Potent, Orally Active Corticotropin-Releasing Factor Receptor-1 Antagonists Containing a Tricyclic Pyrrolopyridine or Pyrazolopyridine Core

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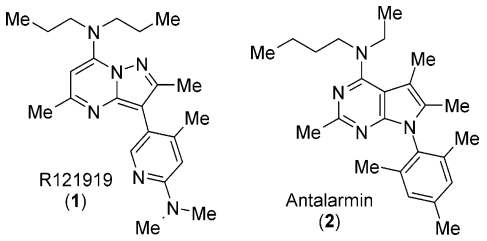
Two new classes of tricyclic-based corticotropin-releasing factor (CRF_1) receptor-1 antagonists were designed by constraining known 1*H*-pyrrolo[2,3-*b*]pyridine and 1*H*-pyrazolo[3,4-*b*]pyridine ligands. Pyrrole- and pyrazole-based molecules **19g** and **22a**, respectively, were discovered that potently bind the recombinant CRF_1 receptor (K_i = 3.5, 2.9 nM) and inhibit adrenocorticotropic hormone (ACTH) release from rat pituitary cell culture (IC_{50} = 14, 6.8 nM). These compounds show good oral bioavailabity (F = 24%, 7.0%) and serum half-lives in rats ($t_{1/2}$ = 6.3, 12 h) and penetrate the rat brain ([brain]/[plasma] = 0.27, 0.52) but tend toward large volumes of distribution (V_D = 38, 44 L kg^{-1}) and rapid clearances (CL = 70, 43 mL min^{-1} kg^{-1}). When given orally, both the pyrazole and the pyrrole leads dose-dependently inhibit stress-induced ACTH release in vivo. ACTH reductions of 84−86% were observed for 30 mg kg^{-1} doses.

Introduction

Corticotropin-releasing factor (CRF) is a 41-amino acid peptide that has a multitude of effects in both the central nervous system and periphery but has the primary purpose of coordinating an organism's response to exogenous stressors.[1−5] This action is mediated through the binding of the CRF peptide on the pituitary, which triggers secretion of adrenocorticotropic hormone (ACTH), the key hormone governing stress response. The effect of CRF on the hypothalamic−pituitary−adrenal (HPA) axis occurs via interaction with two distinct G-protein-coupled receptors and, in particular, via high-affinity binding to the first of these receptors (CRF_1), which is expressed in the pituitary. Considerable clinical evidence substantiates that hypersensitivity of the CRF signaling pathway results in anxiety and stress-related disorders and that antagonism of the CRF_1 receptor represents a potential treatment for such pathologies. Elevated levels of CRF have been found in the cerebrospinal fluid (CSF) of patients suffering from depression[6,7] and post-traumatic stress disorder,[8,9] and the efficacious treatment of clinically depressed patients led to a measurable decrease in these levels.[7,10] Postmortem analysis of suicide victims shows a reduced presence of CRF_1 receptors in the frontal cortex, presumed to be a consequence of down-regulation in response to the elevated CRF levels.[11] Finally, R121919 (**1**), a non-peptide and selective CRF_1 receptor antagonist, proved to be effective in treating severely depressed patients in an open labeled phase IIA clinical trial.[12,13]

Several companies have disclosed potent CRF_1 receptor antagonists based on monocyclic and bicyclic cores, including substituted azines **3** and their bicyclic counterparts **4**[4,5] (Figure 1). Both the above-mentioned clinical compound (**1**) and the extensively researched antalarmin (**2**)[14] fall within this broad structural class. A limited number of tricyclic derivatives have also been reported.[4] We envisioned synthesizing compounds with a third ring installed (i.e., **5** and **6**), anticipating that the additional constraint would not only produce a distinct SAR for this series relative to compounds of classes **3** and **4** but may force conjugation of the 4-amino nitrogen atom lone pair to the pyridine ring, potentially affecting the hydrogen-bonding potential at this site.[15] Although the precise nature of the interaction between the pyridine or pyrimidine nitrogen atom and the receptor is not known,[16] its presence is essential for high-affinity binding, and the basicity of this nitrogen atom is important for the pharmacokinetics of the molecule.[17]



Results and Discussion

Chemistry. We sought a general synthetic method for the preparation of these compounds that would allow late-stage differentiation to either the pyrazole **5** or the pyrrole **6** and that would also permit rapid variation of the aryl substituent to produce libraries of related compounds. The facility with which nitrogen nucleophiles displace chloride from 2-chloropyridines, particularly when the 3-position of the pyridine is substituted with an electron-withdrawing group, suggested that ketone **7** would be a suitable key intermediate.[18]

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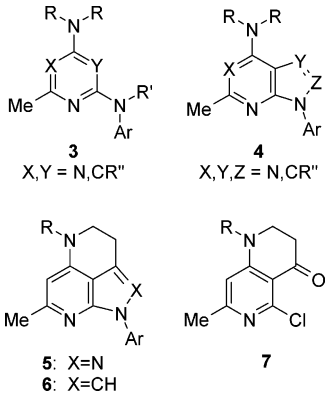
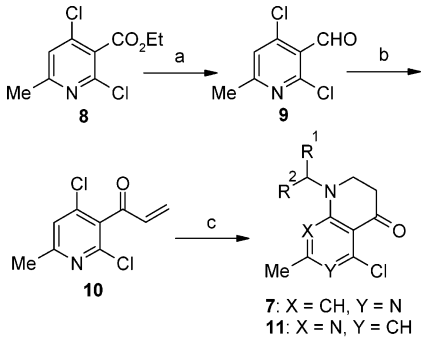


Figure 1. Design of tricyclic CRF_1 receptor antagonists.

Scheme 1[a]



[a] Reagents and conditions: (a) (i) LAH, THF, −78 °C; (ii) oxalyl chloride, DMSO, TEA, DCM, −70 °C to room temp; (b) (i) CH_2=CHMgBr, THF, −78 °C; (ii) Jones' reagent; (c) $R^1R^2CHNH_2$, ethanol, room temp.

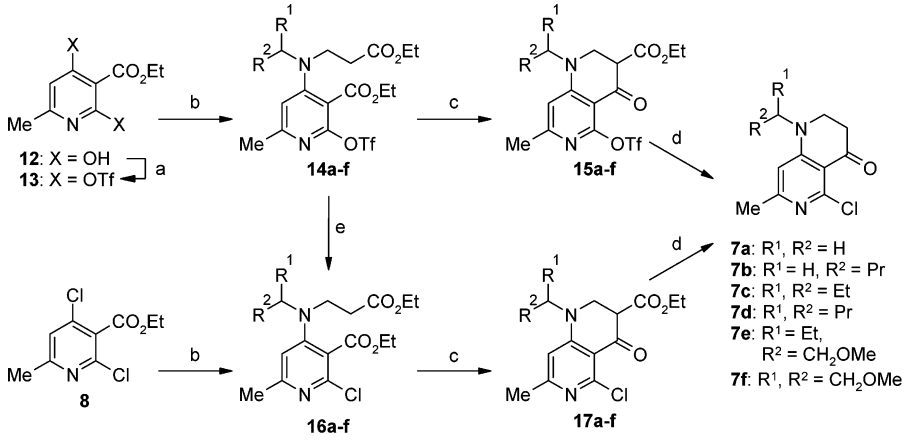
The initial synthetic route to compound **7** is outlined in Scheme 1. Ethyl 2,4-dichloro-6-methylnicotinate (**8**) was converted to aldehyde **9** via lithium aluminum hydride reduction and Swern oxidation of the resulting alcohol. Addition of vinylmagnesium bromide to aldehyde **9**, followed by oxidation of the resulting allylic alcohol, afforded enone **10**. This material readily cyclized when treated with primary amines to generate a mixture of regioisomers of the newly formed piperidine ring. For example, when 4-heptylamine was used in this reaction, the cyclized products were formed in 42% combined yield and the 4-chloropyridine **11** was present in slight excess.

However, the modest yield for the cyclization step in Scheme 1 prompted us to pursue an alternative synthesis. Toward this end, bis(triflate) **13** was prepared as a chemical equivalent of dichloride **8** by treating hydroxypyridinone **12**[19] with trifluoroacetic anhydride (Scheme 2). When **13** was mixed with ethyl-*N*-(4-methyl)-*β*-alanine under acid catalysis, the desired 4-aminopyridine **14a** was formed in a 3-fold excess over the 2-aminopyridine isomer. Upon reaction with potassium *tert*-butoxide, diester **14a** underwent a Dieckmann cyclization to give ketoester **15a**. Heating this compound with concentrated hydrochloric acid resulted in decarboxylation and exchange of the trifluoromethanesulfonyl group for chlorine. The ketone **7a** prepared by this route was identical in every respect to that prepared in Scheme 1.

The chloropyridine **16a**, prepared either by addition of the *β*-alanine derivatives to **8** or chlorinolysis of sulfonate **14a**, undergoes the cyclization/decarboxylation procedure in substantially better yield. In general, the intermediates **16** have the added advantage that they are typically crystalline and preferentially crystallize over their 4-chloropyridine isomers. As such, this manipulation is more suitable to large-scale synthesis and purification of these intermediates. The bicyclic ketones **7** prepared from **16** are indistinguishable from those prepared from sulfonates **14**.

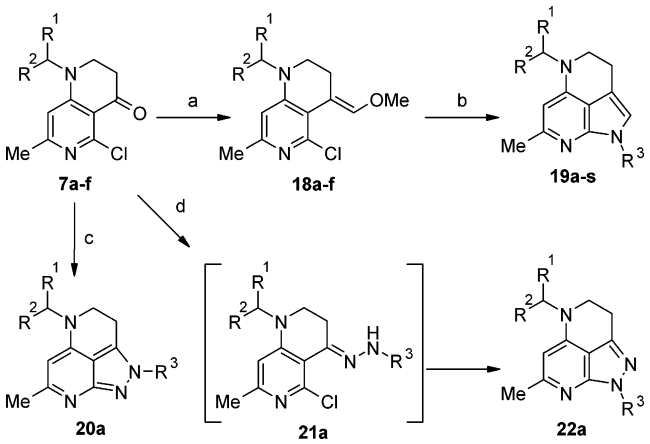
After generation of our prospective key intermediate **7**, it remained to independently anneal both a pyrazole and a pyrrole ring to give compounds **5** and **6**, respectively. In the pyrrole case, an additional carbon atom was introduced via a Wittig reaction, which generated the penultimate enol ether **18** (Scheme 3). Conversion of **18** to the tricyclic compound **19** was effected in several different ways depending on the choice of the R^3 substituent. Typically the cyclization was conducted by thermolysis in the presence of a suitable aniline or amine. In some instances, the yield for this transformation was improved by using a microwave, and in the case of particularly unreactive anilines, recourse was made to palladium catalysis. Unfortunately, none of these conditions were uniformly successful for all substrates. However, despite the low yields for this process, the fact that the R^3 group is introduced in the final step

Scheme 2[a]



[a] Reagents and conditions: (a) trifluoroacetic anhydride, TEA, DCM, 10 °C; (b) ethyl-*N*-alkyl-*β*-alanine, TEA, ACN; (c) *t*-BuOK, THF; (d) 6 M HCl, reflux; (e) 4 M HCl in dioxane.

