2-Aryloxy-4-alkylaminopyridines: Discovery of Novel Corticotropin-Releasing Factor 1 Antagonists

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An orally active clinical candidate of corticotropin-releasing factor 1 (CRF_1) antagonist **1** showed a significant positive food effect in dog and human after oral administration. Efforts to address the food effect issue led us to explore and discover compounds in series **2** as orally active CRF_1 receptor antagonists, in which some compounds showed improved physicochemical properties while retaining desired pharmacological properties. Compound **3a** (CP-376395) was selected for further development, due not only to its reduced food effects but also its greater efficacy in CNS models. Compound **3a** was advanced to the clinic. The synthesis of representative potential candidates and their *in vitro*, *ex vivo*, and *in vivo* data are described.

Introduction

CRF, a 41 amino acid peptide, was identified as a key regulator of the hypothalamus−pituitary−adrenal (HPA) axis.[1] Published preclinical data support the hypothesis that a small-molecule CRF_1 receptor antagonist, such as **4a** (CP-154526) or **4b** (antalarmin), is effective in blocking neuroendocrine effects, autonomic responses, or increased heart rate or behavioral changes induced by exogenous or endogenous CRF.[2,3] Since the first nonpeptide small-molecule CRF_1 receptor subtype antagonist **4a** was published as a research tool,[2] many related chemical series have been disclosed as selective CRF_1 receptor subtype antagonists by several laboratories over the past 13 years. We have independently discovered many CRF_1 antagonist chemotypes; for example, our published pyridine series was found to be safe and free of liver toxicity[4] which has been an issue for some other series.[5,6] An orally active CRF_1 receptor antagonist **1**[4] was advanced to phase II. Compound **1** showed a significant food effect in dog and human.[7] It has low solubility and basicity which is thought to account for a 10- to 20-fold positive food effect observed in dogs and humans. It was thought that increased basicity may lead to improved solubility and reduced food effects. In a continuation of our efforts to improve physicochemical properties, compounds in series **2** were designed, in which the oxygen at either the alkoxy or aryloxy portion of **1** was replaced with an aminoalkyl or arylamino group, respectively (see Figure 1). It is expected that this change will lead to compounds with greater basicity along with improvements in solubility. Efforts to further optimize physicochemical properties by adding a polar group or blocking metabolic sites have led to the discovery of several potential candidates **3a**,**b** and **5a**,**b** that met the desired pharmacological and pharmacokinetic profile and were selected for exploratory toxicology evaluations. Among these, compound **3a** demonstrated unexpected potency in several *in vivo* models and was advanced to the clinic. Compound **3a** showed reduced fed-fasted food effects, only 2–3-fold in dogs and humans[7] as predicted compared to 10–20-fold for **1**. The synthesis, pharmacology, and pharmacokinetic data of the representative compounds are presented.

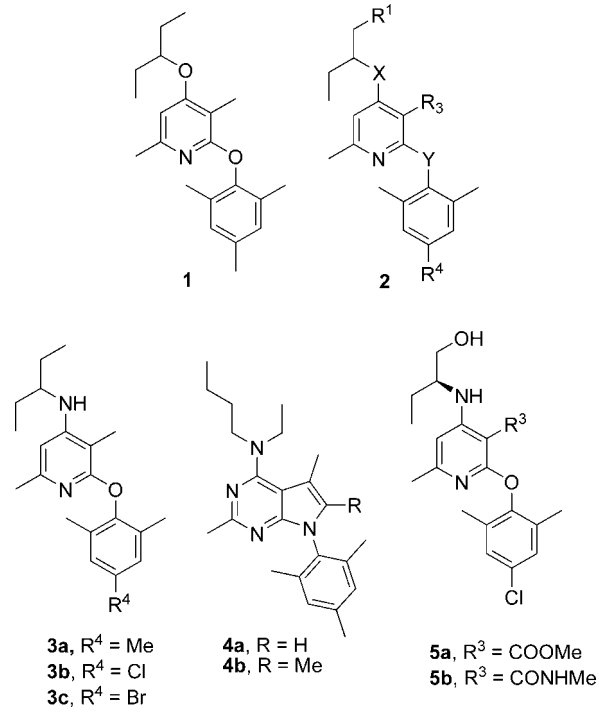

Figure 1. Structures of CRF_1 selective antagonists.

Chemistry

The syntheses of compounds in series **2** are illustrated in Schemes 1−4. Scheme 1 describes a method to control regioselectivity via a pyridine 1-oxide **7**[8] to provide the desired isomer in an excellent yield. Compound **6** was prepared according to the literature procedure[9] with a small modification in which 1 equiv of *N*,*N*-diethylaniline was added to improve the yield from 33%[9] to a maximum of 72% yield. Standard oxidation of compound **6** with *m*-CPBA provided the desired 1-oxide **7** in a low yield along with recovered starting material, which remained unreacted even after 2 days. Changing the

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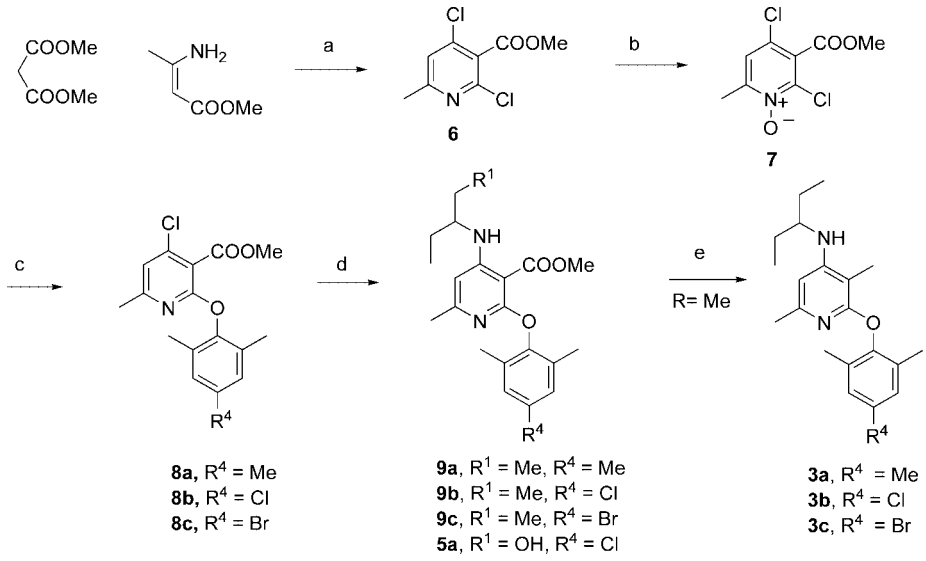
Scheme 1[a]



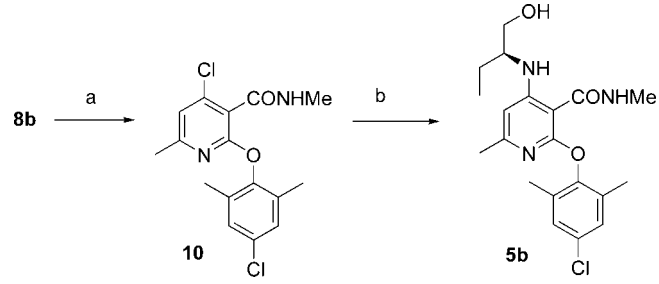
[a] Reagents and conditions: (a) NaOMe in xylene, reflux, 62%; $POCl_3$, diethylaniline, 95%; (b) urea−HOOH, CF_3COOH, rt, 18 h; 93%; (c) (i) trimethylphenol, a base (NaH, KH, or *t*-BuOK) in THF, rt, 97%, (ii) PCl_3 in methylene chloride, 93%; (d) $NH_2CH(Et)(CH_2R^1)$ in DMSO or NMP; (e) Method A (R = Cl): (i) 1 N DIBALH in THF, toluene, 67%, (ii) $SOCl_2$ in methylene chloride, 100%, (iii) 1 N BH_3 in THF, room temperature, 58%. Method B (R = Me and Br): 1 equiv of $AlCl_3$, 3–4 equiv of $LiAlH_4$ in THF, reflux, 2 h (100 and 90%, respectively).

