Synthesis and Structure−Activity Relationships of 8-(Pyrid-3-yl)pyrazolo[1,5-*a*]-1,3,5-triazines: Potent, Orally Bioavailable Corticotropin Releasing Factor Receptor-1 (CRF_1) Antagonists

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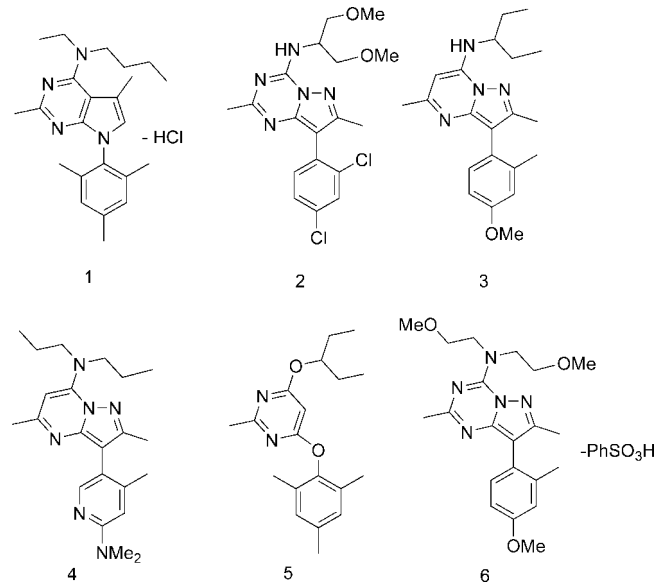
This report describes the syntheses and structure−activity relationships of 8-(substituted pyridyl)pyrazolo[1,5-*a*]-1,3,5-triazine corticotropin releasing factor receptor-1 (CRF_1) receptor antagonists. These CRF_1 receptor antagonists may be potential anxiolytic or antidepressant drugs. This research resulted in the discovery of compound **13-15**, which is a potent, selective CRF_1 antagonist ($hCRF_1$ $IC_{50} = 6.1 \pm 0.6$ nM) with weak affinity for the CRF-binding protein and biogenic amine receptors. This compound also has a good pharmacokinetic profile in dogs. Analogue **13-15** is orally effective in two rat models of anxiety: the defensive withdrawal (situational anxiety) model and the elevated plus maze test. Analogue **13-15** has been advanced to clinical trials.

Corticotropin-releasing factor (CRF[a]), also known as corticotropin-releasing hormone (CRH), is a 41-amino acid residue peptide that has been implicated in the pathophysiology of anxiety and depression.[1−4] CRF functions as a pituitary ACTH secretagogue[5,6] and a neurotransmitter.[7−9] Preclinical studies have documented the essential role of CRF in regulation of endocrine, autonomic, and behavioral responses to stress.[3] Peptidic CRF antagonists, such as α-helical CRF_{9-41}, not only block the effects of exogenous CRF but also block the effects of various natural stressors. Preclinical studies with selective, non-peptide CRF_1 receptor antagonists, such as **1** (CP 154526-1),[10,11] **2** (DMP696),[12] and **3** (DMP 904),[13] provide strong support for the hypothesis that this receptor subtype may be involved in anxiety and depression. Some clinical studies have implicated CRF in the pathophysiology of both depression and anxiety. Maladaptation to chronic stress and related chronic elevation of corticosteroids may be a major pathway leading to some, but not all, forms of depression.[14] Hypercortisolemia in these forms of depression appears to be a direct result of chronic hypersecretion of hypothalamic CRF.[15] The small molecule CRF_1 receptor antagonist **4** (R121919, Scheme 1) had some efficacy in a small open label clinical study in anxiety and depression.[16] However, another CRF_1 antagonist **5** (CP316311) failed in a trial for depression.[17] Structurally diverse CRF_1 antagonists may have different clinical profiles; additional studies may be needed to define the clinical utility of CRF antagonists.

This report describes the syntheses and structure−activity relationships of 8-(pyrid-3-yl)pyrazolo[1,5-*a*]-1,3,5-triazine CRF_1 receptor antagonists, which may have utility as anxiolytic and antidepressant drugs. Previous work on bicyclic CRF antagonists led to the discovery of compounds such as **1**[10,11] and **2**[12] with high lipophilicity (HPLC log $P > 5.0$) or low water solubility (<1 μg/mL, pH 7.0). We have independently reported on several bicyclic CRF_1 receptor antagonist chemotypes including compound **6** (BMS-561388), which was advanced into phase 1 clinical trials.[18] We sought ways to improve water solubility and to improve pharmacokinetic profiles by introduction of substituted pyridyl groups on a pyrazolotriazine core. Our structure−activity studies led to improved physical properties and good efficacy profiles when a 2-methyl-6-methoxypyrid-3-yl group was introduced at the 8-position of the pyrazolotriazine core. One of these compounds, **13-15**, was advanced into clinical trials.

Scheme 1



Results and Discussion

Chemistry. Pyridyl compounds were prepared according to procedures that are outlined in Schemes 2 and 3.[19,20] The pyridyl

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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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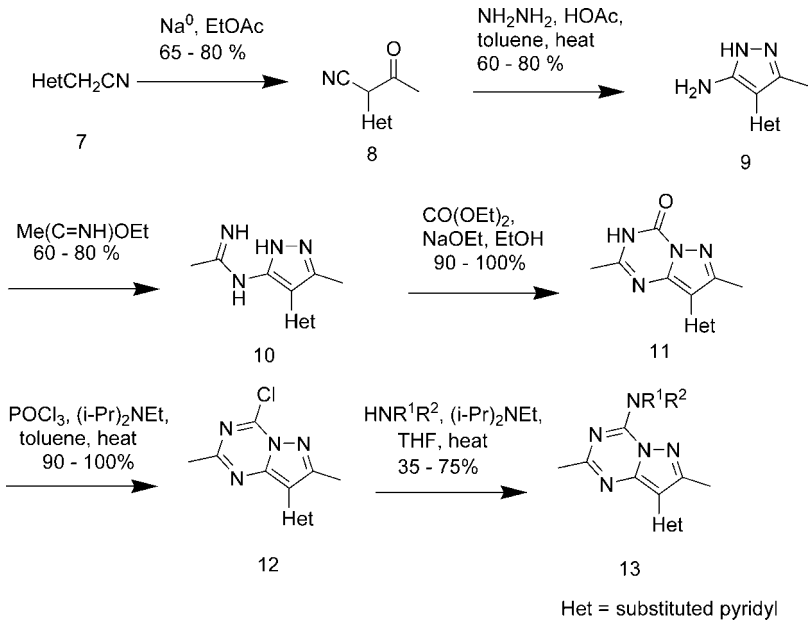
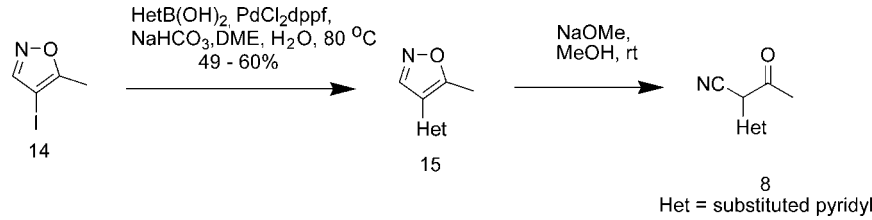
Scheme 2



Scheme 3



starting materials were obtained from commercial sources or by custom synthesis as described in the Experimental Section. Cyanoketones **8** were prepared by acylation of pyridylacetonitriles **7** or by cleavage of isoxazoles **15**. Aminopyrazoles **9** were prepared by condensation of hydrazine with cyanoketones **8**. Treatment of these amines with ethyl acetamidate, followed by cyclization with diethyl carbonate, generated pyrazolotriazinones **11**. Conversion to the chloropyrazolotriazines by treatment with phosphorus oxychloride, followed by displacement of the chloride with various amines, gave the final products **13**.

