Synthesis, Structure–Activity Relationships, and in Vivo Properties of 3,4-Dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-ones as Corticotropin-Releasing Factor-1 Receptor Antagonists

Carolyn D. Dzierba,[*,⊥] Amy G. Takvorian,[†] Maria Rafalski,[†] Padmaja Kasireddy-Polam,[†] Harvey Wong,[‡] Thaddeus F. Molski,[§] Ge Zhang,[§] Yu-Wen Li,[§] Snjezana Lelas,[§] Yong Peng,[§] John F. McElroy,[§] Robert C. Zaczek,[§] Rebecca A. Taub,[§] Andrew P. Combs,[†] Paul J. Gilligan,[⊥] and George L. Trainor[⊥]

Discovery Chemistry, Metabolism and Pharmacokinetics Discovery, Neuroscience Biology, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, Connecticut 06492

Received April 6, 2004

Corticotropin releasing factor (CRF) is the primary regulator of the hypothalamus–pituitary–adrenal (HPA) axis, coordinating the endocrine, behavioral, and autonomic responses to stress. It has been postulated that small molecules that can antagonize the binding of CRF_1 to its receptor may serve as a treatment for anxiety-related and/or affective disorders. Members within a series of 3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-ones, exemplified by compound **2** (IC_{50} = 0.70 nM), were found to be very potent antagonists of CRF_1. Compound **8w** showed high CRF_1 receptor binding affinity and was examined further in vivo. The compound was efficacious in a defensive withdrawal model of anxiety in rats and had a long half-life and reasonable oral bioavailability in dog pharmacokinetic studies.

Introduction

Corticotropin releasing factor (CRF), a 41 amino acid peptide first isolated in 1981,[1] is the primary regulator of the hypothalamus-pituitary-adrenal (HPA) axis, coordinating the endocrine, behavioral and autonomic responses to stress.[2–5] There are two known receptor subtypes of CRF, CRF_1, and CRF_2, the latter having three splice variants, α, β, and γ.[6] Of the two subtypes, the role of CRF_1 has been more extensively studied, and numerous small molecule antagonists of CRF_1 have been reported.[3,6]

There is both preclinical and clinical evidence to suggest that CRF_1 plays a role in anxiety-related diseases.[4,7–9] It has been shown that intracerebroventricular (icv) injection of CRF in rats produces behavioral and physiological changes that mimic the effects of stress.[9] Moreover, icv administration of antagonists of CRF, such as α-helical ovine CRF_{9-41}, blocks the effects of exogenously administered CRF and the effects of a variety of environmental sources of stress.[7,8,10–12]

It has also been postulated that hypersecretion of CRF_1 may be involved in affective disorders including depression and anxiety.[13–20] Patients suffering from depression have elevated levels of CRF in the cerebral spinal fluid (CSF). These levels are reduced when the depression symptoms have been successfully treated.[17,21–23] Additionally, a small molecule CRF_1 antagonist, CP-154,526 (Figure 1), has been shown to block the behavioral effects of CRF_1 in rat models of anxiety.[24–26] Moreover, an open-labeled clinical trial studying the effects of CRF_1 antagonist R121919 (Figure 1) in depressed patients demonstrated reductions in depression and anxiety scores.[27] This evidence strongly suggests that small molecule antagonists of CRF_1 would serve as a treatment for anxiety-related disorders and/or depression.

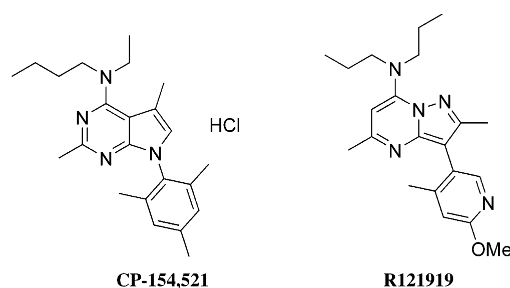


Figure 1.

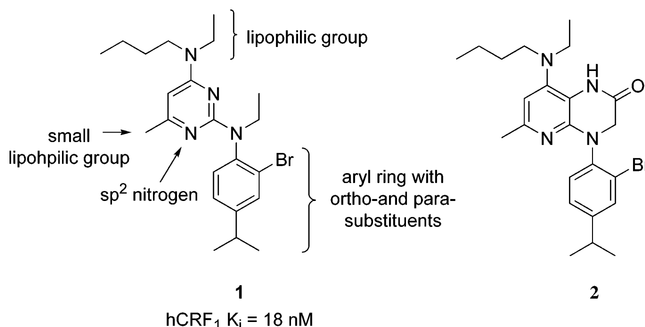


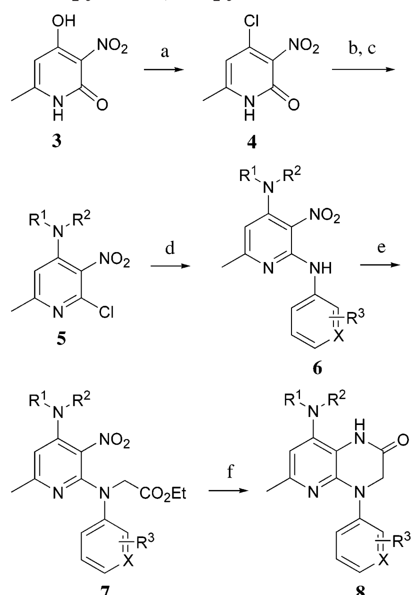
Figure 2.

Design of a new series of small molecule CRF_1 antagonists began with examination of the SAR of the known monocyclic 2-anilinopyrimidine CRF_1 antagonist **1** (Figure 2),[28] which contains features found in several known classes of mono- and bicyclic CRF antagonists.[3,29] These features include (1) an sp^2 nitrogen that may accept a hydrogen bond from a residue on the receptor, (2) an aryl ring that is minimally substituted in the

* Corresponding author: Tel: 203-677-7661. Fax: 203-677-7702, E-mail carolyn.dzierba@bms.com.
⊥ Discovery Chemistry.
‡ Metabolism and Pharmacokinetics Discovery.
§ Neuroscience Biology.
† Present address: Discovery Chemistry, Incyte Corporation, Wilmington, DE 19880.

10.1021/jm049737f CCC: $27.50 © 2004 American Chemical Society
Published on Web 09/29/2004

Scheme 1. Synthesis of 3,4-Dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-ones[a]



[a] Reagents and conditions: (a) Cyclohexylamine, MeOH, room temperature, 40 min, then $POCl_3$, 20 °C, 80 h, 81%; (b) R^1R^2NH, DIEA, CH_3CN, reflux, 3–5 h; (c) $POCl_3$, reflux, 3–5 h, 65–90%; (d) R^3-aniline (X = CH) or R^3-3-aminopyridine (X = N), 130 °C, 6–12 h, 40–90% or R^3-aniline (X = CH), NaH, DMA, 0 °C, then **5**, 100 °C, 10 h, 40–75%; (e) NaH, ethyl iodoacetate, DMF, 0 °C → room temperature, 2 h, 70–95%; (f) Fe°, HCl, EtOH, 70 °C, 12–24 h, 80–95%.

ortho- and para-positions, and (3) a lipophilic group attached to the core para to the sp^2 nitrogen. SAR studies of this series suggest that the ethyl group on the lower anilinic nitrogen is necessary to achieve good binding affinity to the CRF_1 receptor. By tying the ethyl group back into a ring, we hoped to reduce the flexibility of both the ethyl and heteroaryl groups, thereby constraining these groups into an optimal conformation and increase binding affinity. Thus, a series of 3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-ones, exemplified by compound **2** (Figure 2), was prepared. These compounds were then examined for their ability to act as CRF_1 receptor antagonists.

Results and Discussion

Chemistry. Synthesis of the desired pyridopyrazinones started from commercially available 4-hydroxy-6-methyl-3-nitro-2-pyridone, **3** (Scheme 1).[30] The 4-hydroxyl was selectively converted into a chloride by formation of the cyclohexylamine salt followed by treatment with $POCl_3$ to afford chloropyridine **4**. The 4-amino side chain was installed by displacement of the chloride with a primary or secondary amine. The resulting 2-pyridone was converted into the 2-chloropyridine **5** by treatment with $POCl_3$. The aryl ring was installed by heating **5** with an aniline to give anilinopyridine **6.** For less reactive anilines, including those with electron-withdrawing groups in the 4-position (para to the nitrogen), it was necessary to initially deprotonate the aniline with NaH in *N*,*N*-dimethylacetamide (DMA) and then heat to 100 °C with 2-chloropyridine **5** to affect conversion. Alkylation of the anilinic nitrogen with ethyl iodoacetate gave nitro ester **7**. Reduction of the nitro group with Fe° and acetic acid in refluxing ethanol also affected cyclization to form the desired six-membered ring of **8**.

Table 1. SAR Studies of the 8-Amino Group of the 3,4-Dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-ones

compd	R^1	R^2	R^3	R^4	mean $rCRF_1$ IC_{50}[a] (nM)
8a[b]	Bu	Et	OMe	H	0.30 ± 0.05
8b[b]	Et	H	OMe	H	>1000
8c[b]	Bu	H	OMe	H	>1000
8d	Bu	Et	Cl	H	0.49 ± 0.07
8e[b]	3-pentyl	H	Cl	H	198 ± 23
8f[b]	$MeOCH_2(Et)CH$	H	Cl	H	68 ± 2
8g[b]	Bu	Et	OMe	F	0.50 ± 0.07
8h	*i*Pr	Et	OMe	F	1.5 ± 0.1
8i	Bu	Et	CN	H	2.2 ± 0.1
8j	Pr	Pr	CN	H	1.2 ± 0.2
8k	cyc-$BuCH_2$-	Pr	CN	H	1.8 ± 0.3

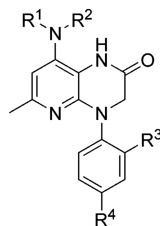
[a] IC_{50} values (n = 2, 3, or 4) ± SEM. [b] Trifluoroacetic acid salt.