oxidizing agent to urea−HOOH in CF_3COOH improved the yield dramatically (93%). On the other hand, we have found that oxidation of 3,6-dimethyl-2,4-dichloropyridine with *m*-CPBA provided a better yield[4] than urea−HOOH, indicating urea−HOOH may only be suitable for oxidation of an electron-deficient pyridine, where *m*-CPBA may be a better choice for an electron-rich pyridine. Coupling of **7** with an aryloxy compound followed by PCl_3 reduction provided the corresponding aryloxypyridine (**8a–c**) in excellent yield. Reaction of a 4-chloronicotinic acid methyl ester with an appropriate alkylamino compound yielded the corresponding 4-alkylamino-2-aryloxypyridine derivatives (**9a−c** and **5a**). Two reduction methods were used for conversion of the 3-COOMe-pyridine **9a−c** to the corresponding 3-Me derivatives **3a−c**. The first method involved a three-step synthesis to give the corresponding 3-methyl analogue **3b** from **9b** in moderate yield as shown in Scheme 1 (see footnote e, method A). Efforts to improve the process led us to identify a one-step method for conversion of a 3-methyl ester−pyridine to the corresponding 3-Me-pyridine (e.g., **3a** and **3c**) using $LiAlH_4/AlCl_3$ in THF in excellent yield (>90%). This method also worked well for conversion of 3-COOH to 3-Me as shown in Schemes 3 and 4 (conversion of **12** to **13** and **15** to **16**) or in the presence of a halogen group as seen in conversion of **9c** to **3c** (R^4 = Br) in Scheme 1.

Scheme 2 illustrates the method for the synthesis of compound **5b** in which the final 2-aminobutanol coupling with **10** was achieved by direct heating at 150 °C in good yield.

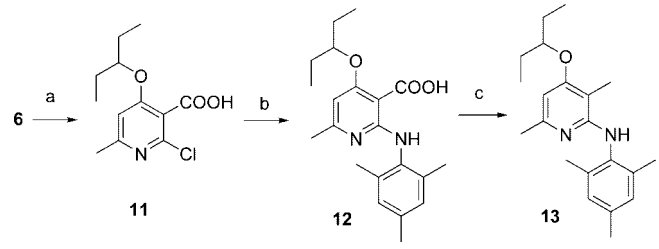
Scheme 3 describes the synthesis of compound **13**. Direct coupling of the 2,4-dichloropyridine **6** with an alkoxide provides a 2:1 mixture of regioisomers in favor of the desired 4-alkyl ether product **11**. Introduction of the trimethylanilino group to the 2-Cl-pyridine proved to be challenging. Coupling of the aniline via a pyridine *N*-oxide failed to provide the desired product due to self-dimerization/oxidation of the trimethylaniline. The best method we have identified after exploring various conditions for generating **12** afforded the desired product in about 30% yield by coupling **11** with trimethylaniline in the presence of Cu and K_2CO_3 in DMF. Final reduction with $AlCl_3$/$LiAlH_4$ gave the desired compound **13** for biological assay. Compound **16** was prepared in a similar way in which the

Scheme 2[a]



[a] Reagents and conditions: (a) (i) LiOH H_2O in *p*-dioxane/H_2O, (ii) $SOCl_2$ in methylene chloride, (iii) $MeNH_2$(g) in methylene chloride; (b) (*S*)-2-amino-1-butanol in *N*-methylpyrrolidone at 150 °C.

Scheme 3[a]



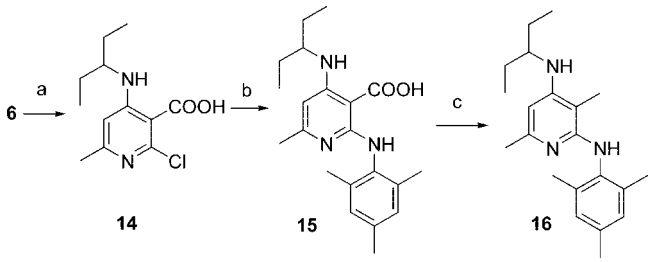
[a] Reagents and conditions: (a) (i) 3-pentanol, NaH in THF, 22% isolated yield, (ii) NaOH in MeOH/H_2O, reflux, 92%; (b) 1 equiv of trimethylaniline, 1 equiv of K_2CO_3, 0.2 equiv of Cu in DMF, 29.4%; (c) 3 equiv of $LiAlH_4$, 1 equiv of $AlCl_3$ in THF, reflux, 78%.

4-alkylamino group was introduced first, followed by trimethylaniline coupling and reduction as shown in Scheme 4.

Results and Discussion

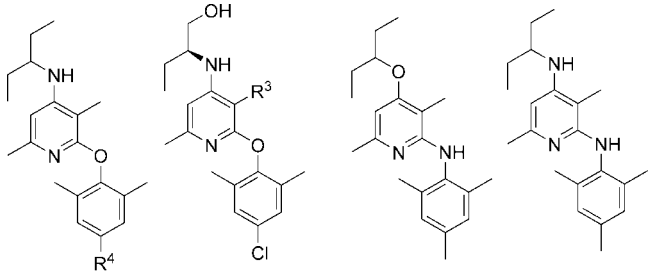
Initial SAR exploration by replacing the oxygen in the alkoxy or aryloxy substituents of **1** provided compounds in series **2** as shown in Table 1. Compound **1** has a low pK_a (~3.6) where the mesylate was found to be the only appropriate salt that can form a crystalline solid; however, it dissociates easily in aqueous solution. The mesylate salt of **1** is quite insoluble at pH 7 and

Scheme 4[a]



[a] Reagents and conditions: (a) (i) 1-ethylpropylamine in DMSO, 110 °C, 42%, (ii) LiOH in dioxane/H_2O, (iii) 1 N HCl, 75%; (b) 1 equiv of trimethylaniline, 1 equiv of K_2CO_3, 0.2 equiv of Cu in DMF, 32.5%; (c) 3 equiv of $LiAlH_4$, 1 equiv of $AlCl_3$ in THF, reflux, 100%.

Table 1. *In Vitro* Binding Affinity and *Ex Vivo* Data of Compounds in Series **2**



	in vitro[a]		*ex vivo*[b] at 3.2 mg/kg (oral)	
compd	pIC_{50} ± SEM (M)	IC_{50} (nM)	cortex (% inhibition ± SD)	pituitary (% inhibition ± SD)
1	8.17 ± 0.04	6.8	75 ± 7	72 ± 11
3a	8.29 ± 0.05	5.1	81 ± 17	49 ± 16
3b	8.14 ± 0.06	7.2	79 ± 23	72 ± 4
3c	8.25 ± 0.19	5.6	86 ± 4	81 ± 4
5a	8.19 ± 0.08	6.4	111 ± 17	86 ± 4
5b	7.51 ± 0.05	31	86 ± 2	63 ± 2
13	8.43 ± 0.02	3.7	NT	NT
16	6.13 ± 0.08	750	NT	NT

[a] Values reported are the geometric mean of at least three experimental runs using rat cortex.[2a] [b] Compounds were administered orally. ^{125}I-ovineCRF (^{125}I-oCRF) binding to brain and pituitary tissues in rats was measured using an *ex vivo* brain homogenate binding assay. NT: not tested.

