Non-Peptide Corticotropin-Releasing Hormone Antagonists: Syntheses and Structure−Activity Relationships of 2-Anilinopyrimidines and -triazines

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Screening of our chemical library using a rat corticotropin-releasing hormone (CRH) receptor assay led to the discovery that 2-anilinopyrimidine **15-1** weakly displaced [^{125}I]-0-Tyr-oCRH from rat frontal cortex homogenates when compared to the known peptide antagonist α-helical CRH(9−41) (K_i = 5700 nM vs 1 nM). Furthermore, **15-1** weakly inhibited CRH-stimulated adenylate cyclase activity in the same tissue, but it was less potent than α-helical CRH(9−41) (IC_{50} = 20 000 nM vs 250 nM). Systematic structure−activity relationship studies, using the cloned human CRH_1 receptor assay, defined the pharmacophore for optimal binding to $hCRH_1$ receptors. Several high-affinity 2-anilinopyrimidines and -triazines were discovered, some of which had superior pharmacokinetic profiles in the rat. This paper describes the structure−activity studies which improved $hCRH_1$ receptor binding affinity and pharmacokinetic parameters in the rat. Compound **28-17** (mean $hCRH_1$ K_i = 32 nM) had a significantly improved pharmacokinetic profile in the rat (19% oral bioavailability at 30 mg/kg) as well as in the dog (20% oral bioavailability at 5 mg/kg) relative to the early lead structures.

Introduction

Corticotropin-releasing hormone (or factor, CRH or CRF) is the prime regulator of the hypothalamus−pituitary−adrenal (HPA) axis, coordinating endocrine, autonomic, behavioral, and immune responses to stress.[1−3] Synthesis of this 41-amino acid peptide by corticotrophs in the hypothalamus, followed by transport via the portal system to the anterior pituitary, stimulates secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the general circulation. ACTH stimulates secretion of glucocorticoids from the adrenal glands. Glucocorticoids exert negative feedback inhibition on the HPA axis at the levels of the hypothalamus and pituitary. In CRH knockout mice, levels of ACTH and corticosterone have been reported to be only 25% of those concentrations in normal mice, which testifies to the importance of CRH in regulation of the HPA axis.[4]

CRH receptors have become targets for drug design since dysfunction in the HPA axis has been correlated with various disorders. Hypersecretion of CRH in the central nervous system (CNS) has been proposed to underlie depression and a spectrum of anxiety-related disorders.[5,6] Plasma ACTH and cortisol levels are elevated in a large segment of the depressed patient population.[1,7] Similarly, levels of CRH in the cerebrospinal fluid (CSF) of depressed patients have been reported to be higher than those levels in age- and sex-matched control patients.[8] Postmortem studies employing immunochemical techniques have shown that CRH-secreting cells are increased 4-fold in the hypothalami of depressed patients.[9] Arguments for a role of CRH in anxiety disorders have also been advanced.[10−14] Intracerebroventricular (icv) injection of CRH in rats elicits many behavioral and physiological events, which may be termed "stress-related".[15−17] Moreover, these effects may be blocked by the peptide antagonist α-helical CRH(9−41). Chronic restraint stress in rodents or primates causes long-term activation of the HPA axis. Transgenic mice, which overexpress CRH, have elevated plasma ACTH and glucocorticoid levels and manifest stress-related behaviors.[18,19] On the basis of the above findings, CRH antagonists may be useful for the treatment of depression or anxiety.

There are currently two known subtypes of CRH receptors, one of which has splice variants.[1,20,21] These subtypes are distinguished by the rank order of potencies of peptide agonists in a coupled adenylate cyclase assay, as well as their anatomical localization. Rat and human CRHs have very similar amino acid sequences; rat and human receptor sequences are highly homologous. The in vitro pharmacology of rat CRH receptors was originally studied in brain homogenates; in recent

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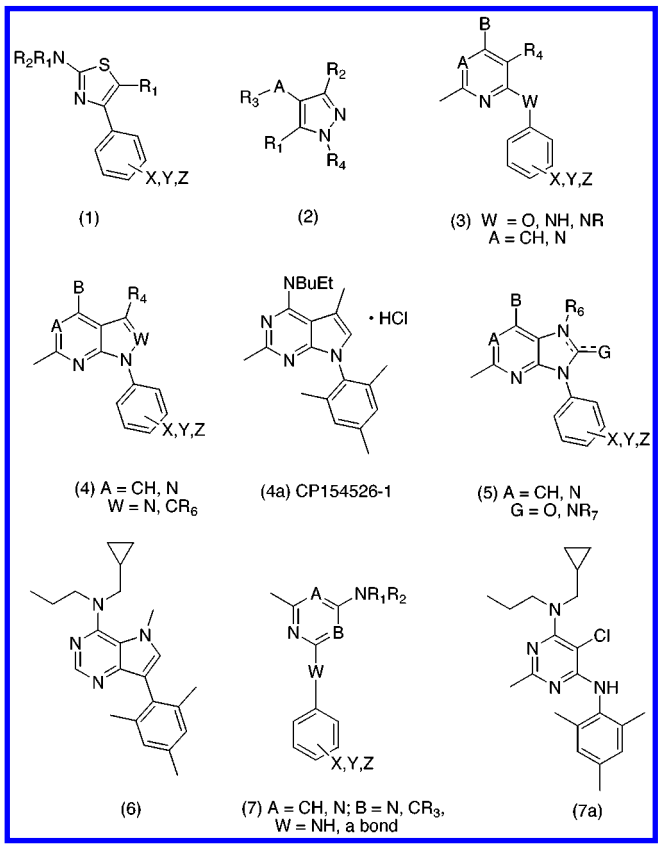
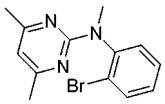
Scheme 1



years, the rodent and human receptors have been cloned and expressed in stable cell lines for binding assays. CRH_1 receptors are found primarily in the rat cortex, hypothalamus, amygdala, cerebellum, and pituitary. The CRH_1 receptors substantially outnumber CRH_2 sites in the pituitary. The $CRH_{2\alpha}$ receptor is a 411-amino acid protein, which is similar to the CRH_1 subtype (71% overall homology, 83% homology in the seven-transmembrane regions). $CRH_{2\alpha}$ receptors are also positively coupled to adenylate cyclase; the rank order of agonist potencies is sauvagine = urotensin > r/hCRH > ovine CRH (oCRH). $CRH_{2\alpha}$ receptors are primarily found in the rat lateral septum, ventromedial hypothalamus, amygdala, and entorhinal cortex, while they are virtually absent in the cerebellum, cortex, and pituitary. A splice variant, the $CRH_{2\beta}$ receptor, is a 431-amino acid protein, which is longer than $CRH_{2\alpha}$ at the amino terminus and is primarily expressed in rat heart, lung, and skeletal muscle. However, in human tissues, the $CRH_{2\alpha}$ subtype is also found in heart, lung, and skeletal muscle. The functional role of CRH receptor subtypes in specific diseases is still under investigation.[1,22,23]

There are reports in the literature on non-peptide CRH antagonists, with some biological data on their affinity for rCRH or $hCRH_1$ receptors. Aminothiazole derivatives of general formula **1** (Scheme 1) have been described as CRH antagonists, but no biological data were provided.[24] A series of substituted pyrazoles of formula **2** and fused and unfused pyrimidines and pyridines of formulas **3**−**5** have been reported.[25−31] The pharmacology of one of these compounds, **4a** (CP154526-1, rat CRH K_i = 5.7 nM vs 33 nM for α-hel-CRH(9−41)), has been described in detail.[32,33] Deazapurines[34] represented by compound **6** have been described as CRH_1 ligands. Specifically, the binding affinity of compound **6** has been reported (IC_{50} = 0.110 μM). We have reported, in preliminary form, our work on pyrimidine and triazine CRH antagonists **7** and fused derivatives thereof.[35−39] Finally, others[40,41] have reported on related pyrimidines and triazines of general formula **7**, providing pharmacology data on some derivatives. Compound **7a** was reported to have high affinity for $hCRH_1$ receptors (K_i = 1.7 nM vs 20 nM for D-Phe-CRH(12−41)). To date, no reports on CRH_2-selective ligands have been published.

