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$CRHR_1$ Receptor Binding and Lipophilicity of Pyrrolopyrimidines, Potential Nonpeptide Corticotropin-Releasing Hormone Type 1 Receptor Antagonists

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Abstract—A series of compounds related to *N*-butyl-*N*-ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (**1**, antalarmin, Fig. 1) have been prepared and evaluated for their $CRHR_1$ binding affinity as the initial step in the development of selective high affinity hydrophilic nonpeptide corticotropin-releasing hormone type 1 receptor ($CRHR_1$) antagonists. Calculated log P (Clog P) values were used to evaluate the rank order of hydrophilicity for these analogues. Introducing oxygenated functionalities (δ-hydroxy or bis-β-ethereal) into **1** gave more hydrophilic compounds, which had good affinity for the receptor. Introducing an amino group or shortening the alkyl side chain was detrimental to $CRHR_1$ affinity. The alcohol 4-[ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]butan-1-ol (**3**), bearing a terminal hydroxyl group on an *N*-alkyl side-chain, showed the highest $CRHR_1$ binding affinity among these compounds ($K_i = 0.68$ nM), and is one of the highest affinity $CRHR_1$ ligands known. Compounds **3**–**5**, and **8,** which are likely to be less lipophilic than **1**, have high $CRHR_1$ affinity and may be valuable probes to further study the CRH system.

Introduction

Corticotropin-releasing hormone (CRH) was isolated in 1981 from ovine hypothalamus extracts and characterized as a 41-amino acid neuropeptide.[1] CRH stimulates the activity of the hypothalamic-pituitary-adrenal (HPA) axis through the release of adrenocorticotropic hormone (ACTH),[2,3] coordinating the overall response of the body to stress. It mediates pituitary ACTH release, which in turn triggers the secretion of the adrenal steroid, cortisol. In addition, CRH is found throughout the central nervous system where it acts as a peptide neurotransmitter.[4–6] The actions of CRH as a neurotransmitter are not fully understood but diverse neuropsychiatric and neurodegenerative diseases show changes in CRH secretion and action. In addition to its

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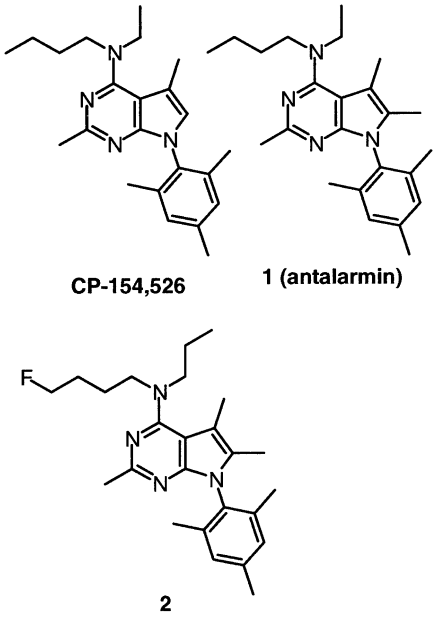


Figure 1.

central effects, CRH also acts in the periphery as an immune cytokine. CRH acts in concert with cortisol to regulate inflammatory responses found in both infection and autoimmune disorders.[7–11]

To date, there are two classes of G-protein-coupled CRH receptors ($CRHR_1$ and $CRHR_2$) that have been characterized and cloned from mouse, rat, and human,[12–17] and there is a distinct regional distribution for them in the central nervous system (CNS). The broad central distribution of CRH and $CRHR_1$ and $CRHR_2$ supports CRH as an important peptide neurotransmitter within the CNS.[4–6] Overproduction of CRH in the brain has been associated with mental disorders such as anxiety,[18] depression,[19] and substance abuse.[20] Treatment of depressed patients with antidepressant or electroconvulsive therapy resulted in decreased cerebrospinal fluid CRH as well as in improvement in the clinical conditions of these patients. Overproduction of CNS CRH may also underlie some of the symptoms of affective and addictive disorders. Selective $CRHR_1$ antagonists may be efficacious for the treatment of these disorders by blocking the effects of excess CRH on $CRHR_1$.[21] Furthermore, $CRHR_1$ antagonists may prove to be useful research tools to probe CRH and stress related disorders.[22]

Several peptide CRHR antagonists including α-helical CRH (9–41), [D-Phe^{12}, Nle^{22}]CRH (12–41), and astressin have been synthesized and studied extensively.[23–25] However, their potential clinical utility is limited because of poor $CRHR_1$ selectivity and poor penetration through the blood–brain barrier (BBB). Therefore, a potent and selective nonpeptide $CRHR_1$ antagonist with good BBB penetration could be a versatile tool to further investigate the biological effects of CRH and $CRHR_1$. In addition, such compounds would aid the discovery and development of future CRH-specific medications.

The pharmaceutical industry has led the development of $CRHR_1$ antagonists as medications.[26] The structure–activity relationships (SAR) for nonpeptide $CRHR_1$ antagonists in a wide variety of structural classes have been investigated.[26–34] Among them, the (4-dialkylamino)pyrrolo[2,3-*d*]pyrimidines, CP-154,526 (Fig. 1) and antalarmin (**1**, first described by Chen at Pfizer),[35] show high affinity and selectivity for $CRHR_1$ in binding assays and significant pharmacological effects in animal behavioral studies.[30,36–41] Non-peptide $CRHR_1$ receptor ligands, such as antalarmin[41] and its analogue, CP154, 526,[29] have been identified which are selective antagonists for $CRHR_1$ receptors; they inhibit CRH-stimulation of cAMP or CRH-stimulated ACTH release from cultured rat anterior pituitary cells. However, these pyrimidines have poor solubility, possibly due to their high lipophilicity, which restricts their practical use as medications and as radiotracers. Our efforts[42] to develop a positron emission tomography (PET) radiotracer for $CRHR_1$ resulted in the synthesis of a fluoro-substituted analogue of **1** (Fig. 1). This analogue (**2**) showed subnanomolar affinity for $CRHR_1$ ($K_i = 0.91$ nM).[42] However, only a small fraction of this compound rapidly penetrated the BBB, as shown by studies with [^{3}H]**2**.[43] As the initial step towards the important goal of obtaining $CRHR_1$-selective antagonists capable of crossing the BBB, we have synthesized a number of new ligands in the pyrrolopyrimidine series and examined their affinity for the $CRHR_1$ receptor.

High affinity ligands have been obtained in the pyrrolopyrimidines series, but attempts to decrease their lipophilicity resulted in lowering receptor affinity.[28] In order to find high affinity $CRHR_1$ antagonists, which were less lipophilic, we examined several of our formerly prepared pyrrolopyrimidine intermediate compounds, **3**, **4**,[42] **5**,[43] and **6**–**7**.[42] These, except for **6**, had not been previously evaluated as $CRHR_1$ ligands, and none were previously described chemically. We also synthesized a more oxygenated compound (**8**) that subsequently appeared in the literature.[28] Further, several compounds in this series which we have synthesized (**9**–**11**) have been mentioned, and their NMR data listed, in a patent,[35] but neither their other chemical properties, nor any pharmacological data were mentioned therein.

