4-(1,3-Dimethoxyprop-2-ylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)-pyrazolo[1,5-*a*]-1,3,5-triazine: A Potent, Orally Bioavailable CRF_1 Receptor Antagonist

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Structure−activity studies in the pyrazolo[1,5-*a*]-1,3,5-triazine series led to the discovery that compound **11i** (DMP696) is a potent $hCRF_1$ receptor antagonist (K_i = 1.7 nM vs 7.5 nM for α-hel-CRF(9−41), $hCRF_1$ adenylate cyclase IC_{50} = 82 nM vs 286 nM for α-hel-CRF(9−41)). Compound **11i** has excellent oral pharmacokinetic profiles in rats and dogs (37% and 50% oral bioavailabilities, respectively). This compound displays good activity in the rat situational anxiety model (MED = 3 mg/kg (po)), whereas a literature standard **1** (CP154526-1) was inactive (MED > 30 mg/kg (po)). Analogue **11i** reduced stereotypical mouth movements in rhesus monkeys by 50% at 21 mg/kg (po) using the human intruder paradigm. Overall, the profile of pyrazolotriazine **11i** indicates that $hCRF_1$ receptor antagonists may be anxiolytic agents, which have reduced motor side effect profiles.

Introduction

Corticotropin-releasing factor (CRF), a 41-amino acid peptide, is the primary physiological regulator of pro-opiomelanocortin (POMC)-derived peptide secretion from the anterior pituitary gland.[1−3] This hormone also has a broad extrahypothalamic distribution in the central nervous system, apart from the pituitary gland, and produces a broad spectrum of autonomic, electrophysiological, and behavioral effects consistent with a neurotransmitter or neuromodulator role in the brain.[4−6] CRF also plays a significant role in the coordination of immune system responses to physiological, psychological, and immunological stressors.[7,8]

Clinical studies have provided evidence that CRF may have a role in several psychiatric disorders and neurological diseases including depression, anxiety, and feeding disorders.[3] Hyposecretion of CRF has been postulated in the etiology and pathophysiology of Alzheimer's disease and Parkinson's disease.[9] Postmortem studies on the brains of Alzheimer's patients uncovered a deficit in CRF immunoreactivity in key cortical regions.[10] Hypersecretion of CRF has been proposed to underlie depression and anxiety-related disorders. In major depression, the concentration of CRF is significantly increased in the cerebrospinal fluid (CSF) of medication-free individuals.[11−14] Immunohistochemical studies have shown that CRF-secreting cells are increased 4-fold in the hypothalami of depressed patients.[15] In addition, there is a blunted effect of intravenous (iv) administration of CRF in depressed patients on adrenocorticotropin (ACTH) plasma levels.[16−18] Behavioral studies in rats and non-human primates provide additional support for the hypothesis that hypersecretion of CRF may be involved in the symptoms seen in human depression.[19]

Preclinical studies support a role for CRF in human anxiety disorders. CRF produces "anxiogenic" effects in animals after direct injection into the cerebral ventricles (icv administration), and interactions between anxiolytic drugs and CRF have been demonstrated in a variety of behavioral models for anxiety.[20,21] These "anxiogenic" effects may be blocked with the peptidic CRF receptor antagonist α-helical CRF(9−41) in a variety of behavioral paradigms, demonstrating that the antagonist produces "anxiolytic-like" effects that are qualitatively similar to those of the benzodiazepines.[22,23] Chlordiazepoxide modulates the "anxiogenic" effects of CRF in both the conflict test[24,25] and the acoustic startle test[26] in rats. Behavioral and physiological studies with transgenic mice which overexpress CRF and with mutant mice which are null for CRF or one of its receptor subtypes demonstrate a pivotal role for CRF in coordinating neuronal, endocrine, and immune responses to stress.[3,27]

The effects of CRF are mediated through seven-transmembrane receptors, which are coupled to G-proteins and are located in the central nervous system (CNS) as well as the periphery.[3,28] There are two subtypes of CRF receptors: CRF_1 and CRF_2; three splice variants have been found for the latter subtype: $CRF_{2\alpha}$, $CRF_{2\beta}$, and $CRF_{2\gamma}$. These receptor subtypes have been cloned and expressed in cell systems.[3] The distribution of these subtypes in the CNS has been elucidated.[3] The function of these receptor subtypes in disease is currently the subject of intense research, and the evaluation of subtype-selective compounds is necessary for progress.

Nonpeptidic CRF_1-selective receptor antagonists have been reported in the literature,[3] and the most exten-

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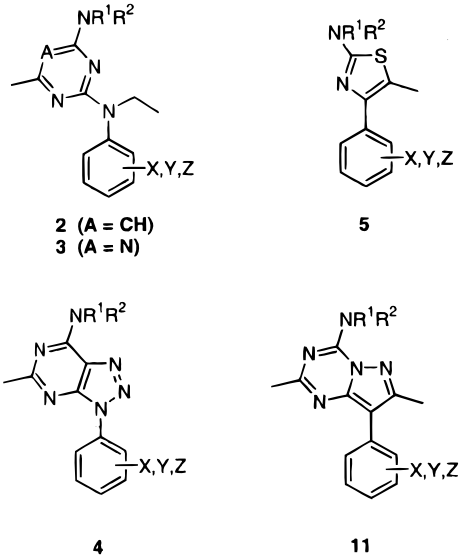
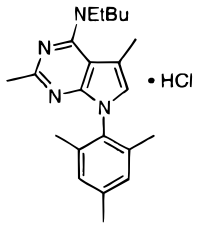
Chart 1