Biology. Our screening strategy began with evaluating the compounds in a receptor binding assay, by measuring displacement of [^{125}I]Tyr-*o*-CRF from rat frontal cortex homogenate. Compounds with high binding affinities were evaluated in the defensive withdrawal model of anxiety in rats. Compounds that showed behavioral efficacy were examined in an ex vivo binding assay to determine receptor occupancy and in vivo plasma levels after oral dosing. Lead compounds were then taken into pharmacokinetic (PK) studies to evaluate their in vivo properties.

Structure–Activity Relationships. We set out to examine the structure–activity relationships for this series by evaluating the compounds in a CRF_1 receptor binding assay. We initially examined the effect of varying the 8-amino group on binding potency (Table 1). It was found that a dialkylamino group attached to the upper portion of the core led to better potency than a monoalkylamino group. For example, *N*-ethylamino-substituted (**8b**) or *N*-butylamino-substituted (**8c**) compounds were both inactive (IC_{50} > 1 μM), while the corresponding *N*-butyl-*N*-ethylamino compound (**8a**) was highly potent (IC_{50} = 0.30 nM). Branching somewhat improved the binding affinity of the monosubstituted amino analogues (compare **8e**, IC_{50} = 198 nM and **8f**, IC_{50} = 68 nM). Although these branched analogues showed modest binding affinity, they were still 2 orders of magnitude less potent than the corresponding disubstituted amino analogue **8d** (IC_{50} = 0.49 nM). Branching on one of the amino substituents of a disubstituted amino analogue did not have a detrimental effect on binding affinity (compare **8g**, IC_{50} = 0.50 nM and **8h**, IC_{50} = 1.5 nM). Other small aliphatic groups were tolerated in this position as seen by comparing **8i** (IC_{50} = 2.2 nM) with **8j** and **8k**, which bind to CRF_1 with IC_{50} values of 1.3 and 1.8 nM, respectively.

To determine the effect of lipophilicity changes on binding affinity, a series of analogues incorporating

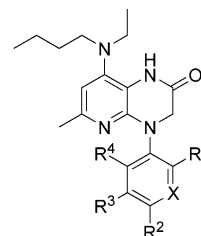
Table 2. SAR Studies of the 8-Amino Group of the 3,4-Dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-ones



compd	R^1	R^2	R^3	R^4	mean $rCRF_1$ IC_{50}[a] (nM)
8l[b]	Bu	Et	Cl	*i*-Pr	0.91 ± 0.31
8m[b]	$MeOCH_2CH_2$	Pr	Cl	*i*-Pr	2.6 ± 0.3
8n[b]	$MeOCH_2CH_2$	$MeOCH_2CH_2$	Cl	*i*-Pr	18 ± 3
8o	(*rac)*furanylCH_2	Et	Br	*i*-Pr	4.1 ± 1.3
8p	$NCCH_2CH_2CH_2$	Et	Me	OMe	92 ± 21
8q[b]	$MeSO_2CH_2CH_2CH_2$	Et	Br	*i*-Pr	305 ± 92
8r	morpholino		Br	*i*-Pr	126 ± 12

[a] IC_{50} values (n = 2, 3 or 4) ± SEM. [b] Trifluoroacetic acid salt.

Table 3. SAR Studies of the 4-Aryl Group of the 3,4-Dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-ones



compd	R^1	X	R^2	R^3	R^4	mean $rCRF_1$ IC_{50}[a] (nM)
2	Br	CH	*i*-Pr	H	H	0.70 ± 0.20
8s	Br	CH	H	H	H	7.1 ± 0.4
8t[b]	H	CH	*i*-Pr	H	H	49 ± 12
8d	Cl	CH	Cl	H	H	0.49 ± 0.07
8a[b]	Cl	CH	OMe	H	H	0.30 ± 0.05
8g[b]	Cl	CH	OMe	F	H	0.50 ± 0.07
8u	Me	CH	OMe	H	H	0.92 ± 0.29
8v[c]	Me	N	OMe	H	H	4.2 ± 0.8
8w	OMe	N	OMe	H	H	0.82 ± 0.09
8i	Cl	CH	CN	H	H	2.2 ± 0.1
8x[b]	CN	CH	Cl	H	H	10.0 ± 0.1
8y[b]	Cl	CH	CN	H	Cl	3.3 ± 0.3

[a] IC_{50} values (n = 2, 3, or 4) ± SEM. [b] Trifluoroacetic acid salt. [c] Bis-trifluoroacetic acid salt.

heteroatoms into the 8-amino group was examined (Table 2). Addition of a methyl ether onto the amino side chain (compound **8m**) led to a small decrease in binding affinity (IC_{50} = 2.6 nM) versus the corresponding *N*-butyl-*N*-ethylamino analogue **8l** (IC_{50} = 0.91 nM). Addition of a second methyl ether (compound **8n**) led to an even greater decrease in affinity (IC_{50} = 18 nM). Constraining the ether substituent into a furanyl ring was tolerated (**8o**, IC_{50} = 4.1 nM). Incorporation of polar groups such as cyano (**8p**) or methanesulfonyl (**8q**) led to a significant loss in potency (IC_{50} = 92 and 305 nM, respectively). Constraining the heteroatoms into a ring such as morpholine (**8r**, IC_{50} = 126 nM) did not improve the binding affinity. From examination of the SAR it was determined that an 8-amino group disubstituted with small lipophilic substituents is preferred to obtain good CRF_1 binding affinity.

Next we turned our attention to SAR studies around the lower aryl ring, holding the 8-amino group constant as *N*-butyl-*N*-ethylamino (Table 3). With many CRF antagonists, for example in the pyrimidine series (compound **1**, Figure 2), it has been observed that both ortho- and para-substitution is required for good binding affinity. The group in the ortho-position helps to induce a twist between the core ring system and lower aryl group, while the substituent in the para-position helps to increase binding affinity. This is exemplified by comparison of compounds **2**, **8s**, and **8t**. The 2-bromo-4-isopropylphenyl analogue **2** has excellent potency, with a binding affinity of 0.70 nM. Removal of the 4-isopropyl group (**8s**) led to a 10-fold loss in potency, while removal of the 2-bromo substituent (**8t**) led to a 70-fold loss in potency. This suggests that both the ortho- and para-substituents are required to obtain good binding affinity.

Lipophilic groups such as isopropyl (compound **2**) and chloro (compound **8d**) were preferred in the para-position (IC_{50} = 0.70 and 0.49 nM, respectively). However, mildly polar substituents, such as methoxy (compound **8a**), could also be incorporated into the para-position without loss in activity ($rCRF_1$ IC_{50} = 0.30 nM). Substitution at the 5-position was limited to small groups, such as fluoro (**8g**, IC_{50} = 0.50 nM).

Introduction of a pyridyl group in the lower aryl position was also examined. 2-Methyl-4-methoxypyridyl analogue **8v** (IC_{50} = 4.2 nM) was 5-fold less active than the corresponding phenyl analogue **8u** (IC_{50} = 0.92 nM). However, the more electron rich 2,4-dimethoxypyridyl analogue **8w** (IC_{50} = 0.82 nM) was of similar potency to analogue **8u**.

Other polar substituents could be incorporated into the phenyl group but were better tolerated in the para-position than in the ortho-position. For example, 2-chloro-4-cyanophenyl analogue **8i** (IC_{50} = 2.2 nM) was more potent than the 4-chloro-2-cyanophenyl analogue **8x** (IC_{50} = 10.0 nM). Addition of a second ortho-substituent on the aryl ring was not detrimental to the overall potency. For example, the 2,6-dichloro-4-cyanophenyl analogue **8y** (IC_{50} = 3.3 nM) was essentially equipotent to analogue **8i**. Examination of the SAR for the lower aryl group suggests that the optimal substitution pattern is 2,4-disubstitution. Small groups are also tolerated in the 5- and 6-positions, while slightly larger groups, such as isopropyl, are tolerated in the 4-position. Polarity could be incorporated in this region either in the para-position of the phenyl or as a pyridyl group.

In Vivo Studies. Compound **8w** was selected for further examination in a rat behavioral model based on its high CRF_1 binding affinity. Behavioral efficacy and efficacious plasma concentrations were determined using the defensive withdrawal model of anxiety in rats.[31-33] Briefly, rats were placed in a darkened chamber located in an unfamiliar, brightly lit chamber (a situation representing a heightened state of anxiety). The amount of time required by each rat to emerge from the chamber and explore the open field was measured. A compound was considered efficacious if the latency to emerge from the chamber was significantly reduced relative to vehicle-treated animals.