We believed that if a sufficiently hydrophilic compound could be obtained it would be better able to cross the BBB than antalarmin (**1**), and if it also had comparable affinity with **1** for the $CRHR_1$ receptor it might, then, be a useful template for further investigation. We hoped to find compounds with decreased lipophilicity while still retaining good affinity for the $CRHR_1$ receptor, and to do that we assumed that the relative lipophilicities of the compounds were related to their calculated log P values (Clog P); these calculated values were used as a rough numerical descriptor to indicate the trend in lipophilicity for the various molecules. In our comparison of the lipophilicities of the compounds in Table 1, we anticipated that an increase in Clog P would indicate that we had obtained a more lipophilic compound, and a decrease would imply that we had prepared a more hydrophilic compound.

Table 1. $CRHR_1$ affinity, lipophilicity, and physical properties

Compd	K_i (nM)[a]	Clog P[b]	mp (°C)	% Yield
3	0.68±0.38	5.17±1.31	150–152	90
4	3.0±0.6	6.23±1.31	139–141	100
5	1.8±0.1	5.37±1.31	151–152	96
6	2.3±0.2	6.82±1.31	139–140	100
7	55[d]	5.15±1.31	Oil	95
8	1.7±0.2	4.26±1.33	126–128	99
9	>1000	5.52±1.31	182[c]	71
10	>1000	5.61±1.31	226[c]	24
11	>1000	4.86±1.30	145–146	85
17	17[d]	3.73±1.33	95–98[e]	91
18	8[d]	3.73±1.33	95–98[e]	92
19	97[d]	5.32±1.32	218[c]	92
20	>1000	6.30±1.32	181–184	95
21	6.4±5.1	6.45±1.30	160–162	74
22	24[d]	4.66 ±1.30	155–157	91
1	2.5±0.6	6.98±1.30		

[a]The $CRHR_1$ binding affinity for the test compounds were determined using competitive displacement of [^{125}I]Tyr^0–sauvagine binding in membrane homogenates of rat cerebellum as described in the text. Except for **7**, **17**–**19**, and **22**, three binding curves conducted in duplicate were generated for each compound and the K_i values represent the mean of the three experiments±SEM.
[b]Calculated log P values. For detail, see Experimental.
[c]Decomposed.
[d]The results of a single determination.
[e]Amorphous solid.

Chemistry

4-Chloro-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidine (**12**) was prepared by modification of the route previously described by Chen.[35] The amino alcohols, 4-ethylamino-1-butanol (**13**), 4-allylamino-1-butanol (**14**), and 3-(cyclopropylmethyl)amino-1-propanol (**15**), were prepared in 80–90% yield as previously described.[42,43]

The preparation of β-hydroxyantalarmin **9** is shown in Scheme 1. Treating **12** with an excess amount of (2-butylamino)ethanol in dimethylformamide (DMF) at 150 °C for 2 h produced a mixture of four products, which were isolated and characterized. The yield of the desired product **9** was 32% and the major product was the alkyl aryl ether **10**. A possible mechanism for the formation of **10** is an acid-catalyzed rearrangement of alcohol **9** (Fig. 2). Hydrogen chloride was produced during the formation of **9** and the protonated compound **9** could easily form a five-membered ring transition state necessary for the rearrangement. In support of this mechanism, (1) heating an ethyl acetate solution of **9,** in an attempted recrystallization of its hydrochloride salt, readily transformed alcohol **9** into ether **10**, and, (2) no analogous by-products were formed when either (3-alkylamino)propanol or (4-alkylamino)butanol were used instead of (2-butylamino)ethanol in this coupling reaction.

The origin of byproducts **11** and **16** could be due either to the reaction of **12** with dimethylamine from decomposition of DMF, or a trace of water in DMF. Therefore, dimethylsulfoxide (DMSO) was substituted for DMF and **12** was reacted with (2-butylamino)ethanol at 130 °C for 5 h to afford desired alcohol **9** as the major product in 71% yield. The yield of the rearranged by-product **10** in DMSO is much lower than that in DMF.

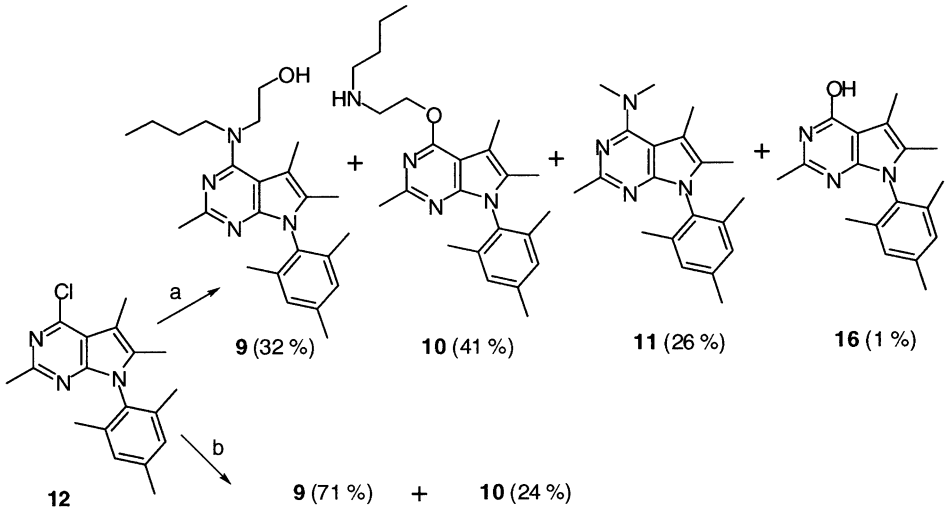
The other potentially more polar analogues of **1** (**3**–**8**, **17**–**22**), were synthesized in a similar manner as in the preparation of **9** (Scheme 2). Reaction of **12** with an excess of the appropriate alkylamine in DMSO at 130 °C for 5 to 6 h afforded secondary and tertiary amines (**3**–**8**, **17**–**22**) in good yield as shown in Table 1.

Pharmacology and Discussion

The $CRHR_1$ binding affinity and the calculated log P (Clog P) values of these compounds are listed in Table 1. The effect of a hydroxyl group on binding to the $CRHR_1$ receptor was determined by its position on the nitrogen atom's side-chain. Thus, compound **9**,[35] the *N*-2-hydroxyethyl relative of antalarmin **1**, had little or no affinity, and the isomeric *N*-4-hydroxybutyl compound **3**[42] had the highest affinity (K_i = 0.68 nM) of all of the evaluated compounds. Appropriate placement of the polar group appears to be crucial for optimum $CRHR_1$ binding affinity. It is likely that the major difference in affinity between these isomers is related to their interaction with specific amino acids in the receptor's binding site. Also, since a hydroxyl moiety is likely to increase hydrophilicity [the Clog P value calculated for compounds **9** and **3** was 5.52 and 5.17, respectively, as compared with 6.98 for antalarmin (**1**)], these data may indicate that we can find less lipophilic compounds than **1** which also have higher affinity for the $CRHR_1$ receptor. Previous SAR studies demonstrated that increasing hydrophilicity of nonpeptide $CRHR_1$ antagonists resulted in reducing $CRHR_1$ affinity.[27,28,44,45] In contrast, in this series of δ-hydroxyl analogues, compound **3** with a lower Clog P than **1** showed high receptor affinity. Thus, a highly lipophilic side-chain is not a requirement for high $CRHR_1$ binding affinity.