sively studied compound is pyrrolopyrimidine **1** (CP154526-1).[29−33] This high-affinity CRF_1 antagonist (human K_i = 2.4 nM (IMR32 neuroblastoma cells)) blocks the behavioral effects of CRF in rats; it reverses the r/hCRF-enhanced acoustic startle responses of rats (dose of r/hCRF = 1 mg in 2 mL (icv), complete block at 17.8 mg/kg (ip)). In comparison, the peptide antagonist D-Phe-CRF(12−41) (3.3 mg (icv)) completely blocked this effect when co-administered with the same dose of r/hCRF. Compound **1** also antagonizes the fear-potentiated acoustic startle response in rats (total block at 17.8 mg/kg (ip)). In the rat learned helplessness test, a putative model for depression, acute administration of **1** (32 mg/kg (ip), 60-min pretest, single dose) reverses the effects of exposure to inescapable foot shocks for 3 consecutive days. In the same paradigm, treatment with the antidepressant imipramine (17.8 and 32 mg/kg (sc)) has no significant effect. The effects of **1** on behavior suggest a potential anxiolytic or antidepressant indication for CRF_1 antagonists.



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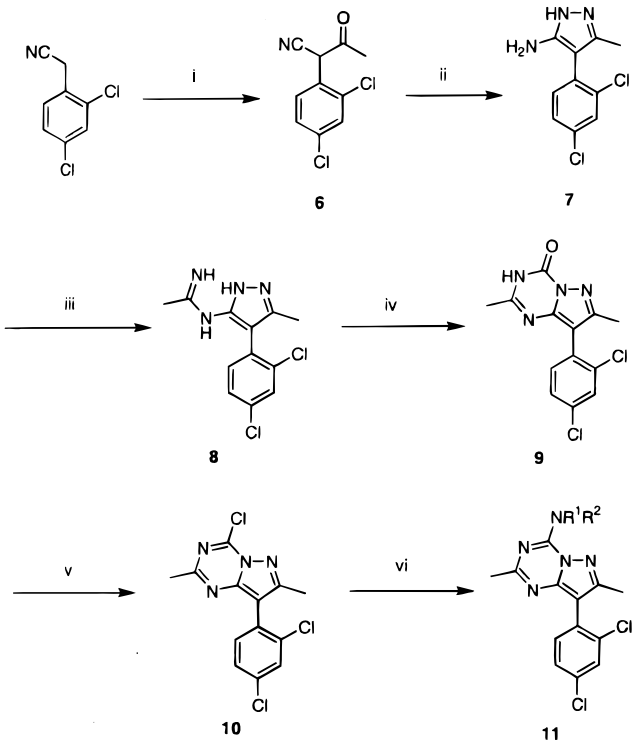
We focused on pyrazolo[1,5-*a*]-1,3,5-triazines **11** as potential CRF receptor antagonists based on an analysis of the structure−activity relationships (SARs) for anilinopyrimidines/triazines **2** and **3**, triazolopyrimidines **4,** and arylthiazoles **5** (Chart 1).[34−37] Overlay of these structures suggested that alternate bicyclic nuclei, such as the pyrazolo[1,5-*a*]-1,3,5-triazine core, might hold the peripheral functional groups in the correct orientation to promote binding affinity to the CRF_1 receptor.[38]

Results and Discussion

Chemistry. The chemical syntheses of pyrazolo[1,5-*a*]-1,3,5-triazines **11** are illustrated in Scheme 1. 2,4-

Scheme 1[a]



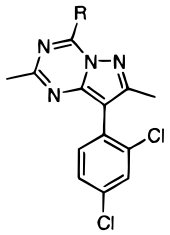
[a] (i) Na(0), EtOAc (65−77%); (ii) NH_2NH_2, HOAc, toluene (60−70%); (iii) Me(C=NH)OEt (69−79%); (iv) $CO(OEt)_2$, NaOEt, EtOH (90−100%); (v) $POCl_3$, Et_2NPh, toluene (46−68%); (vi) HNR^1R^2, THF (46−68%).

Dichlorophenylacetonitrile was acetylated in the presence of sodium metal and ethyl acetate to yield ketonitrile **6**. Treatment of **6** with hydrazine monohydrate in refluxing toluene afforded aminopyrazole **7**. The amidine acetate salt **8** was formed from condensation of intermediate **7** with ethyl acetimidate in acetonitrile in the presence of 1 equiv of glacial acetic acid. The reaction of **8** with diethyl carbonate in the presence of sodium ethoxide in refluxing ethanol formed pyrazolotriazinone **9**. Chlorination with phosphorus oxychloride in the presence of 2.0 equiv of *N*,*N*-diethylaniline at reflux temperature generated the key intermediate **10**. The coupling of this water-sensitive intermediate with various amines provided the final products.

Biology. The pharmacological evaluation of compounds started with testing analogues in a cloned human CRF_1 ($hCRF_1$) receptor binding assay to assess receptor binding affinity, in which the displacements of [^{125}I]0-Tyr-ovine CRF (oCRF) by our test compounds were measured. Promising compounds in terms of affinity were also evaluated in a rat ex vivo binding assay to estimate plasma levels as a function of time. This bioassay cannot distinguish parent compounds from their active metabolites. Therefore, leading compounds were studied in rat or dog pharmacokinetic studies. Select analogues with good binding affinity (K_i ≤ 10 nM) were then evaluated in a CRF-stimulated adenylate cyclase assay to measure antagonist potency.