Table 4 summarizes the results of the defensive withdrawal model following oral dosing of compound **8w** at 1, 3, and 10 mg/kg. CRF_1 receptor occupancy in the parietal cortex was determined by ex vivo autoradiography.[33] The compound was effective at reducing exit

Table 4. Mean Total and Free Plasma Exposures and CRF_1 Receptor Occupancies Following Oral Doses of Compound **8w** in the Defensive Withdrawal Model in Rats

oral dose (mg/kg)	mean total plasma concn (nM)	mean free plasma concn[a] (nM)	CRF_1 receptor occupancy (%)	% decrease in exit latency (p vs vehicle)
1	3.1 ± 1.0	0.05 ± 0.02	17 ± 13	0
3	21.7 ± 24.6	0.36 ± 0.41	23 ± 17	14 (0.43)
10	61.5 ± 21.8	1.02 ± 0.36	59 ± 19	81 (<0.0001)

[a] Based upon a rat unbound fraction in plasma of 1.66% determined by equilibrium dialysis.

latency by 81% (relative to vehicle-treated control) at 10 mg/kg, ($n = 8$ per dose). This dose provided a mean CRF_1 receptor occupancy of 59% ($n = 5$) and a mean free plasma concentration (plasma free fraction = 1.66%) similar to the in vitro CRF_1 IC_{50} (i.e. 0.82 nM). A similar correspondence between free plasma concentration, occupancy, and behavioral efficacy has been reported for the CRF_1 antagonist DMP696.[33]

Compound **8w** was examined in PK studies to further characterize the in vivo properties of this CRF_1 antagonist. On the basis of the in vitro metabolic properties of this class of compounds in liver microsomes from different species, dog was observed to be the species that most closely correlated with human and was thus chosen for the following study. Compound **8w** was administered to beagle dogs at an intravenous dose of 1.0 mg/kg ($n = 2$) and at an oral dose of 5.0 mg/kg ($n = 2$). The following pharmacokinetic parameters were derived noncompartmentally from the study: Cl = 1.67 ± 0.03 L/h/kg, V_{ss} = 40.6 ± 1.8 L/kg, $t_{1/2}$ = 27.6 ± 3.4 h, F = 24%. Upon the basis of the pharmacokinetic parameters, compound **8w** appeared to be a moderate/high clearance compound with a large volume of distribution, a long half-life, and reasonable oral bioavailability in dogs. The long half-life of this compound is likely due to its large volume of distribution. Although the systemic clearance of **8w** is not optimal, this compound provides a reasonable starting point for further optimization.

The antagonist properties of compound **8w** were assessed in a cell-based functional assay by measuring the ability of the compound to inhibit CRF-stimulated ACTH secretion in cultured rat pituitary cells.[34] Compounds **8w** produced a concentration-dependent inhibition of CRF (0.3 nM)-induced ACTH release from rat pituitary cells (IC_{50} of 38.7 ± nM, $n = 3$ for CRF) and completely suppressed CRF-stimulated ACTH secretion at higher concentrations, indicating that this compound behaves as an antagonist at pituitary CRF_1 receptor. To test for agonist activity, compound **8w** was exposed alone to pituitary cells. Basal secretion of ACTH from cells was not altered, indicating a lack of agonist properties. Compound **8w** was also shown to be highly selective for CRF_1 over $hCRF_{2\alpha}$ ($hCRF_{2\alpha}$ IC_{50} > 10 μM) and against a broad panel of receptors (CEREP, Celle L'Evescault, France). At 10 μM no affinity was observed for monoamine (adrenergic (α_1, α_2, β_1), dopaminergic, or serotonergic receptors), histaminergic H_1, muscarinic, GABA, oxytocin, CGRP, or opioid receptors. Additionally, no affinity was detected at 10 μM for ion channels (glutamate (NMDA), calcium type L, or calcium-activated potassium sites). Only modest affinity was observed for adenosine A_3, canabanoid CB_1 and vasopressin V_1 (49%, 53%, and 47% inhibition @ 10 μM, respectively).

Conclusion

In conclusion, the structure–activity relationships for this series of 3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-ones was established by systematic variation of substituents on the 8-amino side chain and the 4-aryl ring. A number of highly potent analogues were identified, supporting the hypothesis that reducing the conformational flexibility of the system improves the receptor binding affinity. Compound **8w** was efficacious at 10 mg/kg in a rat behavioral model for situational anxiety and was found to occupy 59% of the CRF receptors at this dose. This compound was further examined in dog pharmacokinetic studies and found to have a long half-life and reasonable oral bioavailability in this species. Additionally, **8w** was shown to be a selective antagonist of CRF_1 against a wide range of other receptors.

Experimental Section

Chemistry. All reactions were performed under nitrogen with continuous magnetic stirring unless otherwise stated. Anhydrous solvents from commercial sources were used for all reactions. All other reagents and solvents were reagent grade and used as received from commercial sources. Flash chromatography was performed with E. Merck Kieselgel 60 silica gel (230–400 mesh). All ^1H NMR and ^{13}C NMR spectra were obtained on a Varian Inova spectrometer (operating at 300 MHz) or a Bruker Avance (operating at 400 or 500 MHz), and the signals are reported in ppm relative to TMS. All high-resolution mass spectra (HRMS) were obtained on a VG 70-VSE instrument with NH_3 as the carrier gas for chemical ionization. Compounds were purified using silica gel chromatography (hexanes/ethyl acetate as the elutant) or by reverse phase high-pressure liquid chromatography (HPLC) (0.1% TFA in water–0.1% TFA in acetonitrile gradient–20–80% acetonitrile over 30 min). Purity measurements were carried out using reverse phase HPLC using two different eluting systems. Method a: 0.1% TFA in water–0.1% TFA in acetonitrile gradient (0–100% acetonitrile over 40 min) on an Agilent HP1100 HPLC with a YMC C18 column; Method b: 0.2% phosphoric acid and 10% methanol in water–0.2% phosphoric acid and 10% water in methanol gradient (0–100% methanol over 28 min) on a Shimadzu LC10 HPLC with a Zorbax SB–C18 column.

4-Chloro-6-methyl-3-nitro-2-pyridone (4). To a 2-L round-bottomed flask equipped with a magnetic stir bar was added 4-hydroxy-6-methyl-3-nitro-2-pyridone (163.3 g, 0.96 mol) in MeOH (1.2 L). To the resulting slurry was added cyclohexylamine (135.1 mL, 1.2 mol), and the mixture was stirred for 40 min. During this time, the mixture became homogeneous, followed by precipitation of a yellow solid. The mixture was then cooled in an ice bath and filtered. The mother liquor was concentrated in vacuo (25–30 °C and 25 mmHg), affording a yellow solid that was combined with the previous precipitate and dried in a vacuum oven at room temperature. The solids were added slowly in small portions to a 3-L round-bottomed flask equipped with a mechanical stirrer, temperature probe, N_2 inlet, and $POCl_3$ (1 L, 10.7 mol). The internal temperature of the reaction was maintained <20 °C with external cooling. The reaction was then stirred at room temperature for ~80 h and then poured slowly into a 5 gallon bucket containing 1 kg of ice and 1 L of H_2O. Additional ice was added to maintain the temperature of the solution at room temperature. The resultant slurry was filtered and the solids were dissolved in EtOAc (1 L). The solution was dried over $MgSO_4$, filtered, and concentrated in vacuo (25–30 °C and 25 mmHg), affording pyridone **4** (146.0 g, 81% yield) as a pale yellow solid. ^1H NMR (300 MHz, DMSO-d_6) δ 2.25 (s, 3H), 6.40 (s, 1H), 12.95 (br s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 18.7, 105.0, 137.8, 138.8,

150.3, 154.7; HRMS Calcd for ($C_6H_5ClN_2O_3$) [M + H]$^+$ 189.0067; found 189.0061.

4-(*N*-Butyl-*N*-ethylamino)-2-chloro-6-methyl-3-nitropyridine (5a). To a 2-L three-neck round-bottom flask equipped with a mechanical stirrer, condenser, and N_2 inlet were added 4-chloro-6-methyl-3-nitro-2-pyridone **4** (29.7 g, 157.4 mmol), *N*-butyl-*N*-ethylamine (26.1 mL, 191.4 mmol), CH_3CN (800 mL), and DIEA (55 mL, 314.8 mmol). The mixture was heated at reflux for 3 h. The mixture was cooled to room temperature and concentrated in vacuo. The mixture was treated with H_2O and extracted with EtOAc (3×). The organic fractions were combined, washed with saturated aqueous NaCl, and concentrated to afford the aminopyridine as a yellow powder (30.7 g, 77%). To a 1-L round-bottom flask equipped with a magnetic stir bar and condenser was added $POCl_3$ (174 mL, 1.8 mol). The aminopyridine (30.7 g, 121.9 mmol) was added to the solution slowly at 0 °C (ice/H_2O bath). The mixture was heated at reflux for 3 h. The solution was cooled and slowly added to ice/H_2O (1.5 L). The mixture was neutralized with 50% aq NaOH and extracted with EtOAc. The combined organic layers were washed with brine and concentrated to afford aminopyridine **5a** as yellow crystals (25.5 g, 72%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.93 (t, *J* = 7.3 Hz, 3H), 1.16 (t, *J* = 7.0 Hz, 3H), 1.30 (sextet, *J* = 7.3 Hz, 2H), 1.53 (pentet, *J* = 7.3 Hz, 2H), 2.42 (s, 3H), 3.17 (t, *J* = 7.3 Hz, 2H), 3.27 (q, *J* = 7.0 Hz, 2H), 6.51 (s, 1H); HRMS Calcd for ($C_{12}H_{18}ClN_3O_2$) [M + H]$^+$ 272.1166; found 272.1174.