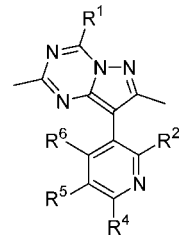
Pharmacology. Pyrazolo[1,5-*a*]-1,3,5-triazines **13** were studied in a series of in vitro and in vivo tests to identify preclinical candidates. The compounds were first tested for their binding affinity to rat cortical homogenate CRF receptors.[21] Compounds with high receptor binding affinity ($IC_{50} \leq 20$ nM) were then evaluated in dog pharmacokinetic studies (1 mg/kg, po) using a cassette dosing paradigm. Compounds that had AUC (po) values greater than 1000 nM·h and $t_{1/2}$ (po) values greater than 10 h were advanced to secondary in vitro biochemical experiments, rat behavioral pharmacology studies, and rat and chimpanzee pharmacokinetic studies with individual compounds. The leading compounds were tested for their affinity to CRF_1 receptors endogenously expressed in human IMR32 neuroblastoma cells,[10] since rat cortical membranes contain CRF_1 and, to a much lesser extent, CRF_2 sites. Antagonist vs agonist function was determined by measuring a compound's effects on CRF-stimulated ACTH production in rat pituitary cells. Anxiolytic efficacy was assessed in the rat defensive withdrawal and elevated plus maze models.[22−24]

The rat CRF receptor binding data for compounds **13** are summarized in Table 1. Introduction of a 2,4-dimethyl-6-dimethylaminopyrid-3-yl group at the 8-position of the pyrazolotriazine core affords analogues with moderate binding affinity (compounds **13-1** to **13-7**). The most potent analogues in this subseries, **13-5**, **13-6**, and **13-7**, have tertiary amine side chains at the 4-position. Replacement of the 2-methyl group with hydrogen on the pyrid-3-yl portion led to modest improvements in receptor binding affinity in two cases (compare **13-9** with **13-3** as well as **13-8** with **13-4**). Replacement of the dimethylamino group with a methoxy substituent at the 6-position of the pyrid-3-yl region gives small improvements in receptor binding affinity in two cases (compare **13-11** with **13-9**, **13-12** with **13-8**, and **13-13** with **13-10**). However, introduction of the 2-methyl-6-methoxypyridyl group at the 8-position of the pyrazolotriazine core affords analogues with superior receptor binding affinity (compounds **13-16** to **13-30**, Table 1).

Compounds with CRF binding IC_{50} values less 20 nM were submitted to dog cassette dosing pharmacokinetic studies (1 mg/kg, po). Data for only the leading compounds are presented in Table 2, all of which have the 2-methyl-6-methoxypyrid-3-yl substituent. Compounds **13-15**, **13-16**, **13-18**, and **13-24** have high AUC, C_{max}, and $t_{1/2}$ values. Analogue **13-15** has the best AUC and C_{max} values in this subset (AUC = 2020.0 ± 558.6 nM·h and C_{max} = 301.3 ± 117.2 nM). The reference compound **6** has a comparable profile (AUC = 1661.0 ± 210.6 nM·h and C_{max} = 369.0 ± 92.6 nM).[25]

Compound **13-15** was profiled further in other receptor binding assays to test for cross-reactivity with other sites. This analogue potently displaces ^{125}I-Tyr^0-ovine-CRF (150 pM) from human CRF_1 receptors which are endogenously expressed in human IMR32 cells[10] (mean IC_{50} values (±standard deviation) equal to 10.6 ± 4.5 nM (n = 8) vs IC_{50} = 2.0 ± 0.5 nM (n =

Table 1. Rat CRF Receptor Binding Affinity Data[a]



compd	R^1	R^2	R^4	R^5	R^6	rat IC_{50} + SD (nM)	*n*
13-1	$NHCHEt_2$	Me	NMe_2	H	Me	25.6 ± 0.6	3
13-2	$NHCH_2CH_2OMe$	Me	NMe_2	H	Me	>10000	3
13-3	$N(CH_2CH_2OMe)_2$	Me	NMe_2	H	Me	133.3 ± 18.8	4
13-4	NEt_2	Me	NMe_2	H	Me	58.0 ± 17.6	3
13-5	NPr_2	Me	NMe_2	H	Me	14.9 ± 4.0	3
13-6	NEtBu	Me	NMe_2	H	Me	32.8 ± 13.9	3
13-7	NPrBu	Me	NMe_2	H	Me	12.8 ± 1.6	3
13-8	NEt_2	H	NMe_2	H	Me	13.8 ± 3.0	3
13-9	$N(CH_2CH_2OMe)_2$	H	NMe_2	H	Me	91.9 ± 4.5	3
13-10	$NHCHEt_2$	H	NMe_2	H	Me	24.8 ± 5.0	3
13-11	$N(CH_2CH_2OMe)_2$	H	MeO	H	Me	23.8 ± 15.4	4
13-12	NEt_2	H	MeO	H	Me	14.5 ± 5.4	3
13-13	$NHCHEt_2$	H	MeO	H	Me	3.0 ± 2.0	3
13-14	$NHCHEt_2$	Me	MeO	H	H	3.1 ± 0.1	3
13-15	(*R*)-NHCHMeEt	Me	MeO	H	H	6.1 ± 0.6	6
13-16	(*S*)-NHCHMeEt	Me	MeO	H	H	7.0 ± 0.6	4
13-17	(*S*)-NHCHMePr	Me	MeO	H	H	4.5 ± 1.6	3
13-18	(*R*,*S*)-NHCHMePr	Me	MeO	H	H	3.5 ± 0.5	4
13-19	NHCHMeBu	Me	MeO	H	H	5.1 ± 0.9	3
13-20	NEt_2	Me	MeO	H	H	12.0 ± 3.0	4
13-21	NPr_2	Me	MeO	H	H	5.7 ± 1.4	4
13-22	$N(CH_2CH_2OMe)_2$	Me	MeO	H	H	8.5 ± 1.2	3
13-23	$NEt(CH_2CH_2OMe)$	Me	MeO	H	H	6.6 ± 1.3	3
13-24	$N(CH_2\text{-}c\text{-}C_3H_5)CH_2CH_2OMe$	Me	MeO	H	H	3.1 ± 0.4	3
13-25	$N(CH_2\text{-}c\text{-}C_3H_5)Pr$	Me	MeO	H	H	1.6 ± 1.5	3
13-26	NMeEt	Me	MeO	H	H	81.6 ± 20.7	3
13-27	NMePr	Me	MeO	H	H	17.6 ± 4.3	3
13-28	NMeBu	Me	MeO	H	H	8.1 ± 7.2	3
13-29	NEtBu	Me	MeO	H	H	4.9 ± 1.4	4
13-30	NPrBu	Me	MeO	H	H	2.4 ± 1.2	3
α-helical CRF_{9-41}						1.24 ± 0.8	8

[a] SD is the standard deviation, and *n* is the number of measurements.

Table 2. Dog Casette Dosing Pharmacokinetic Data (1 mg/kg, po)[a]

compd	mean AUC (nM·h)	mean C_{max} (nM)	mean $t_{1/2}$ (h)
13-15	2020.0 ± 558.6	301.3 ± 117.2	10.7 ± 1.8
13-24	1726.9 ± 565.7	313.7 ± 143.6	11.4 ± 4.8
13-22	1465.1 ± 261.3	275.0 ± 106.4	10.3 ± 2.5
13-18	955.4 ± 347.6	155.7 ± 62.6	11.8 ± 2.9
13-16	916.3 ± 159.5	168.3 ± 46.9	7.9 ± 0.8
6	1661.0 ± 210.6	369.0 ± 92.6	10.1 ± 1.7

[a] Vehicle = Labrafil; $n = 3$ dogs for each compound except reference compound **6** where $n = 54$ (18 separate studies, 3 dogs each). Standard errors of the mean are reported.

20) for α-helical CRF_{9-41}). Compound **13-15** does not displace ^{125}I-Tyr^0-sauvagine (150 pM) from $CRF_{2\beta}$ receptors expressed in pig choroid plexus membranes (IC_{50} > 1000 nM).[26] This analogue does not bind to the human CRF binding protein (IC_{50} > 1000 nM).[26] Analogue **13-15** was sent to NovaScreen (Hanover, MD) for an extensive screening in a variety of assays including receptor binding, ion channel effects, regulatory sites, second messengers, uptake sites, and enzymes. This compound was evaluated at 10^{-7} and 10^{-5} M to determine if it exhibited significant activity, i.e., greater than 50% inhibition or enhancement of binding, in 99 assays. Compound **13-15** at 10 μM inhibited ligand binding in the adenosine A_1 assay by 90% and in the neurokinin 2 assay by 80%. This compound was then tested twice in a dose response study for the adenosine A_1 receptor using the cloned rat receptor transfected into CHO cells.[26] The resultant K_i values were 2660 and 3520 nM. The IC_{50} for the human neurokinin 2 receptor was determined to be 4.9 μM, based on two experiments.[26]

Introduction of the 2-methyl-6-methoxypyrid-3-yl group at the 8-position of the pyrazolotriazine core also afforded improved solubility in the case of compound **13-15**. The key solubilities were 16 μg/mL in water (pH 7.4), 16.3 mg/mL in 0.01 N HCl (pH 2.5), 300 mg/mL in ethanol, and 460 mg/mL in acetone. The solubilities for the reference compound **2** were <1 μg/mL in water (pH 7.4) and 0.23 mg/mL in dilute HCl (pH 2.3). Reference compound **6** had solubilities of <1 μg/mlL in water (pH 7.4) and 2.5 mg/mL in dilute HCl (pH 2.3). HPLC log *P* values are 4.32, 4.97, and 4.76 for compounds **13-15**, **2**, and **6**, respectively.