2 with solubility of 0.2 and 3 μg/mL, respectively. Replacement of the 3-alkoxy group with a 3-alkylamino group resulted in compound **3a** with increased basicity (pK_a of 6.9) and solubility in simulated gastrointestinal fluid or at low pH (0.1 μg/mL at pH 7 and 5.4 mg/mL at pH 2.4), which also made a significant impact on reduction of food effects from 10- to 20-fold seen with **1** down to 2–3 fold in dogs and humans as expected. A very basic 2,4-diaminopyridine **16** (pK_a of 9.3) reduced CRF_1 binding affinity dramatically (IC_{50} value of 750 nM). During the course of our CRF program, a CRF_1 pharmacophore was developed based on the SAR from several chemotypes, in which the lone pair electron of the "N" in the core template (e.g., pyrido in series **2**) is essential for a compound to exhibit potent CRF_1 binding affinity. The basic nitrogen in **16** exists as a protonated form in the pyrido ring rather than amino side chain (X-ray structural analysis) at physiological pH; thus, the interaction of the "N" with the CRF receptor is diminished, which may account for the poor CRF_1 binding affinity. The polar 3-COOH intermediate **12** was found to be inactive as expected. Except for the very basic 2,4-diaminopyridine **16**, all compounds in Table 1 showed low nanomolar binding affinity. Replacing the

Table 2. Pharmacokinetics of CRF_1 Antagonists **1** and **3a** in Beagle Dogs

pharmacokinetic parameter	**1**	**3a**
intravenous dose (mg/kg)	1.0	1.0
CL (mL/min/kg)[a]	4.1 ± 0.4	17 ± 5
VD_{ss} (L/kg)[a]	1.1 ± 0.3	5.3 ± 2.2
VD_γ (L/kg)[a]	3.1 ± 0.8	12 ± 7
$t_{1/2\gamma}$ (hr)[b]	8.3	8.8
oral dose (mg/kg) fasted state	5.0	1.0
C_{max} (ng/mL)[a]	187 ± 170	46.2 ± 25.3
T_{max} (h)[a]	1.5 ± 0.6	1.8 ± 1.3
F (%)[a]	3.6 ± 2.6	22 ± 19
estimated F_a (%)[b]	4.4	73
oral dose (mg/kg) fed state	5.0	1.0
C_{max} (ng/mL)[a]	1740 ± 290	131 ± 62
T_{max} (h)[a]	1.9 ± 1.0	2.0 ± 1.4
F (%)[a]	37 ± 19	64 ± 10
estimated F_a (%)[b]	46	100

[a] Values reported are mean + standard deviation. [b] Values reported are harmonic mean.

p-methyl group of the trimethylphenoxy of **3a** to help reduce metabolic clearance with a chloro resulted in compound **3b** with no improvement of clearance; the *in vitro* $t_{1/2}$ values in human liver microsomes were 38 and 30 min for **3a** and **3b**, respectively. Introduction of a polar group such as OH at the C_4-side chain and COOMe of R^3 of **3b** resulted in compound **5a** with increased polarity and decreased protein binding (increased unbound fraction from 0.22% to 1.3%). Compound **5a** seemed to have good stability when incubated with human liver microsomes (intrinsic clearance = 0.047 mL/min/mg protein); however, the possibility existed that the 3-methyl ester could be hydrolyzed by esterases in liver and other tissues. Replacing the 3-COOMe with a more polar 3-CONHMe led to compound **5b** with slightly weaker *in vitro* potency but reduced protein binding (4.5% free), which may account for good *ex vivo* activity. All tested compounds in Table 1 demonstrated good brain penetrability and oral activity measured by *ex vivo* binding in animals treated at 3.2 mg/kg. In general, compounds that exhibit good brain penetration as measured by *ex vivo* binding also demonstrate efficacy in animal behavior models as seen with these compounds.

Most compounds demonstrated similar *in vivo* potency compared to **1**,[4] with the exception of compound **3a**. Compound **3a** was unexpectedly more efficacious than others in the fear-potentiated startle and locus coeruleus excitation models induced by icv CRF, despite having a similar intrinsic affinity for the CRF_1 receptor. Several potential candidates (**3a**,**b**, **5a**,**b**) met the desired pharmacological and pharmacokinetic profile and were selected for exploratory toxicology evaluation. Among these, compound **3a** passed exploratory toxicology studies and was selected for further development.

Pharmacology Results of 3a. Compound **3a** fully antagonizes oCRF-stimulated adenylate cyclase activity in rat cerebral cortex and at human CRF_1 receptors with an apparent K_i value of 12 nM, indicating antagonist functional activity. It is highly selective for the human CRF_1 receptor subtype; affinity for the CRF_2 receptor is >10000 nM. It showed affinities greater than 1 μM against 40 neurotransmitter receptor and ion channels (Nova Screen). Compound **3a** significantly attenuated activation of the HPA axis (MED = 10 mg/kg, p.o., $p < 0.05$ versus CRF alone, One-Way ANOVA, Dunnett's post hoc test, $N = 10$), as measured by increased plasma adrenocorticotropin hormone (ACTH) levels, in response to i.v. CRF (4 μg/kg) administration. In the CNS, systemically administered **3a** blocks the effects of both exogenous and endogenous CRF. Pretreatment with **3a** reversed the excitation of locus coeruleus neurons induced by

icv CRF (3 μg) with an ID_{50} of <0.01 mg/kg, i.v. Compound **3a** completely blocked the enhanced startle response induced by icv CRF (1 μg) at 17.8 mg/kg, p.o. and partially blocked at 10 mg/kg, p.o. without significantly altering baseline startle. The attenuation of fear-potentiated startle was statistically significant at lower doses (0.32–3.2 mg/kg, p.o., with 62–83% blockade) and completely reversed by **3a** at 10 mg/kg, p.o. In conclusion, **3a** has been shown to behave as a CRF_1 receptor antagonist in several different *in vivo* paradigms.

Pharmacokinetics of CRF_1 Receptor Antagonists (1 and 3a) in Dogs. Both compounds **1** and **3a** were studied for their pharmacokinetic behavior in beagle dogs after intravenous and oral administration. Following i.v. administration, the clearance of **1** was low relative to hepatic blood flow (4.1 vs 35 mL/min/kg), while the clearance of **3a** was moderate (17 vs 35 mL/min/kg). The kinetics were multiphasic for both compounds and demonstrated volumes of distribution during the terminal elimination phase that were considerably greater than the steady-state volumes of distribution. Such an observation can be explained by partitioning into a deep pharmacokinetic compartment (e.g., adipose tissue) from which the compound slowly leeches back into the circulation, and is a common phenomenon for lipophilic compounds. (In fact, in a tissue distribution study conducted in rats, sequestration of **1** into adipose tissue was observed; data not shown.)

Following oral administration, the bioavailability of **1** was lower than **3a**, despite the lower systemic clearance. This is consistent with **3a** being better absorbed than **1**. Back calculations of estimates of the fraction absorbed suggest that **3a** is 73% absorbed while **1** is only 4% absorbed in animals that have been fasted. When these compounds were administered to animals 1 h following feeding, the oral bioavailability of **1** was markedly increased (10-fold), while the oral bioavailability increased 3-fold for **3a**. Positive food effects can arise by several different mechanisms, including possible inhibition of intestinal efflux transporters by food constituents or enhanced dissolution of the compound by food. In the case of **1** and **3a**, the latter explanation is more plausible because administration of these compounds in triglyceride formulations also resulted in increased oral bioavailability in the fasted state (data not shown). These results clearly show that in progressing from compound **1** to compound **3a**, increasing the basicity in this series improved the oral pharmacokinetics and reduced the magnitude of the positive food effect.