***N*^{2}-(2-Bromo-4-isopropylphenyl)-*N*^{4}-butyl-*N*^{4}-ethyl-6-methyl-3-nitropyridine-2,4-diamine (6).** 4-(*N*-*n*-Butyl-*N*-ethylamino)-2-chloro-6-methyl-3-nitropyridine **5a** (1.0 g, 3.7 mmol) and 2-bromo-4-isopropylaniline (1.6 g, 7.4 mmol) were heated neat at 130 °C for 16 h. The viscous black oil was taken up in EtOAc (50 mL) and washed with H_2O (2×). The combined aqueous layers were extracted with EtOAc (1×). The combined organic layers were dried ($MgSO_4$) and concentrated in vacuo. The crude black oil was purified by silica gel chromatography (98% hexanes/2% AcOH → 88% hexanes/10% ethyl acetate/2% AcOH) to afford 1.2 g (68% yield) of **5a** as an orange oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.91 (t, *J* = 7.3 Hz, 3H), 1.19 (t, *J* = 7.0 Hz, 3H), 1.22 (d, *J* = 6.7 Hz, 6H), 1.31 (m, 4H), 2.34 (s, 3H), 2.84 (septet, *J* = 7.0 Hz, 1H), 3.19 (t, *J* = 6.8 Hz, 2H), 3.26 (q, *J* = 6.8 Hz, 2H), 6.23 (s, 1H), 7.13 (d, *J* = 8.6 Hz, 1H), 7.40 (s, 1H), 8.31 (d, *J* = 8.6 Hz, 1H), 9.52 (s, 1H); HRMS Calcd for ($C_{21}H_{29}BrN_4O_2$) [M + H]$^+$ 449.1552; found 449.1552.

{(2-Bromo-4-isopropylphenyl)-[4-(butylethylamino)-6-methyl-3-nitropyridin-2-yl]amino}acetic Acid Ethyl Ester (7). A solution of **5** (1.56 mmol, 701 mg) in DMF (3.1 mL) was cooled to 0 °C. NaH (94 mg of a 60% dispersion in oil, 2.34 mmol) was added in portions and the red solution stirred at 0 °C for 30 min. Ethyl iodoacetate (277 mL, 2.34 mmol) was added dropwise over 5 min, and the resultant solution was allowed to warm to room temperature and was stirred for an additional 2 h. The reaction was quenched with H_2O (10 mL) and extracted with EtOAc (3×). The combined organic layers were washed with H_2O (5×), dried ($MgSO_4$), and concentrated in vacuo. The crude red-orange oil was purified by silica gel chromatography (100% hexanes → 75% hexanes/25% ether) to afford 677 mg (81% yield) of **7** as a red orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (t, *J* = 7.3 Hz, 3H), 1.00 (t, *J* = 7.1 Hz, 3H), 1.21 (d, *J* = 6.9 Hz, 6H), 1.26 (t, *J* = 7.1 Hz, 3H), 1.38 (sextet, *J* = 7.1 Hz, 2H), 1.59 (pentet, *J* = 7.3 Hz, 2H), 2.33 (s, 3H), 2.85 (septet, *J* = 6.9 Hz, 1H), 2.94 (t, *J* = 7.3 Hz, 2H), 3.02 (q, *J* = 7.1 Hz, 2H), 4.16 (q, *J* = 7.1 Hz, 2H), 4.38 (m, 2H), 6.34 (s, 1H), 7.04 (dd, *J* = 8.3, 2.0 Hz, 1H), 7.27 (d, *J* = 8.3 Hz, 1H), 7.43 (d, *J* = 2.0 Hz, 1H); HRMS Calcd for ($C_{25}H_{35}BrN_4O_4$) [M + H]$^+$ 535.1920; found 535.1923.

4-(2-Bromo-4-isopropylphenyl)-8-(butylethylamino)-6-methyl-3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-one (2). A solution of ester **7** (200 mg, 0.37 mmol) and iron powder (104 mg, 1.87 mmol) in EtOH (4 mL) was heated at reflux for 12 h using a heating mantle and boiling chips. The mixture was allowed to cool to room temperature and filtered through Celite eluting with EtOAc. The organics were washed with 1 N NaOH (2×) then brine (1×), dried ($MgSO_4$), and concentrated in vacuo. The crude yellow oil was purified by silica gel chromatography (90% hexanes/10% ethyl acetate → 75% hexanes/25% ethyl acetate) to afford 145 mg (81% yield) of **2** as a white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.86 (t, *J* = 7.3 Hz, 3H), 1.03 (t, *J* = 7.3 Hz, 3H), 1.23 (d, *J* = 7.3 Hz, 6H), 1.27 (sextet, *J* = 7.3 Hz, 2H), 1.38−1.47 (m, 2H), 2.18 (s, 3H), 2.94 (septet, *J* = 7.3 Hz, 1H), 3.12−3.36 (m, 4H), 3.96−4.14 (m, 1H), 4.26−4.42 (m, 1H), 6.56 (br s, 1H), 7.37 (br d, *J* = 7.5 Hz, 1H), 7.42 (br d, *J* = 7.5 Hz, 1H), 7.59 (br s, 1H); ^{13}C NMR (300 MHz, CD_3OD) δ 13.0, 14.1, 19.0, 21.1, 24.0, 24.0, 30.7, 35.0, 47.1, 51.9, 55.1, 108.7, 111.5, 122.5, 129.4, 130.6, 133.6, 138.2, 143.8, 145.0, 151.7, 153.7, 164.2; HRMS Calcd for ($C_{23}H_{31}BrN_4O$) [M + H]$^+$ 459.1759; found 459.1766. HPLC: (a) >99%; (b) 97%.

Compounds **8a**−**c**, **8g**−**h**, and **8l**−**w** were prepared in an analogous fashion to compound **2**.

8-(Butylethylamino)-4-(2-chloro-4-methoxyphenyl)-6-methyl-3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-one, Trifluoroacetic Acid Salt (8a). ^{1}H NMR (300 MHz, CD_3OD) δ 0.92 (t, *J* = 7.3 Hz, 3H), 1.11 (t, *J* = 7.3 Hz, 3H), 1.33 (sextet, *J* = 7.3 Hz, 2H), 1.51 (pentet, *J* = 7.3 Hz, 2H), 2.24 (s, 3H), 3.22 (t, *J* = 7.3 Hz, 2H), 3.27 (q, *J* = 7.3 Hz, 2H), 3.85 (s, 3H), 4.78 (m, 2H), 6.55 (s, 1H), 6.99 (dd, *J* = 2.9, 8.8 Hz, 2H), 7.12 (d, *J* = 2.9 Hz, 1H), 7.38 (d, *J* = 8.8 Hz, 1H); HRMS Calcd for ($C_{21}H_{27}ClN_4O_2$) [M + H]$^+$ 403.1901; found 403.1903; HPLC: (a) 99%; (b) >99%.

4-(2-Chloro-4-methoxyphenyl)-8-(ethylamino)-6-methyl-3,4-dihydropyrido[2,3-*b*]pyrazin-2(1*H*)-one, Trifluoroacetic Acid Salt (8b). ^{1}H NMR (300 MHz, CD_3OD) δ 1.31 (t, *J* = 7.3 Hz, 3H), 2.28 (s, 3H), 3.37 (q, *J* = 7.3 Hz, 2H), 3.83 (s, 3H), 4.31 (m, 2H), 6.44 (s, 1H), 7.03 (dd, *J* = 2.9, 8.8 Hz, 1H), 7.17 (d, *J* = 2.9 Hz, 1H), 7.43 (d, *J* = 8.8 Hz, 1H); HRMS Calcd for ($C_{17}H_{19}ClN_4O_2$) [M + H]$^+$ 347.1275; found 347.1276; HPLC: (a) >98%; (b) >98%.

8-(Butylamino)-4-(2-chloro-4-methoxyphenyl)-6-methyl-3,4-dihydropyrido[2,3-*b*]pyrazin-2(1*H*)-one, Trifluoroacetic Acid Salt (8c). ^{1}H NMR (300 MHz, CD_3OD) δ 0.97 (t, *J* = 7.3 Hz, 3H), 1.45 (sextet, *J* = 7.3 Hz, 2H), 1.64 (pentet, *J* = 7.3 Hz, 2H), 2.28 (s, 3H), 3.34 (t, *J* = 7.3 Hz, 2H), 3.83 (s, 3H), 4.84 (m, 2H), 6.44 (s, 1H), 7.03 (dd, *J* = 2.9, 8.8 Hz, 1H), 7.17 (d, *J* = 2.9 Hz, 1H), 7.42 (d, *J* = 8.8 Hz, 1H); HRMS Calcd for ($C_{19}H_{23}ClN_4O_2$) [M + H]$^+$ 375.1588; found 375.1579; HPLC: (a) 95%; (b) 98%.

***N*^{4}-Butyl-*N*^{2}-(2,4-dichlorophenyl)-*N*^{4}ethyl-6-methyl-3-nitropyridine-2,4-diamine (6d).** To 2,4-dichloroaniline (1.2 g, 7.4 mmol) in *N*,*N*-dimethylacetamide (35 mL) cooled to 0 °C was added NaH (588 mg of 60% NaH in oil, 14.7 mmol) in small portions. The solution was stirred at 0 °C for 15 min, and then 2-chloro-4-(*N*-ethyl-*N*-butylamino)-5-methyl-3-nitropyridine **5a** (1.0 g, 3.68 mmol) in *N*,*N*-dimethylacetamide (2 mL) was added slowly. The mixture was allowed to warm to ambient temperature then heated at 100 °C for 3 h. The reaction was carefully quenched with H_2O and extracted with EtOAc (3×). The combined organic layers were washed with H_2O (4×), dried ($MgSO_4$), and concentrated in vacuo. The crude brown oil was purified by silica gel chromatography (98% hexanes/2% AcOH → 88% hexanes/10% ethyl acetate/2% AcOH) to afford 949 mg (65% yield) of **6d** as an orange oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, *J* = 7.3 Hz, 3H), 1.20 (t, *J* = 7.1 Hz, 3H), 1.31 (sextet, *J* = 7.3 Hz, 2H), 1.58 (pentet, *J* = 7.3 Hz, 2H), 2.36 (s, 3H), 3.20 (dd, *J* = 7.3, 7.3 Hz, 2H), 3.27 (q, *J* = 7.1 Hz, 2H), 6.28 (s, 1H), 7.20 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.38 (d, *J* = 2.5 Hz, 1H), 8.55 (d, *J* = 8.9 Hz, 1H), 9.68 (br s, 1H). HRMS Calcd for ($C_{18}H_{22}Cl_2N_4O_2$) [M + H]$^+$ 397.1198; found 397.1196.