Scheme 3[a]



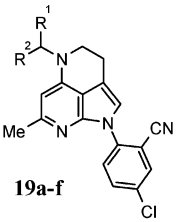
[a] Reagents and conditions: (a) $Ph_3P{=}CHOMe$, THF, reflux; (b) R^3NH_2, PTSA, tetramethylene sulfone, 225 °C; (c) R^3NHNH_2, ethanol, reflux; (d) (i) R^3NHNH_2, methanol, 60 °C; (ii) diphenyl ether, 200 °C.

from readily available anilines and amines makes this synthetic route amenable to parallel synthesis.

For the pyrazole compound class, direct cyclization of **7** with substituted hydrazines in refluxing ethanol resulted in almost exclusive formation of the 1-aryl isomers **20**. Under these conditions, the reaction appears to proceed by initial displacement of chloride by the more nucleophilic primary nitrogen of the hydrazine, followed by cyclization and dehydration. To drive the reaction to the desired 2-aryl isomer **22**, ketone **7** and the hydrazine were allowed to react at low temperature until formation of the corresponding hydrazone **21** was complete. Thermolysis of this intermediate then afforded **22**, which was accompanied by smaller amounts of **20**. The use of these modified conditions allowed the preparation of a variety of tricyclic-based pyrazolopyridines **22** for evaluation in the CRF_1 receptor binding assay.

In Vitro Results. When comparing the biological activity of these new tricyclic compounds to previously reported monocyclic and bicyclic analogues, we anticipated that the alkyl bridge forming the new fused ring would compensate for one of the alkyl chains on the sp^3 nitrogen atom of compounds such as **3** and **4**. To evaluate this hypothesis, a number of representatives containing alternative substitutions in this position were synthesized, and the binding constants of some key examples are presented in Table 1. The simple *N*-methyl analogue **19a** is inactive, but the introduction of an *N*-butyl group (**19b)** or a 3-pentyl group (**19c)** results in binding constants of 49 and 62 nM, respectively. When the size of this substituent is increased to a 4-heptyl group, as in **19d**, binding potency improves to 13 nM. Regrettably, the relatively lower potency of **19b** indicates that in the fused tricyclic series, the new alkyl bridge is incapable of compensating for one of the substituents on the exocyclic nitrogen atom of bicyclic ligands such as **4**, and branched aliphatic groups on the piperidine ring nitrogen atom are essential for high-affinity binding. In an attempt to reduce the significant impact of a heptyl group on the log *D* of these molecules, oxygen atoms were incorporated into one (**19e**) or both (**19f**) arms of the branched group. This leads to significant loss of binding potency. Other attempts to introduce more hydrophilic groups into this portion of the molecule also met with limited success.

Table 1. Reference Compounds and SAR of R^1 and R^2 Substitution for Tricyclic Compounds



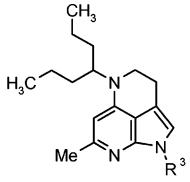
Cmpd	R^1, R^2	Ki (nM)[a] (S.D.)
1[b]	-	7.8 (5)
2[c]	-	3.1 (2)
19a	H, H	N.A.[d]
19b	H, Pr	49 (11)
19c	Et, Et	62 (46)
19d	Pr, Pr	13 (4)
19e	Et, CH_2OMe	40 (5)
19f	CH_2OMe, CH_2OMe	120 (11)

[a] Averaged from a minimum of two replicates. [b] R121919. [c] Antalarmin. [d] Not active (<30% inhibition at 10 μM).

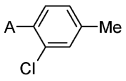
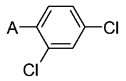
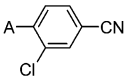
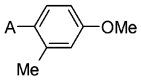
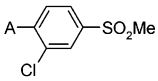
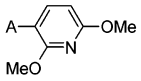
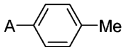
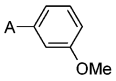
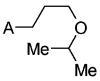
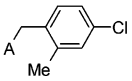
On the other hand, when the group R^3 is considered, the SAR of these new compounds demonstrates a similarity to that of the bicyclic counterparts (Table 2).[20] In particular, 2,4-disubstituted aryl groups provide the most potent analogues, particularly when the substituents are small (**19g**−**l**). The electronic nature of these groups does not appear to have a significant impact on the binding potency. For example, a cyano, a methoxy, or a methanesulfonyl group are all well-tolerated (**19i**−**k**). In fact, even a dimethoxypyridine group can be used to replace this substituted phenyl, as evidenced by **19l**. This provides an opportunity to balance the hydrophobicity inherent to the α-branched alkyl group on the piperidine ring. As one would expect from the previously reported SAR of the bicycle-based compounds, deletion of the 4-substituent (**19m**) or the 2-substituent (**19n**) reduces inhibition constants by more than 5-fold, and 3-substituted examples (**19o**) are typically 50-fold less potent. Moreover, attempting to replace the aryl group with an alternative lipophilic counterpart drastically alters the affinity of these compounds as exemplified by **19p**, and even introducing a methylene spacer between the aryl ring and the tricyclic core reduces the potency by several orders of magnitude (**19q**).

Binding data for a representative pyrazole-based compound **22a** and its regioisomer **20a** are presented in Table 3. The SAR of the pyrazole-based tricycles parallels that of their pyrrole counterparts and does not need to be discussed in detail. Hence, the analogue containing a 2,4-dichlorophenyl group and a 4-heptyl group as the pyrazole and piperidine substituents, respectively, has a K_i of 2.9 nM (**22a**). The regioisomer **20a**, formed during preliminary investigations into the

Table 2. SAR of R^3 Substitution for Tricyclic Compounds

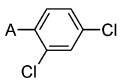
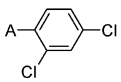


19g-q

Cmpd	R^{3a}	Ki (nM)[b] (S.D.)
19g		3.5 (3)
19h		4.2 (6)
19i		7.2 (7)
19j		6.5 (4)
19k		13 (3)
19l		5.4 (3)
19m		55 (33)
19n		30 (16)
19o		190 (190)
19p		4100 (4900)
19q		1900 (420)

[a] A: site of attachment to tricyclic core. [b] Averaged from a minimum of two replicates.

Table 3. CRF_1 Binding Data for **20a** and **22a**

Cmpd	R^1, R^2	R^{3a}	Ki (nM)[b] (S.D.)
20a	Pr, Pr		N.A.[c]
22a	Pr, Pr		2.9 (2)

[a] A: site of attachment to tricyclic core. [b] Averaged from a minimum of two replicates. [c] Not active (<30% inhibition at 10 μM).

synthesis of **22a**, is inactive in the CRF_1 receptor binding assay.

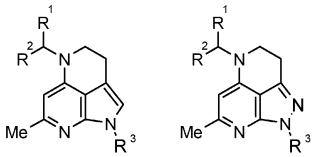
Pharmacokinetics. On the basis of their in vitro profiles, several of the tricyclic CRF_1 receptor ligands were evaluated in rat pharmacokinetic studies (Table 4). Pyrrole **19g** is simultaneously one of the most lipophilic compounds from the series and the most potent, with a K_i of 3.5 nM and an IC_{50} of 14 nM in a functional assay of ACTH release in rat pituitary cell culture. When administered orally to conscious rats, the compound showed good bioavailability (F = 24%) and nearly an ideal serum half-life ($t_{1/2}$ = 6.3 h). The molecule was rapidly cleared (CL = 70 mL min^{-1} kg^{-1}) and demonstrated a very high volume of distribution (V_D = 38 L kg^{-1}), assuming linear pharmacokinetics at these doses. In an attempt to gauge how the volume of distribution and clearance of these compounds relate to their substituents, less lipophilic analogues were similarly evaluated. Surprisingly, introduction of more polar groups into the R^3 site failed to lower the volume of distribution, and the clearance of these molecules was either increased or unchanged (**19j**−**l**). For **19j** and **19l**, the increased clearance may be a result of the metabolically labile methoxy groups. Indeed, when the methoxy substituent was replaced with the trifluoromethoxy group as in **19r**, the clearance dropped to 28 mL min^{-1} kg^{-1}, which also resulted in the highest oral bioavailability of the group (76%). In terms of the R^1 and R^2 sites of substitution, the introduction of less lipophilic substituents had a somewhat unpredictable effect on the pharmacokinetics of the molecules (**19r** and **19s**). In some cases, these substitutions lead to lower volumes of distribution (**19s**) or clearances (**19r**) but never both. Again, the presence of a labile methoxy group, this time in the R^1 substituent, may be the reason for the extensive clearance of **19s**. In addition, compounds **19r** and **19s** were evaluated in a cassette paradigm in lower doses and in the presence of other compounds. This may have influenced the determined pharmacokinetic properties, and these data are not necessarily directly comparable to the data from noncassette studies.

Pyrazole **22a** possesses a more moderate clearance, a longer half-life, but a lower bioavailability than pyrrole **19g**. This may indicate that the pyrazole class is not as easily absorbed as the corresponding pyrrole-based compounds. As well, most of the tricyclic compounds tested are able to penetrate the brain. Pyrrole **19g** and pyrazole **22a** show brain-to-plasma ratios of 0.27 and 0.52, respectively, when administered intravenously.

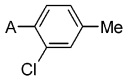
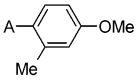
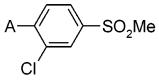
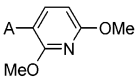
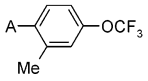
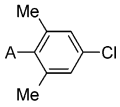
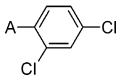
In Vivo Efficacy. Compound **19g** demonstrates high potency in binding and ACTH release and good oral bioavailability and serum half-life and penetrates the blood−brain barrier. It is rapidly cleared but still shows the best overall profile of the various pyrrole compounds. Pyrazole **22a** has lower oral bioavailability than **19g**, but is modestly more potent and has better clearance and brain penetration values. The complementary nature of these compounds prompted us to evaluate them in vivo. A number of different animal efficacy models have been reported in the literature, although the performance of CRF_1 antagonists in these models is somewhat indiscriminate.[21] In our hands, the most predictive models are those involving the antagonist-mediated suppression of elevated serum ACTH levels induced by an exogenous stressor. As such, we chose to evaluate the tricyclic compounds in a restraint-induced anxiety model.