Our research focused on the discovery of CRH_1 antagonists for the possible treatment of depression or anxiety disorders. At the time we initiated our program, the reports cited above had not appeared in print. Empirical screening of our chemical library using a rat receptor assay led to the discovery that **15-1** had weakly displaced [^{125}I]-0-Tyr-oCRH from rat frontal cortex homogenates when compared to the known peptide antagonist α-helical CRH(9−41) (K_i = 5700 nM vs 1 nM). Furthermore, **15-1** weakly inhibited CRH-stimulated adenylate cyclase activity in the same tissue, when compared to α-helical CRH(9−41) (IC_{50} = 20 000 nM vs 250 nM). We report herein on systematic structure−activity relationship studies to optimize CRH receptor binding affinity and pharmacokinetic parameters, wherein substituents on the heteroaryl and phenyl rings as well as the central nitrogen were varied. Triazine variants of **15-1** were also studied. Molecular modeling studies and research on the effects of restriction of conformation on biological activity have been reported separately.[37,39,42,43]



(15-1)

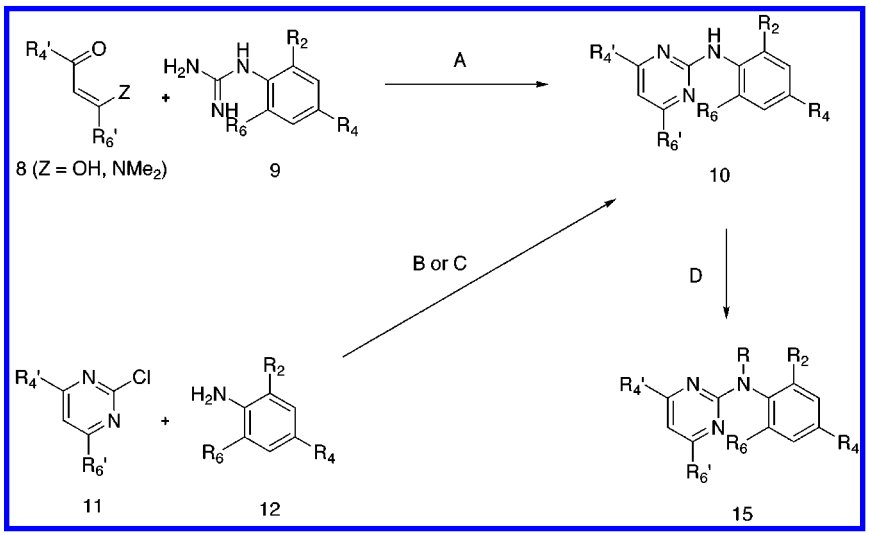
Results and Discussion

Chemistry. We sought general routes for the syntheses of analogues of **15-1** to enable rapid exploration of the SAR.

Scheme 2 illustrates a method starting with 1,3-dicarbonyl compounds or 3-(dimethylamino)propanone derivatives **8**.[44] These precursors were readily condensed with arylguanidines of formula **9** in refluxing toluene or *N*,*N*-dimethylformamide (DMF) solutions to afford 2-anilinopyrimidines of formula **10** in 40−80% yields. Alkylation of the central nitrogen produced the desired targets **15** in 70−90% yields. Alternatively, the coupling of various substituted anilines **12** with 2-chloro-4,6-disubstituted pyrimidines **11** under thermal or base-mediated conditions generated intermediates **10**, which were then transformed to compounds **15** as described above.

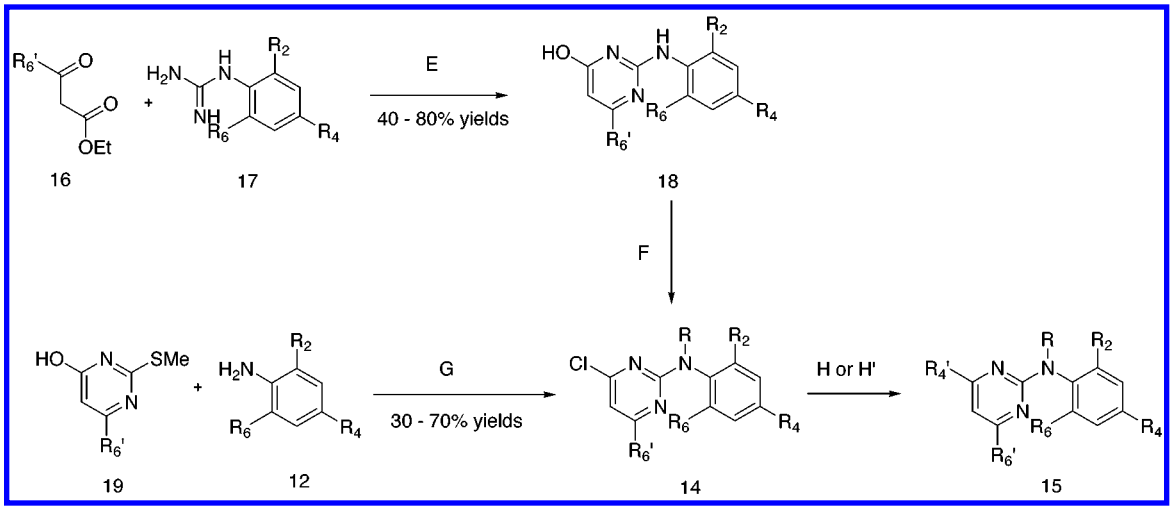
Scheme 3 depicts variations of the above conversions where the 4-substituents on the pyrimidine ring were introduced at later stages in the synthetic sequence. Condensation of 3-keto esters **16** with arylguanidines **17** under thermal or base-mediated conditions led to the formation of 2-anilino-4-hydroxypyrimidines **18** in 40−80% yields. Reaction with $POCl_3$ and subsequent alkylation of the central nitrogen generated precursors **14**

Scheme 2[a]



[a] Reactions: (A) DMF or toluene, Δ, 40−80% yields; (B) $(CH_2OH)_2$ or dioxane, Δ (50−60% yields); (C) NaH, THF, DMF or toluene, Δ (50−70% yields); (D) NaH, RX, THF or DMF, Δ, 70−90% yields.