Conclusions

Compounds in series **2** were designed to address physicochemical properties. All compounds exhibited potent CRF_1 binding affinity except **16** because of its high basicity. These low nanomolar compounds in series **2** also demonstrated good brain *ex vivo* displacement activity after oral administration, indicating good oral activity and brain penetrability. Several potential candidates **3a**,**b** and **5a**,**b** met the desired pharmacological and pharmacokinetic profile. Compound **3a** is clearly more potent than **1** in relevant *in vivo* models, despite having a similar intrinsic affinity for the CRF_1receptor subtype. In dogs, a moderate food effect was observed; oral bioavailability was 22% in fasted animals and 64% in fed animals. As a result, **3a** was selected to be a back up candidate and advanced to the clinic, where it may be useful in evaluating the utility of CRF_1 receptor antagonists in stress-related disorders in the clinic.

Experimental Section

The syntheses of compounds in series **2** are described below. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. High-field ^{1}H NMR spectra were recorded on a Varian XL-300, XL-400, Bruker AM 250, or Bruker AM 300 instrument. Elemental analyses were carried out by Schwarzkopf Microanalytical, Woodside, NY.

2,4-Dichloro-6-methyl-1-oxynicotinic Acid Methyl Ester (7). Urea hydrogen peroxide addition compound (98% pure) (3.860 g, 40.9 mmol) was added to a stirring solution of 2,4-dichloro-6-methylnicotinic acid methyl ester (3.000 g, 13.6 mmol) in trifluoroacetic acid (15 mL) under nitrogen. The reaction mixture was stirred at room temperature for 18 h. Additional urea−hydrogen peroxide (0.640 g, 6.8 mmol) was added and the reaction stirred for an additional 3 h. The reaction mixture was poured over ice–water and stirred rapidly. The mixture was treated with sodium thiosulfate, neutralized with 2 N aqueous NaOH to pH 11, and extracted three times with chloroform. The combined chloroform extracts were washed with water andbrine, dried over sodium sulfate, and concentrated to give 2.98 g (93%) of the desired product as a white crystal: mp 90–91.5 °C; ^{1}H NMR ($CDCl_3$) δ 7.26 (s, 1H), 3.98 (s, 3H), 2.54 (s, 3H). Anal. ($C_8H_7NO_2Cl_2$) C, H, N.

4-Chloro-6-methyl-2-(2,4,6-trimethylphenoxy)-1-nicotinic Acid Methyl Ester (8a). A mixture of 2,4-dichloro-6-methyl-1-oxynicotinic acid methyl ester (2.240 g, 9.49 mmol) and trimethylphenol (1.290 g, 9.49 mmol) in dry THF was cooled in an ice bath, and 60% NaH in oil (380 mg, 9.49 mmol) was added portionwise during a period of 5 min. The reaction mixture was stirred at room temperature for 6 h. The mixture was quenched with water and saturated ammonium chloride and extracted twice with chloroform. The organic layer was dried and concentrated to give 3.120 g (97%) of the title compound, which was used directly in the next step: ^{1}H NMR ($CDCl_3$) δ 7.04 (s, 1H), 6.78 (s, 2H), 3.48 (s, 3H), 2.52 (s, 3H), 2.22 (s, 3H), 2.08 (s, 6H).

To a solution of crude 4-chloro-6-methyl-2-(2,4,6-trimethylphenoxy)-1-oxynicotinic acid methyl ester (3.100 g) in 30 mL of dry methylene chloride was added 2 M PCl_3 in methylene chloride (5.3 mL, 10.6 mmol) at room temperature. The resulting mixture was heated at reflux for 1 h. The mixture was concentrated to dryness, and the residue was poured over ice–water and extracted with $CHCl_3$. The organic layer was dried and concentrated to give 2.820 g (93%) of the crude title compound. The crude material was purified through silica gel column chromatography using $CHCl_3$/hexane (1:1) as eluent to give 1.452 g of yellow crystals: mp 127–128.5 °C; ^{1}H NMR ($CDCl_3$) δ 6.84 (s, 2H), 6.82 (s, 1H), 3.94 (s, 3H), 2.27 (s, 3H), 2.25 (s, 3H), 2.04 (s, 6H). Anal. $C_{17}H_{18}ClNO_3$ (C,H,N).

4-Chloro-6-methyl-2-(2,6-dimethyl-4-chlorophenoxy)nicotinic Acid Methyl Ester (8b). The title compound was prepared by the method analogous to that described for compound **8a** as a white crystal: mp 125–128 °C; ^{1}H NMR ($CDCl_3$) δ 7.03 (s, 2H), 6.86 (s, 1H), 3.96 (s, 3H), 2.25 (s, 3H), 2.05 (s, 6H).

4-Chloro-6-methyl-2-(2,6-dimethyl-4-bromophenoxy)nicotinic Acid Methyl Ester (8c). The title compound was prepared by the method analogous to that described for compound **8a** to give white crystals: mp 108–110 °C. Anal. ($C_{16}H_{15}BrClNO_3$) C, H, N.

4-(1-Ethylpropylamino)-6-methyl-2-(2,4,6-trimethylphenoxy) nicotinic Acid Methyl Ester (9a). A mixture of 4-chloro-6-methyl-2-(2,4,6-trimethylphenoxy)nicotinic acid methyl ester (3.434 g, 10.74 mmol) and 1-ethylpropylamine (10 mL) in 10 mL of DMSO was heated at 120 °C for 15 h. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried and concentrated to give a yellow solid. The yellow solid was recrystallized with hexane to give 2.519 g (63%) of the title compound as white crystals: mp 106–107.5 °C; ^{1}H NMR ($CDCl_3$) δ 8.07 (d, 1H), 6.87 (s, 2H), 6.08 (s, 1H), 3.87 (s, 3H), 3.34 (m, 1H), 2.30 (s, 3H), 2.12 (s, 3H), 2.09 (s, 6H), 1.64 (m, 4H), 0.97 (t, 6H). Anal. ($C_{22}H_{30}N_2O_3$) C, H, N.

4-(1-Ethylpropylamino)-6-methyl-2-(2,6-dimethyl-4-chlorophenoxy)nicotinic Acid Methyl Ester (9b). A mixture of 4-chloro-6-methyl-2-(4-chloro-2,6-dimethylphenoxy)nicotinic acid methyl ester (77 mg, 0.226 mmol) and 1-ethylpropylamine in DMSO was heated at 120 °C for 4 h. The mixture was quenched with saturated ammonium chloride, water, and brine and extracted with ethyl

acetate. The organic layer was dried and concentrated to give 140 mg of yellow solid: ^{1}H NMR ($CDCl_3$) δ 8.10 (d, 1H), 7.03 (s, 2H), 6.09 (s, 1H), 3.88 (s, 3H), 3.35 (m, 1H), 2.10 (s, 3H), 2.08 (s, 6H), 1.5–1.7 (m, 4H), 0.96 (t, 6H).

4-(1-Ethylpropylamino)-6-methyl-2-(2,6-trimethyl-4-bromophenoxy)nicotinic Acid Methyl Ester (9c). A mixture of 4-chloro-6-methyl-2-(4-bromo-2,6-dimethylphenoxy)nicotinic acid methyl ester and 1-ethylpropylamine in DMSO was heated at 120 °C for 16 h. The mixture was quenched with water and brine and extracted with ethyl acetate. The organic layer was dried and concentrated to dryness. The residue was purified by silica gel column chromatography using hexane to 3% ethyl acetate in hexane as eluent to give the title compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 8.1 (d, 1H), 7.18 (s, 2H), 6.08 (s, 1H), 3.87 (s, 3H), 3.35 (m, 1H), 2.10 (s, 3H), 2.08 (s, 6H), 1.4–1.7 (m, 4H), 0.96 (t, 6H).