It is obviously not always correct to situate a hydrophilic moiety on the *N*-alkyl side-chain, as we have learned from the evaluation of compounds **6** and **7**.[42] The latter compound, like **3**, has an *N*-hydroxybutyl side-chain, albeit the other *N*-substituent is a little more bulky than in **3**. Unfortunately, **7** was found to have about 25 times less affinity for the receptor than the comparable non-hydroxylated, more lipophilic, compound **6**. Thus, while it is logical to want to decrease lipophilicity in order to obtain drugs capable of crossing the BBB, it is clear from even these limited data that finding such compounds which are also potent and selective may not be a simple task.

In recent SAR studies,[27,28] replacing the dialkylamino substituents in $CRHR_1$ antagonists by alkyl ether groups afforded compounds that retained good $CRHR_1$ affinity. Therefore, we examined the $CRHR_1$ affinity of our byproduct β-amino ether **10**; it did not show significant affinity. Shortening the alkyl side chain to a methyl group, while retaining the tertiary amino substituent, afforded compound **11**, which also did not



Scheme 1. (a) Excess (2-butylamino)ethanol, DMF 150 °C; (b) excess (2-butylamino)ethanol, DMSO,130 °C.

display any $CRHR_1$ affinity. Lengthening the ethyl group in alcohol **3** afforded derivatives **4**[42] and **5**,[43] which showed slightly decreased $CRHR_1$ affinity. The affinity of the butyl analogue **4** was comparable to **1**, and the allyl analogue **5** possessed slightly better $CRHR_1$ affinity than **1**. Constrained chiral β-alcohols **17** and **18** were found to have much better $CRHR_1$ affinity (>58-fold) than the unconstrained β-alcohol **9**. These results indicate that proper placement of polar groups can maintain high $CRHR_1$ affinity or even increase the ligand's affinity. Since the secondary amino ether **10** did not display $CRHR_1$ affinity, we prepared the tertiary amino analogues **19** and **20**. Amine **19** did show higher $CRHR_1$ affinity than **10**. However, compared with **1**, **19** demonstrated significantly decreased $CRHR_1$ affinity, and that affinity was completely lost in the amine **20**. These data and previous SAR studies[28,44] suggest that the strongly basic alkylamino functionality is detrimental to $CRHR_1$ binding affinity in this series.

The bis(2-methoxyethyl)amino analogue **8**[28] showed increased $CRHR_1$ affinity and a much lower Clog P value than **1** (Table 1), and is in sharp contrast to the poor affinity of the β-alcohol **9** and the aminoether **10**. Compound **8** was previously noted[28] to have much lower affinity ($K_i = 7.1$ nM). This may be due to the fact that it was examined as an oil, rather than as the crystalline solid, which we obtained, and the noted affinity was the result of a single determination.[28] Our data indicate that the bis-β-ether may be a useful substituent for the development of polar and high affinity $CRHR_1$ ligands, as shown in this ring system and others.[28,33]

Shortening the *N*-butyl side-chain of **1** afforded compounds **21**[35] and **22**; these displayed reduced $CRHR_1$ affinity relative to **1**. Interestingly, the secondary amine **22** displayed much higher affinity than the tertiary amine **11**, which is an isomer of **22** with a similar Clog P value. This suggests that an alkyl group larger than a methyl is important for high binding affinity to the $CRHR_1$ receptor.

Since the (cyclopropylmethyl)propylamino analogue **6** possessed about the same $CRHR_1$ affinity and Clog P value as **1**,[41] we introduced a γ-hydroxyl group in **6** to afford alcohol **7**. The $CRHR_1$ affinity of **7** is much

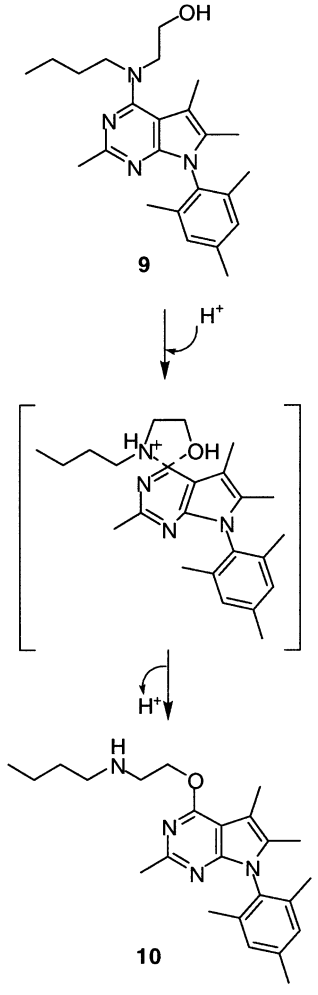
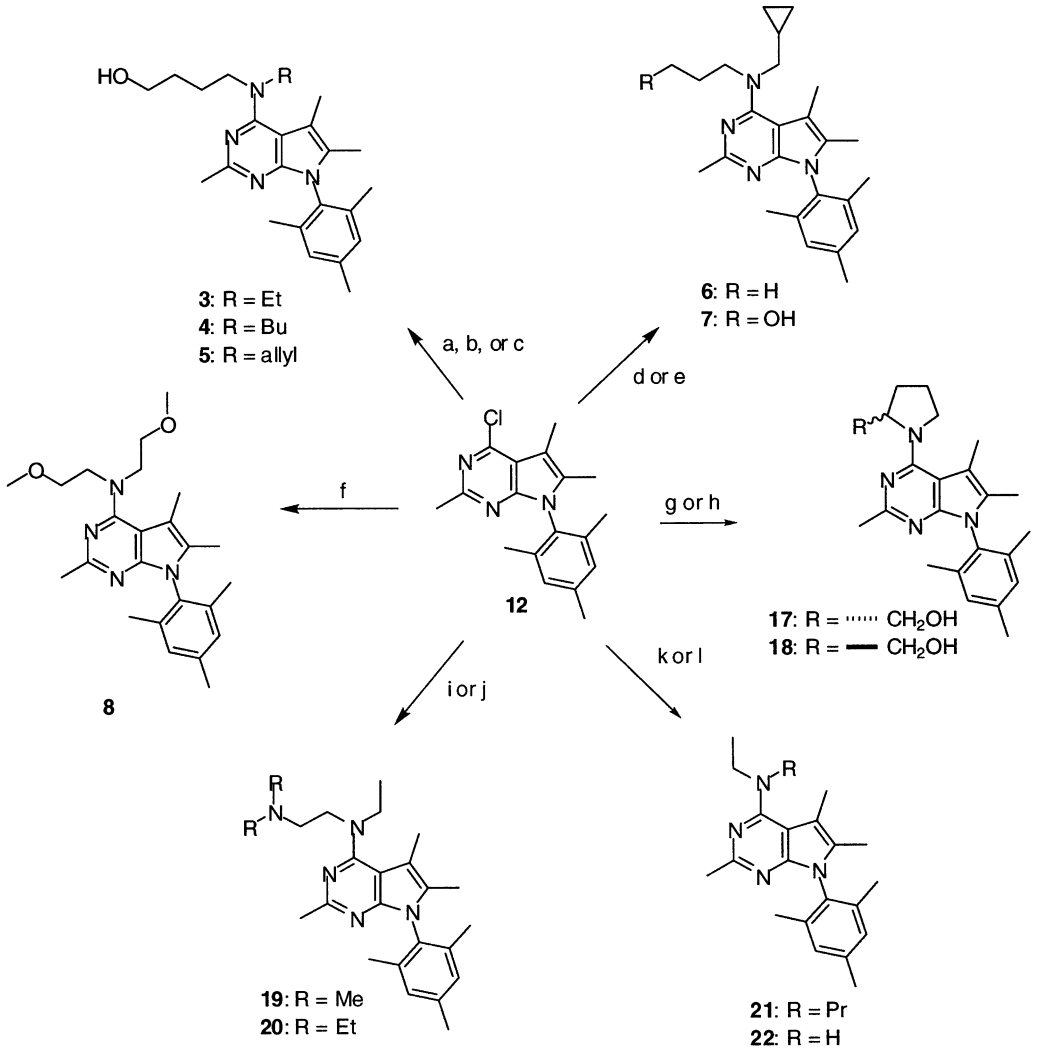


