), 1.62 (m, 2H), 1.44 (m, 2H), 1.33 (t, 3H, $J = 7$), 0.95 (m, 3H). CI-HRMS calcd for $C_{21}H_{30}N_5O_2$: 384.5056. Found: 384.2396 (M + H). Anal.: HPLC.

12-18: solid. NMR ($CDCl_3$, 300 MHz): δ 6.53 (d,1H, $J = 8$), 6.47 (d, 1H, $J = 2$), 6.39 (d, 1H, $J = 2$), 4.76 (m, 1H), 3.83 (s, 3H), 3.70 (s, 3H), 2.49 (m, 2H), 2.46 (s, 3H), 2.22 (s, 3H), 2.14 (m, 2H), 2.09 (s, 3H), 1.81 (m, 2H). CI-HRMS calcd for $C_{20}H_{26}N_5O_2$: 368.4625. Found: 368.2100 (M + H). Anal.: HPLC.

12-19: solid; mp 114 °C. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d, 1H, $J = 2$), 6.38 (d, 1H, $J = 2$), 4.05 (m, 4H), 3.80 (s, 3H), 3.70 (s, 3H), 2.38 (s, 3H), 2.18 (s, 3H), 2.10 (s, 3H), 1.34 (t, 6H, $J = 7$). CI-HRMS calcd for $C_{20}H_{28}N_5O_2$: 370.4785. Found: 370.2200 (M + H). Anal. ($C_{20}H_{28}N_5O_2$) C, H, N.

12-20: oil. NMR ($CDCl_3$, 300 MHz): δ 6.46 (d, 1H, $J = 2$), 6.38 (d, 1H, $J = 2$), 3.95 (m, 4H), 3.82 (s, 3H), 3.71 (s, 3H), 2.37 (s, 3H), 2.17 (s, 3H), 2.10 (s, 3H), 1.77 (m, 4H), 0.98 (t, 6H, $J = 7$). CI-HRMS calcd for $C_{22}H_{32}N_5O_2$: 398.2553. Found: 398.5326 (M + H). Anal.: HPLC.

12-21: oil. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d, 1H, $J = 2$), 6.39 (d, 1H, $J = 2$), 6.01 (m, 2H), 5.29 (m, 4H), 4.66 (m, 4H), 3.83 (s, 3H), 3.71 (s, 3H), 2.39 (s, 3H), 2.18 (s, 3H), 2.09 (s, 3H). CI-HRMS calcd for $C_{22}H_{28}N_5O_2$: 394. Found: 394 (M + H). Anal.: HPLC.

12-22: oil. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d, 1H, $J = 2$), 6.38 (d, 1H, $J = 2$), 4.03 (m, 4H), 3.83 (s, 3H), 3.71 (s, 3H), 2.38 (s, 3H), 2.18 (s, 3H), 2.10 (s, 3H), 1.26 (m, 1H), 0.97 (t, 3H, $J = 7$), 0.55 (m, 2H), 0.38 (m, 2H). CI-MS calcd for $C_{22}H_{28}N_5O_2$: 410. Found: 410 (M + H). Anal.: HPLC.

12-23: oil. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d,1H, $J = 3$), 6.38 (d, 1H, $J = 3$), 4.06 (m, 2H), 3.82 (s, 3H), 3.70 (s, 3H), 3.56 (s, 3H), 2.39 (s, 3H), 2.18 (s, 3H), 2.09 (s, 3H), 1.78 (m, 2H), 0.97 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{20}H_{28}N_5O_2$: 370.2246. Found: 370.4785 (M + H). Anal.: HPLC.

12-24: oil. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d,1H, $J = 3$), 6.39 (d, 1H, $J = 3$), 5.98 (m, 1H), 5.28 (m, 2H), 4.76 (m, 2H), 3.83 (s, 3H), 3.70 (s, 3H), 3.52 (s, 3H), 2.40 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H). CI-HRMS calcd for $C_{20}H_{26}N_5O_2$: 368.2090. Found: 368.4625 (M + H). Anal.: HPLC.

12-25: oil. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d,1H, $J = 2$), 6.38 (d, 1H, $J = 2$), 4.04 (m, 4H), 3.83 (s, 3H), 3.71 (s, 3H), 2.37 (s, 3H), 2.17 (s, 3H), 2.10 (s, 3H), 1.76 (m, 2H), 1.42 (m, 2H), 1.33 (t, 3H, $J = 7$), 1.01 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{22}H_{32}N_5O_2$: 398.5326. Found: 398.2559 (M + H). Anal.: HPLC.

12-26: oil. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d,1H, $J = 2$), 6.39 (d, 1H, $J = 2$), 4.10 (m, 4H), 3.83 (s, 3H), 3.78 (t, 2H, $J = 6$), 3.71 (s, 3H), 3.40 (s, 3H), 2.38 (s, 3H), 2.17 (s, 3H), 2.10 (s, 3H), 1.33 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{21}H_{30}N_5O_3$: 400.2343. Found: 400.2353 (M + H). Anal.: HPLC.