Scheme 3[a]



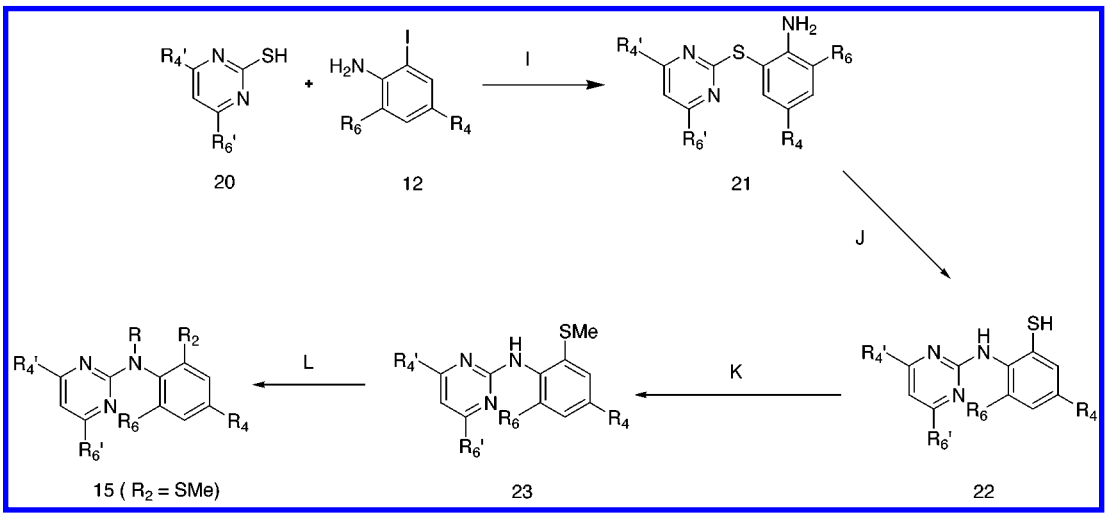
[a] Reactions: (E) neat, DMF or toluene, Δ or K_2CO_3, EtOH (40−80% yields); (F) (i) $POCl_3$, Δ, (ii) NaH, RX, DMF (40−80% yields); (G) (i) neat, Δ, (ii) NaH, RX, DMF, Δ, (iii) $POCl_3$, Δ (30−70% yields); (H) $R_4'H$, *i*-Pr_2NEt, THF or DMF, Δ (30−70% yields); (H′) (where R_4' = NR′R″) (i) excess $R'NH_2$, Δ, (ii) NaH, R″X, DMF.

in 40−80% yields. Nucleophilic displacement of the halogen at the 4-position of the pyrimidine ring gave the target compounds **15** in 30−80% yields. Alternatively, couplings with 2-(methylthio)-4-hydroxy-6-substituted pyrimidines **19** under thermal conditions afforded 2-anilino-4-hydroxy-6-substituted pyrimidines. Alkylation of the central nitrogen, followed by treatment with phosphorus oxychloride ($POCl_3$), gave intermediates **14**, which were then converted to the final products **15** as outlined above. In some cases, it was preferable to react intermediates **14** with primary amines and then alkylate the side chain nitrogen in the presence of a base to give products **15**, where R_4' is NR′R″ (overall yields range from 40% to 80%).

Scheme 4 illustrates a route to 2-anilinopyrimidines, which incorporates a novel variation of the Smiles rearrangement[45,46] as one of its key steps. Copper-mediated reaction of 2-pyrimidinethiols with 2-iodoanilines **12** (R_2 = I) produced 2-(phenylthio)pyrimidines **21**. Base- or acid-catalyzed Smiles rearrangement generated thiols **22** or their disulfide counterparts. Reduction of the disulfide−thiol mixtures and subsequent alkylation of the thiols gave intermediates **23**. Alkylation of the central nitrogen proceeded in straightforward fashion, as described for Scheme 2 above, to provide compounds **15** (where R_2 = SMe, overall yields range from 40% to 80%).

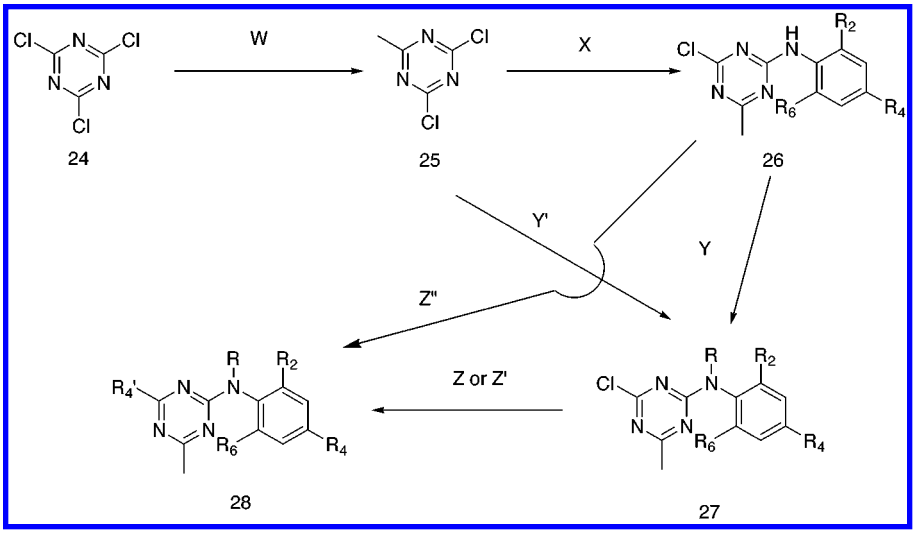
2-Anilinotriazines were prepared by the procedures outlined in Scheme 5.[47] Cyanuric chloride (**24**) was treated with methylmagnesium chloride to afford the 2,4-dichloro-6-methyltriazine (**25**) in 60−70% yields. Condensation of this intermediate with various substituted anilines generated monochlorotriazines **26** in 50−70% yields.[48] Alkylation of the central nitrogen proceeded in 80−95% yields, and subsequent nucleophilic addition of substituted amines in the presence of bases such as Hunig's base produced triazine targets **28** in 60−80% yields. In some cases, it was preferable to treat intermediates **27** with primary amines and then alkylate the side chain nitrogen to provide compounds **28**. Compounds **25** were also treated with *N*-alkylanilines to generate intermediates **27** in 30−70% yields. There were also instances where it was advantageous to react intermediates **26** with amines and alkylate these com-

Scheme 4[a]



[a] Reactions: (I) Cu, K_2CO_3, DMF; (J) HCl, MeOH, Et_2O or NaH, xylene or DMF, Δ; (K) (i) $NaBH_4$, EtOH, (ii) MeI; (L) NaH, RX, DMF (80−90% yields).

Scheme 5[a]



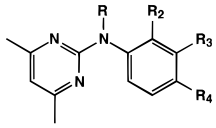
[a] Reactions: (W) MeMgCl, CH_2Cl_2, −30 °C to rt (60−70% yields); (X) **12**, DMF or dioxane, rt or Δ (50−70% yields); (Y) NaH, RX, THF or DMF (80−95% yields); (Y′) *i*-Pr_2NEt, $RNHC_6H_3R_2R_4R_6$, THF, Δ, (60−70% yields); (Z) $R_4'H$, *i*-Pr_2NEt, THF, DMSO or DMF (60−80% yields); (Z′) (for R4′ = NR′R″) (i) $R'NH_2$, *i*-Pr_2NEt, THF, DMSO or DMF (60−80% yields), (ii) NaH, DMSO, R″X, rt (70−90% yields); (Z″) (i) $R_4'H$, *i*-Pr_2NEt, THF, DMSO or DMF (55−67% yields), (ii) NaH, THF, RX, rt (80−95% yields).

pounds to produce products **28** where R_4' is NR′R″ (overall yields range from 40% to 80%).