Analogue **13-15** is an antagonist of rat CRF_1 receptors expressed in rat brain homogenates. It inhibits CRF (0.3 nM)-mediated adrenocorticotropic hormone (ACTH) release from pituitary cell culture with an IC_{50} equal to 129 ± 17 nM ($n = 3$) and has no agonist properties. In the same experiments, the r/hCRF concentration response was also evaluated and a concentration at 50% maximum effect (EC_{50}) equal to 0.3 ± 0.1 nM ($n = 3$) was observed. Analogue **13-15** does not exhibit partial agonist or inverse agonist activity in the absence of CRF stimulation. The reference compound **6** had an IC_{50} value equal to 72.9 ± 10.4 nM ($n = 4$), while r/hCRF had an EC_{50} value equal to 0.3 ± 0.1 nM ($n = 4$).

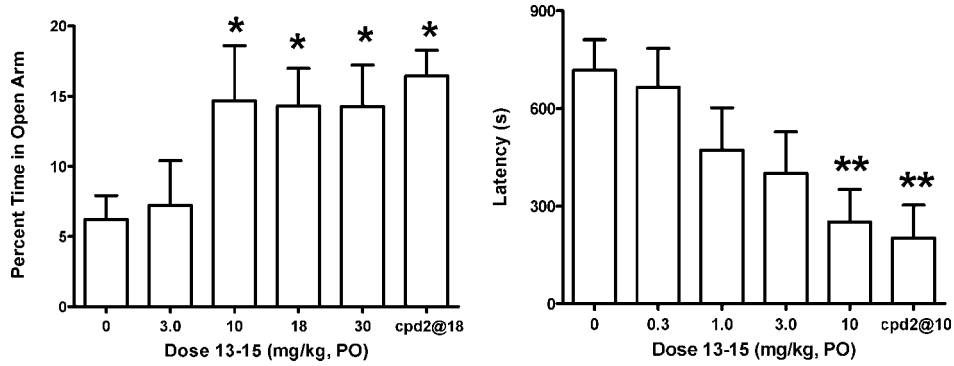


Figure 1. Rat behavioral data. Anxiolytic-like effects of **13-15** in the elevated-plus maze (left) and defensive withdrawal tests (right) in rats. Abscissae indicate the dose (mg/kg). cpd2@18 is compound **2** at 18 mg/kg, and cpd2@10 is compound **2** at 10 mg/kg. Left panel ordinate displays the mean (±SEM) percent time in open arms for $n = 12$ animals per dose. Right panel ordinate displays the mean (±SEM) latency to exit the dark chamber (in seconds) for $n = 8$ animals per dose. Maximum latency is 900 s. Compounds **13-15** and **2** were administered in 0.25% Methocel, po, 60 min prior to testing: (*) $p < 0.05$; (**) $p < 0.01$.

Compound **13-15** was next evaluated in the rat elevated plus maze test[22−24] for possible anxiolytic efficacy. 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 7.3 ± 1.6 s. The positive control, compound **2** at a dose of 18 mg/kg, significantly increased the percent time spent in open arms ($p = 0.05$). Pretreatment with **13-15** increased percent time in open arms [$F(5,66) = 2.35$, $p = 0.05$, analysis of variance (ANOVA) followed by individual mean comparisons using Fisher's least significant difference test]. The lowest effective dose was 10 mg/kg ($p = 0.02$; Figure 1, left panel). Higher doses of **13-15** (18 and 30 mg/kg) also increased percent time in open arms of the elevated-plus maze ($p = 0.03$).[23]

The anxiolytic efficacy of analogue **13-15** was also assessed in the rat defensive withdrawal model (Figure 1, right panel) [22−24] 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 717 ± 94 s (80% of the total test duration). The positive control **2** at a dose of 10 mg/kg decreased exit latency by 72% relative to vehicle control ($p = 0.004$). Pretreatment with **13-15** decreased exit latency ($H(5) = 14.34$, $p = 0.02$, 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 44% relative to vehicle-treated animals ($p = 0.06$; Figure 1, right panel). A higher dose of **13-15** (10 mg/kg) decreased exit latency by 65% ($p = 0.008$).[23]

The CRF receptor occupancy of compound **13-15** 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 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[23] established receptor occupancy relative to vehicle-control animals. The receptor occupancy (±standard error of the mean) was calculated to be 37 ± 6%, 26 ± 15%, 59 ± 12%, 69 ± 9%, and 85 ± 10% 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 59 ± 12% receptor occupancy in ex vivo studies.

Compound **13-15** was evaluated for CNS side effects to determine whether the apparent anxiolytic efficacy may be confounded by other behavioral effects. In the rat spontaneous locomotor activity test,[22−24] compound **13-15** has modest but not statistically significant effects at doses up to 100 mg/kg (po),

Table 3. Rat Spontaneous Locomotor Activity Data[a]

compd	dose, po (mg/kg)	N	total distance ± SEM (cm)
13-15	0	8	5389 ± 950
	1.0	8	4106 ± 407
	3.0	8	5639 ± 942
	10	8	3695 ± 244
	30	8	4846 ± 627
	100	8	4041 ± 419
chlordiazepoxide	0	8	5760 ± 963
	3.0	8	4624 ± 738
	10	8	3981 ± 259[b]
	30	8	2162 ± 264[c]
	100	8	1062 ± 161[c]

[a] The vehicle was 0.25% Methocel. Compounds were administered 1 h pretest. SEM is the standard error of the mean, and N is the number of rats. [b] Latency value differs significantly from that of the associated vehicle group (zero dose) with $p < 0.05$. [c] Latency value differs significantly from that of the associated vehicle group (zero dose) with $p < 0.001$.

Table 4. Rat Rotorod Performance Data[a]

compd	dose, po (mg/kg)	N	time on rotorod ± SEM (s)
13-15	0	8	158 ± 30
	1.0	8	133 ± 26
	3.0	8	162 ± 14
	10	8	158 ± 16
	30	8	157 ± 27
	100	8	121 ± 16
chlordiazepoxide	0	8	205 ± 23
	3.0	8	227 ± 20
	10	8	128 ± 35
	30	8	114 ± 23[b]

[a] The vehicle was 0.25% Methocel. Compounds were administered 1 h pretest. SEM is the standard error of the mean, and N is the number of rats. [b] Latency value differs significantly from that of the associated vehicle group (zero dose) with $p < 0.05$.

while chlordiazepoxide has pronounced effects at 30 and 100 mg/kg (po) (Table 3). In the rat rotorod test,[22−24] compound **13-15** has no significant effect on rotorod performance up to 100 mg/kg (po) while chlordiazepoxide impaired performance at 10 and 30 mg/kg (po) (Table 4). Thus, **13-15** has a side effect profile in the locomotor and rotorod tests, which is superior to chlordiazepoxide. Furthermore, the efficacy of **13-15** does not appear to be influenced by motoric side effects.

Compound **13-15** was evaluated in rat pharmacokinetic studies. Rats were dosed intravenously at 1 mg/kg and orally at 5 mg/kg ($n = 3$, Table 5). Reference compound **6** was also administered separately. Compounds **13-15** and **6** have good maximal oral exposure ($C_{max} = 1260.8 \pm 552.5$ nM and 851 ± 195 nM, respectively), good oral bioavailability (40% and 51%,

Table 5. Rat Pharmacokinetic Parameters for Compounds **6** and **13-15** (1 mg/kg (iv), 5 mg/kg (po))[a]

parameter	**6**	**13-15**
CL ((mL/min)/kg)	20.0 ± 3.3	17.9 ± 4.8
V_{ss} (L/kg)	14.6 ± 3.9	14.9 ± 1.0
$t_{1/2}$ (h)	9.7 ± 2.8	13.5 ± 3.1
C_{max} (po, nM)	851 ± 195	1260.8 ± 552.5
t_{max} (po, h)	0.5	0.75
AUC (μM·h)	5330 ± 2070	5814.6 ± 1306.7
F (po, %)	51	40

[a] $n = 3$. Oral vehicle = 0.5% methylcellulose. iv vehicle =10% DMAC, 70% propylene glycol, and deionized water.