Figure 2. Acid-catalyzed rearrangement of **9**.



Scheme 2. (a) **13**; (b) 4-(butylamino)butan-1-ol; (c) **14**; (d) (cyclopropylmethyl)propylamine; (e) **15**; (f) bis(2-methoxyethyl)amine; (g) L-prolinol; (h) D-prolinol; (i) *N*,*N*-dimethyl-*N*′-ethylethylenediamine; (j) *N*,*N*,*N*′-triethylethylenediamine; (k) ethylpropylamine; (l) ethylamine.

higher than the β-alcohol **9** and lower than δ-alcohols **3**, **4**,[42] and **5**.[43] Thus, in this series of hydroxylated analogues of **1**, the position of the hydroxyl group appears to effect their $CRHR_1$ binding affinity [δ-OH (**3**, **4**, **5**) > > γ-OH (**7**) > > β-OH (**9**)].

In summary, we have examined the receptor affinity of compounds with diverse side-chains on the nitrogen atom with the hope of finding one which would have a lower Clog P value than antalarmin (and, thus, likely to be more hydrophilic and more capable of crossing the BBB), and yet retain high affinity for the $CRHR_1$ receptor. Among the compounds which we have evaluated, **3**–**5**, and the bis(2-methoxyethyl)amino analogue **8**, were found to have about the same, or higher, affinity as antalarmin (**1**), and they all had lower Clog P values. The usefulness of these compounds for the continued investigation of the biological effects of CRH and $CRHR_1$ will depend on their ability to cross the BBB. Whether or not they will be found to do so, these compounds can serve as templates for the preparation of a new series of ligands in the future.

Experimental

Melting points (Table 1) were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Atlanta. Chemical ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. Electron ionization mass spectra (EIMS) and high-resolution mass measurements (HR-MS) were obtained using a V. G. Micro Mass 7070F mass spectrometer. Optical rotations were obtained using a Perkin–Elmer 341 polarimeter and are reported at the sodium D-line (589 nm). ^{1}H NMR spectra were recorded using a Varian XL-300 spectrometer. The ^{1}H NMR spectra of **6**, **11**, and **21** were consistent with the literature.[35] Chemical shifts are expressed in parts per million (ppm) on the δ scale relative to a tetramethylsilane (TMS) internal standard. Thin-layer chromatography (TLC) was performed on 250 μm Analtech GHLF silica gel plates. Flash column chromatography was performed with 40–63 μm silica gel 60 (Fluka).

General procedure for the synthesis of 4-alkylamino-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidines. 2-[Butyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]ethan-1-ol (9) and butyl[2-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yloxy]ethyl]amine (10). A mixture of 4-chloro-2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidine (**12**)[35] (3.14 g, 10.0 mmol) and 2-(butylamino)ethanol (8.92 g, 76.1 mmol) in DMSO (16 mL) was heated at 130 °C for 6 h. The reaction mixture was cooled to rt, quenched with water (300 mL), and extracted with EtOAc (300 mL). The extract was washed with water and brine, dried ($MgSO_4$), filtered, and evaporated. The crude product was purified via silica gel flash column chromatography (5% MeOH in CH_2Cl_2) to afford **9** (2.80 g, 71%) as a white glassy solid and **10** (0.93 g, 24%) as a crystalline white solid. **9**: R_f 0.52 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 (broad s, 1H), 6.99 (s, 2H), 3.90 (t, J = 4.4 Hz, 2H), 3.76 (t, J = 4.9 Hz, 2H), 3.56–3.62 (m, 2H), 2.45 (s, 3H), 2.39 (s, 3H), 2.35 (s, 3H), 1.96 (s, 3H), 1.83 (s, 6H), 1.68–1.78 (m, 2H), 1.29–1.39 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.7, 158.0, 152.7, 138.4, 136.8, 132.3, 130.3, 129.1, 105.0, 104.5, 60.5, 52.4, 50.8, 30.6, 25.0, 21.0, 20.1, 17.5, 13.9, 12.2, 9.9; MS (CI) m/z 395 (MH^+); Anal. calcd for $C_{24}H_{34}N_4O{\cdot}HCl{\cdot}0.5H_2O$: C, 65.51; H, 8.25; N, 12.73. Found: C, 65.50; H, 8.16; N, 12.68. **10**: R_f 0.45 (CH_2Cl_2/CH_3OH = 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (s, 2H), 4.62 (t, J = 5.4 Hz, 2H), 3.08 (t, J = 5.4 Hz, 2H), 2.73 (t, J = 7.3 Hz, 2H), 2.51 (s, 3H), 2.39 (s, 3H), 2.35 (s, 3H), 1.94 (s, 3H), 1.83 (s, 6H), 1.48–1.56 (m, 2H), 1.34–1.44 (m, 2H), 0.93 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.4, 159.8, 152.7, 138.4, 137.0, 129.8, 129.2, 105.7, 102.9, 64.9, 49.3, 48.8, 32.3, 25.8, 21.1, 20.3, 17.5, 13.9, 10.6, 9.6; MS m/z calcd for $C_{24}H_{34}N_4O^+$: 394.2733, found 394.2721; 395 (MH^+).