Pharmacology. The screening strategy for our compounds consisted of evaluation of compounds in a receptor binding assay to assess affinity, followed by testing select analogues with good binding affinity ($K_i \leq 50$ nM) in a CRH-coupled adenylate cyclase assay to measure antagonist potency and rat pharmacokinetic experiments to determine plasma levels after different routes of administration. We initially employed a rat receptor binding assay, in which the displacements of [^{125}I]Tyr-o-CRH by our test compounds from rat frontal cortex homogenates were measured, since only this source for the rat receptor was readily available at the time we began our studies. Subsequently, we switched to a cloned human $hCRH_1$ receptor binding assay. We report data from the cloned human receptor assay in Tables 2−9. Antagonist potency for some high-affinity compounds was assessed in an adenylate cyclase inhibition assay using either rat frontal cortex homogenates[55] or HEK293 cells expressing $hCRH_1$ receptors.[56] The most promising compounds in terms of affinity were evaluated in rat or dog pharmacokinetic studies. The general pharmacology profiles of a few compounds have been reported elsewhere.[49]

2-Anilinopyrimidines. Initial SAR studies using the rat receptor binding assay focused on the effects of substitution on the phenyl ring of lead structure **15-1** (Table 1). Replacement of bromine at the 2-position with iodine afforded a modest enhancement in affinity (compare **15-1** vs **15-2**, mean K_i = 5700 nM vs 3655 nM). Movement of the iodine from the 2- to the 4- position retained binding affinity, suggesting that modification of these two positions in tandem might improve affinity for rCRH receptors (compare **15-2** with **15-3**, mean K_i = 3655 and 3301 nM, respectively). Introduction of several other ortho substituents destroyed binding affinity.[36] In the 2-bromophenyl subseries, binding affinity improved as the size of alkyl groups at the 4-position increased from methyl (molar refractivity (MR) = 0.56)[50,51] to isopropyl (MR = 1.50) at the 4-position of the phenyl ring. Replacement of the methyl

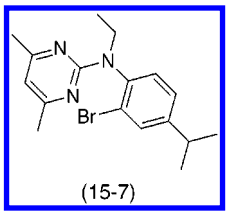
Table 1. Initial SAR Studies on 2-Anilinopyrimidines[a]



example	R	R_2	R_3	R_4	mean rCRH K_i (nM)	scheme, reactions	mp (°C)
α-hel-CRH (9−41)					1		
15-1	Me	Br	H	H	5700	2-B,D	165−167[b]
15-2	Me	I	H	H	3655	2-B,D	amorph
15-3	Me	H	H	I	3301	2-B,D	175−177[b]
15-4	Me	Br	H	Me	1099	2-B,D	oil
15-5	Me	Br	H	Et	187	2-B,D	126−127
15-6	Me	Br	H	*i*-Pr	96	2-B,D	163−164[b]
15-7	Et	Br	H	*i*-Pr	46 (63(h))	2-B,D	151−153[b]
15-8	Et	I	H	*i*-Pr	38	2-B,D	150−153[b]

[a] Note: h, human CRH_1 binding assay result. [b] HCl salt.

group on the central nitrogen with an ethyl moiety also improved binding affinity. Compound **15-7** (mean rat CRH K_i = 46 nM) was a pure CRH antagonist; it inhibited CRH-stimulated adenylate cyclase activity (IC_{50} = 180 nM for inhibition vs IC_{50} = 200 nM for α-hel-CRH(9−41)), but this compound had no effect on isoproterenol-stimulated or basal adenylate cyclase activity in rat frontal brain homogenates. Subsequently, we discovered that **15-7** had moderate affinity for the cloned $hCRH_1$ receptor (mean K_i = 63 nM, n = 3). This compound had no significant affinity for several other receptors (e.g., IC_{50} > 10 000 nM for serotonin receptors (vs [^{3}H]dLSD), adrenergic α_1 or α_2 receptors (vs [^{3}H]-prazosin or [^{3}H]RX781094, respectively), $GABA_a$ (vs [^{3}H]-GABA), and benzodiazepine (vs [^{3}H]flunitrazepam) as well as serotonin reuptake sites (vs [^{3}H]citalopram)).[52] Unfortunately, intraveneous, intraperitoneal, or oral administration of **15-7** to rats at 12 mg/kg (iv) or 30 mg/kg (ip, po) failed to generate detectable levels in plasma. Compound **15-7** became the starting point for studies, which were aimed at improving pharmacokinetic profile without reducing CRH binding affinity. Our operating hypothesis was that the high lipophilicity of **15-7** (HPLC log P > 6.4) was largely responsible for poor plasma levels.



Additional investigations of substituent effects on the phenyl ring, using the cloned human CRH_1 receptor assay, revealed that additional groups at the 2-position were well-tolerated. A methylthio group could replace bromine at the 2-position, affording some analogues with comparable or superior binding affinity (e.g., compare **15-7** ($hCRH_1$ K_i = 63 nM) with **15-15** ($hCRH_1$ K_i = 36 nM)). Introduction of methyl or trifluoromethyl groups also provided analogues with good binding affinity (compare **15-16** ($hCRH_1$ K_i = 20 nM) with **15-19** ($hCRH_1$ K_i = 54 nM) and **15-21** (hCRH K_i = 68 nM)). Removal of an ortho substituent greatly reduced receptor binding affinity (cf. **15-13** ($hCRH_1$ K_i > 1000 nM)).

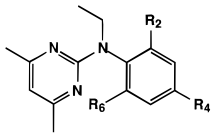
Variation of groups at the 4-position of the phenyl ring was explored next, for a constant substituent at the 2-position. Binding affinity for $hCRH_1$ receptors decreased quickly as the size of the 4-substituent increased beyond a certain value. In the 2-bromophenyl subseries, the replacement of 4-isopropyl group with butyl or *tert*-butyl (MR = 1.50 vs 1.96 for both)[50,51] reduced binding affinity (compare **15-7** vs **15-11** and **15-12** ($hCRH_1$ K_i = 63 nM vs >100 and >100 nM, respectively)). The introduction of moieties, which are approximately isosteric to or smaller than the isopropyl group, afforded derivatives with moderate to good binding affinity in the 2-methylthiophenyl subseries (e.g., NMe_2, OMe, $COCH_3$; MR = 1.55, 0.79, 1.12, respectively)[50,51] (compare **15-15** ($hCRH_1$ K_i = 36 nM) vs **15-16**, **15-17**, and **15-18** ($hCRH_1$ K_i = 20, 57, and 97 nM, respectively)). Removal of the 4-substituent reduced affinity (cf. **15-9** ($hCRH_1$ K_i > 1000 nM)). Previously we reported that the introduction of hydroxyl-substituted groups or very basic amino groups at the 4-position of the phenyl ring decreased binding affinity in the 2-methylthiophenyl set.[36] These data indicate that there are steric bulk, polarity, and basicity constraints on substitution at the 4-position of the phenyl ring.

The SAR at the 6-position of the phenyl ring was briefly explored. Replacement of hydrogen at the 6-position by methoxy or methylthio groups afforded compounds with good to moderate binding affinity (compare **15-7** vs **15-23** and **15-24** ($hCRH_1$ K_i = 63 nM vs 26 and 51 nM, respectively).

Variations in substitution on the central nitrogen in **15-7** have been reported previously.[36] Allyl or ethyl substitutions were optimal; replacement of these two moieties by hydrogen abolished affinity for $hCRH_1$ receptors.