[3,6-Dimethyl-2-(2,4,6-trimethylphenoxy)pyridin-4-yl](1-ethylpropyl)amine (3a). To a mixture of 4-(1-ethylpropylamino)-6-methyl-2-(2,4,6-trimethylphenoxy)nicotinic acid methyl ester (29 mg, 0.078 mmol) and $AlCl_3$ (10 mg, 0.078 mmol) in dry THF was added 1 M $LiAlH_4$ in diethyl ether (0.31 mL, 0.31 mmol) at room temperature. After being sitrred for 10 min, the mixture was heated at reflux for 2 h. The mixture was quenched with 0.2 mL of water, 0.2 mL of 1 N NaOH, 0.4 mL of water, and 10 mL of dry THF and stirred for 15 min. The mixture was filtered through Celite and washed with chloroform. The filtrate was dried over dry sodium sulfate, filtered and concentrated to dryness to give 26 mg (93%) of white crystalline solid: mp 64–68 °C; ^{1}H NMR (free base, $CDCl_3$, 300 MHz) δ 6.82 (s, 2H), 6.05 (s, 1H), 3.70 (d, 1H, NH), 3.32 (m, 1H), 2.29 (s, 3H), 2.15 (s, 3H), 2.11 (s, 3H), 2.08 (s, 6H), 1.45–1.70 (m, 4H), 0.93 (t, 6H). The corresponding HCl salt was prepared and recrystallized from diethyl ether to give a white solid: mp 212–215 °C; ^{1}H NMR (hydrochloride salt, $CDCl_3$, 300 MHz) δ 6.89 (s, 2H), 6.20 (s, 1H), 5.18 (d, 1H,NH), 3.46 (m, 1H), 2.66 (s, 3H), 2.27 (s, 3H), 2.11 (s, 6H), 2.04 (s, 3H), 1.68 (m, 4H), 0.95 (t, 6H). Anal. ($C_{21}H_{30}N_2O\cdot HCl$) C, H, N.

3,6-Dimethyl-2-(2,6-dimethyl-4-bromophenoxy)pyridin-4-yl](1-ethylpropyl)amine (3c). The title compound was prepared by the method analogous to that for **3a** to give a white solid: ^{1}H NMR ($CDCl_3$) δ 7.19 (s, 2H), 6.09 (s, 1H), 3.36 (d, 1H), 3.33 (m, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 2.09 (s, 6H), 1.4–1.8 (m, 4H), 0.97 (t, 6H).

[3,6-Dimethyl-2-(2,6-dimethyl-4-chlorophenoxy)pyridin-4-yl](1-ethylpropyl)amine (3b). To a 0 °C solution of 4-(1-ethylpropylamino)-6-methyl-2-(2,6-dimethyl-4-chlorophenoxy)nicotinic acid methyl ester (145 mg, 0.37 mmol) in toluene (1 mL) was added 1 M diisobutylaluminum hydride in THF (1.2 mL, 1.2 mmol). The mixture was then stirred at room temperature. The mixture was quenched with methanol, stirred for 1 h, filtered through Celite, and washed with methanol. The filtrate was concentrated to dryness to give 140 mg of crude product that was purified by silca gel column chromatography using 1:1 hexane/chloroform to give the desired 3-hydroxymethyl product as a white solid (90 mg, 67%).

To a solution of the 3-hydroxymethyl solid (85 mg, 0.23 mmol) in methylene chloride (0.3 mL) was added $SOCl_2$ (0.3 mL). The mixture was stirred at room temperature for 1 h and then concentrated to dryness to give a light golden oil that was pumped in vacuo to give crude 3-chloromethyl product. To a solution of 3-chloromethyl derivative (75 mg, 0.196 mmol) in dry THF was added 1.0 M BH_3 in THF (0.59 mL, 0.59 mmol) and the mixture stirred for 2 h. The mixture was quenched with dilute HCl and stirred for 5 min. The reaction mixture was neutralized with 2 N NaOH and water and extracted with ethyl acetate. The organic layer was separated, dried, and concentrated to dryness. The residue was purified by silica gel column chromatography to give the title compound as a colorless oil (36 mg): ^{1}H NMR($CDCl_3$) δ 7.03 (s, 2H), 6.08 (s, 1H), 3.73 (d, 1H), 3.3 (m, 1H), 2.15 (s, 3H), 2.12 (s, 3H), 2.08 (s, 6H), 1.4–1.6 (m, 4H), 0.96 (t, 6H). The HCl salt was prepared as a light yellow crystal, mp 194–197 °C. Anal. ($C_{20}H_{27}ClN_2O\cdot HCl$) C, H, N.

2-(4-Chloro-2,6-dimethylphenoxy)-4-(1-hydroxymethylpropylamino)-6-methylnicotinic acid methyl Ester (5a). A mixture of 4-chloro-2-(4-chloro-2,6-dimethylphenoxy)-6-methylnicotinic acid methyl ester (9.0 g, 26.45 mmol) and (*S*)-2-amino-1-butanol (12.7 mL) in 1-methyl-2-pyrrolidinone was heated at 130 °C for 2 h and then at 100 °C overnight. The mixture cooled to room temperature, poured into ice–water, and diluted with ethyl acetate. The organic layer was separated, washed with water, dried over anhydrous sodium sulfate, filtered, and concentrated to dryness to give 13.6 g of crude product as a light yellow oil. The oil was purified by silica gel column chromatography using chloroform to 2% MeOH in chloroform as eluent to give 6.684 g (64%) of the title compound as white glass foam. The glass foam was triturated with hexane to give a white solid. The solid was recrystallized from diisopropyl ether to give white crystals, mp 122.5–124 °C. Anal. ($C_{20}H_{25}ClN_2O_4$) C, H, N.

4-Chloro-2-(2,4,6-trimethylphenoxy)-6,*N*-dimethylnicotinamide (10). To a solution of 4-chloro-6-methyl-2-(2,6-dimethyl-4-chlorophenoxy)nicotinic acid methyl ester (10.00 g, 29.4 mmol) in 60 mL of *p*-dioxane was added a solution of $LiOH\cdot H_2O$ (2.50 g, 103.4 mmol) in 60 mL of water. The resulting mixture was heated at reflux for 2 h and then stirred at room temperature overnight. The mixture was cooled in an ice bath and quenched with 11 mL of concentrated HCl. The resulting mixture was extracted twice with methylene chloride. The organic layer was separated, dried over Na_2SO_4, and filtered. The filtrate was concentrated to give a crude 4-chloro-6-methyl-2-(2,4,6-trimethylphenoxy)nicotinic acid as a yellow oil (9.87 g) that was used as is.

To a solution of 4-chloro-2-(4-chloro-2,6-dimethylphenoxy)-6-methylnicotinic acid (3.002 g, 9.2 mmol) in 10 mL of methylene chloride was added thionyl chloride (4 mL, 54.8 mmol) at room temperature, and the mixture was stirred for 1 h. The resulting mixture was concentrated to dryness to give 3.112 g of 4-chloro-2-(4-chloro-2,6-dimethy-phenoxy)-6-methylnicotinic acyl chloride as a light yellow solid. An excess of methylamine was bubbled into a solution of the crude acyl chloride (0.841 g, 5 mmol) in 6 mL of methylene chloride until a precipitate formed. The mixture was stirred at room temperature for 1 h, quenched with water, and extracted with chloroform. The organic layer was separated, dried over Na_2SO_4, and filtered. The filtrate was concentrated to dryness to give 0.721 g of the desired product as an off-white solid. The solid was recrystallized from EtOAc/Et_2O to give off-white crystals: mp 243.1 °C; ^{1}H NMR ($CDCl_3$) δ 7.04 (s, 2H), 6.87 (s, 1H), 5.87 (brs,1H), 3.06 (d, 3H), 2.25 (s, 3H), 2.07 (s, 6H).