4-Ethylamino-1-butanol (13).[42,43] A solution of γ-butyrolactone (8.61 g, 100 mmol) and ethylamine (9.02 g, 200 mmol) in THF (100 mL) was heated at 50 °C for 22 h. The resulting mixture was evaporated to give *N*-ethyl-4-hydroxybutanamide (13.1 g, 100%) as a light yellow oil, which was used in the following step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.99 (broad s, 1H), 3.69 (t, J = 5.9 Hz, 2H), 3.24–3.33 (m, 2H), 2.35 (t, J = 6.9 Hz, 2H), 1.87 (p, J = 6.1 Hz, 2H), 1.14 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 62.2, 34.4, 33.9, 28.0, 14.6; MS (CI) m/z 132 (MH^+). To a stirred mixture of $LiAlH_4$ (115 mmol) in THF (115 mL) was added a solution of *N*-ethyl-4-hydroxybutanamide (10.1 g, 77.0 mmol) in THF (40 mL) at room temperature. The reaction mixture was brought to reflux for 3 h, and cooled before poured into a 40% NaOH aqueous solution (200 mL). The resulting mixture was extracted with ether (100 mL), and then the organic layer was dried (K_2CO_3), filtered, and evaporated to give **13** (8.82 g, 98%) as a pale yellow oil, which was used in the following step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.57 (t, J = 4.9 Hz, 2H), 2.63–2.70 (m, 4H), 1.62–1.70 (m, 4H), 1.12 (t, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 62.4, 49.3, 43.5, 32.3, 28.6, 14.6; MS (CI) m/z 118 (MH^+).

4-(Prop-2-enylamino)butan-1-ol (14).[42,43] A mixture of allylamine (53.5 g, 937 mmol) and 4-chlorobutan-1-ol (25.4 g, 234 mmol) in THF (30 mL) was refluxed for 22 h, cooled and concentrated. The residue was dissolved in ether (400 mL) and washed with saturated aqueous K_2CO_3. The organic solution was dried ($MgSO_4$), filtered, and evaporated to afford **14** (24.4 g, 81%) as a yellow oil, which was used in the following step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.83–5.96 (m, 1H), 5.18 (dd, J = 15.6, 2.0 Hz, 1H), 5.11 (d, J = 8.8 Hz, 1H), 3.56 (t, J = 5.9 Hz, 2H), 3.24 (dd, J = 4.9, 2.0 Hz, 2H), 2.63 (t, J = 6.4 Hz, 2H), 1.58–1.63 (m, 4H); MS (CI) m/z 130 (MH^+).

3-(Cyclopropylmethyl)amino-1-propanol (15).[42,43] A mixture of (aminomethyl)cyclopropane (21.3 g, 300 mmol) and 3-bromopropan-1-ol (13.9 g, 100 mmol) in THF (200 mL) was heated at 45 °C for 11 h, and then cooled to room temperature. K_2CO_3 (40 g) was added, and the resulting mixture was filtered and the precipitate was washed with ether. The combined filtrate was evaporated to dryness and the residue was distilled to afford the amino alcohol **15** (10.5 g, 84%) as a colorless oil: bp 86–88 °C/2 mm Hg; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.82 (t, J = 4.9 Hz, 2H), 3.21 (broad s, 2H), 2.90 (t, J = 5.9 Hz, 2H), 2.47 (d, J = 6.8 Hz, 2H), 1.67–1.74 (m, 2H), 0.91–0.95 (m, 1H), 0.45–0.51 (m, 2H), 0.09–0.14 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 64.4, 54.7, 49.7, 30.6, 10.9, 3.2; MS m/z calcd for $C_7H_{15}NO^+$: 129.1154, found 129.1156; 130 (MH^+).

4-[Ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]butan-1-ol (3). Synthesized from **13** according to the general procedure as a colorless oil: R_f 0.48 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (s, 2H), 3.72 (t, J = 5.9 Hz, 2H), 3.53–3.62 (m, 4H), 2.92 (broad s, 1H), 2.47 (s, 3H), 2.37 (s, 3H), 2.34 (s, 3H), 1.94 (s, 3H), 1.83 (s, 6H), 1.73–1.83 (m, 2H), 1.59–1.65 (m, 2H), 1.23 (t, J = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.1, 158.8, 152.7, 138.2, 136.9, 132.5, 129.5, 129.0, 104.9, 62.2, 46.8, 46.3, 29.3, 25.6, 23.6, 21.0, 17.6, 12.9, 12.2, 9.9; MS (CI) m/z 395 (MH^+). Anal. calcd for $C_{24}H_{34}N_4O{\cdot}HCl$: C, 66.87; H, 8.19; N,12.99. Found: C, 66.81; H, 8.16; N,12.91.

4-[Butyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]butan-1-ol (4). Synthesized using 4-(butylamino)butan-1-ol according to the general procedure as a colorless oil: R_f 0.48 (EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 2H), 3.67 (t, J = 6.4 Hz, 2H), 3.48–3.58 (m, 4H), 2.46 (s, 3H), 2.36 (s, 3H), 2.34 (s, 3H), 1.93 (s, 3H), 1.83 (s, 6H), 1.55–1.77 (m, 6H), 1.23–1.32 (m, 3H), 0.89 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.4, 158.8, 152.7, 138.2, 137.0, 132.6, 129.5, 129.0, 104.9, 104.8, 62.4, 51.4, 47.9, 29.8, 29.5, 25.7, 23.8, 21.0, 20.2, 17.6, 13.9, 12.2, 9.9; MS (CI) m/z 423 (MH^+). Anal. calcd for $C_{26}H_{38}N_4O{\cdot}HCl$: C, 68.02; H, 8.56; N, 12.20. Found: C, 67.96; H, 8.54; N, 12.12.

4-[Prop-2-enyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)-pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]butan-1-ol (5). Synthesized from **14** according to the general procedure as a

colorless oil: R_f 0.35 ($CH_2Cl_2/CH_3OH = 95:5$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 2H), 5.92–6.05 (m, 1H), 5.22–5.35 (m, 2H), 4.14 (d, $J = 5.8$ Hz, 2H), 3.68 (t, $J = 6.4$ Hz, 2H), 3.52 (t, $J = 7.9$ Hz, 2H), 2.48 (s, 3H), 2.36 (s, 3H), 2.34 (s, 3H), 1.94 (s, 3H), 1.83 (s, 6H), 1.70–1.80 (m, 2H), 1.53–1.62 (m, 2H); MS (CI) m/z 407 (MH^+). Anal. calcd for $C_{25}H_{34}N_4O \cdot HCl \cdot 0.25\ H_2O$: C, 67.09; H, 8.00; N, 12.51. Found: C, 67.31; H, 7.99; N, 12.55.

(Cyclopropylmethyl)propyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine[35] (6). Synthesized from (cyclopropylmethyl)propylamine according to the general procedure as a light yellow oil: R_f 0.59 ($CH_2Cl_2/CH_3OH = 97:3$); ^1H NMR[35] (300 MHz, $CDCl_3$) δ 6.98 (s, 2H), 3.63 (t, $J = 7.3$ Hz, 2H), 3.42 (d, J = 5.8 Hz, 2H), 2.46 (s, 3H), 2.38 (s, 3H), 2.35 (s, 3H), 1.94 (s, 3H), 1.84 (s, 6H), 1.62–1.75 (m, 2H), 1.06–1.12 (m, 1H), 0.92 (t, $J = 7.3$ Hz, 3H), 0.46–0.52 (m, 2H), 0.11–0.16 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.5, 158.8, 152.8, 138.1, 137.0, 132.7, 129.2, 129.0, 104.7, 55.4, 50.9, 25.9, 21.0, 20.5, 17.6, 12.2, 11.5, 9.9, 9.4, 3.5; MS (CI) m/z 391 (MH^+). Anal. for $C_{25}H_{34}N_4 \cdot HCl \cdot 0.25\ H_2O$: C, 69.58; H, 8.29; N,12.98. Found: C, 69.57; H, 8.31; N,12.97.