Substitution on the 4- or 6-position of the pyrimidine ring in **15-7** was evaluated next. Binding affinity for the CRH receptor drops as the size of both the 4- and 6-substituents increases from methyl to ethyl to propyl.[36,50,51] However, if the substituent at the 6-position was held constant as a methyl group, then a wide variety of substituents could be introduced at the 4-position of the pyrimidine ring, generating anilinopyrimidines with moderate to high binding affinity. The 4-(3-pentyl) analogue **15-26** (MR for 3-pentyl = 2.42) had high affinity ($hCRH_1$ K_i = 2nM), indicating large groups may be tolerated at the 4-position in the pyrimidine ring. Structurally diverse amino substituents at this position were also very well-tolerated. Many 4-substituted amino analogues of **15-7** had high binding affinity; for example, **15-35**, **15-36**, **15-37**, **15-28**, **15-29**, and **15-30** had $hCRH_1$ K_i values of 9, 5, 9, 22, 11, and 14 nM, respectively. Therefore, size alone cannot be considered a critical factor for substitution at the 4-position of the pyrimidine ring. The introduction of the dibasic amine piperazine reduced binding affinity (cf. **15-42** ($hCRH_1$ K_i > 1000 nM)) as did the introduction of acidic moieties (compare **15-38**, bearing a tetrazole ring with **15-39**, bearing a *N*-methyltetrazole ring ($hCRH_1$ K_i > 1000 nM vs 41 nM)). Restricting the conformation of the flexible dialkylamino side chains had minimal effect on receptor affinity; cyclic amino analogues had comparable or 2−3-fold weaker affinity

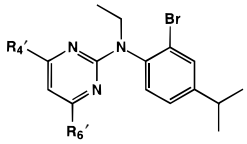
Table 2. SAR Studies on 2-Anilino-4,6-dimethylpyrimidines: The Phenyl Ring



example	R_2	R_4	R_6	mean hCRH$_1$ K_i (nM)	scheme, reactions	mp (°C)
α-hel-CRH (9−41)				1		
15-9	Br	H	H	>1000	2-B,D	94−95
15-7	Br	*i*-Pr	H	63	2-B,D	151−153[b]
15-10	Br	OMe	H	35	2-B,C,D	amorph
15-11	Br	Bu	H	>100	2-B,D	124−126[b]
15-12	Br	*t*-Bu	H	>100	2-B,C	180−185
15-13	H	*i*-Pr	H	>1000	2-B,D	66−68
15-15	SMe	*i*-Pr	H	36	4-I,J,K,L	64−66
15-16	SMe	NMe_2	H	20	4-I,J,K,L	119−120
15-17	SMe	OMe	H	60	*a*	128−130
15-18	SMe	$COCH_3$	H	90	*a*	125−126
15-19	Br	OMe	OMe	12	2-B,D	113−115
15-20	Me	NMe_2	H	54	2-B,D	79−81
15-21	CF_3	NMe_2	H	68	2-B,D	121−123
15-22	Br	NMe_2	OMe	5	2-B,D	amorph
15-23	Br	*i*-Pr	OMe	26	2-C,D	amorph
15-24	Br	*i*-Pr	SMe	51	4-I,J,K,L	117−119[b]

[a] See Experimental Section. [b] HCl salt.

Table 3. SAR Studies on 2-Anilinopyrimidines: The Pyrimidine Ring



example	R_4'	R_6'	mean hCRH$_1$ K_i (nM)	scheme, reactions	mp (°C)
α-hel-CRH(9−41)			1		
15-7	Me	Me	63	2-A,D	151−153
15-25	Et	Et	>100	2-A,D	120−121
15-26	3-pentyl	Me	2	2-A,D	oil
15-27	NHCH-3-pentyl	Me	44	3-G,H	oil
15-28	N(Et)CH_2CH_2OMe	Me	22	3-E,F,H″	oil
15-29	N(Pr)CH_2CH_2OMe	Me	11	3-E,F,H″	127−129[b]
15-30	N(CH_2Ph)CH_2CH_2OMe	Me	14	3-E,F,H″	oil
15-31	N(CH_2CH_2OMe)$_2$	Me	25	3-E,F,H″	oil
15-32	NEt_2	Me	22	3-G,H	150−152[c]
15-33	NBuEt	Me	19	3-G,H	159−160
15-34	N(Et)CH_2Ph	Me	17	3-E,F,H″	oil
15-35	NPr_2	Me	9	3-E,F,H″	oil
15-36	NPr(c-C_3H_5)	Me	5	3-E,F,H″	oil
15-37	NPr(CH_2-c-C_3H_5)	Me	9	3-G,H	138−139
15-38	N(Bu)CH_2-5-tetrazolyl	Me	>1000	3-E,F,H′	oil
15-39	N(Bu)CH_2-(1-Me-5-tetrazolyl)	Me	41	*a*	oil
15-40	morpholino	Me	22	2-B,D	219−222[c]
15-41	piperidinyl	Me	29	2-C,D	211−212[c]
15-42	piperazine	Me	>1000	2-B,D	233−234[c]

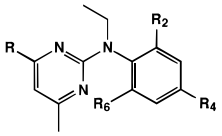
[a] See Experimental Section. [b] Fumarate salt. [c] HCl salt.

than their acyclic counterparts (compare **15-32**, the diethylamino analogue, with **15-41**, the piperidyl derivative (hCRH$_1$ K_i = 22 and 29 nM, respectively)). The data from the above discussion suggest that increasing the lipophilicity of the substituted amino groups at the 4-position of the pyrimidine ring may enhance binding affinity, but increasing the basicity or acidity of these groups diminishes binding affinity.

The SARs for discrete regions of the 2-anilinopyrimidine core structure were not independent for each region. High-affinity analogues could be obtained by simultaneous modification of substituents at both the pyrimidine and phenyl termini (Tables 4 and 5).

The SARs for the 2-anilinopyrimidines based on data reported here and previously[36] may be summarized as depicted in Scheme 6. For the phenyl group, medium-sized lipophilic substituents at the 2-position, preferably Br, I, or MeS moieties, enhance hCRH$_1$ binding affinity. In separate reports,[39,42] molecular modeling and NMR studies indicate that the ortho substituent enforces an approximate orthogonal relationship between the phenyl and pyrimidine rings. Medium-sized lipophilic groups at the 4-position of the phenyl ring, which may or may not be weak hydrogen bond acceptors, contribute to good binding affinity (e.g., *i*-Pr, OMe, NMe_2, $COCH_3$). At the 6-position of the phenyl ring, H, OMe, and SMe appear

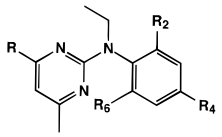
Table 4. SAR Studies on 2-Anilinopyrimidines: Additivity Effects



example	R	R_2	R_4	R_6	mean $hCRH_1$ K_i (nM)	scheme, reactions	mp (°C)
α-hel-CRH(9−41)					1		
15-33	NBuEt	Br	*i*-Pr	H	22	3-G,H	159−160[a]
15-40	morpholinyl	Br	*i*-Pr	H	22	2-B,D	219−222[b]
15-43	NEt_2	Br	I	H	9	2-C,D	oil
15-44	NE*t*-Bu	Br	I	H	14	2-C,D	oil
15-46	NEt_2	Br	$COCH_3$	H	5	2-C,D	amorph
15-47	NE*t*-Bu	Br	$COCH_3$	H	8	2-C,D	118−120[a]

[a] Fumarate salt. [b] HCl salt.