2-(4-Chloro-2,6-dimethylphenoxy)-4(*S*)-(1-hydroxymethylpropylamino)-6,*N*-dimethylnicotinamide (5b). A mixture of 4-chloro-2-(2,4,6-trimethylphenoxy)-6,*N*-dimethylnicotinamide (22.36 g, 65.9 mmol) and (*S*)-2-amino-1-butanol (17.8 mL, 189 mmol) in *N*-methylpyrrolidinone (50 mL) was heated at 150 °C overnight. The mixture was quenched with water and extracted twice with ethyl acetate. The organic layer was washed with brine and separated. The organic layer was dried over Na_2SO_4 and filtered. The filtrate was purified by silica gel column chromatography using 1% methanol in methylene chloride as eluent to give 25.87 g (~100%) of the desired product as a light yellow foam form that was recrystallized from Et_2O/hexane to give white crystals: mp 110.2 °C; ^{1}H NMR($CDCl_3$) δ 9.80 (d, 1H), 8.12 (s, 1H), 7.04 (s, 2H), 6.22 (s, 1H), 3.5–3.8 (m, 3H), 2.93 (d, 3H), 2.06 (s, 9H), 1.8(brs,1H), 1.5–1.7 (m, 2H), 0.99 (t, 3H) ppm. Anal. ($C_{20}H_{26}ClN_3O_3$) C, H, N.

2-Chloro-4-(1-ethylpropoxy)-6-methylnicotinic Acid (11). To a solution of 3-pentanol (11.7 mL, 108.25 mmol) in 200 mL of THF was added 60% NaH in oil (2.60 g, 108.25 mmol) at 0 °C, and stirring was continued for 30 min. 2,4-Dichloro-6-methylnicotinic methyl ester (22.75 g, 103.10 mmol) was added at 0 °C, and the mixture was stirred at room temperature overnight. The mixture was quenched with water (400 mL) and extracted three times with ethyl acetate (400 mL). The organic layer was separated, dried, and concentrated to give 30.59 g of yellow oil as a mixture of isomers with at least four components. The desired product was isolated from silica gel column chromatography using 8% ethyl

acetate in hexane as eluent to give 7.72 g (27.5%) as a clear oil. Anal. ($C_{13}H_{18}ClNO_3$) C, H, N.

A mixture of 2-chloro-4-(1-ethylpropoxy)-6-methylnicotinic acid methyl ester (7.70 g, 28.34 mmol) and NaOH (11.33 g, 283.36 mmol) in 130 mL of MeOH and 75 mL of water was heated at reflux for 3 h. The mixture was concentrated to dryness and diluted with water, adjusted to pH 3 with 1 N HCl, and then extracted with EtOAc. The organic layer was separated, dried, and concentrated to give the desired product which was triturated with EtOAc to give 6.3 g (86%) of white crystals, mp 170–172 °C. Anal. ($C_{12}H_{16}ClNO_3$) C, H, N.

4-(1-Ethylpropoxy)-6-methyl-2-(2,4,6-trimethylphenylamino)nicotinic Acid (12). A mixture of 2-chloro-4-(1-ethylpropoxy)-6-methylnicotinic acid (372 mg, 1.44 mmol), trimethylaniline (195 mg, 1.44 mmol), potassium carbonate (199 mg, 1.44 mmol), and copper (18 mg, 0.29 mmol) in 6 mL of DMF was heated at 110 °C for 24 h. The mixture was quenched with water (200 mL) and extracted with ethyl acetate (3 × 150 mL). The organic layer was separated, dried, and concentrated to give a brown oil (750 mg). The oil was purified by silica gel column chromatography using 3.5% MeOH in chloroform as solvent to give the less polar spot first (210 mg) as an undesired material, and the spot collected next was found to be the desired product (151 mg, 29.4%), mp 195–200 °C. Anal. ($C_{21}H_{28}N_2O_3$) C, H. N.

[4-(1-Ethylpropoxy)-3,6-dimethylpyridin-2-yl](2,4,6-trimethylphenyl)amine (13). To a solution of 4-(1-ethylpropoxy)-6-methyl-2-(2,4,6-trimethylphenylamino)nicotinic acid (240 mg, 0.673 mmol) in dry THF (8 mL) were added aluminum chloride (90 mg, 0.673 mmol) and 1.0 M of lithium aluminum hydride in THF (2.7 mL, 2.7 mmol). The resulting mixture was heated at reflux for 3 h. The mixture was quenched with 0.1 mL of water and 0.1 mL of 2 N NaOH and then quenched with water and ethyl acetate. The organic layer was separated, dried, and concentrated to give 250 mg of brown oil. After silica gel column chromatography, 170 mg (78%) of the title compound was obtained which was prepared as an HCl salt to give a white solid: mp 132–133 °C; ^{1}H NMR ($CDCl_3$) δ 6.87 (s, 2H), 6.09 (s, 1H), 5.39 (brs, 1H), 4.13 (m, 1H), 2.27 (s, 3H), 2.22 (s, 3H), 2.15 (s, 6H), 1.98 (s, 3H), 1.67 (m, 4H), 0.94 (t, 6H).

2-Chloro-4-(1-ethylpropylamino)-6-methylnicotinic Acid (14). A mixture of 2,4-dichloro-6-methylnicotinic acid methyl ester (2.228 g, 10.13 mmol) and 1-ethylpropylamine (1.762 g, 20.26 mmol) in DMSO (4 mL) was heated at 110 °C for 5 h and then at room temperature overnight. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried and concentrated to give 1.796 g of crude material. The crude material was purified by silica gel column chromatography using 1:1 hexane/chloroform, then chloroform to 5% methanol in chloroform gradient as eluent to give 0.309 g of the undesired regioisomer, then 1.167 g (43%) of the desired product as a colorless oil, and then 0.259 g of the bis-1-ethylpropylamino adduct. The desired product: ^{1}H NMR ($CDCl_3$) δ 7.14 (brs, 1H), 6.27 (s, 1H), 3.86 (s, 3H), 3.27 (m, 1H), 2.33 (s, 3H), 1.3–1.6 (m, 4H), 0.88 (t, 6H).

A mixture of 2-chloro-4-(1-ethylpropylamino)-6-methylnicotinic acid methyl ester (1.45 g, 5.36 mmol) and $LiOH \cdot H_2O$ (0.45 g, 10.72 mmol) in 28 mL of a 1:1 mixture of *p*-dioxane/water was stirred at room temperature overnight. The mixture was quenched with 10.7 mL of 1 N HCl and concentrated to dryness. The residue was diluted with chloroform, filtered, and washed with chloroform. The filtrate was dried over anhydrous Na_2SO_4, concentrated, and pumped in vacuo to give a white foam (1.03 g, 75%) of 2-chloro-4-(1-ethylpropylamino)-6-methylnicotinic acid that was used as is.

4-(1-Ethylpropylamino)-6-methyl-2-(2,4,6-trimethylphenylamino)nicotinic Acid (15). A mixture of 2-chloro-4-(1-ethylpropylamino)-6-methylnicotinic acid (4.00 g, 15.58 mmol), trimethylaniline (2.11 g, 15.58 mmol), potassium carbonate (2.15 g, 15.58 mmol), and copper (0.20 g, 3.12 mmol) in DMF was heated at reflux. The mixture was quenched with ammonium chloride, stirred for 20 min, filtered through Celite, and washed with ethyl acetate. The filtrate was extracted with ethyl acetate. The organic layer was separated, dried, and concentrated to dryness. The residue was purified by silica gel column chromatography using 4% methanol in chloroform as eluent to give the desired product (1.8 g, 32.5%) as a solid: mp 130–135 °C; ^{1}H NMR ($CDCl_3$) δ 13.54 (brd s, 1H), 11.90 (brd s, 1H), 8.20 (s, 2H), 5.88 (s, 1H), 3.34 (m, 1H), 2.24 (s, 3H), 2.19 (s, 6H), 2.16 (s, 3H), 1.54–1.67 (m, 4H), 0.96 (t, 6H).