Table 6. Pharmacokinetic Parameters for Compound **13-15** in Chimpanzee (1 mg/kg, po)[a]

parameter	iv chimpanzee	oral chimpanzee	oral chimpanzee
dose (mg/kg)	0.5	1.0	1.0
AUC_{inf} (μM·h)	11.9	11.7	16.1
C_{max} (μM)		0.84	1.3
CL ((L/h)/kg)	0.12		
V_{ss} (L/kg)	4.2		
$t_{1/2}$ (h)	30	26	26
F (po, %)		49	68

[a] Oral vehicle = 50−50 (v/v) mixture of 0.5% methylcellulose and Tang orange juice containing 1% Tween-80. iv vehicle = 10% DMAC, 70% propylene glycol, and deionized water over 10 min (0.2 mL/kg).

Table 7. Pharmacokinetic Parameters for Compound **6** in Chimpanzee (0.5 mg/kg, iv, or 1 mg/kg, po)[a]

parameter	iv	po
N	2	3
dose (mg/kg)	0.5	1.0
AUC_{inf} (μM·h)	2.04	0.212 ± 0.089
C_{max} (μM)		0.027 ± 0.006
CL ((L/h)/kg)	0.61	
V_{ss} (L/kg)	9.52	
$t_{1/2}$ (h)	20.2	16.5 ± 3.4
F (po, %)		5.2 ± 2.2

[a] Oral vehicle = 50−50 (v/v) mixture of 0.5% methylcellulose and Tang orange juice containing 1% Tween-80. iv vehicle = 10% DMAC, 70% propylene glycol, and deionized water over 10 min (0.2 mL/kg).

respectively), and acceptable elimination half-lives (13.5 ± 3.1 h and 9.7 ± 2.8 h, respectively).

Analogue **13-15** also has an excellent oral pharmacokinetic profile in chimpanzees (Table 6). The compound was dosed in two animals at 1 mg/kg (po), using a Tang orange drink−Methocel vehicle. In addition, the compound was administered intravenously to one chimpanzee at a dose of 0.5 mg/kg in 10% dimethylacetamide, 70% propylene glycol, and deionized water over 10 min (0.2 mL/kg). The key parameters are as follows: CL = 0.12 (L/kg)/h, C_{max}(po) = 840 and 1300 nM, elimination $t_{1/2}$ = 26 h, and percent oral bioavailability F = 49% and 68% (animals 1 and 2, respectively). In contrast, reference compound **6**, which was administered at the same doses, had higher clearance (0.61 (L/kg)/h), lower C_{max}(po) (270 ± 6 nM), shorter $t_{1/2}$ (16.5 ± 3.4 h), and lower oral bioavailability (5.2 ± 2.2%) (Tables 6 and 7).

Corticotropin-releasing factor has peripheral as well as central pharmacological effects. It is therefore important to assess the peripheral effects of potential CRF_1 antagonist candidates. Analogue **13-15** was tested for effects on cardiovascular, pulmonary, renal, and gastrointestinal functions.

Compound **13-15** was examined in dogs for effects on cardiovascular function. The procedures followed those described for reference compound **2**.[12] In an anesthetized dog study, mongrel dogs were maintained on sodium pentobarbital anesthesia and mechanically ventilated. Transducers were implanted for measurement of left ventricular and arterial blood pressures, and the ECG was recorded. The following parameters were determined: the first derivative of the left ventricular pressure (to assess cardiac contractility); mean arterial blood pressure; heart rate; and the PR, QRS, QT, and QTc intervals. Each of four dogs received a 6 min continuous intravenous infusion of **13-15** at escalating doses of 0.3, 1, 3, and 5 mg/kg, with a 30 min interval between successive doses. A separate group of four dogs received the vehicle on the same schedule. Blood samples for determination of plasma concentration of **13-15** were obtained after each infusion and at the end of the experiment.

Intravenous administration of **13-15** did not cause a significant change in heart rate or PR, QRS, QT, or QTc interval in the ECG. However, dogs administered 3 and 5 mg/kg doses showed minimal and transient increases in mean arterial pressure of approximately 10 mmHg, coincident with dose escalation. No change in mean arterial pressure was detected in a similar time frame in anesthetized, spontaneously breathing mongrel dogs given **13-15** as a single intravenous dose of 5 mg/kg. Cardiac contractility also increased (approximately 200 mmHg/s) coincident with dose escalation to the two highest doses. In addition, contractility gradually increased in two of four dogs during the last 2 h of the experiment, although the peak increase did not correlate with the peak plasma concentration of **13-15**. Since this change was not observed consistently within the group of dogs and was not accompanied by changes in other cardiovascular parameters, it is probably unrelated to the compound. Total pooled plasma concentrations at the end of each infusion of 0.3, 1, 3, and 5 mg/kg were 149.9, 472.8, 1293, and 2427 nM, respectively (0.1−1.7 times the mean single-dose human C_{max} at 100 mg). In conclusion, **13-15**, when administered intravenously in the anesthetized dog model, produced a minimal and transient increase in mean arterial pressure and cardiac contractility following dose escalation at ≥3 mg/kg. There was no effect on heart rate or ECG parameters.

The effects of **13-15** were examined on respiratory function in spontaneously breathing, anesthetized mongrel dogs ($n = 4$). The procedures followed those previously described for reference compound **2**.[12] Infusion of **13-15** at 5.0 mg/kg to barbiturate-anesthetized dogs over 6 min resulted in a transient (less than 10 min) increase in respiration rate (37.5 ± 21.7%) and in minute volume (28.0 ± 9.5%) when compared to dogs receiving a vehicle infusion. These effects disappeared by 15 min postdose. No other respiratory parameters were affected. Neither mean arterial pressure nor heart rate was affected by compound **13-15** in this study. Total pooled plasma concentrations were 1790, 834.8, 412.1, and 229.0 nM at 6, 15, 30, and 60 min, respectively. The reference compound **2** was previously reported to have no effects in this model, but a different vehicle was employed because of solubility differences.[12] Thus, compound **13-15** (5.0 mg/kg, iv) has a minimal effect acutely on respiratory function with no effect upon mean arterial pressure and heart rate.

The effects of **13-15** on renal function in rats ($n = 9$) were also examined according to previously published procedures.[12] Administration of analogue **13-15** (30 mg/kg, po) resulted in an increase in urine volume (3.0 ± 0.1 mL/100 g body weight vs 1.7 ± 0.2 mL/100 g body weight for vehicle). The compound also caused decreases in the concentration of glucose (4.4 ± 0.4 mg/dL per 100 g body weight vs 9.9 ± 1.9 mg/dL per 100 g body weight for vehicle), urea (166 ± 18 mg/dL per 100 g body weight vs 321 ± 62 mg/dL per 100 g body weight for vehicle), creatinine (8.0 ± 0.9 mg/dL per 100 g body weight vs 16.2 ± 3.3 mg/dL per 100 g body weight for vehicle), sodium (58.4 ±

3.4 mg/dL per 100 g body weight vs 49.9 ± 3.1 mg/dL per 100 g body weight for vehicle), and potassium (32.1 ± 2.8 mg/dL per 100 g body weight vs 60.3 ± 11.1 mg/dL per 100 g body weight for vehicle) in the urine ($p < 0.5$ for all cases). Plasma concentrations were determined in a different cohort of rats at 1 and 5 h postdosing. The mean plasma concentrations were 3994 and 4952 nM at 1 and 5 h after oral administration of 30 mg/kg analogue **13-15** dosed as described above. Treatment with the control compound furosemide affected renal function in a predictable fashion. Furosemide (30 mg/kg) in this model significantly increased urine volume and decreased osmolality, glucose, urea, protein, and potassium (see Supporting Information). The reference compound **2** was previously reported to have no effects in this model.[12] Therefore, analogue **13-15** has an effect on acute renal function at 30 mg/kg (po); furosemide affects renal function in a predictable fashion.