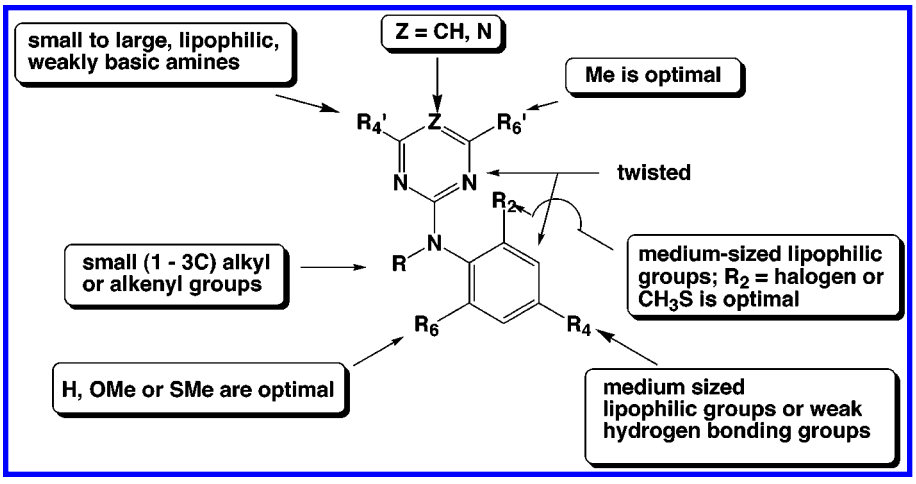
Table 5. SAR Studies on 4-(Diethylamino)-2-anilinopyrimidines



example	R	R_2	R_4	R_6	mean $hCRH_1$ K_i (nM)	scheme, reactions	mp (°C)
15-46	NEt_2	Br	$COCH_3$	H	5	2-C,D	amorph
15-48	NEt_2	Cl	OMe	OMe	10	2-B,D	oil
15-49	NEt_2	CF_3	NMe_2	H	11	2-B,D	106−107
15-50	NEt_2	Cl	OMe	Cl	15	2-C,D	oil
15-51	NEt_2	Br	SMe	H	24	2-C,D	oil
15-52	NEt_2	Br	SO_2Me	H	24	2-C,D	amorph
15-53	NEt_2	Cl	Br	Cl	24	2-C,D	oil
15-54	NEt_2	Br	*i*-Pr	SMe	26	2-C,D	amorph
15-55	NEt_2	Br	NMe_2	H	27	3-G,H	oil
15-56	NEt_2	Br	Br	OMe	31	2-C,D	105−107[b]
15-57	NEt_2	Br	$COCH_3$	OMe	28	*a*	91−94

[a] See Experimental Section. [b] HCl salt.

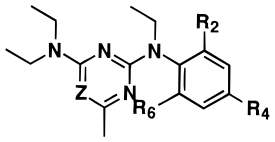
Scheme 6. Structure−Activity Relationships



to be optimal. The minimal studies made at the meta positions of the phenyl ring do not permit any definitive conclusions. Small lipophilic alkyl or alkenyl groups on the central nitrogen contribute to optimal binding affinity (i.e., ethyl or allyl).[36] There is a severe steric constraint on substitution at the 6-position of the pyrimidine ring; a methyl group is the optimal substituent. In contrast, substituents with a wide variety of sizes and shapes may be introduced at the 4-position of the pyrimidine ring. Lipophilic substituents, which may be weakly basic or hydrogen bonding, tend to be favored; very basic or acidic moieties are not well- tolerated.

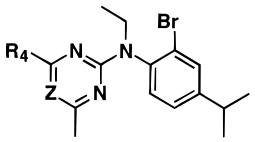
Compound **15-19**, a silent $hCRH_1$ antagonist from this series,[56] was evaluated in rat pharmacokinetic studies. Data are presented in Table 9 (doses = 30 mg/kg (iv, ip, po)). Intraperitoneal administration afforded higher plasma levels than did oral administration (mean C_{max} = 1884 nM vs 366 nM). Bioavailability via the ip route was also superior to that by the oral route (26% vs 4%). The clearance was unusually high (5.32 L/h/kg). None-

Table 6. SAR Studies on Anilinotriazines versus Pyrimidines



example	Z	R_2	R_4	R_6	mean $hCRH_1$ K_i (nM)	scheme, reactions	mp (°C)
28-1	N	Br	NMe_2	H	6	7-W,X,Y,Z	76−78
15-55	CH	Br	NMe_2	H	27	3-G,H	oil
28-2	N	Br	OMe	OMe	10	7-W,X,Y,Z	122−124
15-58	CH	Br	OMe	OMe	48	2-B,D	oil
28-3	N	Br	$COCH_3$	H	17	7-W,X,Y,Z	amorph
15-46	CH	Br	$COCH_3$	H	5	2-C,D	amorph
28-7	N	CF_3	NMe_2	H	20	7-W,X,Z″	oil
15-49	CH	CF_3	NMe_2	H	11	2-B,D	106−107
28-4	N	Br	*i*-Pr	H	27	7-W,X,Y,Z	100−101
15-59	CH	Br	*i*-Pr	H	30	3-G,H	oil

Table 7. SAR Studies on Anilinotriazines versus Pyrimidines



example	Z	R_4	mean $hCRH_1$ K_i (nM)	scheme, reactions	mp (°C)
28-6	N	NBuEt	5	7-W,X,Y,Z	75−77[a]
15-33	CH	NBuEt	18	3-G,H	159−160[b]
28-7	N	$N(CH_2CH_2OMe)_2$	18	7-W,X,Z″	oil
15-31	CH	$N(CH_2CH_2OMe)_2$	25	3-E,F,H′	oil
28-5	N	NEt_2	27	7-W,X,Y,Z	oil
15-32	CH	NEt_2	30	3-G,H	150−152[a]

[a] Fumarate salt. [b] HCl salt.

theless, these parameters were significantly superior to those for the lead structure, **15-7**. The HPLC log *P* values were 4.48 for **15-19** and >6.4 for **15-7**. Reduction in lipophilicity relative to **15-7** improved intraperitoneal and, to a lesser extent, oral pharmacokinetic profiles in this case.

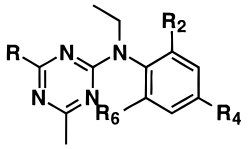
2-Anilinotriazines. Anilinotriazines were then explored in an effort to further reduce lipophilicity and to improve oral absorption. The replacement of CH by N in the heterocycle did not adversely affect receptor binding affinity. The SARs for the triazines were parallel, but not identical, to those for the corresponding pyrimidines (cf. Tables 6 and 7) when the substitution on the phenyl ring was varied and the 4-substituent on the heterocycle was kept constant as a diethylamino group. The $hCRH_1$ K_i values for pairs of congeners rarely varied by more than 3-fold. Similarly, when the phenyl group was kept constant as a 2-bromo-4-isopropylphenyl moiety, the effects of amine substitution at the 4-position of the triazine ring appeared to be parallel to that in the pyrimidine series for this limited set of examples. Table 8 delineates the binding data for 4-substituted triazines with a variety of phenyl ring substitution patterns. A variety of tertiary amine substitutions (e.g., cycloalkylamino or methoxyalkylamino moieties) were well-tolerated at the 4-position of the triazine ring, which is consistent with the observations for the pyrimidine series described above. The following observations were made about the effects of phenyl ring substituents: (1) bromine, methyl, and trifluoromethyl groups at the 2-position of the phenyl ring are compatible with good binding affinity; (2) isopropyl, dimethylamino, cyano, methoxy, trifluoromethoxy, and acetyl substituents at the 4-position of the phenyl ring contribute to good binding affinity; and (3) hydrogen or a methoxy group at the 6-position of the phenyl ring appears to be optimal. All the above data indicate the SARs for the triazine and pyrimidine series to be similar.