12-27: oil. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d,1H, $J = 2$), 6.39 (d, 1H, $J = 2$), 4.34 (m, 2H), 4.04 (m, 2H), 3.86 (s, 3H), 3.79 (t, 2H, $J = 6$), 3.71 (s, 3H), 3.39 (s, 3H), 2.38 (s, 3H), 2.17 (s, 3H), 0.86 (m, 1H), 0.54 (m, 2H), 0.40 (m, 2H). CI-HRMS calcd for $C_{23}H_{32}N_5O_3$: 426.2488. Found: 426.2488 (M + H). Anal.: HPLC.

12-28: oil. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d,1H, $J = 2$), 6.39 (d, 1H, $J = 2$), 4.30 (m, 4H), 3.83 (s, 3H), 3.77 (t, 4H, $J = 6$), 3.71 (s, 3H), 3.39 (s, 6H), 2.37 (s, 3H), 2.16 (s, 3H), 2.09 (s, 3H). CI-HRMS calcd for $C_{22}H_{32}N_5O_4$: 430.2454. Found: 430.2468(M + H). Anal.: HPLC.

12-29: oil. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d,1H, $J = 2$), 6.39 (d, 1H, $J = 2$), 4.15 (m, 4H), 3.83 (s, 3H), 3.71 (s, 3H), 3.01 (t, 2H, $J = 7$), 2.39 (s, 3H), 2.18 (s, 3H), 2.09 (s, 3H), 1.38 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{21}H_{27}N_6O_2$: 395.4883. Found: 395.2192 (M + H). Anal.: HPLC.

12-30: solid. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d,1H, $J = 2$), 6.39 (d, 1H, $J = 2$), 3.83 (s, 3H), 3.20 (s, 3H), 3.20 (m, 1H), 2.39 (s, 3H), 2.19 (s, 3H), 2.10 (s, 3H), 1.80 (m, 8H), 0.94 (dt, 3H, $J = 7, 3$). CI-HRMS calcd for $C_{23}H_{32}N_5O_2$: 410.5438. Found: 410.2558 (M + H). Anal.: HPLC.

12-31: solid; mp 117.7−118 °C. NMR ($CDCl_3$, 300 MHz): δ 6.67 (s, 2H), 4.30 (m, 4H), 3.80 (s, 3H), 3.78 (t, 4H, $J = 8$), 3.40 (s, 6H), 2.37 (s, 3H), 2.12 (s, 3H), 2.02 (s, 6H). CI-HRMS calcd for $C_{22}H_{32}N_5O_3$: 414.2505. Found: 414.2492 (M + H). Anal.: HPLC.

12-32: oil. NMR ($CDCl_3$, 300 MHz): δ 6.47 (d, 1H, $J = 2$), 6.38 (d, 1H, $J = 2$), 4.10 (m, 2H), 3.82 (s, 3H), 3.71 (s, 3H), 3.56 (s, 3H), 2.39 (s, 3H), 2.18 (s, 3H), 2.09 (s, 3H), 1.75 (m, 2H), 1.40 (m, 2H), 0.98 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{21}H_{30}N_5O_2$: 384.2419. Found: 384.2413 (M + H). Anal.: HPLC.

12-33, free base: solid; mp 72 °C. NMR ($CDCl_3$, 300 MHz): δ 6.93 (s, 1H), 6.77 (s, 1H), 4.20−4.45 (m, 4H), 3.84 (s, 3H), 3.76 (t, $J = 8$, 4H), 3.40 (s, 6H), 2.40 (s, 3H), 2.25 (s, 3H), 2.19 (s, 3H), 2.15 (s, 3H). CI-HRMS calcd for $C_{22}H_{32}N_5O3$: 414.2505. Found: 414.2493 (M + H). Anal.: HPLC.

12-33: white crystalline solid, mp 168.6 °C. NMR (DMSO-d_6, 300 MHz): δ 7.59 (m, 2H), 7.31 (m, 3H), 6.90 (d, 2H, $J = 8$), 3.82 (s, 3H), 3.74 (t, 4H, $J = 6$), 3.28 (s, 6H), 2.32 (s, 3H), 2.18 (s, 3H), 2.13 (s, 3H), 2.07 (s, 3H). CI-HRMS (NH_3) calcd for $C_{22}H_{31}N_5O_3$: 414.2505. Found: 414.2509 $(M + H)^+$. Anal. ($C_{22}H_{31}N_5O_3 \cdot C_6H_5SO_3H$) C, H, N.

12-34: oil. NMR ($CDCl_3$, 300 MHz): δ 6.94 (d, 1H, $J = 12$), 6.89 (d, 1H, $J = 9$), 6.18 (d, 1H, $J = 9$), 4.20 (m, 2H), 3.91 (s, 3H), 2.47 (s, 3H), 2.30 (s, 3H), 2.17 (s, 3H), 1.65 (m, 4H), 1.00 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{20}H_{27}FN_5O$: 372.2199. Found: 372.2203 (M + H). Anal.: HPLC.