Compound **13-15** did not significantly affect gastrointestinal motility when compared to vehicle controls. Fasted male Sprague−Dawley (Cesarean-derived, 171−217 g) rats ($n = 10$) were used to determine the effects of **13-15** and standard controls (carbachol and atropine) on gastrointestinal (GI) motility as evaluated by gross observation and transit of an orally administered charcoal suspension (10% w/v charcoal in 0.25% methylcellulose).[10] Carbachol (0.3 mg/kg, ip) significantly increased GI motility from 38.4 ± 3.8% (vehicle group, mean ± SEM) to 55.8 ± 3.6% (carbachol), while atropine (3.0 mg/kg, po) significantly decreased GI motility in this animal model from 42.1 ± 1.8% (vehicle) to 27.5 ± 2.5% (atropine). The reference compound **2** was previously reported to have no effects in this model, similar to compound **13-15**.[12]

Conclusion

Compound **13-15** is a potent, orally bioavailable antagonist of CRF_1 receptors. The compound has anxiolytic efficacy in the rat elevated plus maze and defensive withdrawal models with little effect on locomotor or rotorod activity. It has promising oral pharmacokinetic profiles in rats, dogs, and chimpanzees. The compound has small or no effects on dog cardiovascular and respiratory functions and no effect on rat gastrointestinal function. This compound has a mild acute effect on renal function at 30 mg/kg (po). Compound **13-15** (BMS-562086)[28] has been advanced into clinical trials, the results of which will be reported separately.

Experimental Section

Chemistry. 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 instrument (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 the general procedures outlined by D. Perrin and WLF Armarego.[29] 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_2O, solvent B = 0.1% TFA−CH_3CN, gradient 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_2O, solvent B = 0.1% TFA−MeOH, gradient 5−95% B over 15 min) (method B). Combustion analyses were performed by Quantitative Technologies, Whitehouse, NJ.

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

4-((*R*)-2-Butylamino)-2,7-dimethyl-8-(2-methyl-6-methoxypyrid-3-yl)[1,5-*a*]pyrazolo-1,3,5-triazine (13-15). Step A: 2-Methoxy-6-methylpyridine. Sodium (31.0 g, 1.35 mol) was added portionwise to methanol (500 mL) over 30 min with stirring in a flask equipped with a reflux condenser. After the addition was complete, the reaction mixture was allowed to cool to ambient temperature. 2-Fluoro-6-methylpyridine (50 g, 450 mmol) was added portionwise with stirring. The reaction mixture was then heated to reflux temperature and stirred for 48 h. The mixture was then cooled to ambient temperature and solvent was removed in vacuo to provide 2-methoxy-6-methylpyridine, a yellow oil. The residue was taken up in water (500 mL), and three extractions with ether (200 mL) were performed. The combined organic layers were dried over $MgSO_4$ and filtered, and solvent was removed in vacuo from the filtrate to give a yellow liquid. ^1H NMR ($CDCl_3$, 300 MHz): δ 7.44 (dd, 1H, $J = 8, 7$), 6.71 (d, 1H, $J = 7$), 6.53 (d, 1H, $J = 8$), 3.91 (s, 3H), 2.45 (s, 3H).

Alternatively, a mixture of 2-hydroxy-6-methylpyridine (6.85 g, 62.8 mmol), silver carbonate (22.5 g, 81.6 mmol), iodomethane (39.1 mL, 628 mmol), and chloroform (200 mL) was stirred at ambient temperature for 40 h in the dark. The reaction mixture was filtered through Celite. The collected solid was washed with ether. The combined filtrates were concentrated in vacuo to give 2-methoxy-6-methylpyridine, a liquid (6.25 g), which gave NMR data identical to the product from the above procedure.

Step B: 3-Bromo-6-methoxy-2-methylpyridine. A mixture of 2-methoxy-6-methylpyridine (17.0 g, 138 mmol) and a solution of disodium hydrogen phosphate (0.15 M in water, 250 mL) was stirred at room temperature. Bromine (7.1 mL, 138 mmol) was added dropwise over 15 min via an addition funnel. The reaction mixture was then stirred at room temperature for 4 h. The clear colorless solution was diluted with water (500 mL) and extracted with dichloromethane (200 mL) three times. The combined organic layers were dried over $MgSO_4$ and filtered, and solvent was removed in vacuo from the filtrate to give a yellow liquid. Flash chromatography on silica gel (EtOAc/hexane, 1:20) and removal of solvent from the desired combined fractions afforded 6-methoxy-3-bromo-2-methylpyridine, a clear colorless liquid (15.4 g). ^1H NMR ($CDCl_3$, 300 MHz): δ 7.60(d, 1H, $J = 8$), 6.46 (d, 1H, $J = 8$), 3.89 (s, 3H), 2.54 (s, 3H).

Step C: 6-Methoxy-2-methylpyridine-3-boronic Acid. A solution of 6-methoxy-3-bromo-2-methylpyridine (59.8 g, 296 mmol) in dry THF (429 mL) was cooled with stirring to about −78 °C under a nitrogen atmosphere. A solution of *n*-butyllithium (2.5 M, 130.4 mL, 326 mmol) in hexane was added dropwise over 30 min. The reaction mixture was stirred for 3 h at about −78 °C. A solution of triisopropyl borate (102.7 mL, 445 mmol) in dry THF (100 mL) was added dropwise over 30 min. The reaction mixture was warmed to ambient temperature with stirring over 16 h. Acetic acid (37.35 g, 622 mmol) and then water (110 mL) were added to the reaction mixture with stirring. After 2 h, the layers were separated and the organic layer was concentrated in vacuo. The residue was taken up in 2-propanol (750 mL), and solvent was removed on a rotary

evaporator (bath temperature of ~50 °C). The residue was triturated with ether. The product, 6-methoxy-2-methylpyridine-3-boronic acid, was collected by filtration and dried in vacuo (48.4 g): mp >200 °C. ^{1}H NMR (CD_3OD, 300 MHz): δ 7.83 (d, 1H, J = 8), 6.56 (d, 1H, J = 8), 3.85 (s, 3H), 2.44 (s, 3H). GC−MS: 168 (M^+ + H).

Step D: 2-Methyl-3-(5-methylisoazolyl)-6-methoxypyridine. A mixture of 4-iodo-5-methylisoxazole (18.2 g, 87 mmol), 6-methoxy-2-methylpyridine-3-boronic acid (14.6 g, 87 mmol), sodium bicarbonate (22.0 g, 262 mmol), water (150 mL), and DME (150 mL) was degassed three times with stirring by the application of a vacuum and then introduction of a nitrogen atmosphere. [1,1-Bis(diphenylphosphino)ferrocene]dichloropalladium(II) (2.14 g, 2.6 mmol) was added in one portion. The reaction mixture was degassed as before. The reaction mixture was then stirred at 80 °C for 4 h, and then it was cooled to ambient temperature. Three extractions with EtOAc, drying the combined organic layers over $MgSO_4$, filtration, and removal of solvent in vacuo afforded an oil. Flash chromatography (EtOAc/hexane, 1:9) and removal of solvent in vacuo from the desired fractions gave the product 2-methyl-3-(5-methylisoxazol-4-yl)-6-methoxypyridine (7.15 g). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.16 (s, 1H), 7.33 (d, 1H, J = 8), 6.63 (d, 1H, J = 8), 3.95 (s, 3H), 2.35 (s, 6H). APCI$^+$-MS: 205 (M^+ + H).

Step E: 1-Cyano-1-(2-methyl-6-methoxypyrid-3-yl)propanone, Sodium Salt. A mixture of sodium methoxide (25% w/w, 13 mL, 70 mmol), 2-methyl-3-(5-methylisoxazol-4-yl)-6-methoxypyridine (7.15 g, 35 mmol), and methanol (50 mL) was stirred at room temperature for 16 h. Solvent was removed in vacuo to give a yellow oil. Trituration with ether, filtration, and drying in vacuo afforded the crude product as a white solid (9.3 g).

Step F: 5-Amino-4-(2-methyl-6-methoxypyrid-3-yl)-3-methylpyrazole. A mixture of 1-cyano-1-(2-methyl-6-methoxypyrid-3-yl)propan-2-one, sodium salt (9.3 g), hydrazine hydrate (6 mL, 123.3 mmol), and glacial acetic acid (150 mL) was stirred at room temperature for 4 h. The reaction mixture was concentrated in vacuo. The residue was dissolved in 1 N HCl, and the resulting solution was extracted with EtOAc two times. A 1 N NaOH solution was added to the aqueous layer until pH 12. The resulting semisolution was extracted three times with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and filtered. Solvent was removed in vacuo to give a viscous oil (5.8 g). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.37 (d, 2H, J = 8), 6.62 (d, 2H, J = 8), 3.95 (s, 3H), 2.36 (s, 3H), 2.08 (s, 3H). APCI$^+$-MS: 219 (M^+ + H), 260 (M^+ + CH_3CN).