Early SAR studies[39,40] established that the 2,4-dichlorophenyl group significantly enhanced $hCRF_1$ binding affinity, and subsequent analogue synthesis rapidly focused on amino side chain substituents on the six-membered portion of the bicycle (Table 1). Several high-affinity CRF_1 ligands were discovered. Substituted-

Table 1. hCRF$_1$ Receptor Binding and Physical Data[a]



example	R	mean hCRF$_1$ K_i (nM)	formula	mp (°C)
11a	NEtBu	0.4 ± 0.2 (5)	$C_{19}H_{23}Cl_2N_5$	oil
11b	NPr_2	0.6 ± 0.1 (2)	$C_{19}H_{23}Cl_2N_5$	oil
11c	NPr(CH_2-*c*-Pr)	0.7 ± 0.2 (5)	$C_{20}H_{23}Cl_2N_5$	oil
11d	NH-3-pentyl	1.1 ± 0.4 (7)	$C_{18}H_{21}Cl_2N_5$	139−140
11e	NEt_2	1.2 ± 0.2 (4)	$C_{17}H_{19}Cl_2N_5$	133−134
11f	NH-3-hexyl	1.2 ± 0.5 (3)	$C_{19}H_{23}Cl_2N_5$	130−132
11g	NH-4-heptyl	1.6 ± 0.7 (6)	$C_{20}H_{25}Cl_2N_5$	114−116
11h	NH-3-heptyl	1.6 ± 0.4 (4)	$C_{20}H_{25}Cl_2N_5$	90−92
11i	$NHCH(CH_2OMe)_2$	1.7 ± 0.7 (16)	$C_{18}H_{21}Cl_2N_5O_2$	120−121
11j	$N(CH_2CH_2OMe)_2$	1.8 ± 0.5 (8)	$C_{19}H_{23}Cl_2N_5O_2$	oil
11k	$NHCH(Et)CH_2OMe$	1.9 ± 0.3 (4)	$C_{18}H_{21}Cl_2N_5O$	179−181
11l	$NHCH(CH_2OEt)_2$	6.2 ± 0.3 (2)	$C_{20}H_{25}Cl_2N_5O_2$	oil
11m	NMePh	44.3 ± 6.5 (2)	$C_{20}H_{17}Cl_2N_5$	60−62
α-hel-CRF(9−41)	−	7.5 ± 1.0 (20)	−	−
1	−	1.6 ± 0.9 (11)	−	−

[a] Standard deviations are reported. Parenthetical values are the number of determinations.

Table 2. Rat ex Vivo Binding Data[a]

example	mean hCRF$_1$ K_i (nM)	*N*	% binding inhibition 1 h	% binding inhibition 3 h
11a	0.4	5	73.2 ± 21.3	82.9 ± 14.9
11c	0.7	5	73.3 ± 17.1	95.4 ± 8.9
11d	1.1	8	65.9 ± 13.3	71.8 ± 6.7
11f	1.2	4	46.6 ± 14.8	67.0 ± 6.5
11g	1.6	5	63.9 ± 10.7	74.8 ± 11.1
11i	1.7	7	78.2 ± 13.6	81.3 ± 5.7
11j	1.8	8	85.4 ± 9.2	92.9 ± 6.8
1	1.6	10	<10	<10

[a] Standard deviations are reported. *N* = the number of rats employed in the studies.

alkylamino and di(substituted-alkyl)amino groups greatly enhanced binding affinity for the hCRF$_1$ receptor, while one example of arylamino substitution reduced affinity (cf. **11m**).

Compounds were then evaluated in a rat ex vivo binding assay to estimate plasma exposure after oral administration. In this test, compounds were administered orally at 10 mg/kg and plasma samples were drawn at 1 and 3 h postdose. The plasma samples were diluted 10-fold with assay medium and submitted to the hCRF$_1$ receptor binding assay. Table 2 delineates the percent inhibition values as a function of time. All the analogues had superior exposure in this rat test relative to that of the reference compound **1** (CP154526-1), which had plasma exposures below the limit of detection for this bioassay.

Pyrazolotriazine **11i** (DMP696) (hCRF$_1$ K_i = 1.7 ± 0.7 nM, n = 16) was chosen for detailed in vitro studies based on its high affinity for hCRF$_1$ receptors and its projected duration of exposure in the rat ex vivo binding assay. This compound is a potent antagonist of hCRF$_1$-coupled adenylate cyclase activity in HEK293 cell membranes (IC_{50} = 82 ± 14 nM vs 10 nM for r/hCRF, n = 2) while α-hCRF(9−41) is less potent (IC_{50} = 286 ± 63 nM, n = 3). This analogue is equally potent in a CRF-stimulated adenylate cyclase assay using rat cortical membrane preparations against an identical agonist concentration (IC_{50} = 79 ± 27 nM, n = 2). This compound has no affinity for hCRF$_{2\alpha}$ receptors expressed in HEK293 cells and no affinity for the human CRF-binding protein. Compound **11i** was also submitted to a broad battery of receptor binding assays in the NovaScreen (Hanover, MD) and CEREP (Celle L'Evescault, France) programs. No effects at 10 μM are detected for monoamine (adrenergic (α_1, α_2, α_3, β), dopaminergic, or serotonergic receptors), histaminergic H_1 or H_2, muscarinic, $GABA_a$, $GABA_b$, oxytocin, vasopressin V_1, glycine, CGRP, prostaglandin (LTB_4, LTD_4, and thromboxane A_2), or opioid receptors. Similarly, no affinity is detected at 10 μM for ion channels (glutamate (NMDA or Cl sites), calcium (types L or N), or calcium-activated potassium sites), and no monoamine oxidase A or B inhibition is detected at the same concentration. Very weak binding is observed at adenosine A_1 and A_3 receptors (63% and 47% inhibition of CPA and NECA binding, respectively, at 10 μM).