The effects of oral dosing of **19g** and **22a** in the restraint-induced ACTH release mouse model are pre-

Table 4. In Vitro and in Vivo Properties of Selected Tricyclic CRF_1 Receptor Antagonists



19 **22**

Cmpd	R^1, R^2	R^{3a}	Ki (nM)[b] (S.D.)	ACTH IC_{50} (nM)[b,c] (S.D.)	V_d (L/Kg)	CL (mL/min/Kg)	$t_{1/2}$ (h)	F (%)
19g	Pr, Pr		3.5 (4)	14 (4)	38[d]	70[d]	6.3[d]	24[d]
19j	Pr, Pr		6.5 (4)	39 (26)	53[d]	107[d]	5.7[d]	18[d]
19k	Pr, Pr		13 (3)	250 (87)	66[d]	152[d]	5.0[d]	74[d]
19l	Pr, Pr		5.4 (3)	29 (16)	62[d]	70[d]	10[d]	50[d]
19r	Et, Et		6.0 (3)	48 (21)	80[e]	28[e]	33[e]	76[e]
19s	Et, CH_2OMe		16 (3)	27[f]	19[e]	87[e]	2.5[e]	66[e]
22a	Pr, Pr		2.9 (2)	6.8 (3)	44[g]	43[g]	12[g]	7.0[g]

[a] A: site of attachment to tricyclic core. [b] Averaged from a minimum of two replicates unless otherwise noted. [c] Measuring ACTH release in isolated rat pituitary cell culture. [d] By administration of 10 mg/kg po and 10 mg/kg iv. [e] By coadministration of 2 mg/kg po and 1 mg/kg iv each of five compounds. [f] Single determination. [g] By administration of 10 mg/kg po and 5 mg/kg iv.

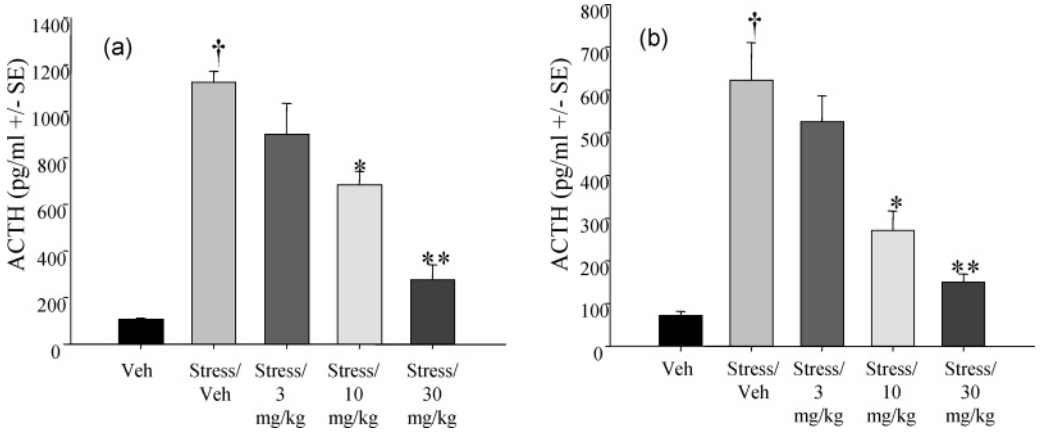


Figure 2. Effect of compounds **19g** (a) and **22a** (b) on stress-induced ACTH release. Data are presented as the mean ± SEM: (†) $p < 0.0001$ vs vehicle with no stress induction; (*) $p < 0.001$ vs vehicle with stress induction; (**) $p < 0.0001$ vs vehicle with stress induction.

sented in Figure 2. In both experiments, a robust increase in ACTH ($p < 0.0001$) is observed when mice are subjected to a 45 min period of restraint. Both compounds attenuate this ACTH elevation in a dose-dependent manner from oral doses of 3−30 mg/kg, and the effect is statistically significant at 10 mg/kg. At 30 mg/kg, the pyrrole **19g** and the pyrazole **22a** showed 84% and 86% reduction in ACTH levels, respectively, with *p*-values below 0.0001. These compounds have clearly demonstrated that they are efficacious attenuators of stress-induced ACTH release.

Conclusion

In summary, we have presented the design, synthesis, pharmacology, pharmacokinetics, and in vivo efficacy of two novel classes of fused tricyclic CRF_1 receptor ligands. Both compound classes have generated potent, functional CRF_1 receptor antagonists, which are orally absorbed, penetrate the brain, and normalize elevated

ACTH levels brought on by exogenous sources of stress. The introduction of a third ring and a branched lipophilic substituent gives these molecules an unusual pharmacokinetic profile in rodents. It is unclear at this stage whether these data will be predictive of the pharmacokinetics in humans, but the synthesis of compounds designed to optimize these properties is currently underway.

Experimental Section

Chemistry. General Methods. ^{1}H and ^{13}C NMR spectra were obtained with a Varian 300 MHz spectrometer (Mercury) or with a Bruker 500 MHz spectrometer at Numega Labs Inc., San Diego, CA. The chemical shifts are reported in parts per million (δ) downfield using TMS as the internal standard and $CDCl_3$ as the solvent except where indicated. Matrix-assisted laser desorption/ionization (MALDI) FTMS experiments were performed on an IonSpec FTMS mass spectrometer at The Scripps Research Institute, San Diego, CA. Samples were irradiated with a nitrogen laser operated at 337 nm, and the laser beam was controlled by a variable attenuator and focused on the sample target. Fast atom bombardment (FAB) analysis was carried out on M-Scan's VG Analytical ZAB 2-SE high-field mass spectrometer at M-Scan Inc., West Chester, PA. A cesium ion gun was used to generate ions for the acquired high-resolution mass spectra. LC−MS analyses were performed on a Perkin-Elmer Sciex API-100 mass spectrometer using the electron spray ionization technique or on a Spectra-System P4000 HPLC system coupled with a Finnigan LCD/Deca mass spectrometer using the electrospray ionization technique. Purification of final compounds was conducted on a prep-LC−MS Dionex system, using an Alpha C18 30 mm × 75 mm reverse-phase column at a flow rate of 45 mL/min. The mobile phase was a gradient of 95/5 to 5/95 A/B; A = H_2O−0.1%TFA, B = CH_3CN−0.1% TFA. All compounds after purification were reanalyzed on a reverse-phase HPLC−MS system (DIONEX with ESI+ ionization mode) and shown to be at least 85% pure on the basis of both UV wavelengths (220 and 254 nM) and total ion current (TIC) integration from the mass spectrum. All commercially available reagents were used without further purification. Microwave reactions were performed using a Personal Chemistry Emrys optimizer. Hydrochloride salts of final compounds used for pharmacokinetic measurements were prepared by treatment of the free bases in DCM with excess HCl in ether, concentration of the mixture, and recrystallization as necessary. Methanesulfonic acid salts were prepared by addition of a single equivalent of the sulfonic acid from a stock solution, concentration, and recrystallization as necessary.

2,4-Dichloro-6-methylpyridine-3-carbaldehyde (9). Ethyl 2,4-dichloro-6-methylnicotinate (8.04 g, 34.3 mmol) was dissolved in THF (40 mL) and added to a stirred suspension of LAH (6.52 g, 0.170 mmol) in THF (80 mL) at −78 °C. The mixture was stirred for 6 h at this temperature and 1 h at −30 °C and treated cautiously with water (5.5 mL), 15% aqueous sodium hydroxide (5.5 mL), and water (17 mL) with vigorous stirring. The mixture was warmed to room temperature and filtered. The white precipitate was washed liberally with ethyl acetate. The combined organic portions were dried ($MgSO_4$) and concentrated under vacuum to afford 6.40 g of the crude alcohol as a colorless oil that solidified on standing.

DMSO (14.2 mL, 200 mmol) was added to a stirred solution of oxalyl chloride (8.7 mL, 99 mmol) in dichloromethane (100 mL) at −70 °C. After 15 min, the above alcohol (6.40 g, 33.3 mmol) in dichloromethane (25 mL) was added, followed by triethylamine (56 mL), and the mixture was allowed to warm to room temperature and stirred for 1 h. The mixture was washed with aqueous sodium bicarbonate (75 mL), dried ($MgSO_4$), and concentrated under vacuum. The residue was purified by column chromatography (elution with 10% ethyl acetate in hexanes) to afford 5.00 g (78%) of **9** as a pale-yellow oil which solidified on standing: ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.45 (s, 1 H), 7.26 (s, 1 H), 2.58 (s, 3 H); LC−MS (APCI) *m/z* 189.9 (MH^+). Anal. ($C_7H_5Cl_2NO$) C, H, N.

1-(2,4-Dichloro-6-methylpyridin-3-yl)prop-2-en-1-ol. Vinylmagnesium bromide in THF (1.0 M, 6.7 mL) was added to a stirred solution of aldehyde **9** (1.15 g, 6.05 mmol) in THF (20 mL) at −78 °C. The mixture was stirred at this temperature for 30 min and warmed to room temperature, and the reaction was quenched with aqueous sodium bicarbonate (40 mL). The mixture was extracted twice with ethyl acetate, and the combined extracts were dried ($MgSO_4$) and concentrated under vacuum to afford 1.37 g of the crude allylic alcohol as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (s, 1 H), 6.18 (ddd, J = 17.1, 10.5, 5.3 Hz, 1 H), 5.94−5.88 (m, 1 H), 5.34−5.27 (m, 2 H), 3.01 (d, J = 9.9 Hz, 1 H), 2.51 (s, 3 H); LC−MS (APCI) *m/z* 218.1 (MH^+). Anal. ($C_9H_9Cl_2NO$) C, H, N.

1-(2,4-Dichloro-6-methylpyridin-3-yl)propenone (10). The allylic alcohol from the previous step and *N*-methylmorpholine *N*-oxide (NMO, 1.06 g, 9.05 mmol) were dissolved in dichloromethane (27 mL) and treated with 4 Å molecular sieves (1.3 g). The mixture was stirred for 20 min, and tetrapropylammonium perruthenate (TPAP, 65 mg) was added. The mixture was stirred for 1 h. Some starting material persisted, so additional NMO (1.06 g) and TPAP (65 mg) were added and stirring was continued for 1 h. The mixture was filtered (Celite) and concentrated under vacuum, and the residue was purified on a silica gel column (elution with 10% ethyl acetate in hexanes) to afford 0.50 g (38%) of **10** as a pale-yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (s, 1 H), 6.60 (dd, J = 17.7, 10.5 Hz, 1 H), 6.22 (d, J = 10.5 Hz, 1 H), 6.02 (d, 17.4 Hz, 1 H), 2.57 (s, 3 H); LC−MS (APCI) *m/z* 215.9 (MH^+). Anal. ($C_9H_7Cl_2NO$) C, H, N.