Step G: 5-Acetamidino-4-(2-methyl-6-methoxypyrid-3-yl)-3-methylpyrazole, Acetic Acid Salt. Ethyl acetamidate hydrochloride (6.46 g, 52.2 mmol) was added quickly to a rapidly stirred mixture of potassium carbonate (6.95 g, 50.0 mol), dichloromethane (60 mL), and water (150 mL). The layers were separated, and the aqueous layer was extracted with dichloromethane (2 × 60 mL). The combined organic layers were dried over $MgSO_4$ and filtered. Solvent was removed by simple distillation, and a clear pale-yellow liquid was used without further purification.

Glacial acetic acid (1.0 mL, 17.4 mmol) was added to a stirred mixture of 5-amino-4-(2-methyl-6-methoxypyrid-3-yl)-3-methylpyrazole (3.8 g, 17.4 mmol), ethyl acetamidate free base, and dichloromethane (100 mL). The resulting reaction mixture was stirred at room temperature for 16 h; at the end of that time, it was concentrated in vacuo. The residue was triturated with ether, and the product was filtered and washed with copious amounts of ether. The white solid was dried in vacuo (5.4 g). ^{1}H NMR (CD_3OD, 300 MHz): δ 7.43 (d, 2H, J = 8), 6.69 (d, 2H, J = 8), 4.9 (br s, 2H), 3.93 (s, 3H), 2.31 (s, 3H), 2.24 (s, 3H), 2.13 (s, 3H), 1.88 (s, 3H). APCI$^+$-MS: 260 (M^+ + H).

Step H: 2,7-Dimethyl-8-(2-methyl-6-methoxypyrid-3-yl)[1,5-*a*]pyrazolo[1,3,5]triazin-4(3*H*)-one. Sodium pellets (3.9 g, 169 mmol) were added portionwise to ethanol (200 mL) with vigorous stirring. After all the sodium reacted, 5-acetamidino-4-(2-methyl-6-methoxypyrid-3-yl)-3-methylpyrazole, acetic acid salt (5.4 g, 16.9 mmol) and diethyl carbonate (16.4 mL, 135.3 mmol) were added. The resulting reaction mixture was heated to reflux temperature and stirred for 18 h. The mixture was cooled to room temperature, and solvent was removed in vacuo. The residue was dissolved in water, and a 1 N HCl solution was added slowly until pH ~ 6. The aqueous layer was extracted with EtOAc three times; the combined organic layers were dried over $MgSO_4$ and filtered. Solvent was removed in vacuo to give a solid. Trituration with ether, filtration, and drying in vacuo afforded a white solid (3.9 g). ^{1}H NMR (CD_3OD, 300 MHz): δ 7.49 (d, 2H, J = 8), 6.69 (d, 2H, J = 8), 3.93 (s, 3H), 2.35 (s, 3H), 2.28 (s, 3H), 2.24 (s, 3H). APCI$^+$-MS: 286 (M^+ + H). Anal. ($C_{14}H_{15}N_5O_2 \cdot H_2O$) C, H, N.

Step I: 4-Choro-2,7-dimethyl-8-(2-methyl-6-methoxypyrid-3-yl)[1,5-*a*]pyrazolo-1,3,5-triazine. A mixture of 2,7-dimethyl-8-(2-methyl-6-methoxypyrid-3-yl)[1,5-*a*]pyrazolo-1,3,5-triazin-4-one (example 1, 3.9 g, 13.7 mmol), diisopropylethylamine (9.5 mL, 54.7 mmol), phosphorus oxychloride (5.1 mL, 54.7 mmol), and toluene (75 mL) was stirred at reflux temperature for 4 h. The volatiles were removed in vacuo. The residue was loaded on a pad of silica gel on Celite and eluted with a 1:1 mixture of EtOAc and hexane. Solvent was removed in vacuo from the filtrate to give 4-chloro-2,7-dimethyl-8-(2-methyl-6-methoxypyrid-3-yl)[1,5-*a*]-pyrazolotriazine, an oil, which was used without further purification.

Step J: 4-((*R*)-2-Butylamino)-2,7-dimethyl-8-(2-methyl-6-methoxypyrid-3-yl)[1,5-*a*]pyrazolo-1,3,5-triazine (13-15). A mixture of 4-chloro-2,7-dimethyl-8-(2-methyl-6-methoxypyrid-3-yl)[1,5-*a*]pyrazolotriazine, (*R*)-2-butylamine (2.0 mL, 20.5 mmol), diisopropylethylamine (9.5 mL, 54.7 mmol), and dry THF (25 mL) was stirred at ambient temperature for 18 h. Solvent was removed in vacuo. Column chromatography of the residue (first using EtOAc/hexane, 1:2, then using EtOAc/hexane, 1:4) afforded the product. Removal of solvent in vacuo gave the title product, a white solid (2.3 g): mp 118.3 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.41 (d, 1H, J = 8), 6.63 (d, 1H, J = 8), 6.25 (br d, 1H, J = 9), 4.35−4.30 (m, 1H), 3.95 (s, 3H), 2.49 (s, 3H), 2.35 (s, 3H), 2.30 (s, 3H), 1.76−1.66 (m, 2H), 1.34 (d, 3H, J = 7), 1.02 (t, 3H, J = 7). ^{13}C NMR ($CDCl_3$, 100.52 MHz): δ 163.8, 163.0, 155.7, 153.7, 147.8, 146.6, 141.6, 118.5, 107.4, 106.6, 53.3, 48.2, 29.7, 26.1, 22.9, 20.4, 13.1, 10.3. IR (neat, KBr, cm^{-1}): 3380 (m), 3371 (m), 2968 (m), 2928 (m), 2872 (w), 1621 (s), 1588 (s), 1544 (s), 1489 (s), 1460 (s), 1425 (s), 1413 (s), 1364 (s), 1346 (m), 1304 (s), 1275 (s), 1247 (s), 1198 (m), 1152 (m), 1134 (m), 1112 (m), 1034 (s), 1003 (m). ESI(+)-HRMS calcd for $C_{18}H_{24}N6O$: 341.2089. Found: 341.2093 (M^+ + H). Anal. ($C_{18}H_{24}N_6O$) C, H, N.

The following analogues were prepared according to the methods described above from the appropriate pyridine precursors and amines.

13-1: oil. NMR ($CDCl_3$, 300 MHz): δ 6.31 (s, 1H), 6.17 (d, 1H, J = 10), 4.20 (m, 1H), 3.09 (s, 6H), 2.46 (s, 3H), 2.20 (s, 3H), 2.17 (s, 3H), 1.99 (s, 3H), 1.76 (m, 2H), 1.64 (m, 2H), 1.02 (t, 3H, J = 7). CI-HRMS calcd for $C_{21}H_{32}N_7$: 382.2719. Found: 382.2712 (M + H). Anal.: HPLC.

13-2: oil. NMR ($CDCl_3$, 300 MHz): δ 6.69 (m, 1H), 6.31 (s, 1H), 3.86 (q, 2H, J = 5), 3.65 (t, 2H, J = 5), 3.44 (s, 3H), 3.08 (s, 6H), 2.46 (s, 3H), 2.20 (s, 3H), 2.15 (s, 3H), 1.97 (s, 3H). CI-HRMS calcd for $C_{19}H_{28}N_7O$: 370.2355. Found: 370.2378 (M + H). Anal.: HPLC.

13-3: oil. NMR ($CDCl_3$, 300 MHz): δ 6.31 (s, 1H), 4.33 (s, 4H, J = 5), 3.77 (t, 4H, J = 6), 3.39 (s, 6H), 3.08 (s, 6H), 2.37 (s, 3H), 2.15 (s, 3H), 2.14 (s, 3H), 1.97 (s, 3H). CI-HRMS calcd for $C_{22}H_{34}N_7O_2$: 428.2774. Found: 428.2775 (M + H). Anal.: HPLC.

13-4: oil. NMR ($CDCl_3$, 300 MHz): δ 6.31 (s, 1H), 4.07 (m, 4H), 3.08 (s, 6H), 2.38 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 1.98 (s, 3H), 1.36 (t, 6H, J = 7). CI-HRMS calcd for $C_{20}H_{30}N_7$: 368.2563. Found: 368.2565 (M + H). Anal.: HPLC.