Compound **11i** evinces anxiolytic activity in the rat situational anxiety test.[41,42] Rats placed in a small darkened chamber located in an unfamiliar, brightly lit open arena spend most of the time within the chamber, an action consistent with a heightened state of anxiety.[41] Systemic pretreatment with anxiolytic drugs such as the benzodiazepines or intracerebroventricular injection of the peptide CRF antagonist α-helical CRF(9−41) reduces the latency time to emerge from the chamber and explore the open field. This model does not rely on shocks or other aversive stimuli or conditioning paradigms. Rats were dosed orally 1 h prior to testing with chlordiazepoxide (20 mg/kg (po), n = 16) and **11i** (3, 5.6, 10, 18, and 45 mg/kg (po), n = 8 each) in a methocel suspension. The animals were placed in the chamber, and the latency to exit was measured (Table 3). Total test time was 900 s. The minimum effective dose for **11i**

Table 3. Rat Situational Anxiety Test Data[a]

compd	dose (mg/kg (po))	latency to exit (s)	*p* vs saline
saline	–	784 ± 55	–
chlordiazepoxide	20.0	216 ± 80	<0.001
11i	3.0	482 ± 100	<0.05
11i	5.6	426 ± 134	<0.05
11i	10.0	286 ± 96	<0.001
11i	18.0	408 ± 112	<0.05
11i	45.0	680 ± 100	–

[a] Standard errors of the mean are reported. $N = 8$. The total test time was 900 s.

is 3 mg/kg (po), and the maximal efficacy is observed after 10 mg/kg (po) (63% reduction in latency). The literature compound **1** (CP154526-1; 30 mg/kg (po)) is inactive in this test. Chlordiazepoxide causes a 72% reduction in latency at 20 mg/kg (po). However, **11i** does not inhibit open-field locomotor activity at 10, 30, and 100 mg/kg (po), while chlordiazepoxide does at 30 mg/kg (po) (50% reduction relative to controls). Compound **11i** does not cause any significant neuromuscular effects (e.g. ataxia, sedation, splayed limbs, catalepsy) up to 100 mg/kg (po) in fasted Sprague–Dawley rats over a 6-h observation period, except for loss of lift at 100 mg/kg (po). These data suggest that analogue **11i**, unlike chlordiazepoxide, may be a potent anxiolytic drug, for which there is a large separation between the dose which causes sedation and that which is efficacious.

Compound **11i** has been examined in a primate model for anxiety, the human intruder test.[43] Immature rhesus monkeys display stereotypical behaviors (vocalizations (coo or bark), freezing behavior, and exaggerated movements of mouth muscles) when confronted with an intruder or predator in a confined space. Eye contact between a human technician and the caged monkey immediately evokes these behaviors. Compound **11i** reduces stereotypical mouth movements ("lip smacking") by 50% at 21 mg/kg ((po) $n = 4$), which is statistically significant ($p < 0.01$) when compared to control animals. Qualitative improvements in the other behavioral parameters are noted at this dose. Alprazolam is reported to have efficacy at 1 mg/kg (im) in this model.[46] Both compounds were administered orally in 0.5% methocel at 0.5 h prior to onset of the test.

Some peripheral effects of **11i** have been examined in the rat and dog since CRF receptors populate peripheral as well as central tissues. CRF_1 sites have been found in rat testes, ovaries, and components of the immune system, while CRF_2 receptors have been reported to be present in the rat GI tract, heart, and skeletal muscle.[3] Acute dosing of **11i** (30 mg/kg (po)) has no statistically significant effect on GI motility as evaluated by the transit of an orally administered charcoal suspension (10% w/v charcoal in 0.25% aqueous methyl cellulose) when compared to vehicle control. Carbachol (0.3 mg/kg (ip)), the positive control for this study, significantly increases GI motility (59.5 ± 4.9% increase in the stomach to cecum distance traveled by a charcoal meal ($p < 0.01$ relative to controls treated with 0.25% methocel)), while atropine (3.0 mg/kg (po)), the negative control, decreases GI motility (17.6 ± 3.1% decrease ($p < 0.01$ relative to controls)) in the same paradigm. Agent **11i** (10 mg/kg (po)) has no statistically significant effect on acute renal function up to 24 h postdose in rats, which were volume-loaded with an isotonic saline–0.25% methocel formulation of the test compound. Total urine volume and osmolality as well as glucose, urea, creatinine, sodium, potassium, and chloride concentrations were within the ranges for vehicle-treated animals. In contrast, the positive control furosemide (30 mg/kg (po)) increased renal output volume and decreased osmolality and glucose, urea, creatinine, sodium, potassium, and chloride concentrations. The data for furosemide are: renal ouput volume, 6.9 ± 0.4 mL/100 g body weight vs 2.0 ± 0.2 mL/100 g body weight for vehicle ($p < 0.05$); osmolality, 211 ± 5 mosm/100 g body weight vs 320 ± 37 mosm/100 g body weight for vehicle ($p < 0.05$); urea levels, 78 ± 6 mg/dL/100 g body weight vs 290 ± 55 mg/dL/100 g body weight for vehicle ($p < 0.05$); creatinine levels, 2.3 ± 0.3 mg/dL/100 g body weight vs 9.5 ± 1.7 mg/dL/100 g body weight for vehicle ($p < 0.05$); potassium levels, 26.0 ± 1.2 mmol/dL/100 g body weight vs 53.3 ± 6.7 mmol/dL/100 g body weight for vehicle ($p < 0.05$).