5-Chloro-7-methyl-1-(1-propylbutyl)-2,3-dihydro-1*H*-[1,6]naphthyridin-4-one (7d) and 5-Chloro-7-methyl-1-(1-propylbutyl)-2,3-dihydro-1*H*-[1,8]naphthyridin-4-one (11d). Enone **10** (920 mg, 4.26 mmol) was dissolved in ethanol (20 mL) and treated with 4-heptylamine (0.64 mL, 4.3 mmol). The mixture was heated at 60 °C for 16 h and concentrated under vacuum. The residue was taken up in ethyl acetate (50 mL), washed with aqueous sodium bicarbonate (20 mL), dried ($MgSO_4$), and again concentrated. The residue was purified on a silica gel column (elution with 5% ethyl acetate in hexanes for **11d** and 25% ethyl acetate in hexanes for **7d**) to afford 314 mg (25%) of **11d** as a yellow oil followed by 214 mg (17%) of **7d** as a white solid.

Compound 7d: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.54 (s, 1 H), 3.99 (pent, J = 6.5 Hz, 1 H), 3.40 (dd, J = 7.1, 7.1 Hz, 2 H), 2.64 (dd, J = 7.1, 7.1 Hz, 2 H), 2.39 (s, 3 H), 1.64−1.486 (m, 4 H), 1.38−1.24 (m, 4 H), 0.93 (t, J = 7.1 Hz, 6 H); LC−MS (APCI) *m/z* 295.1 (MH^+). Anal. ($C_{16}H_{23}ClN_2O$) C, H, N. The singlet at 6.54 (pyridyl-*H*) and the multiplet at 3.99 (C*H*Pr$_2$) showed an NOE effect that was absent in **11d**, confirming the identity of this compound as **7d**.

Compound 11d: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.42 (s, 1 H), 5.38−5.22 (m, 1 H), 3.36−3.31 (m, 2 H), 2.63−2.59 (m, 2 H), 2.31 (s, 3 H), 1.58−1.26 (m, 4 H), 1.33−1.21 (m, 4 H), 0.90 (t, J = 7.4 Hz, 6 H); LC−MS (APCI) *m/z* 295.1 (MH^+). Anal. ($C_{16}H_{23}ClN_2O\cdot 0.2H_2O$) C, H, N.

Ethyl 6-Methyl-2,4-bis(trifluoromethanesulfonyloxy)-nicotinate (13). In a 5 L round-bottom three-neck flask equipped with a mechanical stirrer and nitrogen bubbler was charged 1 L of anhydrous dichloromethane. To the solution was charged 320 g (1.62 mol) of **12a** and 490 mL (3.5 mol, 2.2 equiv) of triethylamine. The mixture was cooled below 5 °C with an ice bath, and 950 g (3.37 mol, 2.08 equiv) of trifluoromethanesulfonic anhydride in 500 mL of dichloromethane was added via a 2 L addition funnel over 2 h. The mixture was allowed to warm to ambient temperature and was stirred for an additional 2 h. The reaction was quenched with 500 mL of water, and the organic phase was separated. The organic phase was washed twice with 200 mL of 1 N HCl and 100 mL of brine, dried over magnesium sulfate, and filtered over a pad of silica gel (80 mm × 120 mm), eluting with ethyl ether. The solvent was removed in vacuo to afford 790 g (100%) of **13** as a brown oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.22 (s, 1H), 4.45

(q, J = 6.9 Hz, 2H), 2.65 (s, 3H), 1.42 (t, J = 6.9 Hz, 3H); GC−MS (EI) *m/z* 461.

Ethyl 3-Methylaminoproprionate.[22] The compound was prepared according to the general procedure of Rosenberg and Rapoport.[23] Ethyl acrylate (10 mL, 92 mmol) was dissolved in ethanol (50 mL) and treated with *N*-benzylmethylamine (11 mL, 99 mmol). The mixture was stirred for 18 h, after which LC−MS indicated complete product formation (MH^+, 222.0). Palladium on carbon (10%, 5.2 g) was added, followed by ammonium formate (8.2 g, 124 mmol), and the mixture was placed in a preheated oil bath and heated to reflux for 1 h. The mixture was cooled to room temperature, diluted with ethyl acetate, filtered (Celite), and concentrated under vacuum to afford the title compound as a colorless oil, which was used without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.14 (q, J = 7.1 Hz, 2 H), 2.84 (t, J = 6.5 Hz, 2 H), 2.50 (t, J = 6.5 Hz, 2 H), 2.43 (s, 3 H), 1.26 (t, J = 7.2 Hz, 3 H); GC−MS (EI) *m/z* 131 (M^+).

Ethyl 2-[(2-Ethoxycarbonylethyl)methylamino]-6-methyl-4-trifluoromethanesulfonyloxynicotinate and Ethyl 4-[(2-Ethoxycarbonylethyl)methylamino]-6-methyl-2-trifluoromethanesulfonyloxynicotinate (14a). Method A. Compound **13** (8.79 g, 19.1 mmol) was dissolved in acetonitrile (9 mL) and cooled in an ice bath. Triethylamine (3.2 mL, 23 mmol) was added, followed by ethyl 3-methylaminopropionate (3.00 g, 22.9 mmol) in acetonitrile (5 mL). The mixture was allowed to warm to room temperature over 1 h and was stirred at this temperature for 18 h. It was diluted with ethyl acetate (100 mL), washed with water (75 mL) and aqueous sodium chloride (75 mL), dried ($MgSO_4$), and concentrated under vacuum. The residue was purified by flash chromatography (elution with 20% ethyl acetate in hexanes) to afford 1.33 g (16%) of ethyl 2-[(2-ethoxycarbonylethyl)methylamino]-6-methyl-4-trifluoromethanesulfonyloxynicotinate as a white powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.39 (s, 1 H), 4.36 (q, J = 7.1 Hz, 2 H), 4.12 (q, J = 7.2 Hz, 2 H), 3.86 (t, J = 7.2 Hz, 2 H), 2.95 (s, 3 H), 2.67 (t, J = 7.1 Hz, 2 H), 2.40 (s, 3 H), 1.37 (t, J = 7.2 Hz, 2 H), 2 H), 1.23 (t, J = 7.2 Hz, 2 H); LC−MS (APCI) *m/z* 442.9 (MH^+). Anal. ($C_{16}H_{21}F_3N_2O_7S$) C, H, N. A second more polar fraction afforded 4.01 g (47%) of **14a** as a white powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.49 (s, 1 H), 4.37 (q, J = 7.1 Hz, 2 H), 4.14 (q, J = 7.1 Hz, 2 H), 3.61 (t, J = 7.42 H), 2.96 (s, 3 H), 2.63 (t, J = 7.4 Hz, 2 H), 2.39 (s, 3 H), 1.39 (t, J = 7.1 Hz, 2 H); LC−MS (APCI) *m/z* 442.9 (MH^+). Anal. ($C_{16}H_{21}F_3N_2O_7S$) C, H, N. Compound **14a** showed an NOE signal between the singlet at 2.96 (N−CH_3) and the singlet at 6.49 (pyridyl-*H*). An analogous signal was absent in the other isomer isolated from this reaction.

Ethyl 2-Chloro-4-[(2-ethoxycarbonylethyl)methylamino]-6-methylnicotinate (16a). Using Method A and compound **8** as starting material afforded 23% of **16a** as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.42 (s, 1 H), 4.37 (q, J = 7.1 Hz, 2 H), 4.13 (q, J = 7.2 Hz, 2 H), 3.60 (t, J = 7.5 Hz, 2 H), 2.95 (s, 3 H), 2.60 (t, J = 7.5 Hz, 2 H), 2.41 (s, 3 H), 1.39 (t, J = 7.4 Hz, 2 H), 1.25 (t, J = 7.2 Hz, 2 H); LC−MS (APCI) *m/z* 328.9 (MH^+). Anal. ($C_{15}H_{21}ClN_2O_4$) C, H, N.

Ethyl 2-Chloro-4-[(2-ethoxycarbonylethyl)methylamino]-6-methylnicotinate (16a). Method B. Diester **14a** (2.11 g, 4.77 mmol) was dissolved in 4 M hydrogen chloride in dioxane and stirred at room temperature for 16 h. The mixture was basified to pH 10 with 1 N aqueous sodium hydroxide and extracted three times with DCM. The combined extracts were dried ($MgSO_4$) and concentrated, and the residue was purified by flash chromatography (elution with 50% ethyl acetate in hexanes) to afford 174 mg (11%) of **16a**, which was identical to material prepared using method A.

5-Chloro-1,7-dimethyl-2,3-dihydro-1*H*-[1,6]naphthyridin-4-one (7a). Method C. Potassium *tert*-butoxide (0.293 g, 2.61 mmol) was added to an ice-cooled, stirred solution of **14a** (1.07 mg, 2.41 mmol) in THF (5 mL). The mixture was warmed to room temperature and stirred for 3 h. Hydrochloric acid (6 N, 3 mL) was added, and the mixture was heated to reflux for 18 h. It was cooled, basified with 1 N aqueous sodium hydroxide, and extracted three times with DCM. The combined extracts were dried ($MgSO_4$) and concentrated, and the residue was purified by flash chromatography (elution with 50% ethyl acetate in hexanes) to afford 178 mg (35%) of **7a** as a white powder: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.34 (s, 1 H), 3.60 (t, J = 7.2 Hz, 2 H), 3.07 (s, 3 H), 2.75 (t, J = 7.2 Hz, 2 H), 2.42 (s, 3 H); LC−MS (APCI) *m/z* 210.9 (MH^+). Anal. ($C_{10}H_{11}ClN_2O$) C, H, N. Using the same procedure with **16a** as starting material afforded 86% of the title compound.

5-Chloro-4-[1-methoxymeth-(*E*)-ylidene]-1,7-dimethyl-1,2,3,4-tetrahydro[1,6]naphthyridine (18a). Method D. Potassium *tert*-butoxide (319 mg, 2.86 mmol) and (methoxymethyl)triphenylphosphonium chloride (982 mg, 2.86 mmol) were combined under nitrogen. THF (10 mL) was added, and the mixture was stirred for 10 min to generate a deep-red solution. Ketone **7a** (302 mg, 1.43 mmol) was added, and the mixture was heated to reflux for 1 h. After cooling, the mixture was poured into water and extracted with ethyl acetate. The combined extracts were dried ($MgSO_4$) and concentrated, and the residue was purified by flash chromatography (elution with 50% ethyl acetate in hexanes) to afford 191 mg (56%) of **18a** as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94 (s, 1 H), 6.18 (s, 1 H), 3.72 (s, 3 H), 2.34 (t, J = 6.2 Hz, 2 H), 2.93 (s, 3 H), 2.56 (t, J = 6.3 Hz, 2 H), 2.35 (s, 3 H); LC−MS (APCI) *m/z* 238.9 (MH^+). Anal. ($C_{12}H_{15}ClN_2O$) C, H, N.