Compound **6d** was converted into compound **8d** in the same fashion as compound **5a** was converted into compound **2**.

8-(Butylethylamino)-4-(2,4-dichlorophenyl)-6-methyl-3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-one (8d). ^{1}H NMR (300 MHz, CD_3OD) δ 0.93 (t, *J* = 7.3 Hz, 3H), 1.19 (t, *J* = 7.3 Hz, 3H), 1.32 (sextet, *J* = 7.3 Hz, 2H), 1.58 (pentet, *J* = 7.3 Hz, 2H), 2.33 (s, 3H), 3.47 (t, *J* = 7.3 Hz, 2H), 3.53 (q, *J* = 7.3 Hz, 2H), 4.37 (s, 2H), 6.70 (s, 1H), 7.53 (m, 2H), 7.71 (dd, *J* = 2.6, 1.1 Hz, 1H); ^{13}C NMR (300 MHz, CD_3OD) δ 13.0, 14.1, 18.9, 21.1, 30.8, 47.2, 51.9, 54.8, 109.0, 111.8, 130.7, 131.4,

132.2, 133.7, 136.4, 138.2, 143.4, 145.3, 152.1, 164.3; HRMS Calcd for ($C_{20}H_{24}Cl_2N_4O$) $[M + H]^+$ 407.1405; found 407.1409; HPLC: (a) >99%; (b) >99%.

Compounds **8e**−**f**, **8i**−**k,** and **8x**−**y** were prepared in an analogous fashion to compound **8d**.

4-(2,4-Dichlorophenyl)-6-methyl-8-(pentan-3-ylamino)-3,4-dihydropyrido[2,3-*b*]pyrazin-2(1*H*)-one, Trifluoroacetic Acid Salt (8e). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.84 (t, J = 7.3 Hz, 6H), 1.33−1.59 (m, 4H), 1.97 (s, 3H), 3.21−3.32 (m, 1H), 4.11 (m, 2H), 5.46 (d, J = 8.0 Hz, 1H), 6.03 (s, 1H), 7.36−7.43 (m, 2H), 7.58 (s, 1H), 9.96 (br s, 1H); HRMS Calcd for ($C_{19}H_{22}Cl_2N_4O$) $[M + H]^+$ 393.1249; found 393.1264; HPLC: (a) >99%; (b) 96%.

4-(2,4-Dichlorophenyl)-8-(1-methoxybutan-2-ylamino)-6-methyl-3,4-dihydropyrido[2,3-*b*]pyrazin-2(1*H*)-one, Trifluoroacetic Acid Salt (8f). ^{1}H NMR (300 MHz, CD_3OD) δ 1.01 (t, J = 7.3 Hz, 3H), 1.56−1.72 (m, 2H), 1.72−1.86 (m, 2H), 2.32 (s, 3H), 3.36 (s, 3H), 3.43−3.58 (m, 2H), 3.86−3.92 (m, 1H), 4.40 (m, 2H), 6.12 (s, 1H), 7.53 (m, 2H), 7.73 (s, 1H); ^{13}C NMR (300 MHz, CD_3OD) δ 10.6, 18.8, 25.5, 54.1, 56.2, 59.4, 75.2, 102.4, 107.1, 130.7, 131.2, 132.3, 133.8, 136.2, 138.0, 139.9, 145.4, 147.8, 165.1; HRMS Calcd for ($C_{19}H_{22}Cl_2N_4O_2$) $[M + H]^+$ 409.1198; found 409.1190; HPLC: (a) 95%; (b) 95%.

8-(Butylethylamino)-4-(2-chloro-5-fluoro-4-methoxyphenyl)-6-methyl-3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-one, Trifluoroacetic Acid Salt (8g). ^{1}H NMR (300 MHz, CD_3OD) δ 0.91 (t, J = 7.3 Hz, 3H), 1.17 (t, J = 7.3 Hz, 3H), 1.31 (sextet, J = 7.3 Hz, 2H), 1.55 (pentet, J = 7.3 Hz, 2H), 2.32 (s, 3H), 3.47 (m, 4H), 3.92 (s, 3H), 4.30 (m, 2H), 6.66 (s, 1H), 7.35 (d, J_{H-F} = 8.4 Hz, 1H), 7.41 (d, J_{H-F} = 11.4 Hz, 1H); ^{13}C NMR (500 MHz, CD_3OD) δ 13.0, 14.2, 19.2, 21.2, 30.9, 47.2, 52.0, 54.8, 57.5, 108.9, 111.5, 116.7, 117.9 (d, J_{C-F} = 21.2 Hz), 128.8, 131.0, 144.0, 145.3, 150.7 (d, J_{C-F} = 12.2 Hz), 151.6, 153.1 (d, J_{C-F} = 249.2 Hz), 164.1; HRMS Calcd for ($C_{21}H_{26}ClFN_4O_2$) $[M + H]^+$ 421.1807; found 421.1805; HPLC: (a) >99%; (b) 95%.

4-(2-chloro-5-fluoro-4-methoxyphenyl)-8-(ethyl(isopropyl)amino)-6-methyl-3,4-dihydropyrido[2,3-*b*]pyrazin-2(1*H*)-one (8h). ^{1}H NMR (300 MHz, CD_3OD) δ 0.92 (t, J = 7.3 Hz, 3H), 1.11 (d, J = 7.3 Hz, 6H), 2.16 (s, 3H), 3.05 (q, J = 7.3 Hz, 2H), 3.31 (septet, J = 7.3 Hz, 1H), 3.88 (s, 3H), 4.23 (m, 2H), 6.51 (s, 1H), 7.22 (s, 1H), 7.24 (s, 1H); HRMS Calcd for ($C_{20}H_{24}ClFN_4O_2$) $[M + H]^+$ 407.1650; found 407.1673; HPLC: (a) 95%; (b) 97%.

4-[8-(Butylethylamino)-6-methyl-2-oxo-2,3-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-4-yl]-3-chloro-benzonitrile (8i). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 7.3 Hz, 3H), 1.01 (t, J = 7.3 Hz, 3H), 1.27 (sextet, J = 7.3 Hz, 2H), 1.40 (pentet, J = 7.3 Hz, 2H), 2.21 (s, 3H), 2.90 (t, J = 7.3 Hz, 2H), 2.99 (q, J = 7.3 Hz, 2H), 4.35 (m, 2H), 6.44 (s, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.59 (dd, J = 8.0, 1.9 Hz, 1H), 7.73 (d, J = 1.9 Hz, 1H), 7.84 (br s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 12.2, 13.9, 20.4, 24.1, 29.3, 47.3, 51.9, 52.8, 100.7, 110.1, 114.5, 117.5, 128.1, 131.3, 132.6, 134.2, 143.9, 145.2, 145.8, 151.5, 163.2; HRMS Calcd for ($C_{21}H_{24}ClN_5O$) $[M + H]^+$ 398.1748; found 398.1761; HPLC: (a) >99%; (b) >99%.

3-Chloro-4-(8-(dipropylamino)-6-methyl-2-oxo-2,3-dihydropyrido[2,3-*b*]pyrazin-4(1*H*)-yl)benzonitrile (8j). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 7.3 Hz, 6H), 1.45 (sextet, J = 7.3 Hz, 4H), 2.21 (s, 3H), 2.89 (t, J = 7.3 Hz, 4H), 4.35 (m, 2H), 6.44 (s, 1H), 7.37 (d, J = 8.5 Hz, 1H), 7.59 (dd, J = 8.5, 1.8 Hz, 1H), 7.73 (d, J = 1.8 Hz, 1H), 7.81 (br s, 1H); HRMS Calcd for ($C_{21}H_{24}ClN_5O$) $[M + H]^+$ 398.1748; found 398.1753; HPLC: (a) >99%; (b) >99%.

3-Chloro-4-(8-((cyclobutylmethyl)(propyl)amino)-6-methyl-2-oxo-2,3-dihydropyrido[2,3-*b*]pyrazin-4(1*H*)-yl)-benzonitrile (8k). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 7.3 Hz, 3H), 1.43 (sextet, J = 7.3 Hz, 2H), 1.53−1.68 (m, 2H), 1.71−1.85 (m, 2H), 1.85−2.03 (m, 2H), 2.21 (s, 3H), 2.41 (t, J = 7.3 Hz, 1H), 2.86 (t, J = 7.3 Hz, 2H), 2.95 (d, J = 7.3 Hz, 2H), 4.35 (m, 2H), 6.44 (s, 1H), 7.37 (d, J = 8.0 Hz, 1H), 7.59 (dd, J = 8.0, 1.8 Hz, 1H), 7.72 (d, J = 1.8 Hz, 1H), 7.83 (br s, 1H); HRMS Calcd for ($C_{23}H_{26}ClN_5O$) $[M + H]^+$ 424.1904; found 424.1909; HPLC: (a) >99%; (b) >99%.