No statistically significant cardiovascular effects are noted in pentobarbital-anesthetized dogs ($n = 4$) after intravenous administration of **11i**. Escalating doses of **11i** (0.3, 1.0, 3.0, and 5.0 mg/kg) in mixed solvent vehicle (see Experimental Section) have been administered intravenously at 30-min intervals. No effects are observed on heart rate, mean arterial blood pressure, contractility, and electrocardiographic parameters (e.g. QT interval) relative to vehicle-treated animals.

Intravenous infusion of **11i** (1 and 5 mg/kg) does not change pulmonary function in pentobarbital-anesthetized dogs ($n = 8$). No statistically significant changes in respiration rate, intrapleural pressure, tidal volume, minute volume, peak expiratory flow, arterial pO_2, arterial pCO_2, or arterial pH are observed.

Compound **11i** has a good rat pharmacokinetic profile (Table 4). Administration of this compound to Sprague–Dawley rats (5 mg/kg (iv, po), $n = 4$) affords long duration of plasma levels (mean $t_{1/2} = 12.3$ h (iv), 16.5 h (po)). The volume of distribution at steady state ($V_{d,ss}$) is calculated to be 21.5 ± 3.0 L/kg, indicating that there is very extensive distribution into tissues. Systemic clearance (CL) is 2.0 ± 0.33 L/h/kg. The rat oral peak plasma level is high (mean $C_{max} = 317$ nM at 0.44 h), and the oral bioavailability is 37%. After continuous iv infusion of **11i** in solution (*N*,*N*-dimethylacetamide:ethanol:PEG400: 1:1:8) in male CD rats ($n = 4$) using osmotic minipumps (0.47 mg/kg/h) for 69 h, the mean plasma concentration is equal to 250 ± 53 nM at 69 h, the total brain concentration is 579 ± 125 nM, and the brain:plasma ratio is 2.3 ± 0.3. Thus, **11i** is a highly orally bioavailable and brain-penetrant compound in rats.

In beagle dogs, **11i** (1 mg/kg (iv, po), $n = 4$) also affords good plasma level profiles. Mean $t_{1/2}$, CL, and $V_{d,ss}$ values are 34.7 ± 14.2 h, 0.95 ± 0.25 L/kg/h, and 27.1 ± 8.8 L/kg, respectively, after iv administration. Oral dosing gives mean C_{max}, mean $t_{1/2}$, and bioavailability parameters equal to 240 ± 78 nM, 33 ± 12 h, and 50 ± 5%, respectively.

High plasma levels are also observed in rhesus monkeys ($n = 6$) when **11i** is administered at 5 mg/kg (po) (0.25% methocel). The maximal concentration is 365 + 95 nM at 3 ± 0.6 h, the AUC value is 5576 ± 1974 nM·h, and the half-life is 15 ± 2 h.

Table 4. Single-Dose Pharmacokinetic Data for Compound **11i**[a]

	rat (5 mg/kg)		dog (1 mg/kg)	
parameter	iv	po	iv	po
N	4	4	4	4
C_{5min} (nM)	2879 ± 100	–	1371 ± 335	–
$t_{1/2}$ (h)	12.3 ± 2.5	16.5 ± 4.8	34.7 ± 14.2	33.4 ± 12.0
C_{max} (nM)	–	317 ± 42	–	240 ± 78
T_{max} (h)	–	0.44 (0.25−0.5)	–	0.88 (0.5−1.0)
AUCT (nM•h)	5527 ± 604	1861 ± 354	2275 ± 299	469 ± 105
AUC (nM•h)	6175 ± 939	2263 ± 473	2688 ± 556	555 ± 158
V_{ss} (L/kg)	21.5 ± 3.0	–	27.1 ± 8.8	–
CL (L/h/kg)	2.0 ± 0.33	–	0.95 ± 0.25	–
% bioavail	–	36.7	–	49.7

[a] The mean values are reported. *N* = the number of animals used. Standard deviations are reported. The parenthetical values are the ranges for the values.

Conclusion

Compound **11i** (DMP696) is a high-affinity hCRF$_1$ receptor antagonist, which is potent in rat and primate models for anxiety. In the rat situational anxiety test, this compound appears to be more potent after oral administration than chlordiazepoxide and compound **1** (CP154526-1). In the human intruder test in rhesus monkeys, analogue **11i** inhibits a stress-induced behavior at 21 mg/kg (po). On the basis of the currently available data, the peripheral effects in rats and dogs for this compound appear to be minimal after acute dosing, even though high levels of exposure are achieved in these two species. The overall profile of **11i** supports the proposal that hCRF$_1$ receptor antagonists may be effective anxiolytic drugs.