1-(4-Chloro-2-cyanophenyl)-5,7-dimethyl-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene (19a). Method E. Enol ether **18a** (123 mg, 0.52 mmol), 2-amino-4-chlorobenzonitrile (102 mg, 0.67 mmol), and 4-toluenesulfonic acid monohydrate (10 mg, 0.053 mmol) were dissolved in tetramethylenesulfone (2 mL) and heated to 225 °C for 1 h in a microwave reactor. After cooling, the mixture was poured into 1 N sodium hydroxide solution and extracted with ethyl acetate. The combined extracts were dried ($MgSO_4$) and concentrated, and the residue was purified by flash chromatography followed by preparative TLC (elution with 30% ethyl acetate in hexanes) to afford 24 mg (14%) of **19a** as a pale-yellow solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, J = 9.0 Hz, 1 H), 7.72 (d, J = 2.4 Hz, 1 H), 7.65 (dd, J = 8.7, 2.4 Hz, 1 H), 6.94 (s, 1 H), 6.16 (s, 1 H), 3.40 (t, J = 6.1 Hz, 2 H), 3.09 (t, J = 6.1 Hz, 2 H), 3.00 (s, 3 H), 2.52 (s, 3 H); LC−MS (APCI) *m/z* 322.9 (MH^+). Anal. ($C_{18}H_{15}ClN_4$) C, H, N.

Ethyl 3-Butylaminoproprionate.[24] **Method F.** The compound was prepared according to the general procedure of Holley and Holley:[22] ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.10 (dd, J = 7, 14.1 Hz, 2 H), 2.83 (t, J = 6.4 Hz, 2 H), 2.57 (t, J = 6.8 Hz, 2 H), 3.47 (t, J = 6.4 Hz, 2 H), 1.58 (broad s, 1 H), 1.48−1.15 (m, 4 H), 1.22 (t, J = 7 Hz, 3 H), 0.87 (t, J = 7.2 Hz, 3 H); LC−MS (APCI) *m/z* 173.9 (MH^+).

Ethyl 4-[Butyl-(2-ethoxycarbonylethyl)amino]-6-methyl-2-trifluoromethanesulfonyloxynicotinate (14b). 14b was prepared using method A: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.46 (s, 1 H), 4.35 (q, J = 7.2 Hz, 2 H), 4.13 (q, J = 7.2 Hz, 2 H), 3.57 (q, J = 7.5 Hz, 2 H), 3.23 (q, J = 7.5 Hz, 2 H), 2.38 (s, 3 H), 1.63−1.50 (m, 2 H), 1.38 (t, J = 7.2 Hz, 3 H), 1.37−1.18 (m, 4 H), 1.24 (t, J = 7.2 Hz, 3 H), 0.93 (t, J = 7.4 Hz, 3 H); LC−MS (APCI) *m/z* 405.0 (MH^+). Anal. ($C_{19}H_{27}F_3N_2O_7S\cdot 0.4H_2O$) C, H, N.

1-Butyl-5-chloro-7-methyl-2,3-dihydro-1*H*-[1,6]naphthyridin-4-one (7b). 7b was prepared using method C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.33 (s, 1 H), 3.61−3.52 (m, 1 H), 3.45 (pent, J = 3 Hz, 1 H), 3.37 (t, J = 7.5 Hz, 2 H), 2.70 (dd, J = 3.6, 2.4 Hz, 2 H), 2.39 (s, 3 H), 1.90−1.63 (m, 2 H), 1.43 (hex, J = 7.4 Hz, 2 H), 0.99 (t, J = 7.4 Hz, 3 H); LC−MS (APCI) *m/z* 252.8 (MH^+).

1-Butyl-5-chloro-4-[1-methoxymeth-(*E*)-ylidene]-7-methyl-1,2,3,4-tetrahydro[1,6]naphthyridine (18b). 18b was prepared using method D: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.91 (s, 1 H), 6.18 (s, 1 H), 3.71 (s, 3 H), 3.34 (t, J = 6 Hz, 2 H), 3.24 (t, J = 7.5 Hz, 2 H), 2.52 (t, J = 6 Hz, 2 H), 2.34 (s, 3 H), 1.66−1.50 (m, 2 H), 1.45−1.27 (m, 2 H), 0.96 (t, J = 7.4 Hz, 3 H); LC−MS (APCI) *m/z* 281.0 (MH^+).

5-Butyl-1-(4-chloro-2-cyanophenyl)-7-methyl-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene (19b). 19b was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92

(d, J = 9 Hz, 1 H), 7.71 (d, J = 2.4 Hz, 1 H), 7.64 (dd, J = 9, 2.4 Hz, 1 H), 6.91 (s, 1 H), 6.13 (s, 1 H), 3.46 (t, J = 3.4 Hz, 2 H), 3.35 (t, J = 7.5 Hz, 2 H), 3.05 (t, J = 5.7 Hz, 2 H), 2.51 (s, 3 H), 1.72−1.60 (m, 2 H), 1.43 (pent, J = 4.6 Hz, 2 H), 0.98 (s, 3 H); LC−MS (APCI) *m/z* 365.0 (MH^+). Anal. ($C_{21}H_{21}ClN_4 \cdot {}^4/_3H_2O$) C: calcd, 64.87; found, 65.23. H: calcd, 6.13; found, 6.64. N: calcd, 14.41; found, 13.64.

Ethyl 3-(1-Ethyl-propylamino)proprionate. The compound was prepared using method F: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.15 (q, J = 6.9 Hz, 2 H), 2.85 (t, J = 6.3 Hz, 2 H), 2.60−2.50 (m, 1 H), 2.49 (t, J = 6.3 Hz, 2 H), 1.50−1.30 (m, 4 H), 1.26 (t, J = 6.9 Hz, 3 H), 0.94−0.85 (m, 6 H); GC−MS (EI) *m/z* 187 (M^+).

Ethyl 4-[(2-Ethoxycarbonylethyl)(1-ethylpropyl)amino]-6-methyl-2-trifluoromethanesulfonyloxynicotinate (14c). 14c was prepared using method A and used without further purification in the preparation of **16c**.

Ethyl 2-Chloro-4-[(2-ethoxycarbonylethyl)(1-ethylpropyl)amino]-6-methylnicotinate (16c). 16c was prepared using method B: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51 (s, 1 H), 4.33(q, J = 4.4 Hz, 2 H), 4.12 (q, J = 4.4 Hz, 2 H), 3.44−3.30 (m, 3 H), 2.50−2.47 (m, 2 H), 2.03 (s, 3 H), 1.59−1.51 (m, 4 H), 1.36 (t, J = 4.2 Hz, 3 H), 1.24 (t, J = 3.6 Hz, 3 H), 0.84 (t, J = 4.4 Hz, 6 H); LC−MS (APCI) *m/z* 385.1 (MH^+). Anal. ($C_{19}H_{29}ClN_2O_4 \cdot 0.5C_4H_8O_2$) C, H, N.

5-Chloro-1-(1-ethylpropyl)-7-methyl-2,3-dihydro-1*H*-[1,6]naphthyridin-4-one (7c). 7c was prepared using method C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.54 (s, 1 H), 3.80−3.70 (m, 1 H), 3.37 (t, J = 4.1 Hz, 2 H), 2.61 (t, J = 4.1 Hz, 2 H), 2.36 (s, 3 H), 1.66−1.54 (m, 4 H), 0.89 (t, J = 4.4 Hz, 6 H); LC−MS (APCI) *m/z* 267.0 (MH^+). Anal. ($C_{14}H_{19}ClN_2O$) C, H, N.

5-Chloro-1-(1-ethylpropyl)-4-[1-methoxymeth-(*E*)-ylidene]-7-methyl-1,2,3,4-tetrahydro[1,6]naphthyridine (18c). 18c was prepared using method D: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.88 (s, 1 H), 6.36 (s, 1 H), 3.70 (s, 3 H), 3.70−3.60 (m, 1 H), 3.12 (t, J = 3.6 Hz, 2 H), 2.47 (t, J = 3.6 Hz, 2 H), 2.32 (s, 3 H), 1.55 (p, J = 4.4 Hz, 4 H), 0.84 (t, J = 4.4 Hz, 6 H); LC−MS (APCI) *m/z* 385.1 (MH^+). Anal. ($C_{16}H_{23}ClN_2O \cdot 0.25C_7H_{16}$) C, H, N.

1-(4-Chloro-2-cyanophenyl)-5-(1-ethylpropyl)-7-methyl-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene (19c). 19c was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 (d, J = 8.7 Hz, 1 H), 7.71 (d, J = 2.4 Hz, 1 H), 7.64 (dd, J = 8.7, 2.4 Hz, 1 H), 6.92 (s, 1 H), 6.18 (s, 1 H), 3.63 (pentet, J = 7.5 Hz, 1 H), 3.33 (t, J = 6.2 Hz, 2 H), 3.01 (t, J = 5.7 Hz, 2 H), 2.50 (s, 3 H), 1.69−1.59 (m, 4 H), 0.93 (t, J = 7.4 Hz, 6 H); LC−MS (APCI) *m/z* 379.0 (MH^+). Anal. ($C_{18}H_{15}ClN_4 \cdot 0.5H_2O \cdot 0.5C_3H_6O$) C, H, N.

Ethyl 3-(1-Propylbutylamino)proprionate. The compound was prepared using method F: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.13 (q, J = 7.5 Hz, 2 H), 2.84 (t, J = 6.6 Hz, 2 H), 2.48 (t, J = 6.6 Hz, 2 H), 2.50−2.42 (m, 1 H), 1.38−1.30 (m, 8 H), 1.26 (t, J = 7.5 Hz, 3 H), 0.94−0.86 (m, 6 H); GC−MS (EI) *m/z* 215 (M^+).