12-35: solid. NMR ($CDCl_3$, 300 MHz): δ 7.10 (d, 1H, $J = 11$), 7.08 (d, 1H, $J = 8$), 6.18 (d, 1H, $J = 10$), 4.20 (m, 2H), 3.92 (s, 3H), 2.48 (s, 3H), 2.34 (s, 3H), 1.65 (m, 4H), 1.00 (t, 6H, $J = 7$). CI-HRMS calcd for $C_{19}H_{24}ClFN_5O$: 392.1653 Found: 392.1649 (M + H). Anal. ($C_{19}H_{23}ClFN_5O$) C, H, N.

12-36: solid, mp 96−98 °C. NMR ($CDCl_3$, 300 MHz): δ 7.09 (d, 1H, $J = 12$), 7.08 (d, 1H, $J = 8$), 4.07 (m, 4H), 3.91 (s, 3H), 2.41 (s, 3H), 2.30 (s, 3H), 1.35 (t, 6H, $J = 7$). CI-HRMS calcd for $C_{18}H_{22}ClFN_5O$: 378.1497. Found: 378.1500 (M + H). Anal.: HPLC.

12-37: solid. NMR ($CDCl_3$, 300 MHz): δ 7.10 (d, 1H, $J = 4$), 7.08 (d, 1H, $J = 8$), 4.07 (m, 4H), 3.91 (s, 3H), 2.41 (s, 3H), 2.30 (s, 3H), 1.35 (t, 6H, $J = 7$). CI-HRMS calcd for $C_{20}H_{26}ClFN_5O_3$: 438.1708. Found: 438.1701 (M + H). Anal.: HPLC.

12-38: solid, NMR ($CDCl_3$, 300 MHz): δ 6.97 (s, 1H), 6.84 (s, 1H), 4.00 (m, 4H), 3.90 (s, 3H), 3.86 (s, 3H), 2.40 (s, 3H), 2.30 (s, 3H), 1.75 (m, 4H), 1.40 (m, 4H), 0.99 (t, 6H, $J = 7$). CI-HRMS calcd for $C_{23}H_{33}ClN_5O_2$: 446.2323. Found: 446.2326 (M + H). Anal.: HPLC.

12-39: solid, mp 50−52 °C. NMR ($CDCl_3$, 300 MHz): δ 7.14 (s, 1H), 6.82 (s, 1H), 6.18 (d, 1H, $J = 7$), 4.20 (m, 1H), 3.91 (s, 3H), 3.85 (s, 3H), 2.49 (s, 3H), 2.35 (s, 3H), 1.75 (m, 4H), 1.00 (t, 6H, $J = 7$). CI-HRMS calcd for $C_{20}H_{25}BrN_5O_2$: 448.1348. Found: 448.1355 (M + H). Anal.: HPLC.

12-40: solid, mp 52−58 °C. NMR ($CDCl_3$, 300 MHz): δ 7.14 (s, 1H), 6.82 (s, 1H), 3.91 (s, 3H), 3.85 (s, 3H), 2.96 (q, 4H, $J =$

7), 2.49 (s, 3H), 2.35 (s, 3H), 1.00 (t, 6H, $J = 7$). CI-HRMS calcd for $C_{19}H_{24}BrN_5O_2$: 433.1113. Found: 433.1109 (M, ^{79}Br). Anal.: HPLC.

12-41: oil. NMR ($CDCl_3$, 300 MHz): δ 7.13 (s, 1H), 6.80 (s, 1H), 4.35 (m, 4H), 3.90 (s, 3H), 3.85 (s, 3H), 3.76 (t, 4H, $J = 7$), 3.39 (s, 6H), 2.41 (s, 3H), 2.29 (s, 3H). CI-HRMS calcd for $C_{21}H_{29}BrN_5O_4$: 494.1403. Found: 494.1397 (M + H). Anal.: HPLC.

12-42: oil. NMR ($CDCl_3$, 300 MHz): δ 6.81 (s, 1H), 6.70 (s, 1H), 4.35 (m, 4H), 3.90 (s, 3H), 3.83 (s, 3H), 3.76 (t, 4H, $J = 7$), 3.39 (s, 6H), 2.40 (s, 3H), 2.26(s, 3H), 2.13 (s, 3H). CI-HRMS calcd for $C_{22}H_{32}N_5O_4$: 430.2454. Found: 430.2453 (M + H). Anal.: HPLC.

12-43: oil. NMR ($CDCl_3$, 300 MHz): δ 7.04 (d, $J = 8$, 1H), 6.79 (d, $J = 8$, 1H), 4.11 (m, 4H), 3.83 (s, 3H), 2.39 (s, 3H), 2.22 (s, 3H), 2.20 (s, 3H), 2.07 (s, 3H), 1.35 (t, $J = 8$, 6H). CI-HRMS calcd for $C_{21}H_{28}N_5O$: 354.2216. Found: 354.2211 (M + H). Anal.: HPLC.