Experimental Section

Chemistry. Analytical data were recorded for the compounds described below using the following general procedures. Proton NMR spectra were recorded on Varian VXR or Unity 300 FT-NMR instruments (300 MHz); chemical shifts were recorded in ppm (δ) from an internal tetramethysilane standard in deuteriochloroform or deuteriodimethyl sulfoxide as specified below. Coupling constants (*J*) were recorded in hertz (Hz). Mass spectra (MS) were recorded on a Finnegan MAT 8230 spectrometer or a Hewlett-Packard 5988A model spectrometer (both using chemical ionization (CI) with NH_3 as the carrier gas). Gas chromatography−mass spectrosopy (GC−MS) was occasionally obtained using the former instrument. Chemical ionization high-resolution mass spectra (CI-HRMS) were obtained on a VG 7-VSE instrument with NH_3 as the carrier gas. Combustion analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ. Melting points were measured on a Buchi model 510 melting point apparatus or a Thomas-Hoover capillary apparatus and are uncorrected. Boiling points are uncorrected.

Reagents were purchased from commercial sources and, when necessary, purified prior to use according to the general procedures outlined by Perrin and Armarego.[44] 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. All reactions were performed under a nitrogen atmosphere using magnetic stirring. Reactions requiring anhydrous conditions were performed in glassware, which had been flame-dried or oven-dried with purging under a nitrogen atmosphere. Reactions using aqueous media were run under the ambient atmosphere. Anhydrous magnesium sulfate ($MgSO_4$) was used routinely to dry the combined organic layers from extractions. Solvent was routinely removed in vacuo, using a rotary evaporator, followed by evacuation with vacuum pump.

Commonly used abbreviations are: EtOAc (ethyl acetate), MeOH (methanol), EtOH (ethanol), DMF (*N*,*N*-dimethylformamide), HOAc (acetic acid), THF (tetrahydrofuran), and TLC (thin-layer chromatography).

1-Cyano-1-(2,4-dichlorophenyl)propan-2-one (6). To a solution of 2,4-dichlorophenylacetonitrile (40.0 g, 0.22 mol) in ethyl acetate (200 mL) were added sodium pellets (6.43 g, 0.28 mol) portionwise at room temperature. The reaction mixture was heated to reflux for 1 h. Upon cooling to room temperature, ethyl acetate (400 mL) was added to the reaction suspension to facilitate stirring, and resultant mixture was stirred at room temperature overnight. The collected white solid was washed with copious amounts of ether and dissolved in water. The water solution was acidified by adding acetic acid until pH = 5−6. The mixture was extracted three times with ethyl acetate (200 mL); the combined organic layers were dried over anhydrous magnesium sulfate, filtered, and concentrated to dryness to provide a white solid (34.90 g, 70% yield): ^{1}H NMR ($CDCl_3$) δ 2.38 (s, 3H), 5.15 (s, 1H), 7.36 (dd, 1H, *J* = 8, 1 Hz), 7.25 (d, 1H, *J* = 8 Hz), 7.50 (d, 1H, *J* = 1 Hz); MS (CI) *m*/*z* 228 $(M + H)^+$.

5-Amino-4-(2,4-dichlorophenyl)-3-methylpyrazole (7). A mixture of 1-cyano-1-(2,4-dichlorophenyl)propan-2-one (18.25 g, 80.3 mmol), hydrazine monohydrate (5.8 mL, 120.45 mmol), acetic acid (14.9 mL, 200.75 mmol), and toluene (300 mL) was heated at reflux temperature using a Dean−Stark trap for 5 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was dissolved in a 6 N HCl solution (200 mL) and the resulting solution was extracted with ether three times. The aqueous layer was basified by concentrated ammonium hydroxide solution until pH = 11. The resulting mixture was extracted with EtOAc (300 mL) three times. The combined organic layers were dried and concentrated in vacuo to give a pale brown viscous oil (11.70 g, 61% yield): ^{1}H NMR ($CDCl_3$) δ 2.15 (s, 3H), 3.40−3.90 (brs, 1H), 6.20 (br s, 2H, concentration-dependent), 7.25 (d, 1H, *J* = 7 Hz), 7.30 (dd, 1H, *J* = 7, 1 Hz), 7.52 (d, 1H, *J* = 1.0 Hz): HRMS(CI) calcd 242.0252, found 242.0250 $(M + H)^+$.

5-Acetamido-4-(2,4-dichlorophenyl)-3-methylpyrazole, Acetic Acid Salt (8). Glacial acetic acid (4.0 mL, 70.5 mmol) was added into a stirred mixture of 5-amino-4-(2,4-dichlorophenyl)-3-methylpyrazole (17.0 g, 70.5 mmol), ethyl acetamidate (9.2 g, 105.8 mmol), and acetonitrile (400 mL). The resultant mixture was stirred at room temperature for 16 h. Diethyl ether (150 mL) was added into the reaction mixture. A white solid was collected, washed with diethyl ether, and dried (16.50 g, 69%): ^{1}H NMR (DMSO-d_6) δ 1.82 (s, 3H), 2.05 (s, 3H), 3.20−3.45 (br s, 3H), 7.30 (d, 1H, *J* = 7 Hz), 7.40 (dd, 1H, *J* = 7, 1 Hz), 7.61 (d, 1H, *J* = 1.0 Hz); MS(CI) *m*/*z* 283 (M + H).