3-[Cyclopropylmethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amino]propan-1-ol (7). Synthesized from **15** according to the general procedure as a colorless oil: R_f 0.39 ($CH_2Cl_2/CH_3OH = 95:5$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.00 (s, 2H), 3.97 (t, $J = 5.9$ Hz, 2H), 3.59 (t, $J = 5.4$ Hz, 2H), 3.49 (d, $J = 5.9$ Hz, 2H), 2.45 (s, 3H), 2.43 (s, 3H), 2.35 (s, 3H), 1.96 (s, 3H), 1.87–1.93 (m, 2H), 1.84 (s, 6H), 1.05–1.09 (m, 1H), 0.60–0.66 (m, 2H), 0.19–0.24 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.4, 158.4, 152.7, 138.2, 136.7, 132.4, 129.6, 129.0, 104.8, 103.0, 58.7, 56.2, 43.2, 29.9, 25.0, 21.0, 17.5, 13.2, 10.2, 9.9, 3.6; MS m/z calcd for $C_{25}H_{34}N_4O^+$: 406.2733, found 406.2728; 407 (MH^+).

Bis(2-methoxyethyl)[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine[28] (8). Synthesized from bis(2-methoxyethyl)amine according to the general procedure as a colorless oil[28]: R_f 0.56 ($CH_2Cl_2/CH_3OH = 95:5$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 2H), 3.82 (t, $J = 5.9$ Hz, 4H), 3.65 (t, $J = 5.9$ Hz, 4H), 3.34 (s, 6H), 2.45 (s, 3H), 2.38 (s, 3H), 2.34 (s, 3H), 1.93 (s, 3H), 1.83 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5, 158.7, 153.0, 138.3, 137.0, 132.6, 129.8, 129.1, 104.6, 99.3, 71.1, 58.8, 50.2, 25.8, 21.1, 17.6, 12.3, 9.9; MS (CI) m/z 411 (MH^+). Anal. calcd for $C_{24}H_{34}N_4O_2 \cdot HCl \cdot 0.5\ H_2O$: C, 63.21; H,7.96; N,12.28. Found: C, 63.48; H, 8.04; N,12.28.

Dimethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine[35] (11). Synthesized using *N*,*N*-dimethylamine according to the general procedure, except that *N*,*N*-dimethylamine was added at rt and the mixture was then heated to 100–110 °C. Additional *N*,*N*-dimethylamine was added as needed to complete the reaction. The crude product was recrystallized from petroleum ether to afford **11** as white crystals: R_f 0.59 ($CH_2Cl_2/CH_3OH = 97:3$); ^1H NMR[35] (300 MHz, $CDCl_3$) δ 6.98 (s, 2H), 3.13 (s, 6H), 2.48 (s, 3H), 2.39 (s, 3H), 2.34 (s, 3H), 1.94 (s, 3H), 1.83 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 159.0, 138.2, 137.0, 132.7, 129.3, 129.0, 104.7, 103.8, 41.6, 25.9, 21.1, 17.6, 12.8, 9.9; MS (CI) m/z 323 (MH^+); Anal. calcd for $C_{20}H_{26}N_4$: C, 74.49; H, 8.13; N, 17.37. Found: C, 74.38; H, 8.15; N, 17.27.

(*S*)-[1-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]pyrrolidin-2-yl]methanol (17). Synthesized from L-prolinol according to the general procedure as an amorphous solid: ^1H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 2H), 4.63 (q, $J = 7.8$ Hz, 1H), 3.84–3.92 (m, 3H), 3.70 (dd, $J = 11.3$, 8.2 Hz, 1H), 2.39 (s, 3H), 2.34 (s, 3H), 2.32 (s, 3H), 2.11–2.19 (m, 1H), 1.93–2.00 (m, 1H), 1.92 (s, 3H), 1.85 (s, 3H), 1.77–1.83 (m, 1H), 1.75 (s, 3H), 1.50–1.64 (m, 1H); MS (CI) m/z 379 (MH^+); $[\alpha]_D^{20}$ +89.6° (c = 1.0, CH_3OH). Anal. calcd for $C_{23}H_{30}N_4O$: C, 72.98; H, 7.99; N,14.80. Found: C, 73.10; H, 7.95; N,14.67.

(*R*)-[1-[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]pyrrolidin-2-yl]methanol (18). Synthesized from D-prolinol according to the general procedure as an amorphous solid: MS (CI) m/z 379 (MH^+); $[\alpha]_D^{20}$ −89.3° (c = 1.0, CH_3OH). Anal. calcd for: $C_{23}H_{30}N_4O$: C, 72.98; H, 7.99; N, 14.80. Found: C, 72.75; H, 7.93; N, 14.66.

[2-(Dimethylamino)ethyl]ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (19). Synthesized from *N,N*-dimethyl-*N'*-ethylethylenediamine according to the general procedure as a yellow oil: R_f 0.61 ($CH_2Cl_2/CH_3OH/NH_4OH = 90:10:1$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.99 (s, 2H), 3.69 (t, $J = 7.4$ Hz, 2H), 3.60 (q, $J = 7.2$ Hz, 2H), 2.57 (t, $J = 7.4$ Hz, 2H), 2.45 (s, 3H), 2.37 (s, 3H), 2.34 (s, 3H), 2.31 (s, 6H), 1.94 (s, 3H), 1.83 (s, 6H), 1.24 (t, $J = 6.9$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.7, 158.8, 153.0, 138.3, 137.0, 132.6, 129.5, 129.0, 104.7, 57.1, 46.6, 46.3, 45.8, 25.8, 21.1, 17.6, 13.3, 12.3, 9.9; MS (CI) m/z 394 (MH^+). Anal. calcd for $C_{24}H_{35}N_2 \cdot 2\ HCl$: C, 61.79; H, 8.00; N,15.01. Found: C, 61.80; H, 7.99; N, 14.96.

[2-(Diethylamino)ethyl]ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (20). Synthesized from *N,N,N'*-triethylethylenediamine according to the general procedure as a yellow oil: R_f 0.55 ($NH_4OH/CH_3OH/CH_2Cl_2 = 1:10:90$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.99 (s, 2H), 3.56–3.68 (m, 4H), 2.70 (t, $J = 7.9$ Hz, 2H), 2.61 (q, $J = 6.9$ Hz, 4H), 2.45 (s, 3H), 2.37 (s, 3H), 2.35 (s, 3H), 1.94 (s, 3H), 1.83 (s, 6H), 1.26 (t, $J = 7.4$ Hz, 3H), 1.06 (t, $J = 7.4$ Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.6, 158.8, 152.8, 138.3, 137.0, 132.7, 129.4, 129.0, 104.6, 50.0, 47.6, 46.8, 46.1, 25.7, 21.1, 17.6, 13.4, 12.3, 11.9, 9.9; MS (CI) m/z 422 (MH^+). Anal. calcd for $C_{26}H_{39}N_2 \cdot 2\ HCl$: C, 63.14; H, 8.36; N,14.16. Found: C, 63.07; H, 8.37; N,14.11.

Ethylpropyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine[35] (21). Synthesized from ethylpropylamine according to the general procedure as a colorless oil: R_f 0.33 ($CH_2Cl_2/CH_3OH = 97:3$);

^{1}H NMR[35] (300 MHz, $CDCl_3$) δ 6.98 (s, 2H), 3.60 (q, J = 6.8 Hz, 2H), 3.49 (t, J = 7.4 Hz, 2H), 2.46 (s, 3H), 2.37 (s, 3H), 2.34 (s, 3H), 1.94 (s, 3H), 1.84 (s, 6H), 1.67 (m, 2H), 1.20 (t, J = 7.4 Hz, 3H), 0.92 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.2, 158.8, 152.8, 138.1, 137.0, 132.7, 129.2, 129.0, 104.7, 50.5, 44.8, 25.9, 21.0, 20.6, 17.6, 12.7, 12.1, 11.5, 9.9; MS (CI) m/z 365 (MH^+). Anal. calcd for $C_{23}H_{32}N_4$·HCl: C, 68.89; H, 8.30; N,13.97. Found: C, 68.77; H, 8.31; N,13.87.

Ethyl[2,5,6-trimethyl-7-(2,4,6-trimethylphenyl)pyrrolo[2,3-*d*]pyrimidin-4-yl]amine (22). Synthesized from ethylamine according to the general procedure procedure, except that ethylamine was added at rt and the mixture was then heated to 100–110 °C. Additional ethylamine was added as needed to complete the reaction. The crude product was recrystallized from petroleum ether to afford **22** as light yellow crystals: R_f 0.50 (CH_2Cl_2/CH_3OH = 97:3); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (s, 2H), 4.93 (broad s, 1H), 3.62–3.70 (m, 2H), 2.45 (s, 3H), 2.42 (s, 3H), 2.34 (s, 3H), 1.90 (s, 3H), 1.84 (s, 6H), 1.32 (t, J = 7.3 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 156.5, 150.8, 138.1, 136.9, 132.4, 129.0, 127.6, 104.0, 100.5, 35.4, 26.1, 20.9, 17.5, 15.1, 11.2, 9.3; MS (CI) m/z 323 (MH^+). Anal. calcd for $C_{20}H_{26}N_4$: C, 74.49; H, 8.13; N,17.37. Found: C, 74.35; H, 8.16; N,17.28.

$CRHR_1$ binding assay. Since rat cerebellum predominately contains $CRHR_1$ receptors[46,47] the binding affinities of the various compounds was evaluated using competitive displacement of [^{125}I]–Tyr^0–sauvagine binding[48] to $CRHR_1$ receptors in membrane homogenates of rat cerebellum. The K_i values obtained for antalarmin using [^{125}I]–Tyr^0–sauvagine are identical to those obtained earlier using [^{125}I]–ovine CRH which specifically labels only $CRHR_1$ receptors in the concentrations used in the assay.[41]

Frozen whole rat brains, dissected from male Sprague–Dawley rats, 200–300 g, were purchased from Taconic Farms (Germantown, NY). The brains were rapidly frozen, shipped to NIH on dry ice and stored at −70 °C until the time of the assay. At the time of assay, brains were thawed on ice, cerebellar hemispheres were dissected, weighed, and homogenized in 20 vol ice cold homogenization buffer containing PBS, 10 mM $MgCl_2$, 2 mM EGTA, and 0.1 mM bacitracin, pH 7.0. The homogenate was centrifuged for 10 min at 40,000g. The pellet was resuspended in cold PBS buffer and recentrifuged for 10 min at 40,000g. The final pellet was resuspended in homogenization buffer at 30 mg tissue wet weight/mL buffer and 0.1 mL homogenate was added to 1.5 mL microfuge tubes containing 0.1 mL of [^{125}I]–Tyr^0–sauvagine (New England Nuclear, Boston, MA) in incubation buffer (homogenization buffer plus 0.01% BSA), and 0.1 mL of incubation buffer containing increasing concentrations (10^{-10} to 10^{-6} M) of the CRH antagonist analogues. Nonspecific binding was defined by 1 μM ovine CRH and 1 μM sauvagine (Peninsula, Belmont, CA) which gave identical nonspecific binding values. Binding reactions were incubated for 2 h at room temperature. Radioactivity bound to tissue was separated from free ligand by centrifugation in a microfuge (Beckman, Palo Alto, CA) for 10 min at 12,000g at 4 °C. The supernatant was aspirated and the radioactivity of the pellet was measured in a gamma counter at approximately 80% efficiency.

The computer program GraphPad Prism 2.0a for Power Macintosh (GraphPad Software Inc., San Diego CA) was used to calculate K_i values from the IC_{50} of the [^{125}I]–Tyr^0–sauvagine competitive binding curves for each antagonist analogue. Unless noted in Table 1, three binding curves conducted in duplicate were generated for each analogue and the K_i values represent the mean of the three experiments ± SEM.

Calculation of log P values. The predicted values of log P were obtained using the ACD/ilab web service version 2.6 at http://www.acdlabs.com/ilab.

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References and Notes

1. Vale, W.; Rivier, C.; Rivier, J. *Science* **1981**, *213*, 1394.
2. Rivier, C.; Plotsky, P. M. In *Annual Review of Physiology*; Berne, R. M., Ed.; Academic Press: Palo Alto, 1986; Vol. 48, p 475.3. Chrousos, G. P. *N. Engl. J. Med.* **1995**, *332*, 1351.
4. Chang, C. P.; Pearse, R. V., II; O'Connell, S.; Rosenfeld, M. G. *Neuron.* **1993**, *11*, 1187.
5. Kishimoto, Y.; Pearse, R. V., II; Lin, C. R.; Rosenfeld, M. G. A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 1108.
6. Vita, N.; Laurent, P.; Lefort, S.; Chalon, P.; Lelias, J. M.; Kaghad, M.; Le, F. G.; Caput, D.; Ferrara, P. *FEBS Lett.* **1993**, *335*, 1.
7. Webster, E. L.; Elenkov, I. J.; Chrousos, G. P. *Molec. Psychiatry* **1997**, *2*, 368.
8. Irwin, M. R.; Vale, W.; Britton, K. T. *Brain Behav. Immun.* **1987**, *1*, 81.
9. Koob, G. F.; Heinrichs, S. C.; Pich, E. M.; Menzaghi, F.; Baldwin, H.; Miczek, K.; Britton, K. T. In *Proceedings of the Ciba Foundation Symposium No. 172 on Corticotropin-Releasing Factor*; Chadwick, D. J.; Marsh, J., Eds.; John Wiley & Sons: New York, 1993, p 277.
10. Owens, M. J.; Nemeroff, C. B. *Pharmacol. Rev.* **1991**, *43*, 425.
11. Brown, M. R. In *Stress, Neurobiology and Neuroendocrinology*; Brown, M. R.; Koob, G. F., Eds.; Marcel Dekker: New York, 1991, p 193.
12. Chen, R.; Lewis, K. A.; Perrin, M. H.; Vale, W. W. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8967.
13. Perrin, M.; Donaldson, C.; Chen, R.; Blount, A.; Berggren, T.; Bilezikjian, L.; Sawchenko, P.; Vale, W. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2969.
14. Lovenberg, T. W.; Liaw, C. W.; Grigoriadis, D. E.; Clevenger, W.; Chalmers, D. T.; De Souza, E. B.; Oltersdorf, T. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 836.
15. Perrin, M. H.; Donaldson, C. J.; Chen, R.; Lewis, K. A.; Vale, W. W. *Endocrinology* **1993**, *133*, 3058.