13-5: oil. NMR ($CDCl_3$, 300 MHz): δ 6.31 (s, 1H), 4.18 (m, 4H), 3.08 (s, 6H), 2.37 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 1.98 (s, 3H), 1.79 (m, 4H), 0.98 (t, 3H, J = 7). CI-HRMS calcd for $C_{22}H_{34}N_7$: 396.2875. Found: 396.2878 (M + H). Anal.: HPLC.

13-6: oil. NMR ($CDCl_3$, 300 MHz): δ 6.31 (s, 1H), 4.08 (m, 4H), 3.08 (s, 6H), 2.38 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 1.98 (s, 3H), 1.76 (m, 2H), 1.42 (m, 2H), 1.33 (t, 3H, J = 7), 1.00 (t, 3H,

$J = 7$). CI-HRMS calcd for $C_{22}H_{34}N_7$: 396.2875. Found: 396.2881 (M + H). Anal.: HPLC.

13-7: solid. NMR ($CDCl_3$, 300 MHz): δ 6.31 (s, 1H), 3.98 (s, 4H), 3.08 (s, 6H), 2.37 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 1.98 (s, 3H), 1.76 (m, 4H), 1.42 (m, 2H), 0.99 (m, 6H). CI-HRMS calcd for $C_{23}H_{36}N_7$: 410.3032. Found: 410.3034 (M + H). Anal.: HPLC.

13-8: solid; mp 117 °C. NMR ($CDCl_3$, 300 MHz): δ 7.98 (s, 1H), 6.47 (s, 1H), 4.08 (m, 4H), 3.10 (s, 6H), 2.39 (s, 3H), 2.28 (s, 3H), 2.15 (s, 3H), 1.35 (t, 6H, $J = 8$). CI-HRMS calcd for $C_{19}H_{28}N_7$: 354.2406. Found: 354.2388 (M + H). Anal. Calcd for $C_{19}H_{27}N_7$: C, 64.56; H, 7.70; N, 27.74. Found: C, 64.89; H, 7.57; N, 27.63.

13-9: solid; mp 103−104 °C. NMR ($CDCl_3$, 300 MHz): δ 7.97 (s, 1H), 6.47 (s, 1H), 4.30 (m, 4H), 3.76 (d, 4H, $J = 8$), 3.39 (s, 6H), 3.10 (s, 6H), 2.37 (s, 3H), 2.27 (s, 3H), 2.15 (s, 3H). CI-HRMS calcd for $C_{21}H_{32}N_7O_2$: 414.2617. Found: 414.2600 (M + H). Anal.: HPLC.

13-10: solid; mp 153−154 °C. NMR ($CDCl_3$, 300 MHz): δ 7.99 (s, 1H), 7.26 (s, 1H), 6.16 (d, 1H, $J = 8$), 4.20 (m, 1H), 3.11 (s, 6H), 2.46 (s, 3H), 2.33 (s, 3H), 2.17 (s, 3H), 1.69 (m, 4H). CI-HRMS calcd for $C_{20}H_{30}N_7$: 367.2484. Found: 367.2477 (M + H). Anal.: HPLC.

13-11: oil. NMR ($CDCl_3$, 300 MHz): δ 7.96 (s, 1H), 6.69 (s, 1H), 4.32 (m, 4H), 3.94 (s, 3H), 3.76 (t, 2H, $J = 8$), 3.39 (s, 6H), 2.39 (s, 3H), 2.26 (s, 3H), 2.17 (s, 3H). CI-HRMS calcd for $C_{20}H_{28}N_6O_3$: 401.2301. Found: 401.2302 (M + H). Anal.: HPLC.

13-12: oil. NMR ($CDCl_3$, 300 MHz): δ 7.97 (s, 1H), 6.69 (s, 1H), 4.07 (m, 2H), 3.94 (s, 3H), 2.40 (s, 3H), 2.28 (s, 3H), 2.17 (s, 3H), 1.36 (t, 6H, $J = 8$). CI-HRMS calcd for $C_{18}H_{24}N_6O$: 341.2090. Found: 341.2091 (M + H). Anal.: HPLC.

13-13: oil. NMR ($CDCl_3$, 300 MHz): δ 7.98 (s, 1H), 6.70 (s, 1H), 6.19 (d, 1H, $J = 8$), 4.22 (m, 1H), 3.95 (s, 3H), 2.47 (s, 3H), 2.32 (s, 3H), 2.19 (s, 3H), 1.73 (m, 4H), 1.00 (t, 6H, $J = 8$). CI-HRMS calcd for $C_{19}H_{27}N_6O$: 355.2246. Found: 355.2248 (M + H). Anal.: HPLC.

13-14: oil. NMR ($CDCl_3$, 300 MHz): δ 7.41 (d, 1H, $J = 9$), 6.63 (d, 1H, $J = 9$), 6.19 (d, 1H, $J = 10$), 4.22 (m, 1H), 3.95 (s, 3H), 2.48 (s, 3H), 2.35 (s, 3H), 2.29 (s, 3H), 1.73 (m, 4H), 1.00 (t, 6H, $J = 8$). CI-HRMS calcd for $C_{19}H_{27}N_6O$: 355.2246. Found: 355.2238 (M + H). Anal.: HPLC.

13-16: oil. NMR ($CDCl_3$, 300 MHz): δ 7.41 (d, 1H, $J = 8$), 6.63 (d, 1H, $J = 8$), 6.22 (br d, 1H, $J = 9$), 4.33 (m, 1H), 3.95 (s, 3H), 2.48 (s, 3H), 2.34 (s, 3H), 2.29 (s, 3H), 1.71 (m, 2H), 1.34 (d, 3H, $J = 7$), 1.03 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{18}H_{24}N_6O$: 341.2090. Found: 341.2088 (M + H). Anal.: HPLC.

13-17: oil. NMR ($CDCl_3$, 300 MHz): δ 7.40 (d, 1H, $J = 8$), 6.63 (d, 1H, $J = 8$), 6.20 (br d, 1H, $J = 9$), 4.40 (m, 1H), 3.95 (s, 3H), 2.48 (s, 3H), 2.34 (s, 3H), 2.29 (s, 3H), 1.65 (m, 2H), 1.48 (m, 2H), 1.34 (d, 3H, $J = 7$), 0.97 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{19}H_{27}N_6O$: 355.2246. Found: 355.2244 (M + H). Anal.: HPLC.

13-18: oil. NMR ($CDCl_3$, 300 MHz): δ 7.40 (d, 1H, $J = 8$), 6.63 (d, 1H, $J = 8$), 6.20 (br d, 1H, $J = 9$), 4.40 (m, 1H), 3.95 (s, 3H), 2.48 (s, 3H), 2.34 (s, 3H), 2.29 (s, 3H), 1.65 (m, 2H), 1.48 (m, 2H), 1.34 (d, 3H, $J = 7$), 0.97 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{19}H_{27}N_6O$: 355.2246. Found: 355.2250 (M + H). Anal.: HPLC.

13-19: oil. NMR ($CDCl_3$, 300 MHz): δ 7.40 (d, 1H, $J = 8$), 6.63 (d, 1H, $J = 8$), 6.22 (d, 1H, $J = 8$), 4.37 (m, 1H), 3.95 (s, 3H), 2.48 (s, 3H), 2.34 (s, 3H), 2.29 (s, 3H), 1.65 (m, 2H), 1.38 (m, 4H), 1.34 (d, 3H, $J = 7$), 0.92 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{20}H_{29}N_6O$: 369.2403. Found: 369.2427 (M + H). Anal.: HPLC.

13-20: oil. NMR ($CDCl_3$, 300 MHz): δ 7.40 (d, 1H, $J = 8$), 6.62 (d, 1H, $J = 8$), 4.07 (m, 4H), 3.95 (s, 3H), 2.40 (s, 3H), 2.33 (s, 3H), 2.25 (s, 3H), 1.35 (t, 6H, $J = 7$). CI-HRMS calcd for $C_{18}H_{25}N_6O$: 341.2090. Found: 341.2086 (M + H). Anal.: HPLC.

13-21: oil. NMR ($CDCl_3$, 300 MHz): δ 7.40 (d, 1H, $J = 8$), 6.62 (d, 1H, $J = 8$), 4.00 (m, 4H), 3.95 (s, 3H), 2.39 (s, 3H), 2.33 (s, 3H), 2.24 (s, 3H), 1.79 (m, 4H), 0.98 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{20}H_{29}N_6O$: 369.2403. Found: 369.2397 (M + H). Anal.: HPLC.