2,7-Dimethyl-8-(2,4-dichlorophenyl)[1,5-*a*]pyrazolo-1,3,5-triazin-4(3*H*)-one (9). Sodium pellets (11.0 g 0.048 mol) were added in portions to stirred ethanol (400 mL) so that a gentle reflux occurred. After all sodium reacted and the reaction temperature subsided to the ambient value, 5-acetamido-4-(2,4-dichlorophenyl)-3-methylpyrazole acetic acid salt (16.44 g, 0.048 mol) and diethyl carbonate (46.6 mL, 0.38 mol) were added. The resultant reaction mixture was heated at reflux temperature for 6 h. After cooling, the solvent was

removed in vacuo. The residue was dissolved in water and acidified by slowly adding glacial acetic acid until pH = 5−6. The resulting mixture was extracted with EtOAc (300 mL) three times, and the combined organic layers were dried over magnesium sulfate, filtered, and concentrated to give a tan solid (14.0 g, 95% yield): ^{1}H NMR ($CDCl_3$) δ 2.37 (s, 3H), 2.50 (s, 3H), 7.25 (d, 1H, J = 7 Hz), 7.35 (dd, 1H, J = 7, 1 Hz), 7.55 (d, 1H, J = 1 Hz), 10.58 (s, 1H); HRMS(CI) calcd 308.0232, found 309.0297 $(M + H)^+$.

4-Chloro-2,7-dimethyl-8-(2,4-dichlorophenyl)[1,5-*a*]-pyrazolo-1,3,5-triazine (10). 2,7-Dimethyl-8-(2,4-dichlorophenyl)[1,5-*a*]pyrazolo-1,3,5-triazin-4(3*H*)-one (6.7 g, 21.7 mmol), *N*,*N*-diethylaniline (7.5 mL, 47.2 mmol), and phosphorus oxychloride (100 mL) was stirred at reflux temperature for 23 h. The excess phosphorus oxychloride was removed in vacuo. The residue was poured onto ice and extracted quickly with ethyl acetate (250 mL) three times. The combined organic layers were washed with brine (100 mL) once and dried over magnesium sulfate, filtered, and concentrated. The red brown oil residue was quickly purified by flash chromatography using EtOAc:hexanes (9:1) as eluent to afford a yellow oil (6.09 g, 86%): ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H), 2.68 (s, 3H), 7.30 (d, 1H, J = 7 Hz), 7.38 (dd, 1H, J = 7, 1 Hz), 7.55 (d, 1H, J = 1 Hz); MS(CI) m/z 327 $(M + H)^+$.

4-(1,3-Dimethoxy-2-propylamino)-2,7-dimethyl-8-(2,4-dichlorophenyl)[1,5-*a*]pyrazolo-1,3,5-triazine (11i). A mixture of 4-chloro-2,7-dimethyl-8-(2,4-dichlorophenyl)[1,5-*a*]-pyrazolo-1,3,5-triazine (8.69 g, 26.5 mmol), 1,3-dimethoxypropyl-2-aminopropane (5.4 g, 34.5 mmol), and THF (150 mL) was stirred at room temperature for 45 min. The reaction mixture was poured onto water and extracted with ethyl acetate (250 mL) for three times. The combined organic layer was dried over magnesium sulfate, filtered, and concentrated. The resulting residue was purified by column chromatography using 20% hexane in ethyl acetate as eluent to give a pale yellow solid. The solid was recrystallized from hexane to afford white crystals (5.50 g, 51%): mp 120−121 °C; ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H), 2.50 (s, 3H), 3.43 (s, 6H), 3.55−3.70 (m, 4H), 4.58−4.70 (m, 1H), 6.75 (d, 1H, J = 8 Hz), 7.28 (dd, 2H, J = 8, 1 Hz), 7.50 (s, 1H); HRMS(CI) calcd 410.1151, found 410.1147 $(M + H)^+$. Anal. Calcd for $C_{18}H_{21}Cl_2N_5O$: C, 52.69; H, 5.17; N, 16.62; Cl, 17.28. Found: C, 52.82; H, 5.06; N, 16.77; Cl, 17.50.

Biology. The cloned human receptor binding assay was performed as described previously.[34] The rat and human CRF-coupled adenylate cyclase assays have been reported elsewhere.[45]

The rat ex vivo assay is a variation of the cloned human receptor binding assay. Test compounds (10 mg/kg) were orally administered in a 0.5% methocel suspension. Plasma samples were drawn from Sprague−Dawley rats at 1 and 3 h postdose. Plasma samples were then diluted 10-fold with assay medium and aliquots of these diluted samples were analyzed in the standard binding assay.

Pharmacokinetic parameters were determined in rats after intravenous or oral doses of 5 mg/kg. The iv vehicle (2.5 mg/mL) was a mixture of *N*,*N*-dimethylacetamide, ethanol, propylene glycol 400, and water (1:1:6:2, v/v/v/v). The oral vehicle was a 0.25% methocel suspension. At 15 min, 30 min, 1, 2, 4, 6, 8, 10, 12, 16, 24, and 32 h after dosing, blood samples were collected from the tail vein into heparinized tubes containing EDTA. Steady-state brain-to-plasma ratios in rats were determined at the end of a 69-h iv infusion. Blood and brain tissue were collected at the end of the infusion. Plasma was separated by centrifugation and stored frozen until analysis.

Male beagle dogs (n = 4) were given 1 mg/kg of compound iv in a cosolvent vehicle (*N*,*N*-dimethylacetamide:ethanol: polyethylene glycol 400:water: 1:1:6:2, 2.5 mg/mL) or 1 mg/kg po in 0.25% methylcellulose suspension (2.5 mg/mL). Blood samples were collected from jugular veins at predose, 5, 15, and 30 min, and 1, 2, 4, 8, 10, 12, 16, 24, 32, 48, 56, and 72 h after dosing.