12-44: solid; mp 123−124 °C. NMR ($CDCl_3$, 300 MHz): δ 7.05 (d, $J = 8$, 1H), 6.79 (d, $J = 8$, 1H), 6.15 (d, $J = 8$, 1H), 4.19 (m, 1H), 3.85 (s, 3H), 2.46 (s, 3H), 2.30 (s, 3H), 2.22 (s, 3H), 2.10 (s, 3H), 1.70 (m, 4H). CI-HRMS calcd for $C_{21}H_{30}N_5O$: 368.2372. Found: 368.2372(M + H). Anal. ($C_{21}H_{29}N_5O$) C, H, N.

Biological Testing. Some experimental details for the CRF receptor binding assays (rat cortical membranes and human IMR32 cell membranes), the in vitro adenylate cyclase assay, the dog ex vivo binding assay, the defensive withdrawal test, the elevated plus maze test and receptor occupancy studies, have been reported previously.[46−48,52] Additional details are provided below.

Receptor binding affinity to rat cortical receptors was assayed according to the published methods.[46] Curves of the inhibition of ^{125}I-Tyr^0-ovine-CRF binding to cell membranes at various dilutions of test drug were analyzed by the iterative curve fitting program LIGAND[55] which provides IC_{50} values for inhibition which are then used to assess biological activity.

Inhibition of CRF-stimulated adenylate cyclase activity was performed as described by Battaglia and co-workers.[56] Assays are carried out at 37 °C for 10 min in 200 mL of buffer containing 100 mM Tris-HCl (pH 7.4 at 37 °C), 10 mM $MgCl_2$, 0.4 mM EGTA, 0.1% BSA, 1 mM isobutylmethylxanthine (IBMX), 250 units/mL phosphocreatine kinase, 5 mM creatine phosphate, 100 mM guanosine 5′-triphosphate, 100 nM ovine-CRF, antagonist peptides (concentration range from 10^{-9} to 10^{-6} M), and 0.8 mg of original wet weight tissue (approximately 40−60 mg of protein). Reactions are initiated by the addition of 1 mM ATP/[^{32}P]ATP (approximately 2−4 mCi/tube) and terminated by the addition of 100 mL of 50 mM Tris-HCL, 45 mM ATP, and 2% sodium dodecyl sulfate. In order to monitor the recovery of c-AMP, 1 μL of [^{3}H]c-AMP (approximately 40 000 dpm) is added to each tube prior to separation. The separation of [^{32}P]c-AMP from [^{32}P]ATP is performed by sequential elution over Dowex and alumina columns.

Behavioral Pharmacology. Male Sprague−Dawley rats weighing 180−300 g were purchased from Charles River Laboratories (Wilmington, MA). The rats were housed individually in suspended wire cages (for defensive withdrawal studies) or three to a plastic cage (for elevated-plus maze studies) in colony rooms maintained at constant temperature (21 ± 2 °C) and humidity (50 ± 10%). The rooms were illuminated 12 h per day (lights on at 0600 h). The rats had ad libitum access to food and water throughout the studies. Behavioral studies were conducted between 0600 and 1300 h. Animals were maintained in accordance with the guidelines of the Committee on Animals of the Bristol-Myers Squibb Company, the "Guide for Care and Use of Laboratory Animals" (Institute of Animal Laboratory Resources, 1996), and the guidelines published in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Research protocols were approved by the Bristol-Myers Squibb Company Institutional Animal Care and Use Committee.

Test compounds were prepared as suspensions in an aqueous vehicle of 0.25% Methocel (methyl cellulose, type A15c, Dow Chemicals). Suspensions were bead-milled overnight to ensure even suspension using three layers of 4 mm glass beads. Compounds were administered orally by gavage (po) 1 h before behavioral testing in a volume of 2 mL/kg body weight. Doses of all drugs were calculated and are expressed in terms of the free base weight.

The elevated-plus maze, constructed of black, opaque Plexiglas, consisted of four arms at right angles to each other and was elevated 30 cm off the floor. Two of the arms were enclosed and two arms had no walls (open arms). The illumination was 60 lx in both closed and open arms. Rats were placed individually in the center of the maze facing one of the open arms. Behavior was assessed for 5 min by a trained observer (unaware of treatment assignment) via a video monitor. The time in open arms was recorded (in seconds), and data are expressed as percent time in open arms. Entry into an open or a closed arm was defined as placement of all four paws in the open or closed arm. The maze was cleaned with 1.0% glacial acetic acid between animals to prevent olfactory cues from influencing the behavior of subsequently tested animals. The positive control for both studies was compound **2** at a dose of 18 mg/kg, po, administered 1 h before testing. Analogue **12-3** (1.0, 3.0, 10, or 30 mg/kg) was administered acutely po 1 h prior to behavioral testing.