16. Liaw, C. W.; Lovenberg, T. W.; Barry, G.; Oltersdorf, T.; Grigoriadis, D. E.; De Souza, E. B. *Endocrinology* **1996**, *137*, 72.
17. Chalmers, D. T.; Lovenberg, T. W.; Grigoriadis, D. E.; Behan, D. P.; De Souza, E. B. *Trends Pharmacol. Sci.* **1996**, *17*, 166.
18. Bremner, J. D.; Licinio, J.; Darnell, A.; Krystal, J. H.; Owens, M. J.; Southwick, S. M.; Nemeroff, C. B.; Charney, D. S. *Am. J. Psychiatry* **1997**, *154*, 624.
19. Nemeroff, C. B.; Widerlov, E.; Bissette, G.; Walleus, H.; Karlsson, I.; Eklund, K.; Kilts, C. D.; Loosen, P. T.; Vale, W. *Science* **1984**, *226*, 1342.
20. Zhou, Y.; Spangler, R.; LaForge, K. S.; Maggos, C. E.; Ho, A.; Kreek, M. J. *J. Pharmacol. Exp. Ther.* **1996**, *279*, 351.
21. Holsboer, F. *J. Psychiatric Res.* **1999**, *33*, 181.
22. Habib, K. E.; Weld, K. P.; Rice, K. C.; Pushkas, J.; Champoux, M.; Listwak, S.; Webster, E. L.; Atkinson, A. J.; Schulkin, J.; Contoreggi, C.; Chrousos, G. P.; McCann, S. M.; Suomi, S. J.; Higley, J. D.; Gold, P. W. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 6079.
23. Rivier, J.; Rivier, C.; Vale, W. *Science* **1984**, *224*, 889.
24. Barquist, E.; Zinner, M.; Rivier, J.; Taché, Y. *Am. J. Physiol.* **1992**, *262*, G616.
25. Nozu, T.; Martínez, V.; Rivier, J.; Taché, Y. *Am. J. Physiol.: Gastrointest. Liver Physiol.* **1999**, *39*, G867.
26. McCarthy, J. R.; Heinrichs, S. C.; Grigoriadis, D. E. In *Annual Reports in Medicinal Chemistry*; Doherty, A. M., Ed.; Academic Press: New York, 1999; Vol. 34, pp 11.
27. Arvanitis, A. G.; Gilligan, P. J.; Chorvat, R. J.; Cheeseman, R. S.; Christos, T. E.; Bakthavatchalam, R.; Beck, J. P.; Cocuzza, A. J.; Hobbs, F. W.; Wilde, R. G.; Arnold, C.; Chidester, D.; Curry, M.; He, L. *J. Med. Chem.* **1999**, *42*, 805.
28. Chorvat, R. J.; Bakthavatchalam, R.; Beck, J. P.; Gilligan, P. J.; Wilde, R. G.; Cocuzza, A. J.; Hobbs, F. W.; Cheeseman, R. S.; Curry, M.; Rescinito, J. P.; Krenitsky, P.; Chidester, D.; Yarem, J. A.; Klacz, J. D. *J. Med. Chem.* **1999**, *42*, 833.
29. McCarthy, J. R.; Heinrichs, S. C.; Grigoriadis, D. E. *Current Pharmaceutical Design* **1999**, *5*, 289.
30. 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. *J. Med. Chem.* **1997**, 40.
31. Whitten, J. P.; Xie, Y. F.; Erickson, P. E.; Webb, T. R.; De Souza, E. B.; Grigoriadis, D. E.; McCarthy, J. R. *J. Med. Chem.* **1996**, *39*, 4354.
32. Chen, C.; Dagnino, J. R.; De Souza, E. B.; Grigoriadis, D. E.; Huang, C. Q.; Kim, K.-I.; Liu, Z.; Moran, T.; Webb, T. R.; Whitten, J. P.; Xie, Y. F.; McCarthy, J. R. *J. Med. Chem.* **1996**, *39*, 4358.
33. Beck, J. P.; Curry, M. A.; Chorvat, R. J.; Fitzgerald, L. W.; Gilligan, P. J.; Zaczek, R.; Trainor, G. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1185.
34. Beck, J. P.; Arvanitis, A. G.; Curry, M. A.; Rescinito, J. T.; Fitzgerald, L. W.; Gilligan, P. J.; Zaczek, R.; Trainor, G. L. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 967.
35. Chen, Y. L. 1994, International Patent WO 94/13676.
36. Lundkvist, J.; Chai, Z.; Teheranian, R.; Hasanvan, H.; Bartfai, T.; Jenck, F.; Widmer, U.; Moreau, J.-L. *Eur. J. Pharmacol.* **1996**, *309*, 195.
37. Richter, R. M.; Weiss, F. *Synapse* **1999**, *32*, 254.
38. Shaham, Y.; Erb, S.; Leung, S.; Buczek, Y.; Stewart, J. *Psychopharmacol.* **1998**, *137*, 184.
39. 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. *Endocrinology* **1999**, *140*, 79.
40. Griebel, G.; Perrault, G.; Sanger, D. J. *Psychopharmacol.* **1998**, *138*, 55.
41. Webster, E. L.; Lewis, D. B.; Torpy, D. J.; Zackman, E. K.; Rice, K. C.; Chrousos, G. P. *Endocrinology* **1996**, *137*, 5747.
42. Hsin, L.-W.; Webster, E. L.; Chrousos, G. P.; Gold, P. W.; Eckelman, W. C.; Contoreggi, C.; Rice, K. C. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 707.
43. Hsin, L.-W.; Webster, E. L.; Chrousos, G. P.; Gold, P. W.; Eckelman, W. C.; Contoreggi, C.; Rice, K. C. *J. Labelled Compd. Radiopharm.* **2000**, *43*, 899.
44. Cocuzza, A. J.; Hobbs, F. W.; Arnold, C. R.; Chidester, D. R.; Yarem, J. A.; Culp, S.; Fitzgerald, L.; Gilligan, P. J. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1057.
45. Gilligan, P. J.; He, L.; Culp, S.; Fitzgerald, L.; Tam, S. W.; Wong, Y. N. *Bioorg. Med. Chem.* **1999**, *7*, 2321.
46. Primius, R. J.; Yevich, E.; Baltazar, C. W. G. *Neuropsychopharmacology* **1997**, *17*, 308.
47. Rominger, D. H.; Rominger, C. M.; Fitzgerald, L.; Grzanna, R.; Largent, B. L.; Zaczek, R. *J. Pharmacol. Exp. Ther.* **1998**, *286*, 459.
48. Grigoriadis, D. E.; Liu, X. J.; Vaugn, J.; Palmer, S. F.; True, C. D.; Vale, W. W. N. L.; De Souza, E. B. *Mol. Pharmacol.* **1996**, *50*, 679.