Rhesus monkeys were dosed at 5 mg/kg ((po) n = 6, 3 males, 3 females, 0.25% methocel) via a naso-gastric tube. Blood samples were drawn via an in-dwelling cannula in an arm at 0, 0.25, 0.5, 1, 2, 4, 8, 24, 36, and 48 h.

Plasma samples and brain tissues were analyzed after extraction of test compounds by simple liquid−liquid extraction. LC/MS/MS analysis was performed on a Sciex (Thornhill, Ontario) model APIIII triple quadrapole mass spectrometer interfaced with a turbo ion spray ionization source. The liquid chromatography consisted of a Perkin-Elmer series 200 solvent delivery system (Norwalk, CT), a Perkin-Elmer ISS 200 autoinjector, and a Waters Symmetry octyl minibore column (2.1 × 50 mm).

Cardiovascular pharmacodynamic testing was performed in anesthetized dogs. Mongrel dogs (n = 8) of both sexes (weights = 7.0−12.3 kg; HRP Inc., Cumberland VA) were anesthetized with sodium pentobarbital (30 mg/kg (iv)) and mechanically ventilated (15 breaths/min) using a Harvard respirator (Harvard Apparatus, South Natick, MA). The cephalic veins were cannulated for compound administration and infusion of sodium pentobarbital at 6 mg/kg/h. A micromanometer tip catheter (Millar Instruments, Houston, TX) was placed in the left ventricle to measure left ventricular pressure. The first derivative of the left ventriuclar pressure signal, $LV + dP/dt$, was obtained by the Modular Instruments software. Arterial blood pressure was monitored from a cannulated brachial artery with a Statham pressure transducer (Spectamed-Viggio, Oxnard, CA). Blood pressure, left ventricular pressure, left ventricular dP/dt, and a lead II ECG were recorded on a Modular Instruments polygraph. Compound **11i** was administered iv at 0.3, 1.0, 3.0, and 5.0 mg/kg at intervals of 30 min. The vehicle was composed of *N*,*N*-dimethylacetamide, propylene glycol, ethanol, and water (1:6:1:2) and the infusion rate was 0.033 mL/kg/min over 6 min for the 0.3 and 1.0 mg/kg doses, 0.06 mL/kg/min over 10 min for the 3 mg/kg dose, and 0.0625 mL/kg/min over 16 min for the 5.0 mg/kg dose.

Respiratory function was monitored also in anesthetized dogs. Mongrel dogs were treated with sodium pentobarbital as described above but were allowed to breathe spontaneously. Test compound was administered as described above for the cardiovascular test. The femoral artery and vein were cannulated to collect blood samples for determination of blood gases, pH, and compound plasma levels. Arterial blood pressure was measured as described previously. A balloon was positioned in the lower third of the esophagus to record pleural pressure. The endotracheal tube was attached to a Hans Rudolph pneumotachograph (3500 series; Kansas City, MO) connected to a Validyne differential pressure transducer (DP45-14) for the measurement of air flow. The esophageal balloon was similarly attached to one side of a second differential pressure transducer (Validyne, DP45-24), the other side of which was connected to a sidearm inserted in the tracheal cannula adjacent to the pneumotachograph to measure intrapleural pressure. The vehicle was composed of *N*,*N*-dimethylacetamide, polyethylene glycol 400 (PEG400), ethanol, and water (1:6:1:2) and the infusion rate was 0.033 mL/kg/min over 6 min for 0.3 and 1.0 mg/kg doses. Arterial blood samples were taken at −10, 0, 6, 15, 30, 60, and 90 min to measure arterial pH, pO_2, and pCO_2 using a Ciba-Corning 278 pH/blood gas analyzer (Ciba-Corning Diagnostics Corp., Medfield, MA).

Renal function was assessed in conscious male Sprague−Dawley rats (caesarian-derived, 199−224 g). The animals were volume-loaded with isotonic saline containing compound **11i** (10 mg/kg (po)) or furosemide (30 mg/kg (po)) in methylcellulose at a total volume of 25 mL/kg. The initial urine samples were collected during the first 5-h period postdose, then between 5 and 24 h. Total volume and osmolality (Fiske 2400 osmometer, Fiske Associates, Norwood, MA) were measured and glucose, urea, creatinine, sodium, potassium, and chloride concentrations were determined (dimension clinical analyzer, DuPont, Wilmington, DE).

Gastrointestinal motility was evaluated by gross observation and transit of an orally administered charcoal suspension (10% w/v charcoal in 0.25% methylcellulose) in fasted male Sprague−Dawley rats (178−222 g, caesarian-derived). Compound **11i** (30 mg/kg (po)), atropine (3.0 mg/kg (po)), and

carbachol (0.3 mg/kg (ip)) were administered in the standard vehicles. The charcoal suspension (5 mL/kg) was administered orally 60 min after the dose of SK696 and 30 min after the doses of carbachol or atropine. Five minutes after the charcoal meal, the rats were euthanized with CO_2 and the gastrointestinal tract (stomach to sigmoid colon) was removed. The distance from the pyloric sphincter to the charcoal at its most distal location in the small intestine was measured and compared to the pyloric sphincter to cecum measurement. The results are expressed as a percentage relative to methocel controls.

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Supporting Information Available: Combustion analyses, melting points, and spectral data (NMR or HRMS). This material is available free of charge via the Internet at http://pubs.acs.org.

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