Compound **28-13** ($hCRH_1$ K_i = 32 nM, HPLC log *P* = 3.63) was evaluated in the cloned human CRH_1 adenylate cyclase assay and rat and dog pharmacokinetic studies.[53−55] This compound was found to be a silent $hCRH_1$ antagonist.[56] In the first trial, the compound was administered at 12 mg/kg (iv) and 30 mg/kg (iv, po), while in a second experiment the compound was given at 1 mg/kg (iv, po) (Table 9). In the first trial, the bioavailabilities were 47% (ip) and 19% (po) at 30 mg/kg and the mean peak plasma levels were 2698 nM (ip) and 753 nM (po). The differences in bioavailability between the two routes of administration reflected lower oral absorption. Furthermore, at 1 mg/kg, the oral bioavailability was 2%, and the mean peak plasma level was only 21 nM. Possibly, extensive first-pass metabolism was responsible for the lower plasma levels, but at the higher dose, saturation of the metabolic enzymes occurred. Mass spectral studies suggest that one of the methyl ethers was cleaved, but it was not possible to assign the metabolite's structure based on NMR data. In the dog, **28-13** was evaluated at 5 mg/kg (iv, po). The oral biovailability was 20% and the mean peak oral plasma level was 730 nM at 0.5 h. The elimination half-life after iv administration was 6.0 h. While the rat and dog pharmacokinetic profiles of this compound were superior to those for the lead **15-7**, members from another chemical series showed more promising pharmacokinetic profiles,[37,43] and compound **28-13** was not advanced further.

Conclusion

The SARs for 2-anilinopyrimidines and -triazines have been defined through systematic variation of substituents in each of the three regions of the core structures. A weak lead structure **15-1** was transformed into more potent analogues with significantly improved binding affinity (>1000-fold), and significant improvements in the pharmacokinetic profiles of some analogues

Table 8. SAR Studies on 2-Anilinotriazines



example	R	R_2	R_4	R_6	mean $hCRH_1$ K_i (nM)	scheme, reactions	mp (°C)
α-hel-CRH(9−41)					1		
28-8	NH-allyl	Br	*i*-Pr	H	33	7-W,X,Y,Z	90
28-9	NH-3-pentyl	Br	*i*-Pr	H	41	7-W,X,Y′,Z	oil
28-10	$N(allyl)_2$	Br	*i*-Pr	H	4	7-W,X,Y,Z	oil
28-6	NBuEt	Br	*i*-Pr	H	5	7-W,X,Y,Z	75−77
28-11	NPr_2	Br	*i*-Pr	H	4	7-W,X,Y,Z	oil
28-12	N(allyl)-*c*-C_3H_5	Br	*i*-Pr	H	5	7-W,X,Y,Z′	75−77
28-2	NEt_2	Br	OMe	OMe	10	7-W,X,Y,Z	122−124
28-13	morpholinyl	Br	OMe	OMe	32	7-W,X,Y,Z	164−165[a]
28-14	$N(CH_2CH_2OMe)Bu$	Me	Me	Me	13	7-W,X,Y,Z′	oil
28-15	NBuEt	Me	Me	Me	23	7-W,X,Y,Z	oil
28-16	$N(CH_2CH_2OMe)(CH_2$-*c*-$C_3H_5)$	Me	Me	Me	25	7-W,X,Y,Z′	oil
28-17	$NEt(CH_2CH_2OMe)$	Me	Me	Me	31	7-W,X,Y,Z′	oil
28-18	$N(allyl)_2$	Br	OCF_3	H	4	7-W,X,Y,Z	oil
28-19	NBuEt	Br	OCF_3	H	5	7-W,X,Y,Z	oil
28-20	$N(allyl)_2$	Br	NMe_2	H	3	7-W,X,Y,Z	oil
28-1	NEt_2	Br	NMe_2	H	5	7-W,X,Y,Z	76−78
28-21	$NPr(CH_2$-*c*-$C_3H_5)$	CF_3	NMe_2	H	5	7-W,X,Y,Z	75−78
28-22	$N(CH_2$-*c*-$C_3H_5)_2$	CF_3	NMe_2	H	5	7-W,X,Y,Z	100−103
28-23	$NPr(CH_2CH_2CN)$	CF_3	NMe_2	H	5	7-W,X,Y,Z	oil
28-24	NPr_2	CF_3	NMe_2	H	6	7-W,X,Y,Z	85−87
28-25	$NBuCH_2CN$	CF_3	NMe_2	H	6	7-W,X,Y,Z	oil
28-26	$NPrCH_2CH_2CN$	Me	NMe_2	H	7	7-W,X,Y,Z	oil
28-27	NPr_2	Me	NMe_2	H	7	7-W,X,Y,Z	oil
28-28	$NBuCH_2CH_2CN$	Me	NMe_2	H	8	7-W,X,Y,Z	oil
28-29	$NBuCH_2CN$	Me	NMe_2	H	8	7-W,X,Y,Z	75−78

[a] $MeSO_3H$ salt.

Table 9. Rat and Dog Pharmacokinetic Data[a]

example	species	dose (mg/kg, route)	CL (L/h/kg)	$t_{1/2}$ (h)	C_{max} (nM)	T_{max} (h)	%*F*
15-19	rat	30 (iv)	5.32	5.9			
		30 (ip)			1884	0.25	26
		30 (po)			366	1.2	4
28-13	rat	12 (iv)	2.9	15.6			
		30 (ip)			2698	0.3	47
		30 (po)			753	1.6	19
		1 (iv)	4.0	7.6			
		1 (po)			21	0.3	3
	dog	5 (iv)	1.3	6.0			
		5 (po)			730	0.5	20

[a] For rat studies, $n = 4$; for dog studies, $n = 3$. Mean values are given throughout. 0.5% Methocel−water was the vehicle for oral and ip administrations; a mixed solvent system was employed for iv studies (*N*,*N*′-dimethylacetamide − ethanol − propylene glycol − PEG400 − 0.1 N NaOH, 1:1:3:3:2). HPLC detection was used for all studies except the 1 mg/kg experiment where LC/MS/MS analysis was utilized.

relative to that for an intermediate lead structure **15-7** were also achieved through these SAR studies. Some representative compounds had good intraperitoneal, but modest oral pharmacokinetic profiles in the rat. Compound **28-13**, in particular, had a promising profile in the dog. None of the compounds in this paper were found to have binding affinity for the cloned $hCRH_{2\alpha}$ receptors.[56] These studies served as the basis for design of more potent $hCRH_1$ compounds with superior pharmacokinetic profiles.[37,43]

Experimental Section

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

Reagents were purchased from commercial sources and, when necessary, purified prior to use according to the general procedures outlined by Perrin and Armarego.[57] Chromatography was performed on silica gel using the solvent systems indicated below. For mixed solvent systems, the volume ratios are given. Otherwise, parts and percentages are by weight. All reactions were performed under a nitrogen atmosphere using magnetic stirring. Reactions requiring anhydrous conditions were performed in glassware, which had been flame-dried or oven-dried with purging under a nitrogen atmosphere. Reactions using aqueous media were run under the ambient atmosphere. Anhydrous magnesium sulfate ($MgSO_4$) was used routinely to dry the combined organic layers from extractions. Solvent was routinely removed in vacuo, using a rotary evaporator, followed by evacuation with vacuum pump.