13-22: oil. NMR ($CDCl_3$, 300 MHz): δ 7.39 (d, 1H, $J = 8$), 6.62 (d, 1H, $J = 8$), 4.33 (m, 4H), 3.95 (s, 3H), 3.76 (t, 4H, $J = 6$), 3.39 (s, 6H), 2.40 (s, 3H), 2.33 (s, 3H), 2.23 (s, 3H). CI-HRMS calcd for $C_{20}H_{29}N_6O_3$: 401.2301. Found: 401.2301 (M + H). Anal.: HPLC.

13-23: oil. NMR ($CDCl_3$, 300 MHz): δ 7.40 (d, 1H, $J = 8$), 6.63 (d, 1H, $J = 8$), 4.19 (m, 4H), 3.95 (s, 3H), 3.78 (t, 4H, $J = 6$), 3.40 (s, 3H), 2.40 (s, 3H), 2.33 (s, 3H), 2.24 (s, 3H), 1.34 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{19}H_{27}N_6O_2$: 371.2195. Found: 371.2204 (M + H). Anal.: HPLC.

13-24: oil. NMR ($CDCl_3$, 300 MHz): δ 7.40 (d, 1H, $J = 9$), 6.62 (d, 1H, $J = 8$), 4.35 (m, 2H), 4.06 (m, 2H), 3.95 (s, 3H), 3.78 (t, 2H, $J = 6$), 3.39 (s, 3H), 2.41 (s, 3H), 2.33 (s, 3H), 2.25 (s, 3H), 1.28 (m, 1H), 0.55 (m, 2H), 0.41 (m, 2H). CI-HRMS calcd for $C_{21}H_{29}N_6O_2$: 397.2352. Found: 397.2361 (M + H). Anal.: HPLC.

13-25: oil. NMR ($CDCl_3$, 300 MHz): δ 7.41 (d, 1H, $J = 8$), 6.62 (d, 1H, $J = 8$), 4.09 (m, 4H), 3.95 (s, 3H), 2.39 (s, 3H), 2.38 (s, 3H), 2.25 (s, 3H), 1.80 (m, 2H), 1.25 (m, 1H), 0.98 (t, 3H, $J = 7$), 0.55 (m, 2H), 0.40 (m, 2H). CI-HRMS calcd for $C_{21}H_{29}N_6O$: 381.2403. Found: 381.2396 (M + H). Anal.: HPLC.

13-26: oil. NMR ($CDCl_3$, 300 MHz): δ 7.40 (d, 1H, $J = 8$), 6.62 (d, 1H, $J = 8$), 4.16 (m, 2H), 3.95 (s, 3H), 3.56 (s, 3H), 2.41 (s, 3H), 2.33 (s, 3H), 2.26 (s, 3H), 1.34 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{17}H_{23}N_6O$: 327.1933. Found: 327.1928 (M + H). Anal.: HPLC.

13-27: oil. NMR ($CDCl_3$, 300 MHz): δ 7.40 (d, 1H, $J = 9$), 6.62 (d, 1H, $J = 9$), 4.08 (m, 2H), 3.95 (s, 3H), 3.55 (s, 3H), 2.41 (s, 3H), 2.33 (s, 3H), 2.25 (s, 3H), 1.80 (m, 2H), 0.98 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{18}H_{25}N_6O$: 341.2890. Found: 341.2083 (M + H). Anal.: HPLC.

13-28: oil. NMR ($CDCl_3$, 300 MHz): δ 7.40 (d, 1H, $J = 8$), 6.63 (d, 1H, $J = 8$), 4.12 (m, 2H), 3.95 (s, 3H), 3.55 (s, 3H), 2.41 (s, 3H), 2.33 (s, 3H), 2.25 (s, 3H), 1.75 (m, 2H), 1.41 (m, 2H), 0.99 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{19}H_{27}N_6O$: 355.2246. Found: 355.2240 (M + H). Anal.: HPLC.

13-29: oil. NMR ($CDCl_3$, 300 MHz): δ 7.40 (d, 1H, $J = 8$), 6.62 (d, 1H, $J = 8$), 4.04 (s, 4H), 3.95 (s, 3H), 2.40 (s, 3H), 2.33 (s, 3H), 2.24 (s, 3H), 1.76 (m, 2H), 1.43 (m, 2H), 1.33 (t, 3H, $J = 7$), 1.00 (t, 3H, $J = 7$). CI-HRMS calcd for $C_{20}H_{29}N_6O$: 369.2403. Found: 369.2399 (M + H). Anal.: HPLC.

13-30: oil. NMR ($CDCl_3$, 300 MHz): δ 7.41 (d, 1H, $J = 8$), 6.62 (d, 1H, $J = 8$), 3.97 (s, 4H), 3.95 (s, 3H), 2.39 (s, 3H), 2.33 (s, 3H), 2.24 (s, 3H), 1.76 (m, 4H), 1.42 (m, 2H), 0.92 (m, 6H). CI-HRMS calcd for $C_{21}H_{31}N_6O$: 383.2533. Found: 383.2546 (M + H). Anal.: HPLC.

Biological Evaluation. The CRF receptor binding assay, the CRF-stimulated adenylate cyclase assay, the rat defensive withdrawal, the rat elevated plus maze tests, the rat receptor occupancy studies, and the discrete pharmacokinetic studies have been described previously.[18,21−24,27] The dog cardiovascular, dog pulmonary, rat renal, and rat gastrointestinal studies were performed by previously described procedures.[12]

Dog *n*-in-1 Pharmacokinetic Studies. The dog *n*-in-1 pharmacokinetic studies were analyzed with an LC/MS/MS method at Primedica Corporation (Worcester, MA). The test compounds and the reference compound (0.1 mg/kg each (free base weight)) were administered as a homogeneous mixture in Labrafil (test samples were vortexed for 15 min) via oral gavage to each of three beagle dogs. Blood samples were collected into EDTA tubes predose and at 0.5, 1, 2, 4, 6, 9, 12, and 24 h postdose. Samples were extracted using a protein precipitation technique. A sample aliquot was transferred to a plastic test tube, and 25 μL of internal standard was added. Next, 1 mL of acetonitrile was added to the samples and they were vortexed briefly and centrifuged for 5 min. The organic layer was transferred to a clean plastic tube and evaporated to dryness at approximately 65 °C under nitrogen. The dried samples were reconstituted in formic acid/acetonitrile/water (0.1:10:90) and vortexed for approximately 1 min, and the resulting solutions were transferred to autosampler vials.

Gradient reversed-phase LC/MS/MS conditions were employed to quantitate these analytes. A gradient consisting of 0.1% formic acid in water and acetonitrile was used to elute the analytes from

a Phenomonex Luna C8 column (3 μm, 20 mm × 2 mm). Analytes were quantitated with a SCIEX API 3000 LC/MS/MS using TurboIonSpray under positive ion, MRM conditions.

Chimpanzee Pharmacokinetic Studies. The chimpanzee pharmacokinetic studies were analyzed with an LC/MS/MS method at Primedica Corporation (Worcester, MA). Samples were prepared and analyzed as for the dog *n*-in-1 pharmacokinetic studies The following mass transitions were used: compound **13-15**, 341.2 → 285.3; internal standard (8-(5-chloro-2,4-dimethoxyphenyl)-2,7-pyrazolo[1,5-*a*][1,3,5]triazinyl]bis(2-methoxyethyl)amine), 450.3 → 319.1.

Compound **13-15** was administered orally to two alert chimpanzees at 1 mg/kg, and blood samples were collected into EDTA tubes predose and at 2, 4, 6, 9, 12, 24, 36, 48, 72, and 96 h postdose. The oral suspension (1 mg/mL) was prepared in a 50−50 (v/v) mixture of 0.5% methylcellulose and Tang orange juice containing 1% Tween-80. No adverse effects, including emesis, were observed in the chimpanzees. In addition, the compound was administered intravenously to one chimpanzee at a dose of 0.5 mg/kg in 10% DMAC, 70% propylene glycol, and deionized water over 10 min (0.2 mL/kg). Blood sampling was similar to that described after oral dosing but included time points of 6, 15, and 30 min postdose.

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 and the dog cardiovascular function, dog pulmonary function, rat renal function, and rat gastrointestinal motility assessments. This material is available free of charge via the Internet at http://pubs.acs.org.

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