8-(Butylethylamino)-4-(2-chloro-4-isopropylphenyl)-6-methyl-3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-one, Trifluoroacetic Acid Salt (8l). ^{1}H NMR (300 MHz, CD_3OD) δ 0.93 (t, J = 7.3 Hz, 3H), 1.19 (t, J = 7.3 Hz, 3H), 1.28 (d, J = 7.3 Hz, 6H), 1.32 (sextet, J = 7.3 Hz, 2H), 1.58 (pentet, J = 7.3 Hz, 2H), 2.33 (s, 3H), 2.99 (septet, J = 7.3 Hz, 1H), 3.41−3.59 (m, 4H), 4.34 (m, 2H), 6.68 (br s, 1H), 7.38 (dd, J = 8.4, 1.8 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.59 (d, J = 1.8 Hz,1H); ^{13}C NMR (300 MHz, CD_3OD) δ 13.0, 14.2, 19.4, 21.2, 24.1 (2C), 30.8, 35.2, 47.3, 52.0, 55.1, 108.9, 111.8, 128.6, 130.2, 130.4, 132.7, 137.0, 144.2, 145.7, 151.5, 153.2, 164.5; HRMS Calcd for ($C_{23}H_{31}ClN_4O$) $[M + H]^+$ 415.2265; found 415.2254; HPLC: (a) >99%; (b) >99%.

4-(2-Chloro-4-isopropylphenyl)-8-[(2-methoxyethyl)-propylamino]-6-methyl-3,4-dihydro-1*H*-pyrido[2,3-*b*]-pyrazin-2-one, Trifluoroacetic Acid Salt (8m). ^{1}H NMR (300 MHz, CD_3OD) δ 0.96 (t, J = 7.3 Hz, 3H), 1.27 (d, J = 7.3 Hz, 6H), 1.59 (sextet, J = 7.3 Hz, 2H), 2.28 (s, 3H), 2.97 (septet, J = 7.3 Hz, 1H), 3.24−3.34 (m, 3H), 3.48−3.56 (m, 4H), 3.73 (t, J = 7.3 Hz, 2H), 4.31 (m, 2H), 6.61 (br s, 1H), 7.34 (dd, J = 1.8, 8.8 Hz, 1H), 7.41 (d, J = 8.8 Hz, 1H), 7.46 (d, J = 1.8 Hz, 1H); ^{13}C NMR (300 MHz, CD_3OD) δ 11.7, 21.7, 23.6, 24.2, 53.0, 30.7, 54.2, 55.8, 59.2, 71.2, 108.3, 113.9, 127.3, 129.5, 130.2, 133.4, 139.8, 146.8, 147.6, 150.6, 152.2, 166.1; HRMS Calcd for ($C_{23}H_{31}ClN_4O_2$) $[M + H]^+$ 431.2214; found 431.2221; HPLC: (a) 97%; (b) 97%.

8-[Bis-(2-methoxyethyl)amino]-4-(2-chloro-4-isopropylphenyl)-6-methyl-3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-one, Trifluoroacetic Acid Salt (8n). ^{1}H NMR (300 MHz, CD_3OD) δ 1.28 (d, J = 7.3 Hz, 6H), 2.31 (br s, 3H), 2.98 (septet, J = 7.3 Hz, 1H), 3.43 (s, 6H), 3.64−3.76 (m, 8H), 4.32 (m, 2H), 6.77 (br s, 1H), 7.38 (dd, J = 1.8, 8.4 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.49 (d, J = 1.8 Hz, 1H); ^{13}C NMR (300 MHz, CD_3OD) δ 22.2, 22.8, 33.5, 51.8, 52.7, 57.7, 69.8 106.9, 112.3, 125.9, 128.0, 128.7, 131.9, 138.3, 145.2, 146.2, 149.1, 151.0, 164.6; HRMS Calcd for ($C_{23}H_{31}ClN_4O_3$) $[M + H]^+$ 447.2163; found 447.2152; HPLC: (a) >98%; (b) >99%.

4-(2-Bromo-4-isopropylphenyl)-8-[ethyl(tetrahydrofuran-2-ylmethyl)amino]-6-methyl-3,4-dihydro-1*H*-pyrido-[2,3-*b*]pyrazin-2-one (8o). ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.10 (t, J = 7.1 Hz, 3H), 1.26 (d, J = 6.9 Hz, 6H), 1.47 (m, 1H), 1.97 (m, 3H), 2.19 (s, 3H), 2.92 (m, 3H), 3.09 (sextet, J = 6.9 Hz, 1H), 3.24 (sextet, J = 6.9 Hz, 1H), 3.88 (m, 1H), 4.21 (m, 2H), 4.20 (m, 2H), 6.29 (s, 1H), 7.20 (dd, J = 1.7, 8.1 Hz, 1H), 7.22 (d, J = 8.1 Hz, 1H), 7.48 (d, J = 1.7 Hz, 1H), 9.83 (s, 1H); ^{13}C NMR (400 MHz, $CDCl_3$) δ ppm 13.0, 23.8, 23.8, 24.2, 25.9, 29.4, 33.6, 44.7, 53.4, 68.1, 76.3, 77.2, 106.3, 112.3, 122.9, 126.6, 129.6, 131.6, 140.0, 144.4, 145.7, 149.0, 150.6, 163.9; HRMS Calcd for ($C_{24}H_{31}BrN_4O_2$) $[M + H]^+$ 487.1709; found 487.1703; HPLC: (a) >98%; (b) >98%.

4-{Ethyl-[4-(4-methoxy-2-methylphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-pyrido[2,3-*b*]pyrazin-8-yl]amino}-butyronitrile (8p). ^{1}H NMR (300 MHz, CD_3OD) δ 1.04 (t, J = 7.3 Hz, 3H), 1.78 (pentet, J = 7.3 Hz, 2H), 2.06 (s, 3H), 2.16 (s, 3H), 2.49 (t, J = 7.3 Hz, 3H), 3.05−3.22 (m, 4H), 3.78 (s, 3H), 4.00−4.40 (m, 2H), 6.81 (s, 1H), 6.80 (d, J = 8.1 Hz, 1H), 6.82 (s, 1H), 7.14 (d, J = 8.1 Hz, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 12.3, 15.5, 19.0, 23.7, 24.7, 49.1, 50.4, 54.3, 55.7, 107.5, 112.4, 113.8, 116.6, 119.6, 127.8, 135.3, 143.4, 146.5, 152.1, 158.4, 164.0; HRMS Calcd for ($C_{22}H_{27}N_5O_2$) $[M + H]^+$ 394.2243; found 394.2238; HPLC: (a) >98%; (b) >98%.

4-(2-Bromo-4-isopropylphenyl)-8-[ethyl-(3-methanesulfonylpropyl)amino]-6-methyl-3,4-dihydro-1*H*-pyrido-[2,3-*b*]pyrazin-2-one, Trifluoroacetic Acid Salt (8q). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.14 (t, J = 7.1 Hz, 3H), 1.25 (d, J = 6.9 Hz, 6H), 2.10 (m, 2H), 2.35 (s, 3H), 2.92 (s, 3H), 2.92 (septet, J = 6.9 Hz, 1H), 3.11 (dd, J = 7.1, 7.1 Hz, 2H), 3.33 (m, 2H), 3.42 (m, 2H), 4.40 (m, 2H), 6.40 (s, 1H), 7.29 (dd, J = 8.2, 1.7 Hz, 1H), 7.41 (d, J = 8.2 Hz, 1H), 7.52 (d, J = 1.7 Hz, 1H), 8.08 (s, 1H); HRMS Calcd for ($C_{23}H_{31}BrN_4O_3S$) $[M + H]^+$ 523.1378; found 523.1372; HPLC: (a) >98%; (b) 95%.

4-(2-Bromo-4-isopropylphenyl)-6-methyl-8-morpholin-4-yl-3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-one (8r). ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.27 (d, J = 7.3 Hz, 6H), 2.33 (s,

3H), 2.97 (septet, J = 7.3 Hz, 2H), 3.26−3.50 (m, 4H), 3.78−3.96 (m, 4H), 4.20−4.48 (m, 2H), 6.72 (s, 1H), 7.40−7.47 (m, 2H), 7.65 (d, J = 1.4 Hz, 1H); HRMS Calcd for ($C_{21}H_{25}BrN_4O_2$) $[M + H]^+$ 445.1239; found 445.1242; HPLC: (a) >99; (b) 95%.

4-(2-Bromophenyl)-8-(butylethylamino)-6-methyl-3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-one (8s). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (t, J = 7.3 Hz, 3H), 1.18 (t, J = 7.1 Hz, 3H), 1.35 (sextet, J = 7.3 Hz, 2H), 1.53 (pentet, J = 7.3 Hz, 2H), 2.36 (s, 3H), 3.18 (dd, J = 7.3, 7.3 Hz, 2H), 3.27 (q, J = 7.1 Hz, 2H), 4.41 (m, 2H), 6.36 (s, 1H), 7.29 (m, 1H), 7.46 (m, 2H), 7.63 (br s, 1H) 7.68 (d, J = 8.1 Hz, 1H); HRMS Calcd for ($C_{20}H_{25}BrN_4O$) $[M + H]^+$ 417.1290; found 417.1287; HPLC: (a) 97%; (b) >98%.