4-(1-Ethylpropylamino)-3,6-dimethyl-2-(2,4,6-trimethylphenylamino)pyridine (16). To a solution of 4-(1-ethylpropylamino)-6-methyl-2-(2,4,6-trimethylphenylamino)nicotinic acid (100 mg, 0.28 mmol) in 4 mL of dry THF were added 1 M lithium aluminum hydride in diethyl ether (1.12 mL, 1.12 mmol) and aluminum trichloride (38 mg, 0.28 mmol) at room temperature. The resulting mixture was heated at reflux and then cooled to room temperature. The mixture was quenched with 0.8 mL of water, 0.8 mL of 1 N NaOH, and then 1.6 mL of water. The mixture was stirred for 5 min and then filtered through Celite and washed with ethyl acetate. The filtrate was diluted with water, and the organic layer was separated, dried over $MgSO_4$, and concentrated to give the desired product as a golden oil (100 mg, >100%) which was purified by silica gel column chromatography using 0.1 mL of NH_4OH in 500 mL of 20% ethyl acetate in hexane as eluent to give the desired product (98 mg, 100%) as a tan solid: mp 77–79 °C; ^{1}H NMR ($CDCl_3$) δ 6.88 (s, 2H), 5.94 (s, 1H), 5.4 (brd s, 1H), 3.60 (d, 1H), 3.31 (m, 1H), 2.28 (s, 3H), 2.22 (s, 3H), 2.15 (s, 6H) 1.4–1.7 (m, 4H), 0.94 (t, 6H) ppm. Anal. ($C_{21}H_{31}N_3 \cdot 0.5H_2O$) C, H, N.

Biological Evaluation. Experimental details for the CRF receptor binding assay, *in vitro* adenylate cyclase functional assay, effect on elevations in plasma ACTH levels, CRF-induced increases in locus coeruleus firing in the rat, acoustic startle response induced by icv CRF, and fear-potentiated startle induced by electric foot shock have been reported previously.[2a,b]

***Ex Vivo* Receptor Binding Assay with ^{125}I-oCRF.** ^{125}I-oCRF binding to brain and pituitary tissues in rats was measured using brain homogenate assay. Male Sprague–Dawley rats (200–300 g) were treated with a compound or vehicle at the doses indicated. After a specified treatment period (usually 1 h), they were sacrificed via decapitation and the brains and pituitary glands rapidly removed and frozen immediately on dry ice. Tissues were suspended at 25 mg wet weight/mL in buffer (20 mM PIPES [pH 7.0], 10 mM $MgCl_2$, 2 mM EGTA, 0.015% bacitracin, 100 U/mL aprotinin). Aliquots of tissue homogenate (100 μL) were added to assay samples containing 40 pM ^{125}I-oCRF, yielding a final volume of 200 μL. Nonspecific binding was determined using 1 μM rat/human CRF. After a 2 h incubation at room temperature, assay samples were centrifuged for 10 min at 1000*g*. The supernatant was discarded. Samples were rinsed with 100 μL of ice-cold assay buffer and recentrifuged. Pellets were filtered onto betaplate filtermats (A) using a Skatron cell harvester (setting 222). Radioactivity was quantified using a betaplate scintillation counter (Wallac).

Pharmacokinetic Studies in Dogs. All animal studies were conducted using protocols approved by a local Animal Care and Usage Procedure Committee in facilities accredited by IACUC. Four beagle dogs (two male and two female) weighing between 8 and 15 kg were administered **1** mesylate salt or **3a** hydrochloride salt (1.0 mg/kg) into the cephalic vein of the foreleg. The dosing solution for **1** was neat ethanol (15.4 mg/mL) and for **3a** was sterile saline at pH 2 (10 mg/mL). Oral dosing was conducted by gavage with drugs suspended in 0.1% methyl cellulose at pH 2. For the intravenous and fasted oral legs of the study, animals had last eaten approximately 21 h prior to drug administration and were permitted food and water approximately 3.5 h postdose. In the fed oral leg of the study, the animals were given one can of wet dog food 1 h before dosing. Blood samples were collected by venipuncture of the jugular vein prior to drug administration and at time points of 0.083 (i.v. only), 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12, and 24 h postdose, processed to obtain serum and stored frozen until the day of analysis.

Serum samples (0.9 mL) were delivered to silanized test tubes and fortified with internal standard. Samples were alkalinized with NaOH (0.4 mL; 1 M) and extracted with methyl *tert*-butyl ether (4 mL), the aqueous layer was frozen in a dry ice/acetone bath, and the organic layer was decanted into a silanized tube and evaporated

under nitrogen at 35 °C. Sample residues were reconstituted in 20 μL of acetonitrile and analyzed by GC−MS.

The GC−MS consisted of a Hewlett-Packard 5890 model equipped with an HP-5 (5% phenyl methylpolysiloxane) column (length = 12 m; i.d. = 0.25 mm; film thickness = 0.25 μm). Carrier gas (He) was at a flow rate of 1.5 mL/min. The initial oven temperature was at 150 °C for 2 min, followed by an increase of 40 °C/min to 220 °C for 0.20 min, and followed by a second increase of 25 °C/min to 290 °C for 1.5 min. Injection port and transfer line temperatures were 250 and 280 °C, respectively. The MS was operated in SIM mode for the ion current of the base peaks for analytes and internal standards. The sample volume injected was 3 μL. The lower limit of quantitation for the assay was 1.0 ng/mL.

AUC(0→t_{last}) and AUMC(0→t_{last}) were calculated using the linear trapezoid approximation. The elimination rate constant (k_{el}) was determined by the slope of linear regression of elimination phase observed on the plot of the log of the serum concentration vs time. AUC(0→∞) was comprised of the sum of AUC(0→t_{last}) and AUC(t_{last}→∞). The latter value was calculated by dividing C_{tlast} estimated from the aforementioned regression by k_{el}. With i.v. administration, $C_{t=0}$ was estimated by extrapolation of the log-linear regression of the data to $t = 0$ and used in the determination of AUC(t_{last}→∞). Elimination half-life ($t_{1/2}$) was calculated as 0.693/k_{el}. Values of clearance (CL), volume of distribution at steady state (VD_{ss}), volume of distribution during the terminal elimination phase (VD_{γ}), and oral bioavailability were calculated by

$$\mathrm{CL} = \frac{\mathrm{dose}}{\mathrm{AUC}(0 \rightarrow \infty)} \quad (1)$$

$$\mathrm{VD_{ss}} = \frac{\mathrm{dose} \cdot \mathrm{AUMC}(0 \rightarrow \infty)}{\mathrm{AUC}(0 \rightarrow \infty)} \quad (2)$$

$$\mathrm{VD}_{\gamma} = \frac{\mathrm{CL}}{k_{\mathrm{el}}} \quad (3)$$

$$F = \frac{\mathrm{dose}_{\mathrm{i.v.}} \cdot \mathrm{AUC}(0 \rightarrow \infty)_{\mathrm{p.o.}}}{\mathrm{dose}_{\mathrm{p.o.}} \cdot \mathrm{AUC}(0 \rightarrow \infty)_{\mathrm{i.v.}}} \quad (4)$$

Estimates of the fraction absorbed were done by calculating the fraction extracted by the liver during first pass. A value of 35 mL/min/kg was used for hepatic blood flow (Q_h) in the dog. Serum clearance values were corrected to blood values using blood/plasma (B/P) values of 0.61 and 0.69 for **1** and **3a**, respectively.