The defensive withdrawal procedure was used with minor modifications.[47] The testing apparatus consisted of an opaque plexiglass open field (106 cm length × 92 cm width × 50 cm height) containing a cylindrical galvanized chamber (14 cm length, 10 cm diameter) that was positioned lengthwise against one wall, with the open end 40 cm from the corner. The open field was illuminated by a 60 W incandescent bulb, and illumination was titrated by a powerstat transformer to a 50 lx reading at the entrance to the cylinder. Rats were habituated to handling by gently stroking their dorsal surface for approximately 1 min the day before testing. To initiate testing, each rat was placed within the cylinder that was then secured to the floor. Behavior was assessed for 15 min by a trained observer (unaware of treatment assignment) via a video monitor in an adjacent room. The latency to exit the chamber, defined by the placement of all four paws into the open field, was recorded (in seconds). The plexiglass chamber and the cylinder were cleaned with 1.0% glacial acetic acid between animals to prevent olfactory cues from influencing the behavior of subsequently tested animals. The positive control for both studies was compound **2** at 10 mg/kg, po, administered 1 h before testing. Analogue **12-3** (1.0, 3.0, 10, or 30 mg/kg) was administered acutely po 1 h prior to behavioral testing.

Results for percent time in open arms and exit latency are expressed as the mean ± SEM. The percent time in open arms data were subjected to analysis of variance (ANOVA) followed by individual mean comparisons using Fisher's least significant difference test (Kirk, 1968) where appropriate. The significance level was set at $p < 0.05$. The exit latency data were subjected to the Kruskal−Wallis test, followed by individual comparisons using the Mann−Whitney U test. The significance level was set at $p < 0.05$.

Rat Pharmacokinetic Studies. The intravenous (iv) bolus dose consisted of compound **12-3** at 0.5 mg/mL (free base equivalent) in 20% ethanol in physiological saline. This was administered to six rats (three male, three female). The oral dose consisted of **12-3** at 1 mg/mL in 0.5% aqueous methylcellulose containing 1% Tween-80. This was administered the three male rats. Blood was collected from the jugular vein into EDTA containing tubes. Samples were collected at predose, 6, 15, and 30 min and 1, 2, 4, 6, 8, 12, 24, and 32 h postdose. The dosage of **12-3** is expressed as free base equivalents. Individual rat plasma samples were analyzed by analysts in the Drug Metabolism and Pharmacokinetics (DM&PK) Section of DuPont Pharmaceuticals Company, Newark, DE. The free base of **12-3** was quantified using a validated LC/MS method. The assay method involved robotic extraction using a Chemelute solid phase extraction cartridge followed by LC/MS detection. [8-(4-Methoxy-2,3-dimethylphenyl)-2,7-dimethylpyrazolo[1,5-*a*]-[1,3,5]triazinyl]bis(2-methoxyethyl)amine, a structural analogue, was used as the internal standard. The assay range was 5 to 5000 nM using 0.25 mL of rat plasma. The pharmacokinetic parameters were calculated using a noncompartmental model on the WATSON

program, version 5.4 v2, 1998, by Pharmaceutical Software Systems, Inc., Wayne, PA. Systemic clearance (CL), volume of distribution at steady state (V_{ss}), apparent half-life ($t_{1/2}$), and area under the plasma concentration time curve from time 0 to infinity ($AUC_{0-\infty}$) were determined. Harmonic mean and pseudostandard deviation were calculated for the half-lives using Excel software (MS Office 97).

Dog Pharmacokinetic Studies. The test compounds were administered to male beagle dogs (8.0−11.0 kg) at 1 mg/kg intravenously or 5 mg/kg orally ($n = 4$/group), and blood was collected up to 72 h postdose. The iv solution (25 mg/mL) was formulated in a mixture of *N*,*N*-dimethylacetamide/propylene glycol/0.12 N sodium hydroxide (10/65/25, v/v/v) with a final pH of 3.0. The oral suspension (5 mg/mL) was prepared in 0.5% methylcellulose containing 1% Tween-80. All plasma concentrations are expressed as the free base concentrations. Dog plasma samples were collected at 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h postdose and analyzed by LC/MS/MS. Sodium hydroxide (0.5 mL, 0.1 N) and 0.1 mL of the internal standard, **2** (500 nM), were added to the plasma (250 μL) and buffer (500 μL) samples. The test compound was extracted with 7 mL of hexane/ether (80/20, v/v). After shaking for at least 10 min at low speed and centrifuging for 5 min at ~3000 rpm, the organic layer was transferred to a 15 mL conical tube and evaporated under nitrogen. The residue was reconstituted with 200 μL of mobile phase (acetonitrile/10 mM ammonium acetate/acetic acid, 70/30/0.1, v/v/v), and test compound concentrations were determined by LC/MS/MS using a Waters Symmetry C_8 column (2.1 mm × 150 mm) and a Sciex API 300 mass spectrometer. The quantitation limit was 1 ng/mL (2.42 nM). The calculation of pharmacokinetic parameters followed the general methods described for the rat pharmacokinetic studies described above.

Note Added after ASAP Publication. An author name was omitted in the version of this paper released to the web on April 10, 2009. The revised version posted on April 15, 2009.

Supporting Information Available: HPLC purity data of final products. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dzierba, C. D.; Hartz, R. A.; Bronson, J. J. Recent advances in corticotropin-releasing factor antagonists. *Annu. Rep. Med. Chem.* **2008**, *43*, 3–23.

(2) Tellew, J. E.; Luo, Z. Small molecule antagonists of the corticotropin-releasing factor (CRF) receptor: recent medicinal chemistry developments. *Curr. Top. Med. Chem.* **2008**, *8*, 506–520.