8-(Butylethylamino)-4-(4-isopropylphenyl)-6-methyl-3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-one, Trifluoroacetic Acid Salt (8t). ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.84 (t, J = 7.3 Hz, 3H), 1.04 (t, J = 7.3 Hz, 3H), 1.18 (d, J = 7.3 Hz, 6H), 1.23 (sextet, J = 7.3 Hz, 2H), 1.43 (pentet, J = 7.3 Hz, 2H), 2.25 (s, 3H), 2.89 (septet, J = 7.3 Hz, 1H), 3.25−3.45 (m, 4H), 4.28 (m, 2H), 6.67 (br s, 1H), 7.22 (d, J = 8.8 Hz, 2H), 7.27 (d, J = 8.8 Hz, 2H). HRMS Calcd for ($C_{22}H_{32}N_4O$) $[M + H]^+$ 381.2654; found 381.2650; HPLC: (a) 97%; (b) >95%.

8-(Butylethylamino)-4-(4-methoxy-2-methylphenyl)-6-methyl-3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-one (8u). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 7.3 Hz, 3H), 1.00 (t, J = 7.3 Hz, 3H), 1.27 (sextet, J = 7.3 Hz, 2H), 1.40 (pentet, J = 7.3 Hz, 2H), 2.09 (s, 3H), 2.18 (s, 3H), 2.89 (t, J = 7.3 Hz, 2H), 2.96 (q, J = 7.3 Hz, 2H), 3.80 (s, 3H), 4.15−4.42 (m, 2H), 6.60 (s, 1H), 6.76 (dd, J = 7.7, 2.9 Hz, 1H), 6.79 (dd, J = 2.9, 1.1 Hz, 1H), 7.13 (dd, J = 7.7, 1.1 Hz, 1H), 7.91 (br s, 1H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 12.4, 13.9, 18.6, 20.4, 24.3, 29.5, 47.7, 52.3, 54.0, 55.3, 107.6, 112.0, 113.5, 116.2, 127.3, 135.1, 137.5, 144.7, 145.9, 151.3, 157.9, 163.6; HRMS Calcd for ($C_{22}H_{30}N_4O_2$) $[M + H]^+$ 383.2447; found 383.2451; HPLC: (a) 97%; (b) >95%.

8-(Butylethylamino)-4-(6-methoxy-2-methylpyridin-3-yl)-6-methyl-3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-one, bis-Trifluoroacetic Acid Salt (8v). ^{1}H NMR (300 MHz, CD_3OD) δ 0.91 (t, J = 7.3 Hz, 3H), 1.16 (t, J = 7.3 Hz, 3H), 1.31 (sextet, J = 7.3 Hz, 2H), 1.55 (pentet, J = 7.3 Hz, 2H), 2.30 (s, 3H), 2.31 (s, 3H), 3.43 (t, J = 7.3 Hz, 2H), 3.47 (q, J = 7.3 Hz, 2H), 3.92 (s, 3H), 4.29 (m, 2H), 6.63 (s 1H), 6.76 (d, J = 8.8 Hz, 1H), 7.63 (d, J = 8.8 Hz, 1H); HRMS Calcd for ($C_{21}H_{29}N_5O_2$) $[M + H]^+$ 384.2400; found 384.2389; HPLC: (a) 97%; (b) >98%.

8-(Butylethylamino)-4-(2,6-dimethoxypyridin-3-yl)-6-methyl-3,4-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-2-one (8w). ^{1}H NMR (300 MHz, CD_3OD) δ 0.93 (t, J = 7.3 Hz, 3H), 1.17 (t, J = 7.3 Hz, 3H), 1.32 (sextet, J = 7.3 Hz, 2H), 1.55 (pentet, J = 7.3 Hz, 2H), 2.32 (s, 3H), 3.42 (t, J = 7.3 Hz, 2H), 3.48 (q, J = 7.3 Hz, 2H), 3.97 (s, 3H), 3.98 (s, 3H), 4.27 (m, 2H), 6.46 (d, J = 8.4 Hz, 1H), 6.64 (s 1H), 7.63 (d, J = 8.4 Hz, 1H); ^{13}C NMR (300 MHz, CD_3OD) δ 13.0, 14.1, 18.8, 21.1, 30.8, 47.0, 48.2, 51.8, 54.5, 54.7, 104.1, 108.5, 111.2, 115.9, 140.9, 144.1, 144.4, 151.3, 159.8, 164.4, 164.6; HRMS Calcd for ($C_{21}H_{29}N_5O_3$) $[M + H]^+$ 400.2349; found 400.2364; HPLC: (a) >98%; (b) >95%.

2-[8-(Butylethylamino)-6-methyl-2-oxo-2,3-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-4-yl]-5-chloro-benzonitrile, Trifluoroacetic Acid Salt (8x). ^{1}H NMR (300 MHz, CD_3OD) δ 0.87 (t, J = 7.3 Hz, 3H), 1.02 (t, J = 7.3 Hz, 3H), 1.28 (sextet, J = 7.3 Hz, 2H), 1.42 (pentet, J = 7.3 Hz, 2H), 2.20 (s, 3H), 3.06 (t, J = 7.3 Hz, 2H), 3.12 (q, J = 7.3 Hz, 2H), 4.34 (m, 2H), 6.57 (s, 1H), 7.37 (d, J = 8.8 Hz, 1H), 7.67 (dd, J = 8.8, 2.6 Hz, 1H), 7.72 (d, J = 2.6 Hz, 1H), 7.84 (br s, 1H); ^{13}C NMR (300 MHz, DMSO-d_6) δ 12.5, 13.8, 20.3, 20.6, 29.5, 46.2, 50.8, 53.6, 109.5, 111.1, 113.1, 115.1, 127.8, 133.3, 133.8, 135.0, 142.1, 142.8, 147.9, 149.7, 162.4; HRMS Calcd for ($C_{21}H_{24}ClN_5O_2$) $[M + H]^+$ 398.1748; found 398.1749; HPLC: (a) >98%; (b) >98%.

4-[8-(Butylethylamino)-6-methyl-2-oxo-2,3-dihydro-1*H*-pyrido[2,3-*b*]pyrazin-4-yl]-3,5-dichloro-benzonitrile, Trifluoroacetic Acid Salt (8y). ^{1}H NMR (300, $CDCl_3$) δ 0.88 (t, J = 7.3 Hz, 3H), 1.01 (t, J = 7.3 Hz, 3H), 1.29 (sextet, J = 7.3 Hz, 2H), 1.37 (pentet, J = 7.3 Hz, 2H), 2.16 (s, 3H), 2.91 (t, J = 7.3 Hz, 2H), 2.98 (q, J = 7.3 Hz, 2H), 4.31 (m, 2H), 6.37 (s, 1H), 7.69 (s, 2H); HRMS Calcd for ($C_{21}H_{23}Cl_2N_5O$) $[M + H]^+$ 432.1358; found 432.1353; HPLC: (a) 98%; (b) 97%.

Biology. Rat CRH Receptor Binding Assay. Frozen rat frontal cortex (source of CRF_1 receptor) or frozen porcine choroid plexus (source of CRF_2 receptor) were thawed rapidly in assay buffer containing 50 mM Hepes (pH 7.0 at 23 °C), 10 mM $MgCl_2$, 2 mM EGTA, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin A, 0.005% Triton X-100, 10 U/mL bacitracin, and 0.1% ovalbumin and homogenized. The suspension was centrifuged at 32000*g* for 30 min. The resulting supernatant was discarded and the pellet resuspended by homogenization in assay buffer and centrifuged again. The supernatant was discarded and the pellet resuspended by homogenization in assay buffer and frozen at −70 °C. On the day of the experiment aliquots of the homogenate were thawed quickly and homogenate (25 μg/well rat frontal cortex or 10 μg/well porcine choroid plexus) added to ligand (150 pM ^{125}I-ovine-CRF for CRF_1 binding or 100 pM ^{125}I-sauvagine for CRF_2 binding) and drugs in a total volume of 100 μL assay buffer. The assay mixture was incubated for 2 h at 21 °C. Bound and free radioligand were then separated by rapid filtration, using glass fiber filters (Whatman GF/B, pretreated with 0.3% PEI) on a Brandel Cell Harvester. Filters were then washed multiple times with ice cold wash buffer (PBS w/o Ca^{2+} and Mg^{2+}, 0.01% Triton X-100 (pH 7.0 at 23 °C)). Nonspecific binding was defined using 1 μM DMP696 in the CRF_1 binding assay and 1 μM α-helical CRF (9−41) in the CRF_2 binding assay. Filters were then counted in a Wallac Wizard gamma counter.

Behavioral Studies. Subjects. 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 in a colony room maintained at constant temperature (21 ± 2 °C) and humidity (50 ± 10%). The room was illuminated 12 h per day (lights on at 0600 h). The rats had ad libitum access to food and water throughout the study. 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.

Defensive Withdrawal. The defensive withdrawal procedure was used as previously described.[32] Briefly, 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 23-lux reading at the entrance to the cylinder. Rats were habituated to handling by gently stroking their dorsal surface for approximately one minute daily for 5−6 consecutive days 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. All compounds were prepared in 0.25% methocel suspension and beadmilled overnight. They were administered po, 1 h before testing in a volume of 2 mL/kg body weight. Data were analyzed using an analysis of variance, followed by individual mean comparisons using Fisher's Least Significant Difference Test. The significance level was set at $p < 0.05$.

Pharmacokinetic Studies. Pharmacokinetic parameters were estimated in beagle dogs following intravenous (1 mg/kg; $n = 2$) and oral (5 mg/kg; $n = 2$) doses. Intravenous doses were prepared in a vehicle consisting of propylene glycol:water: *N*,*N*-dimethylacetamide, 80:10:10 (v/v). The oral vehicle consisted of Labrafil:ethanol:Tween 80, 85:10:5 (v/v). Blood samples were collected via jugular venipuncture at 0, 0.1, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h post-dose for the intravenous experiment, and at 0, 0.5, 1, 2, 4, 6, 8, and 24 h post-dose for the oral experiment. Plasma was separated by centrifugation and stored frozen at −20 °C until analysis. Concentrations of compound were determined by LC/MS/MS.