Ethyl 4-[(2-Ethoxycarbonylethyl)(1-propylbutyl)amino]-6-methyl-2-trifluoromethanesulfonyloxynicotinate (14d). 14d was prepared using method A: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.53 (s, 1 H), 4.31 (q, J = 4.3 Hz, 2 H), 4.12 (q, J = 4.3 Hz, 2 H), 3.50−3.44 (m, 3 H), 2.49−2.45 (m, 2 H), 2.37 (s, 3 H), 1.54−1.46 (m, 4 H), 1.35 (t, J = 4.3 Hz, 3 H), 1.24 (t, J = 4.3 Hz, 3 H), 1.24−1.18 (m, 4 H), 0.86 (t, J = 4.3 Hz, 6 H); GC−MS (EI) *m/z* 526 (M^+). Anal. ($C_{22}H_{33}F_3N_2O_7S$) C, H, N.

5-Chloro-1-(1-propylbutyl)-7-methyl-2,3-dihydro-1*H*-[1,6]naphthyridin-4-one (7d). 7d was prepared using method C. This material was identical to the material prepared according to Scheme 1.

Ethyl 2-Chloro-4-[(2-ethoxycarbonylethyl)(1-propylbutyl)amino]-6-methylnicotinate (16d). 16d was prepared using method B: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.49 (s, 1 H), 4.32 (q, J = 4.3 Hz, 2 H), 4.12 (q, J = 4.3 Hz, 2 H), 3.54 (p, J = 4.2 Hz, 1 H), 3.44−3.40 (m, 2 H), 2.48−2.45 (m, 2 H), 2.40 (s, 3 H), 1.52−1.45 (m, 4 H), 1.36 (t, J = 4.2 Hz, 3 H), 1.25 (t, J = 4.2 Hz, 3 H), 1.26−1.20 (m, 4 H), 0.87 (t, J = 4.2 Hz, 6 H); LC−MS (APCI) *m/z* 413.0 (MH^+). Anal. ($C_{21}H_{33}ClN_2O_4$) C, H, N.

5-Chloro-1-(1-propylbutyl)-4-[1-methoxymeth-(*E*)-ylidene]-7-methyl-1,2,3,4-tetrahydro[1,6]naphthyridine (18d). 18d was prepared using method D: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.83 (s, 1 H), 6.31 (s, 1 H), 3.85−3.80 (m, 1 H), 3.65 (s, 3 H), 3.07 (t, J = 3.5 Hz, 2 H), 2.41 (t, J = 3.7 Hz, 2 H), 2.27 (s, 3 H), 1.50−1.41 (m, 2 H), 1.41−1.38 (m, 2 H), 1.23−1.17 (m, 4 H), 0.83 (t, J = 4.4 Hz, 6 H); LC−MS (APCI) *m/z* 323.0 (MH^+). Anal. ($C_{18}H_{27}ClN_2O$) C, H, N.

1-(4-Chloro-2-cyanophenyl)-5-(1-propylbutyl)-7-methyl-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene Hydrochloride (19d). 19d was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82−7.81 (m, 1 H), 7.79 (s, 1 H), 7.72−7.71 (m, 1 H), 6.72 (s, 1 H), 6.20 (s, 1 H), 4.00−3.90 (m, 1 H), 3.50 (t, J = 5.0 Hz, 2 H), 3.05 (t, J = 4.8 Hz, 2 H), 2.81 (s, 3 H), 1.72−1.55 (m, 4 H), 1.36−1.27 (m, 4 H), 0.94 (t, 5.7 Hz, 6 H); LC−MS (APCI) *m/z* 407.1 (MH^+). Anal. ($C_{24}H_{27}ClN_4 \cdot HCl \cdot H_2O$) C, H, N.

Ethyl 3-(1-Methoxymethylpropylamino)proprionate. The compound was prepared using method F: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.13 (q, J = 6.9 Hz, 2 H), 3.39−3.23 (m, 2 H), 3.33 (s, 3 H), 2.91−2.85, (m, 2 H), 2.67−2.58, (m, 1 H), 2.49 (t, J = 6.3 Hz, 2 H), 1.47−1.41 (m, 2 H), 1.26, (t, J = 7.2 Hz, 3 H), 0.89 (t, J = 7.5 Hz, 3 H).

Ethyl 4-[(2-Ethoxycarbonylethyl)(1-methoxymethylpropyl)amino]-6-methyl-2-trifluoromethanesulfonyloxynicotinate (14e). 14e was prepared using method A: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.69 (s, 1 H), 4.35 (q, J = 6.9 Hz, 2 H), 4.13 (q, J = 7.2 Hz, 2 H), 3.69−3.59 (m, 1 H), 3.55−3.42 (m, 4 H), 3.30 (s, 3 H), 2.62−2.37 (m, 2 H), 2.40 (s, 3 H), 1.65−1.60 (m, 2 H), 1.38 (t, J = 6.9 Hz, 3 H), 1.25 (t, J = 7.1 Hz, 3 H), 0.87 (t, J = 7.2 Hz, 3 H); LC−MS (APCI) *m/z* 515.1 (MH^+). Anal. ($C_{20}H_{29}F_3N_2O_8S$) C, H, N.

5-Chloro-1-(1-methoxymethylpropyl)-7-methyl-2,3-dihydro-1*H*-[1,6]naphthyridin-4-one (7e). 7e was prepared using method C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.54 (s, 1 H), 4.08−4.03 (m, 1 H), 3.54−3.45 (m, 4 H), 3.33 (s, 3 H), 2.68−2.62 (m, 2 H), 2.39 (s, 3 H), 1.74−1.61 (m, 2 H), 0.98 (t, J = 7.2 Hz, 3 H); LC−MS (APCI) *m/z* 283.1 (MH^+). Anal. ($C_{14}H_{19}ClN_2O_2$) C, H, N.

5-Chloro-1-(1-methoxymethylpropyl)-4-[1-methoxymeth-(*E*)-ylidene]-7-methyl-1,2,3,4-tetrahydro[1,6]-naphthyridine (18e). 18e was prepared using method D: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90 (s, 1 H), 6.37 (s, 1 H), 3.96−3.88 (m, 1 H), 3.71 (s, 3 H), 3.64−3.37 (m, 2 H), 3.32 (s, 3 H), 3.25−3.20 (m, 2 H), 2.49 (t, J = 6.6 Hz, 2 H), 2.33 (s, 3 H), 1.65−1.58 (m, 2 H), 0.90 (t, J = 7.2 Hz, 3 H); LC−MS (APCI) *m/z* 311.1 (MH^+). Anal. ($C_{16}H_{23}ClN_2O_2$) C, H, N.

1-(4-Chloro-2-cyanophenyl)-5-(1-methoxymethylpropyl)-7-methyl-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene (19e). 19e was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, J = 9.0 Hz, 1 H), 7.72 (d, J = 2.4 Hz, 1 H), 7.64 (dd, J = 8.7, 2.4 Hz, 1 H), 6.91 (s, 1 H), 6.21 (s, 1 H), 3.93−3.88 (m, 1 H), 3.61−3.35 (m, 4 H), 3.35 (s, 3 H), 3.02−2.98 (m, 2 H), 2.50 (s, 3 H), 1.75−1.68 (m, 2 H), 0.95 (t, J = 7.5 Hz, 3 H); LC−MS (APCI) *m/z* 395.0 (MH^+). Anal. ($C_{22}H_{23}ClN_4O \cdot 0.5C_3H_6O$) C, H, N.

Ethyl 3-(2-Methoxy-1-methoxymethylethylamino)proprionate. The compound was prepared using method F: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.16 (q, J = 7.1 Hz, 2 H), 3.71−3.55 (m, 5 H), 3.37 (s, 6 H), 3.22 (t, J = 6.2 Hz, 2 H), 2.67 (t, J = 6.2 Hz, 2 H), 1.27 (t, J = 7.1 Hz, 3 H); LC−MS (APCI) *m/z* 220.0 (MH^+).

Ethyl 4-[(2-Ethoxycarbonylethyl)(2-methoxy-1-methoxymethylethyl)amino]-6-methyl-2-trifluoromethanesulfonyloxynicotinate (14f). 14f was prepared using method A: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.70 (s, 1 H), 4.36 (q, J = 7.1 Hz, 2 H), 4.13 (q, J = 7.1 Hz, 2 H), 3.87−3.80 (m, 1H), 3.63−3.48 (m, 6 H), 3.28 (s, 6 H), 2.48 (t, J = 7.7 Hz, 2 H), 2.40 (s, 3 H), 1.37 (t, J = 7.1 Hz, 3 H), 1.24 (t, J = 7.1 Hz, 3 H); LC−MS (APCI) *m/z* 531.0 (MH^+).

5-Chloro-1-(2-methoxy-1-methoxymethylethyl)-7-methyl-2,3-dihydro-1*H*-[1,6]naphthyridin-4-one (7f). 7f was prepared using method C: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.51 (s, 1 H), 4.27 (p, J = 6.1 Hz, 1 H), 3.66−3.56 (m, 6 H), 3.35 (s, 6 H), 2.65 (t, J = 7.0 Hz, 2 H), 2.38 (s, 3 H); LC−MS (APCI) m/z 298.9 (MH^+). Anal. ($C_{14}H_{19}ClN_2O_3$) C, H, N.

5-Chloro-1-(2-methoxy-1-methoxymethylethyl)-4-[1-methoxymeth-(*E*)-ylidene]-7-methyl-1,2,3,4-tetrahydro-[1,6]naphthyridine (18f). 18f was prepared using method D: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.90 (s, 1 H), 6.35 (s, 1 H), 4.16 (p, J = 6.1 Hz, 1 H), 3.71 (s, 3 H), 3.62−3.51 (m, 4 H), 3.36−3.32 (m, 8 H), 2.48 (t, J = 6.3 Hz, 2 H), 2.33 (s, 3 H); LC−MS (APCI) m/z 326.8 (MH^+).

1-(4-Chloro-2-cyanophenyl)-5-(2-methoxy-1-methoxymethylethyl)-7-methyl-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene (19f). 19f was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, J = 8.7 Hz, 1 H), 7.73 (d, J = 2.4 Hz, 1 H), 7.68−7.64 (m, 1 H), 6.90 (s, 1 H), 6.22 (s, 1 H), 4.22−4.17 (m, 1 H), 3.72−3.63 (m, 4 H), 3.57 (t, J = 5.8 Hz, 2 H), 3.37 (s, 6 H), 3.02 (t, J = 5.8 Hz, 2 H), 2.52 (s, 3 H); LC−MS (APCI) m/z 411.1 (MH^+). Anal. ($C_{22}H_{23}ClN_4O_2\cdot H_2O$) C, H, N.