(3) Gilligan, P. J.; Robertson, D. W.; Zaczek, R. Corticotropin releasing factor (CRF) receptor modulators: progress and opportunities for new therapeutic agents. *J. Med. Chem.* **2000**, *43*, 1641–1660.

(4) McCarthy, J. R.; Heinrichs, S. C.; Grigoriadis, D. E. Recent advances with the CRF_1 receptor: Design of small molecule inhibitors, receptor subtypes and clinical indications. *Curr. Pharm. Des.* **1999**, *5*, 289–315.

(5) Gilligan, P. J. Corticotropin-releasing factor receptor antagonists. *Expert Opin. Ther. Pat.* **2006**, *16*, 913–924.

(6) Dunn, A. J.; Berridge, C. W. Physiological and behavioral responses to corticotropin-releasing factor administration: Is CRF a mediator of anxiety or stress responses? *Brain Res. Rev.* **1990**, *15*, 71–100.

(7) Stenzel-Poore, M. P.; Heinrichs, S. C.; Rivest, R.; Koob, G. F.; Vale, W. W. Overproduction of corticotropin-releasing factor in transgenic mice: a genetic model of anxiogenic behavior. *J. Neurosci.* **1994**, *14*, 2579–2584.

(8) Majzoub, J. A.; Muglia, L. J.; Martinez, C.; Jacobson, L. Molecular and transgenic studies of the corticotropin-releasing hormone gene. *Ann. N.Y. Acad. Sci.* **1995**, *771*, 293–300.

(9) Gulyas, J.; Rivier, C.; Perrin, M.; Koerber, S. C.; Sutton, S.; Corrigan, A.; Lahrichi, S. L.; Craig, A. J.; Vale, W.; Rivier, J. Potent, structurally constrained agonists and competitive antagonists of corticotropin-releasing factor. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 10575–10579.

(10) Perrin, M. H.; Sutton, S. W.; Cervini, L. A.; Rivier, J.; Vale, W. W. Comparison of an agonist, urocortin, and an antagonist, astressin, as radioligands for characterization of corticotropin-releasing factor receptors. *J. Pharmacol. Exp. Ther.* **1999**, *288*, 729–734.

(11) 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.

(12) Valentino, R. J.; Foote, S. L. Brain Noradrenergic Neurons, Corticotropin Releasing Factor and Stress. In *Neural and Endocrine Peptides and Receptors*; Moody, T., Ed.; Plenum Press: New York, 1986; pp 101−120.

(13) Davies, M. The role of the amygdala in fear and anxiety. *Annu. Rev. Neurosci.* **1993**, *15*, 353–375.

(14) Lehnert, H.; Schulz, C.; Dieterich, K. Physiological and neurochemical aspects of corticotropin-releasing factor actions in the brain: the role of the locus coeruleus. *Neurochem. Res.* **1998**, *23*, 1039–1052.

(15) Chalmers, D. T.; Lovenberg, T. W.; De Souza, E. B. Localization of novel corticotropin-releasing factor receptor (CRF_2) mRNA expression to specific subcortical nuclei in rat brain; comparison with CRF_1 receptor mRNA expression. *J. Neurosci.* **1995**, *15*, 6340–6350.

(16) 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.

(17) Kishimoto, T.; Pearse, R. V.; Lin, C. R.; Rosenfeld, M. G. A sauvagine/corticotropin releasing factor receptor expressed in heart and skeletal muscle. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 1108–1112.

(18) Kostich, W. A.; Chen, A.; Sperle, K.; Largent, B. L. Molecular identification and analysis of a novel human corticotropin-releasing factor (CRF) receptor: the $CRF_{2\gamma}$ receptor. *Mol. Endocrinol.* **1998**, *12*, 1077–1085.

(19) 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.

(20) Lovenberg, T. W.; Chalmers, D. T.; Liu, C.; De Souza, E. B. $CRF_{2\alpha}$ and $CRF_{2\beta}$ receptor mRNAs are differentially distributed between the rat central nervous system and peripheral tissues. *Endocrinology* **1995**, *136*, 4139–4142.

(21) Lovenberg, T. W.; Liaw, C. W.; Grigoriadis, D. E.; Clevenger, W.; Chalmers, D. T.; De Souza, E. B.; Oltersdorf, T. Cloning and characterization of a functionally distinct corticotropin releasing factor receptor subtype from rat brain. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 836–840.

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

(23) Chalmers, D. T.; Lovenberg, T. W.; Grigoriadis, D. E.; Behan, D. P.; DeSouza, E. B. Corticotropin-releasing factor receptors: from molecular biology to drug design. *Trends Pharmacol. Sci.* **1996**, *17*, 166–172.

(24) Millan, M. A.; Jacobowitz, D. M.; Hauger, R. L.; Catt, K. J.; Aguilera, G. Distribution of corticotropin-releasing factor receptors in the primate brain. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1921–1925.