Acknowledgment. The authors thank Gail K. Mattson and Anne P. Marshall for performing the in vitro binding studies.

References

(1) 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.
(2) Gilligan, P. J.; Hartig, P. R.; Robertson, D. W.; Zaczek, R. Corticotropin-releasing hormone (CRH) receptors and the discovery of selective non-peptide CRH1 antagonists. *Annu. Rep. Med. Chem.* **1997**, *32*, 41−50.
(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) Owens, M. J.; Nemeroff, C. B. Physiology and pharmacology of corticotropin-releasing factor. *Pharmacol. Rev.* **1991**, *43*, 425−473.
(5) De Souza, E. B.; Grigoriadis, D. E. Corticotropin-Releasing-Factor. Physiology, Pharmacology and Role in Central Nervous System and Immune Disorders. In *Psycopharmacology; The Fourth Generation of Progress*; Bloom, F. E., Kupfer, D. J., Eds.; Raven Press: New York, 1995; pp 505−517.
(6) McCarthy, J. R.; Heinrichs, S. C.; Grigoriadis, D. E. Recent advances with the CRF1 receptor: design of small molecule inhibitors, receptor subtypes and clinical indications. *Curr. Pharm. Des.* **1999**, *5*, 289−315.
(7) Britton, K. T.; Lee, G.; Vale, W.; Rivier, C.; Koob, G. F. Corticotropin-releasing factor (CRF) receptor antagonist blocks activating and "anxiogenic" actions of CRF in the rat. *Brain Res.* **1986**, *369*, 303−306.
(8) Berridge, C. W.; Dunn, A. J. A corticotropin-releasing factor antagonist reverses the stress-induced change in exploratory behavior in mice. *Horm. Behav.* **1987**, *21*, 393−401.
(9) 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.
(10) Swerdlow, N. R.; Britton, K. T.; Koob, G. F. Potentiation of acoustic startle by corticotropin-releasing factor (CRF) and by fear are both reversed by alpha-helical CRF_{9-41}. *Neuropsychopharmacology* **1989**, *2*, 285−292.
(11) Adamec, R. E.; Sayin, U.; Brown, A. The effects of corticotropin releasing factor (CRF) and handling stress on behavior in the elevated plus maze test of anxiety. *J. Psychopharmacol.* **1991**, *5*, 175−186.
(12) Liang, K. C.; Melia, K. R.; Miserendino, M. J.; Falls, W. A.; Campeau, S.; Davis, M. Corticotropin-releasing factor: long-lasting facilitation of the acoustic startle reflex. *J. Neurosci.* **1992**, *12*, 2303−2312.
(13) Owens, M. J.; Nemeroff, C. B. The role of corticotropin-releasing factor in the pathophysiology of affective and anxiety disorders: Laboratory and clinical studies. In *Corticotropin Releasing-Factor*, *Ciba Foundation Symposium 172*; Chadwick, D. J., Marsh, J., Ackrill, K., Eds.; J. Wiley and Sons: Chichester, U.K., 1993; pp 296−316.
(14) Banki, C. M.; Bisette, G.; Arato, M.; O'Conner, L.; Nemeroff, C. B. CSF corticotropin-releasing factor-like immunoreactivity in depression and schizophrenia. *Am. J. Psychiatry* **1987**, *144*, 873−877.
(15) France, R. D.; Urban, B.; Krishnan, R. R.; Bisette, G.; Banki, C. M.; Nemeroff, C. B.; Spielman, F. J. CSF corticotropin-releasing factor-like immunoreactivity in chronic pain patients with and without major depression. *Biol. Psychiatry* **1988**, *23*, 86−88.
(16) Arato, M.; Banki, C. M.; Bisette, G.; Nemeroff, C. B. Elevated CSF CRF in suicide victims. *Biol. Psychiatry* **1989**, *25*, 355−359.
(17) 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.
(18) Gold, P. W.; Chrousos, G.; Kellner, C.; Post, R.; Roy, A.; Augerinos, P.; Schulte, H.; Oldfield, E.; Loriaux, D. L. Psychiatric implications of basic and clinical studies with corticotropin-releasing factor. *Am. J. Psychiatry* **1984**, *141*, 619−627.
(19) Holsboer, F.; Mueller, O. A.; Doerr, H. G.; Sippell, W. G.; Stalla, G. K.; Gerken, A.; Steiger, A.; Boll, E.; Benkert, O. ACTH and multisteroid responses to corticotropin-releasing factor in depressive illness: relationship to multisteroid responses after ATCH stimulation and dexamethasone suppression. *Psychoneuroendocrinology* **1984**, *9*, 147−160.
(20) 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.; Oldfield, E. H.; Cutler, G. B.; Chrousos, G. P. Reponses to corticotropin-releasing hormone in the hypercortisolism of depression and Cushing's disease. *N. Engl. J. Med.* **1986**, *314*, 1329−1335.
(21) Nemeroff, C. B.; Widerlov, E.; Bisette, G.; Walleus, H.; Karlsson, I.; Eklund, K.; Kilts, C. D.; Loosen, P. T.; Vale, W. Elevated concentration of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. *Science* **1984**, *226*, 1342−1344.
(22) Arato, M.; Banki, C. M.; Nemeroff, C. B. Hypothalamic-pituitary-adrenal axis and suicide. *Ann. N.Y. Acad. Sci.* **1986**, *487*, 263−270.
(23) Nemeroff, C. B.; Bisette, G.; Akil, H.; Fink, M. Cerebrospinal fluid neuropeptides in depressed patients treated with ETC: corticotropin-releasing factor, B-endorphin and somatostatin. *J. Psychiatry* **1991**, *158*, 59−63.
(24) Chen, Y. L.; Mansbach, R. S.; Winter, S. M.; Brooks, E.; Collins, J.; Corman, M. L.; Dunaiskis, A. R.; Faraci, W. S.; Gallaschun, R. J.; Schmidt, A.; Schulz, D. W. Synthesis and Oral Efficacy of a 4-(Butylethylamino)pyrrolo[2,3-d]pyrimidine: A Centrally Active Corticotropin-Releasing Factor1 Receptor Antagonist. *J. Med. Chem.* **1997**, *40*, 1749−1754.
(25) Mansbach, R. S.; Brooks, E. N.; Chen, Y. L. Antidepressant-like effects of CP-154526, a selective CRF1 receptor antagonist. *Eur. J. Pharmacol.* **1997**, *323*, 21−26.
(26) Griebel, G.; Perrault, G.; Sanger, D. J. Characterization of the behavioral profile of the nonpeptide CRF receptor antagonist CP-154,526 in anxiety models in rodents. Comparison with diazepam and buspirone. *Psychopharmacology* **1998**, *138*, 55−66.
(27) Zobel, A. W.; Nickel, T.; Kunzel, H. E.; Ackl, N.; Sonntag, A.; Ising, M.; Holsboer, F. Effects fo high-affinity corticotropin-releasing hormone receptor 1 receptor antagonist R121919 in major depression: the first 20 patients treated. *J. Psychiatric Res.* **2000**, *34*, 171−181.
(28) Arvanitis, A. G.; Gilligan, P. J.; Chorvat, R. J.; Cheeseman, R. S.; Christos, T. E.; Bakthavatchalam, R.; Beck, J. P.; Cocuzza, A. J.; Hobbs, F. W.; Wilde, R. G.; Arnold, C.; Chidester, D.; Curry, M.; He, L.; Hollis, A.; Klaczkiewicz, J.; Krenitsky, P. J.; Rescinito, J. P.; Scholfield, E.; Culp, S.; De Souza, E. B.; Fitzgerald, L.; Grigoriadis, D.; Tam, S. W.; Wong, Y. N.; Huang, S.-M.; Shen, H. L. Non-Peptide Corticotropin-Releasing Hormone Antagonists: Syntheses and Structure−Activity Relationships of 2-Anilinopyrimidines and -triazines. *J. Med. Chem.* **1999**, *42*, 805−818.
(29) McCarthy, J. R.; Heinrichs, S. C.; Grigoriadis, D. E. Recent progress in corticotropin-releasing factor receptor agents. *Annu. Rep. Med. Chem.* **1999**, *34*, 11−20.
(30) Wilde, R. G. Preparation of tetrahydropteridines and pyridylpiperazines for treatment of neurological disorders. International Patent Publication Number 6083948, 1997.
(31) Takahashi, L. K.; Kalin, N. H.; VandenBurgt, J. A.; Sherman, J. E. Corticotropin-releasing factor modulates defensivw-withdrawal and exploratory behavior in rats. *Behav. Nuerosci.* **1989**, *103*, 648−654.
(32) McElroy, J. F.; Ward, K. A.; Zeller, K. L.; Jones, K. W.; Gilligan, P. J.; He, L.; Lelas, S. The CRF1 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.
(33) 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.
(34) Zhang, G.; Huang, N.; Li, Y.-W.; Qi, X.; Marshall, A. P.; Yan, X.-X.; Hill, G.; Rominger, C.; Prakash, S. R.; Bakthavatchalam, R.; Rominger, D. H.; Gilligan, P. J.; Zaczek, R. Pharmacological characterization of a novel nonpeptide antagonist radioligand, (+-)-N-[2-methyl-4-methoxyphenyl]-1-(1-(methoxymethyl) propyl)-6-methyl-1H-1,2,3-triazolo[4,5-c]pyridin-4-amine ([^{3}H]SN003) for corticotropin-releasing factor1 receptors. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 57−69.

JM049737F