1-(2-Chloro-4-methylphenyl)-7-methyl-5-(1-propylbutyl)-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene Hydrochloride (19g). 19g was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46−7.40 (m, 2 H), 7.30−7.26 (m, 1 H), 6.60 (s, 1 H), 6.14 (s, 1 H), 4.00−3.88 (m, 1 H), 3.51−3.47 (m, 2 H), 3.07−3.03 (m, 2 H), 2.81 (s, 3 H), 2.44 (s, 3 H), 1.75−1.54 (m, 4 H), 1.39−1.27 (m, 4 H), 0.93 (t, J = 7.2 Hz, 6 H); LC−MS (APCI) m/z 396.2 (MH^+). Anal. ($C_{24}H_{30}ClN_3\cdot 2HCl\cdot 1.5H_2O$) C, H, N.

1-(2,4-Dichlorophenyl)-7-methyl-5-(1-propylbutyl)-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene Hydrochloride (19h). 19h was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, J = 8.7 Hz, 1 H), 7.53 (d, J = 2.1 Hz, 1 H), 7.33 (dd, J = 9, 2.4 Hz, 1 H), 6.67 (s, 1 H), 6.12 (s, 1 H), 3.90−3.78 (m, 1 H), 3.33 (t, J = 6.3 Hz, 2 H), 2.99 (t, J = 5.7 Hz, 2 H), 2.47 (s, 3 H), 1.76−1.42 (m, 8 H), 0.93 (t, J = 7.5 Hz, 6 H); LC−MS (APCI) m/z 416.0 (MH^+). Anal. ($C_{23}H_{27}Cl_2N_3\cdot 0.5H_2O$) C, H, N.

1-(2-Chloro-4-cyanophenyl)-7-methyl-5-(1-propylbutyl)-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene Hydrochloride (19i). 19i was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, J = 1.4 Hz, 1 H), 7.74 (dd, J = 6.0, 1.4 Hz, 1 H), 7.67 (d, J = 6.4 Hz, 1 H), 6.61 (s, 1 H), 6.19 (s, 1 H), 3.95−3.93 (m, 1 H), 3.35 (t, J = 5.0 Hz, 2 H), 3.05 (t, J = 5.0 Hz, 2 H), 2.75 (s, 3 H), 1.69−1.56 (m, 4 H), 1.34−1.27 (m, 4 H), 0.92 (t, J = 5.9 Hz, 6 H); LC−MS (APCI) m/z 407.1 (MH^+). Anal. ($C_{24}H_{27}ClN_4\cdot HCl\cdot 1.67H_2O$) C, H, N.

1-(4-Methoxy-2-methylphenyl)-7-methyl-5-(1-propylbutyl)-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene Hydromethanesulfonate (19j). 19j was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (d, J = 7.5 Hz, 1 H), 6.92−6.86 (m, 2 H), 6.54 (s, 1 H), 6.16 (s, 1 H), 4.00−3.88 (m, 1 H), 3.87 (s, 3 H), 3.50−3.45 (m, 2 H), 3.06−3.03 (m, 2 H), 2.74 (s, 3 H), 2.47 (s, 3 H), 2.10 (s, 3 H), 1.74−1.53 (m, 4 H), 1.40−1.26 (m, 4 H), 0.93 (t, J = 6.0 Hz, 6 H); LC−MS (APCI) m/z 392.2 (MH^+). Anal. ($C_{25}H_{33}N_4O\cdot CH_4SO_3$) C, H, N.

1-(2-Chloro-4-methanesulfonylphenyl)-7-methyl-5-(1-propylbutyl)-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene Hydrochloride (19k). 19k was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.14 (d, J = 1.9 Hz, 1 H), 8.00 (dd, J = 8.5, 1.5 Hz, 1 H), 7.69 (d, J = 8.5 Hz, 1 H), 6.69 (s, 1 H), 6.17 (s, 1 H), 3.94−3.92 (m, 1 H), 3.50 (t, J = 6.1 Hz, 2 H), 3.20 (s, 3 H), 3.06 (t, J = 5.7 Hz, 2 H), 2.69 (s, 3 H), 1.69−1.57 (m, 4 H), 1.34−1.27 (m, 4 H), 0.92 (t, J = 7.2 Hz, 6 H); LC−MS (APCI) m/z 460.1 (MH^+). Anal. ($C_{24}H_{30}ClN_3O_2S\cdot HCl\cdot 1.5H_2O$) C, H, N.

1-(2,6-Dimethoxypyridin-3-yl)-7-methyl-5-(1-propylbutyl)-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene Hydrochloride (19l). 19l was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (d, J = 6.4 Hz, 1 H), 6.56 (s, 1 H), 6.45 (d, J = 6.2 Hz, 1 H), 6.13 (s, 1 H), 4.00 (s, 3 H), 3.98 (s, 3 H), 3.98−3,88 (m, 1 H), 3.47 (t, J = 5.0 Hz, 2 H), 3.03 (t, J = 4.8 Hz, 3 H), 2.81 (s, 3 H), 1.67−1.59 (m, 4 H), 1.34−1.30 (m, 4 H), 0.93 (t, J = 6.0 Hz, 6 H); LC−MS (APCI) m/z 409.2 (MH^+). Anal. ($C_{24}H_{32}N_4O_2\cdot 2HCl\cdot 0.5H_2O$) C, H, N.

1-(2-Methoxyphenyl)-7-methyl-5-(1-propylbutyl)-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene Hydrotrifluoroacetate (19m). 19m was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60 (m, 1 H), 7.30 (m, 1 H), 7.04−7.09 (m, 2 H), 6.75 (s, 1 H), 6.11 (s, 1 H), 3.83 (s, 3 H), 3.35 (dd, J = 5.4, 6.0 Hz, 2 H), 3.01 (dd, J = 5.4, 6.0 Hz, 2 H), 2.51 (s, 3 H), 1.25−1.80 (m, 8 H), 0.92 (t, J = 7.5 Hz, 6 H); LC−MS (APCI) m/z 378.0 (MH^+). Anal. ($C_{24}H_{31}N_3O\cdot 0.13C_2HF_3O_2\cdot 0.13H_2O$) C, H, N.

1-(4-Methylphenyl)-7-methyl-5-(1-propylbutyl)-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene (19n). 19n was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, J = 8.1 Hz, 2 H), 7.19 (d, J = 8.1 Hz, 2 H), 6.73 (s, 1 H), 6.05 (s, 1 H), 3.78 (m, 1 H), 3.27 (dd, J = 6.0, 6.0 Hz, 2 H), 2.93 (dd, J = 6.0, 6.0 Hz, 2 H), 2.48 (s, 3 H), 2.30 (s, 3 H), 1.18−1.64 (m, 8 H), 0.844 (t, J = 7.2 Hz, 6 H); LC−MS (APCI) m/z 362.1 (MH^+). Anal. ($C_{24}H_{31}N_3\cdot 0.25H_2O$) C, H, N.

1-(3-Methoxyphenyl)-7-methyl-5-(1-propylbutyl)-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene (19o). 19o was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56−7.54 (m, 1 H), 7.36−7.32 (m, 2 H), 6.85 (s, 1 H), 6.77−6.74 (m, 1 H), 6.13 (s, 1 H), 3.85 (s, 3 H), 3.84−3.82 (m, 1 H), 3.32 (t, J = 6.0 Hz, 2 H), 2.99 (t, J = 5.1 Hz, 2 H), 2.53 (s, 3 H), 1.63−1.30 (m, 8 H), 0.91 (t, J = 7.2 Hz, 6 H); LC−MS (APCI) m/z 378.0 (MH^+). Anal. ($C_{24}H_{31}N_3O\cdot 0.2H_2O$) C, H, N.

1-(3-Isopropoxypropyl)-7-methyl-5-(1-propylbutyl)-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene (19p). 19p was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.48 (s, 1 H), 6.02 (s, 1 H), 4.23 (t, J = 7.2 Hz, 2 H), 3.85−3.75 (m, 1 H), 3.56−3.52 (m, 1 H), 3.42 (t, J = 6.3 Hz, 2 H), 3.27 (t, J = 6.0 Hz, 2 H), 2.92 (t, J = 5.4 Hz, 2 H), 2.52 (s, 3 H), 2.15−2.04 (m, 2 H), 1.60−1.23 (m, 8 H), 1.16 (d, J = 6.3 Hz, 6 H), 0.89 (t, J = 7.2 Hz, 6 H); LC−MS (APCI) m/z 372.1 (MH^+). Anal. ($C_{23}H_{37}N_3O\cdot 0.2H_2O$) C, H, N.

1-(4-Chloro-2-methylbenzyl)-7-methyl-5-(1-propylbutyl)-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene (19q). 19q was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17 (m,1 H), 7.09 (dd, J = 8.1, 2.1 Hz, 1 H), 6.89 (d, J = 8.1 Hz, 1 H), 6.27 (s, 1 H), 6.08 (s, 1 H), 5.32 (br s, 2 H), 3.82 (m, 1 H), 3.30 (dd, J = 6.0, 6.0 Hz, 2 H), 2.91 (dd, J = 6.0, 6.0 Hz, 2 H), 2.55 (s, 3 H), 2.30 (s, 3 H), 1.26−1.80 (m, 8 H), 0.91 (t, J = 7.5 Hz, 6 H); LC−MS (APCI) m/z 410.1 (MH^+). Anal. ($C_{25}H_{32}ClN_3\cdot 0.33H_2O$) C, H, N.

5-(1-Ethylpropyl)-7-methyl-1-(2-methyl-4-trifluoromethoxyphenyl)-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene Hydrochloride (19r). 19r was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 8.5 Hz, 1 H), 7.22 (s, 1 H), 7.17 (d, J = 8.3 Hz, 1 H), 6.54 (s, 1 H), 6.18 (s, 1 H), 3.78−3.74 (m, 1 H), 3.49 (t, J = 5.8 Hz, 2 H), 3.05 (t, J = 5.8 Hz, 2 H), 2.76 (s, 3 H), 2.17 (s, 3 H), 1.76−1.64 (m, 4 H), 0.92 (t, J = 7.2 Hz, 6 H); LC−MS (APCI) m/z 418.1 (MH^+). Anal. ($C_{23}H_{26}F_3N_3O\cdot 0.25HCl\cdot 2H_2O$) C, H, N.

1-(4-Chloro-2,6-dimethylphenyl)-5-(1-methoxymethylpropyl)-7-methyl-1,3,4,5-tetrahydro-1,5,8-triazaacenaphthylene Hydrochloride (19s). 19s was prepared using method E: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (s, 2 H), 6.42 (s, 1 H), 6.21 (s, 1 H), 4.08−4.00 (m, 1 H), 3.61−3.53 (m, 4 H), 3.35 (s, 3 H), 3.08−3.04 (m, 2 H), 2.75 (s, 3 H), 2.02 (d, J = 3.9 Hz, 6 H), 1.77−1.71 (m, 2 H), 0.99 (t, J = 7.2 Hz, 3 H); LC−MS (APCI) m/z 398.1 (MH^+). Anal. ($C_{23}H_{28}ClN_3O\cdot HCl\cdot 1.2H_2O$) C, H, N.