(25) Grigoriadis, D. E.; Dent, G. W.; Turner, J. G.; Uno, H.; Shelton, S. E.; De Souza, E. B.; Kalin, N. H. Corticotropin-releasing factor (CRF) receptors in infant rhesus monkey brain and pituitary gland: biochemical characterization and autoradiographic localization. *Dev. Neurosci.* **1995**, *17*, 357–367.

(26) Grigoriadis, D. E.; Lovenberg, T. W.; Chalmers, D. T.; Liaw, C.; De Souza, E. B. Characterization of corticotropin-releasing factor receptor subtypes. *Ann. N.Y. Acad. Sci.* **1996**, *780*, 60–80.

(27) Miranda, A.; Lahrichi, S. L.; Gulyas, J.; Koerber, S. C.; Craig, A. G.; Corrigan, A.; Rivier, C.; Vale, W.; Rivier, J. Constrained corticotropin-releasing factor antagonists with *i*-(*i*+3) Glu−Lys bridges. *J. Med. Chem.* **1997**, *40*, 3651–3658.

(28) Rivier, J.; Lahrichi, S. L.; Gulyas, J.; Erchegyi, J.; Koerber, S. C.; Craig, A. G.; Corrigan, A.; Rivier, C.; Vale, W. W. Minimal size-constrained corticotropin-releasing factor agonists with *i*−(*i*+3) Glu−Lys and Lys−Gln bridges. *J. Med. Chem.* **1998**, *41*, 2614–2620.

(29) Ruhmann, A.; Bonk, I.; Lin, C. R.; Rosenfeld, M. G.; Spiess, J. Structural requirements for peptidic antagonists of the corticotrpoin-releasing factor receptor (CRFR): development of $CRFR_{2\beta}$-selective antisauvagine-30. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15264–15269.

(30) Schulz, D. W.; Mansbach, R. S.; Sprouse, J.; Braselton, J. P.; Collins, J.; Corman, M.; Dunaiskis, A.; Faraci, S.; Schmidt, A. W.; Seeger, T.; Seymour, P.; Tingley, F. D.; 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.

(31) Mansbach, R. S.; Brooks, E. N.; Chen, Y. L. Antidepressant-like effects of CP154526: a selective CRF_1 receptor antagonist. *Eur. J. Pharmacol.* **1997**, *323*, 21–26.

(32) He, L.; Gilligan, P. J.; Zaczek, R.; Fitzgerald, L. W.; McElroy, J. F.; Shen, H.-S. L.; Saye, J. A.; Kalin, N. H.; Shelton, S.; Christ, D.; Trainor, G.; Hartig, P. 4-(1,3-Dimethoxypropyl-2-amino)-2,7-dimethyl-8-(2,4-dichlorophenyl)pyrazolo-[1,5-*a*]-1,3,5-triazine: a potent, orally