$$F_a = 100\left(\frac{F}{\left(1 - \frac{\mathrm{CL}}{\left(\frac{\mathrm{B}}{\mathrm{P}}\right) \cdot Q_h}\right)}\right) \quad (5)$$

Protein Binding. Protein binding was determined using the technique of equilibrium dialysis. Test compounds were added to fresh serum and dialyzed against 67 mM KH_2PO_4, pH 7.4 with 0.9% (w/v) NaCl in a 20-cell Spectrum equilibrium dialysis apparatus using Teflon cells and #4 membranes with a molecular weight cutoff of 12–14 kDa. The samples were rotated at 20 rpm for 5 h at 37 °C. Individual experiments were conducted in replicates of three. When using radiolabeled compounds, the concentrations were determined by liquid scintillation counting of portions of serum and buffer fractions and when using nonlabeled compounds, analysis was conducted by GC−MS. The fraction bound to protein (f_b) was calculated using the following formula

$$f_b = \frac{(C_s - C_b) \cdot \text{volume correction factor}}{(C_s - C_b) \cdot \text{volume correction factor} + C_b} \quad (6)$$

where C_s and C_b represent drug concentrations in the serum and buffer fractions, respectively, and the volume correction factor represents the quotient of the final serum volume/initial serum volume. (This difference is due to osmotic flow.) Unbound fractions were calculated as $1 - f_b$. Bound and unbound fractions were multiplied by 100 and reported as percentages.

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Supporting Information Available: Elemental analysis for final testing compounds **3a**,**b**, **5a**,**b**, and **16** and intermediates **7**, **8a**,**c**, **9a**, **11**, and **12**, as well as the X-ray structural analysis of the HCl salt of **16**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Vale, W.; Spiess, J.; Rivier, C.; Rivier; J. Characterization of a 41 residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta endorphin. *Science* **1981**, *213*, 1394. (b) Owens, M. J.; Nemeroff, C. B. Physiology and pharmacology of corticotropin-releasing factor. *Pharmacol. Rev.* **1991**, *43*, 425.

(2) (a) 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. (b) Schulz, W. D.; Mansbach, R. S.; Sprouse, J.; Braselton, J. P.; Collins, J.; Corman, M.; Dunaiskis, A.; Faraci, S.; Schmidt, A.; Seeger, T.; Seymour, P.; Tingley, F. D., III; Winston, E. N.; Chen, Y. L.; Heym, J. CP-154,526: a potent and selective nonpeptide antagonist of corticotropin releasing factor receptors. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 10477. (c) Mansbach, R. S.; Winston, E. N.; Chen, Y. L. Antidepressant-like effects of CP-154,526, a selective CRF1 receptor antagonist. *Eur. J. Pharmacol.* **1997**, *323*, 21–26. (d) Shaham, Y.; Erb, S.; Leung, S.; Buczek, Y.; Stewart, J. CP-154,526, a selective, nonpeptide antagonist of the corticotropin-releasing factor1 receptor attenuates stress-induced relapse to drug seeking in cocaine- and heroin-trained rats. *Psychopharmacology* **1998**, *137*, 184. (e) Iredale, P. A.; Alvaro, J. D.; Lee, Y.; Terwilliger, R.; Chen, Y. L.; Duman, R. S. Role of corticotropin-releasing factor receptor-1 in opiate withdrawal. *J. Neurochem.* **2000**, *74*, 199. (f) Arborelius, L.; Skelton, K. H.; Thrivikraman, K. V.; Plotsky, P. M.; Schulz, D. W.; Owens, M. J. Chronic administration of the selective corticotropin-releasing factor 1 receptor antagonist CP-154,526: behavioral, endocrine and neurochemical effects in the rat. *J. Pharmacol. Exp. Ther.* **2000**, *294*, 588. (g) Hikichi, T.; Akiyoshi, J.; Yamamoto, Y.; Tsutsumi, T.; Isogawa, K.; Nagayama, H. Suppression of conditioned fear by administration of CRF receptor antagonist CP-154,526. *Pharmacopsychiatry* **2000**, *33*, 189. (h) Le, A. D.; Harding, S.; Juzytsch, W.; Watchus, J.; Shalev, U.; Shaham, Y. The role of corticotrophin-releasing factor in stress-induced relapse to alcohol-seeking behavior in rats. *Psychopharmacology* **2000**, *150*, 317. (i) Maillot, C.; Million, M.; Wei, J. Y.; Gauthier, A.; Tache, Y. Peripheral corticotropin-releasing factor and stress-stimulated colonic motor activity involve type 1 receptor in rats. *Gastroenterology* **2000**, *119*, 1569.

(3) Webster, E. L.; Lewis, D. B.; Torpy, D. J.; Zachman, E. K.; Rice, K. C.; Chrousos, G. P. *In vivo* and *in vitro* characterization of antalarmin, a nonpeptide corticotropin-releasing hormone (CRF) receptor antagonist: suppression of pituitary ACTH release and peripheral inflammation. *Endocrinology* **1996**, *137*, 5747. (b) Bornstein, S. R.; Webster, E. L.; Torpy, D. J.; Richman, S. J.; Mitsiades, N.; Igel, M.; Lewis, D. B.; Rice, K. C.; Joost, H. G.; Tsokos, M.; Chrousos, G. P. Chronic effects of a nonpeptide corticotropin-releasing hormone type I receptor antagonist on pituitary-adrenal function, body weight, and metabolic regulation. *Endocrinology* **1998**, *139*, 1546. (c) Deak, T.; Nguyen, K. T.; Ehrlich, A. L.; Watkins, L. R.; Spencer, R. L.; Maier, S. F.; Licinio, J.; Wong, M. L.; Chrousos, G. P.; Webster, E.; Gold, P. W. The impact of the nonpeptide corticotropin-releasing hormone antagonist antalarmin on behavioral and endocrine responses to stress. *Endocrinology* **1999**, *140*, 79.

(4) Chen, Y. L.; Braselton, J.; Forman, J.; Gallaschun, R. J.; Mansbach, R.; Schmidt, A.; Seeger, T. F.; Sprouse, J.; Tingley, F. D.; Winston, E.; Schulz, D. W. Synthesis and SAR of 2-aryloxy-4-alkoxy-pyridines as potent orally active corticotrophin-releasing factor 1 antagonists. *J. Med. Chem.* **2008**, ASAP Article, DOI: 10.021/jm070578k.

(5) Unpublished data. A representative compound in pyrrolo[2,3-*d*]pyrimidine series, e.g., 2-[7-(4-bromo-2,6-dimethylphenyl)-2,5-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-ylamino]-(*S*)-butan-1-ol, was found to produce liver toxicity in 90 day toxicology studies in rats and dogs.

(6) Chen, C.; Grigoriadis, D. E. NBI 30775 (R121919), an orally active antagonist of the corticotropin-releasing factor (CRF) type-1 receptor

for the treatment of anxiety and depression. *Drug Dev. Res.* **2005**, *65*, 216.
(7) Unpublished phase I data.
(8) Because the pyridine *N*-oxide intermediates may show exothermic decomposition, it is recommended to test the differential scanning calorimeter prior to scale-up synthesis. Pyridine *N*-oxide intermediates are not recommended for large-scale preparation, and caution is required to ensure safety in the laboratory. In general, a 100 °C difference between process temperature and decomposition temperature is considered to be acceptable.
(9) Chlorination yield was improved in the presence of 1 equiv of *N*,*N*-diethylaniline. Croisy-Delcey, M.; Bisagni, E. Aza analogs of lucanthone: synthesis and antitumor and bactericidal properties. *J. Med. Chem.* **1983**, *26*, 1329.

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