1-(2,4-Dichlorophenyl)-4-methyl-6-(1-propylbutyl)-1,6,7,8-tetrahydro-1,2,3,6-tetraazaacenaphthylene (20a). Ketone **7d** (308 mg, 1.04 mmol) and 2,4-dichlorophenylhydrazine hydrochloride (231 mg, 1.08 mmol) were dissolved in ethanol (5 mL) and heated to reflux for 18 h. The mixture was cooled, poured into aqueous sodium bicarbonate (20 mL), and extracted three times with ethyl acetate (10 mL). The combined extracts were dried ($MgSO_4$) and concentrated, and the residue was purified by flash chromatography (elution with 1% methanol and 0.5% aqueous ammonia in DCM) to afford

195 mg (45%) of **20a** as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.57 (d, J = 2.1 Hz, 1 H), 7.52 (d, J = 8.4 Hz, 1 H), 7.36 (dd, J = 8.4, 2.4 Hz, 1 H), 6.08 (s, 1 H), 3.87−3.78 (m, 1 H), 3.51 (dd, J = 6.3, 6.3 Hz, 2 H), 3.15 (dd, J = 6.5, 6.5 Hz, 2 H), 2.51 (s, 3 H), 1.72−1.50 (m, 4 H), 1.42−1.25 (m, 4 H), 0.93 (t, 7.2 Hz, 6 H); LC−MS (APCI) m/z 417.0 (MH^+). Anal. ($C_{22}H_{26}Cl_2N_4 \cdot 0.25CH_2Cl_2$) C, H, N. A second fraction afforded 82 mg (19%) of **22a**, which was identical to the material prepared below. The two regioisomers were identified by the characteristic downfield shift of the pyridine hydrogen atoms in the ^{1}H NMR spectra of 2-substituted pyrazolo[3,4-*b*]-pyridines relative to their 1-substituted isomers.[25]

2-(2,4-Dichlorophenyl)-4-methyl-6-(1-propylbutyl)-2,6,7,8-tetrahydro-1,2,3,6-tetraazaacenaphthylene (22a). Pyridine **7d** (503 mg, 1.63 mmol) and 2,4-dichlorophenylhydrazine hydrochloride (390 mg, 1.83 mmol) were dissolved in methanol (5 mL) and heated at 60 °C for 18 h. The mixture was concentrated under vacuum, the residue was diluted with diphenyl ether (2 mL), and the mixture was placed in a preheated oil bath at 200 °C. After 2 h at this temperature, the mixture was cooled, diluted with DCM, and purified by flash chromatography (elution with 3% methanol and 0.5% aqueous ammonia in DCM) to afford 439 mg (65%) of **22a** as a tan solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.56 (d, J = 9.0 Hz, 1 H), 7.55 (d, J = 1.8 Hz, 1 H), 7.40 (dd, J = 8.4, 2.1 Hz, 1 H), 5.92 (s, 1 H), 3.92−3.82 (m, 1 H), 3.57 (dd, J = 6.3, 6.3 Hz, 2 H), 3.09 (dd, J = 6.3, 6.3 Hz, 2 H), 2.66 (s, 3 H), 1.72−1.52 (m, 4 H), 1.40−1.25 (m, 4 H), 0.93 (t, 7.2 Hz, 6 H); LC−MS (APCI) m/z 417.0 (MH^+). Anal. ($C_{22}H_{26}Cl_2N_4 \cdot 0.33H_2O$) C, H, N.

This material was converted to the methanesulfonic acid salt as described in General Methods: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61 (s, 1 H), 7.58 (d, J = 8.3 Hz, 1 H), 7.48 (dd, J = 8.6, 1.5 Hz, 1 H), 6.20 (s, 1 H), 4.00−3.85 (m, 1 H), 3.69 (t, J = 6.7 Hz, 2 H), 3.22 (t, J = 6.7 Hz, 2 H), 2.79 (s, 3 H), 2.46 (s, 3 H), 1.72−1.64 (m, 4 H), 1.36−1.29 (m, 4 H), 0.95 (t, J = 7.3 Hz, 6 H); LC−MS (APCI) m/z 417.1 (MH^+). Anal. ($C_{22}H_{26}Cl_2N_4 \cdot CH_4SO_3$) C, H, N.

Biology. In Vitro Binding and Functional Studies. Radioligand binding assays and functional inhibition of CRF-induced cAMP production were performed essentially as previously described for the cloned CRF_1 receptor in heterologously expressed cell membranes.[26] Equilibrium binding of unlabeled ligands was measured in duplicate by inhibition of radioligand binding ([^{125}I]sauvagine) to LtK^- cells expressing the human CRF_1 receptor. Thirty microliters of assay buffer (DPBS, 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4, 2.7 mM KCl, 138 mM NaCl) supplemented with 10 mM $MgCl_2$, 2 mM ethylene glycol bis[β-aminoethyl]-*N*,*N*,*N*′,*N*′-tetraacetic acid, pH 7.4), 20 μL of unlabeled ligand, 50 μL of radioligand, and 100 μL of L-CRF_1 receptor cell membranes were sequentially added to low-protein-binding 96-well plates (Corning no. 3605). The final concentration of radioligand was approximately 90 pM for [^{125}I]sauvagine with a total of 5 μg of membrane. Unlabeled compounds were serially diluted for final concentrations of 10 pM to 1 μM. Following a 2 h incubation at room temperature, bound and free radioligands were separated by rapid vacuum filtration. In all assays total radioligand bound to the filter (total binding) was less than 20% of the total amount of radioligand added. Nonspecific binding was determined in the presence of an excess of the unlabeled analogue of the radioligand. Bound and nonspecific radioactivity was monitored using a Packard Cobra II γ counter (78% efficiency) and analyzed using the nonlinear curve-fitting algorithm software Prism (GraphPad Inc., CA).

CRF-Stimulated ACTH Release from Cultured Rat Anterior Pituitary Cells. For the inhibition of ACTH release from primary rat pituitary cell cultures, five whole pituitaries are collected from 7-week-old female Sprague Dawley rats. Pituitaries are washed six times with HEPES buffer (2.5 g/L BSA, 10 mg/L deoxyribonuclease I, 8.0 g/L NaCl, 0.37 g/L KCl, 100 mg/L sodium phosphate dibasic, 6.0 g/L HEPES, 2.0 g/L glucose) and minced. The tissue is then digested with 10 mL of collagenase for 1.5 h at 37 °C, with trituration every 30 min. The digest is then transferred to a 50 mL conical centrifuge tube and centrifuged at 1000 rpm for 4 min. The supernatant is discarded, and the pellet was resuspended in 10 mL of neuraminidase solution and incubated for 9 min at 37 °C. The suspension is centrifuged at 1000 rpm for 5 min, and the pellet was washed once with 10 mL of BBM-T medium (11.49 g/L custom media mixture, Irvine Scientific, CA; 1.83 g of Na_2CO_3/L; 2.4 g of HEPES/L; 2.0 g/L BSA; 10.0 mg/L transferrin; 50 000 IU/L penicillin and streptomycin; 1 μg/L insulin; 0.1 μg/L EGF; 0.4 μg/L T3; 0.7 μg/L PTH; 10 μg/L glucagon). The resulting pellet is finally resuspended in 3% FCS/BBM-T medium and cultured in 96-well tissue culture plates for 2−3 days at a density of 40 000 cells/well in a final volume of 200 μL of medium.

For the assay of antagonists, cells are washed once with BBM-T, test samples are added in various concentrations (1 μM to 1 pM) with 0.5 nM r/hCRF in 200 μL of BBM-T and incubated for 4 h at 37 °C. The medium is then aspirated and assessed for ACTH release using a standard RIA kit (MP Biomedicals, NY). Again, all data were analyzed using the nonlinear curve-fitting algorithm software Prism as above.

Restraint-Stress-Induced ACTH Release in Mice. Male CD-1 mice (24−26 g, 10/group) were weighed and handled once a day prior to the restraint stress. On the day of testing, animals were dosed by oral gavage with compound or vehicle (10 mL/kg 5% D-mannitol (w/v) in water) 60 min prior to the initiation of the stressor. Restraint stress was evoked by allowing the mice to enter a 50 mL plastic conical tube (tip removed to allow free flow of air) and to be held in place for 45 min. The animals cannot alter their position once inside the tube. Following the 45 min of restraint stress, the animals are removed and immediately placed in an isoflurane chamber for a maximum of 2 min, and blood is collected via cardiac puncture into 0.5 M EDTA-coated tubes. The blood is kept on ice, and plasma is separated by centrifugation (6000*g* at 4 °C for 15 min). Mice in the nonstress control group remained in their home cages for the duration of the experiment following oral gavage with the vehicle. Plasma ACTH concentration was measured using a standard ACTH RIA kit (MP Biomedicals, NY). The ACTH values over time were analyzed using repeated measures, and mixed design ANOVA with peak ACTH values was analyzed using a one-way ANOVA with post hoc Fischer's test for group differences.

Rat Pharmacokinetics. The pharmacokinetics and blood−brain barrier (BBB) penetrations were determined in male Sprague Dawley rats following an intravenous (iv, N = 3/time point) and oral (po, N = 3/time point) dose. The dosing solution was prepared in purified water and filtered through a 0.2 μm Nylon filter before administration (2 mL/kg) via tail vain (iv) or a gavage (po). Terminal blood and brain tissue samples were taken at predetermined time for composite sampling. All plasma and tissue samples were flash-frozen in liquid nitrogen within 10 min of sampling and stored in −70 °C or below until analysis. The bioanalytical method applied for the measurement of test articles in plasma along with added internal standard consisted of precipitation with 200 μL of acetonitrile from 50 μL of plasma, centrifugation, recovery of the supernatant, drying down in a vacuum, and then reconstitution in acetonitrile−water solutions before introduction into an LC−MS/MS system for analysis. The lower limit of quantification (LLOQ) for the analytical methods was 5 ng/mL of test article in plasma. The bioanalytical method applied for the measurement of test articles in brain tissue along with added internal standard consisted of homogenization of half of the brain tissue (longitude cut) in 2 mL of acetonitrile/water (50:50), centrifugation, and recovery of the supernatant before introduction into an LC−MS/MS system for analysis. The lower limit of quantification for the analytical methods was 5 ng/g of test article in brain tissue. All pharmacokinetic parameters were calculated from a noncompartmental model in WinNonlin program. Brain-to-plasma ratio was obtained by comparing brain AUC to plasma AUC.

Supporting Information Available: Table of elemental analysis data for reported compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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