bioavailable CRF_1 antagonist. *J. Med. Chem.* **2000**, *430*, 449–456.
(33) Gilligan, P. J.; Baldauf, C.; Cocuzza, A.; Chidester, D.; Zaczek, R.; Fitzgerald, L. W.; McElroy, J. F.; Smith, M. A.; Shen, H.-S.L.; Saye, J. A.; Christ, D.; Trainor, G.; Robertson, D. W.; Hartig, P. The discovery of 4-(3-pentylamino)-2,7-dimethyl-8-(2-methyl-4-methoxyphenyl)pyrazolo-[1,5-*a*]-pyrimidine: a corticotropin-releasing factor antagonist ($hCRF_1$) antagonist. *Bioorg. Med. Chem.* **2000**, *8*, 181–189.
(34) Gold, P. W.; Loriaux, D. L.; Roy, A.; Kling, M. A.; Calkabrese, J. R.; Kellner, C. H.; Nieman, L. K.; Post, R. M.; Pickar, D.; Gallucci, W.; Augerinos, P.; Paul, S.; Oldfiled, E. H.; Cutler, G. B.; Chrousos, G. P. Responses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease. *New Engl. J. Med.* **1986**, *314*, 1329–1335.
(35) Carroll, B. J.; Curtis, G. C.; Davies, B. M.; Mendels, J.; Sugarman, A. A. Urinary free cortisol secretion in depression. *Psychol. Med.* **1976**, *6*, 43–50.
(36) Raadsheer, F. C.; Hoogendijk, W. J. G.; Stam, F. C.; Tilders, F. J. H.; Swaab, D. F. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Neuroendocrinology.* **1994**, *60*, 436–444.
(37) Nemeroff, C. B.; Bisette, G.; Akil, H.; Fink, M. Neuropeptide concentrations in the cerebrospinal fluid of depressed patients treated with electroconvulsive therapy: corticotropin-releasing factor, β-endorphin,and somatostatin. *Br. J. Psychiatry* **1991**, *158*, 59–63.
(38) Banki, C. M.; Karmacsi, L.; Bissette, G.; Nemeroff, C. B. CSF corticotropin releasing hormone and somatostatin in major depression: response to antidepressant treatment and relapse. *Eur. Neuropsychopharmacol.* **1992**, *2*, 107–113.
(39) DeBellis, M. D.; Gold, P. W.; Geracioti, T. D.; Listwak, S. J.; Kling, M. A. Association of fluoxetine treatment with reductions in CSF concentrations of corticotropin-releasing hormone and arginine vasopressin in patients with major depression. *Am. J. Psychiatry.* **1993**, *150*, 656–657.
(40) Kling, M. A.; Geracioti, T. D.; Licinio, D.; Michelson, E. H.; Oldfield, E. H.; Gold, P. W. Effects of electroconvulsive therapy on the CRH-ACTH-cortisol sytem in melancholic depression: preliminary findings. *Psychopharmacol. Bull.* **1994**, *30*, 489–494.
(41) Zobel, A. W.; Nickel, T.; Kunzel, H. E.; Ackl, N.; Sonntag, A.; Ising, M.; Holsboer, F. Effects of the high affinity corticotropin releasing hormone receptor 1 antagonist R121919 in major depression: the first 20 patients treated. *J. Psychiatr. Res.* **2000**, *34*, 171–181.
(42) Binneman, B.; Feltner, D.; Kolluri, S.; Shi, Y.; Qiu, R.; Stiger, T. A 6-week randomized, placebo-controlled trial of CP-316,311 (a selective CRH1 antagonist) in the treatment of major depression. *Am. J. Psychiatry* **2008**, *165*, 617–620.
(43) Chen, Y. L.; Braselton, J.; Forman, J.; Gallaschun, R. J.; Mansbach, R.; Schmidt, A. W.; Seeger, T. F.; Sprouse, J. S.; Tingley, F. D.; Winston, E.; Schulz, D. W. Synthesis and SAR of 2-aryloxy-4-alkoxypyridines as potent orally active corticotropin-releasing factor 1 receptor antagonists. *J. Med. Chem.* **2008**, *51*, 1377–1384.
(44) He, L.; Gilligan, P. J.; Fitzgerald, L.; Zaczek, R.; Shen, H.; Trainor, G. L.; Hartig, P. R. Pyrazolo-[1,5-*a*]-*s*-triazine as Novel hCRF1 Receptor Antagonists: Design, Synthesis and Structure Activity Relationships. Presented at the American Chemical Society National Meeting, Anaheim, CA, March 21−25, 1999; MEDI 107.
(45) Zhou, J.; Oh, L. M.; Ma, P. Convergent Synthesis of α-Aryl-β-ketonitriles from Aryl Boronic Acids and Isoxazoles. US 6562965, 2003.
(46) DeSouza, E. B. Corticotropin-releasing factor receptors in the rat central nervous system: characterization and regional distribution. *J. Neurosci.* **1987**, *7*, 88–100.
(47) McElroy, J. F.; Ward, K.; Zeller, K. L.; Jones, K. W.; Gilligan, P. J.; He, L.; Lelas, S. The CRF_1 receptor antagonist DMP696 produces anxiolytic effects and inhibits the stress-induced hypothalamic-pituitary-adrenal axis activation without sedation or ataxia in rats. *Psychopharmacology* **2002**, *165*, 86–92.
(48) Li, Y.-W.; Fitzgerald, L.; Wong, H.; Lelas, S.; Zhang, G.; Lindner, M. D.; Wallace, T.; McElroy, J.; Lodge, N. J.; Gilligan, P. J.; Zaczek, R. The pharmacology of DMP696 and DMP904, non-peptidergic CRF_1 receptor antagonists. *CNS Drug Rev.* **2005**, *11*, 21–52.
(49) Sutton, S. W.; Behan, D. P.; Lahrichi, S. L.; Kaiser, R.; Corrigan, A.; Lowry, P.; Potter, E.; Perrin, M. H.; Rivier, J.; Vale, W. Ligand requirements of the human corticotropin-releasing factor-binding protein. *Endocrinology* **1995**, *136*, 1097–1102.
(50) Fitzgerald, L. Unpublished results.
(51) Gilligan, P. J.; Rabel, S. R.; Meenan, P. A. Salt and Crystalline Form Thereof of a Corticotropin Releasing Factor Receptor Antagonist. U.S. Patent Application US2005113375, 2005.
(52) Li, Y.-W.; Hill, G.; Wong, H.; Kelly, N.; Ward, K.; Pierdomenico, M.; Ren, S.; Gilligan, P.; Grossman, S.; Trainor, G.; Taub, R.; McElroy, J.; Zazcek, R. Receptor occupancy of nonpeptide corticotropin-releasing factor 1 antagonist DMP696: correlation with drug exposure and anxiolytic efficacy. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 86–96.
(53) He, L.; Gilligan, P. J.; Arvanitis, A. G.; Chorvat, R. J. Azolo Triazines and Pyrimidines. US 6124289, 2000.
(54) Perrin, D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: New York, 1988.
(55) Munson, P. J.; Rodbard, D. LIGAND: a versatile computerized approach for characterization of ligand binding systems. *Anal. Biochem.* **1980**, *107*, 220–239.
(56) Battaglia, G.; Webster, E. L.; De Souza, E. B. Characterization of corticotropin-releasing factor (CRF) receptor-mediated stimulation of adenylate cyclase activity in rat brain. *Synapse* **1987**, *1*, 572–581.

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