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

***N*-[2-Bromo-4-(1-methylethyl)phenyl]-*N*-ethyl-4-methyl-6-morpholinyl-2-pyrimidinamine, Hydrochloride Salt (15-40) (Scheme 2, Steps B, D).** A mixture of 2,4-dichloro-6-methylpyrimidine (4 g, 24.5 mmol), morpholine (2.14 mL, 24.5 mmol), and diisopropylethylamine (4.52 mL, 26.0 mmol) in ethanol (60 mL) was stirred at 0 °C for 3 h, at 25 °C for 24 h, and at reflux temperature for 1 h. The solvent was then removed in vacuo, and the residue was taken up in a 1 M NaOH solution (50 mL) and extracted with EtOAc three times (200 mL). The combined organic layers were washed with water and then brine; then they were dried, filtered, and concentrated in vacuo to afford a solid. Recrystallization from EtOAc−hexanes gave 2-chloro-4-morpholinyl-6-methylpyrimidine (3.8 g): ^{1}H NMR ($CDCl_3$) δ 6.24 (s, 1H), 3.78−3.74 (m, 2H), 3.64−3.61 (m, 2H), 2.35 (s, 3H).

A mixture of the above intermediate (1 g, 4.7 mmol) and 2-bromo-4-isopropylaniline (1 g, 4.7 mmol) in ethylene glycol (6 mL) was heated at reflux temperature for 1.5 h. After being cooled to ambient temperature, it was partitioned between EtOAc (100 mL) and a 1 M NaOH solution (20 mL). The organic layer was washed with water and brine, dried, filtered, and concentrated in vacuo. The crude product was chromatographed (EtOAc−hexanes, 1:3) to give 2-*N*-[2-bromo-4-(1-methylethyl)phenyl]-4-morpholinyl-6-methylpyrimidamine-(1.5 g): ^{1}H NMR ($CDCl_3$) δ 8.34 (d, 1H, J = 8), 7.38 (d, 1H, J = 2), 7.20 (s, 1H), 5.92 (s, 1H), 3.80−3.76 (m, 2H), 3.61−3.57 (m, 2H), 2.90−2.78 (m, 1H), 2.30 (s, 3H), 1.23 (d, 6H, J = 7).

A solution of the above intermediate (1.0 g, 2.56 mmol) in THF (10 mL) was treated with NaH (60% in oil, 0.15 g, 3.75 mmol) at room temperature for 20 min, and then EtI (0.32 mL, 4 mmol) was added. The mixture was stirred for 24 h and then at reflux temperature for 5 h and was partitioned between EtOAc (100 mL) and water (20 mL). The organic extract was washed with brine, dried, filtered, and concentrated in vacuo. The crude product was chromatographed (EtOAc−hexanes, 12:88) to give the title product (0.94 g) as the free base: ^{1}H NMR ($CDCl_3$) δ 7.48 (s, 1H), 7.16 (s, 1H), 7.16 (s, 1H), 5.75 (s, 1H), 4.2−4.0 (br, 1H), 3.80−3.60 (br, 1H), 3.70−3.60 (br, 2H), 3.50−3.30 (br, 2H), 2.96−2.80 (m, 1H), 2.2 (br, 3H), 1.26 (d, 6H, J = 7), 1.19 (t, 3H, J = 7).

The free base was dissolved in ether (10 mL) and treated with a solution of HCl in ether (1 M, 4 mL, 4 mmol). The precipitate was collected and dried in vacuo (0.94 g, 98% yield): mp 219−222 °C. Anal. ($C_{20}H_{27}N_4BrO\cdot HCl\cdot 0.5H_2O$) C, H, N, Br, Cl.

2-*N*-[2-Bromo-4-(1-methylethyl)phenyl]-*N*-ethyl-4,6-dimethyl-2-pyrimidinamine, Hydrochloride Salt (15-7) (Scheme 2, Steps A, D). 2-Bromo-4-isopropylaniline (6 g, 28.2 mmol) and cyanamide (4.7 g, 112.1 mmol) were dissolved in a mixture of EtOAc (100 mL) and EtOH (13 mL). To this mixture was added a solution of HCl in ether (1 M, 38 mL 38 mmol). The reaction mixture was stirred at room temperature for 1 h, and then, the ether was distilled from the reaction mixture. The remaining solution was heated at reflux for 3 h, cooled to ambient temperature, and diluted with ether (120 mL). The precipitate was collected by filtration and dried to give the crude guanidinium hydrochloride (10.4 g), which was used for the next reaction without purification.

The crude product from the previous reaction (5 g, 13.5 mmol), K_2CO_3 (1.86 g, 13.5 mmol), and 2,4-pentanedione (2.8 mL, 27.28 mmol) were mixed in DMF (35 mL) and stirred at reflux temperature for 24 h. After being cooled to room temperature, the reaction mixture was partitioned between EtOAc (120 mL) and a 0.5 M NaOH solution (100 mL). The aqueous layer was extracted with EtOAc (120 mL), and the combined organic layers were washed with water and then brine. Drying, filtration, and removal of solvent in vacuo afforded a residue. Column chromatography (8% EtOAc−hexanes) gave 2-*N*-[2-bromo-4-(1-methylethyl)phenyl]-4,6-dimethyl-2-pyrimidinamine (3.37 g).

The above intermediate (3 g, 9.37 mmol) was reacted with NaH (60% in oil, 0.4 g, 10 mmol, prewashed with hexanes) and EtI (1.7 g, 11 mmol) in THF at ambient temperature for 16 h. After being cooled to room temperature, the reaction mixture was partitioned between EtOAc (120 mL) and a 0.5 M NaOH solution (100 mL). The aqueous layer was extracted with EtOAc (120 mL), and the combined organic layers were washed with water and then brine. Drying, filtration, and removal of solvent in vacuo afforded a residue. Column chromatography (8% EtOAc−hexanes) gave the title product (2.88 g, 73% yield): ^{1}H NMR ($CDCl_3$) δ 7.50 (d, 1H, J = 2), 7.19 (dd, 1H, J = 8, 2), 6.85 (br s, 1H), 6.30 (s, 1H), 4.13−4.10 (m, 1H), 3.82−3.76 (m, 1H), 3.00−2.85 (m, 1H), 2.23 (br s, 6H), 1.28 (d, 6H, J = 7), 1.19 (t, 3H, J = 7).

Treatment of the free base with a solution of HCl in ether (1M, 10 mL, 10 mmol) afforded a solid, which was collected by filtration. Trituration with ether, filtration, and dyring in vacuo afforded a powder: mp 151−153 °C. Anal. ($C_{17}H_{22}BrN_3\cdot$HCl) C, H, N, Br, Cl.

***N*-(2-Bromo-4-methoxyphenyl)-*N*-ethyl-4,6-dimethylpyrimidin-2-amine (15-10) (Scheme 2, Steps C, D).** Sodium hydride (60% in oil, 554 mg, 13.9 mmol) was washed with hexanes and decanted twice. Toluene (15 mL) was added, followed by a solution of 2-bromo-4-methoxyaniline (1.27 g, 6.30 mmol) in toluene (15 mL). After the reaction mixture was stirred for 15 min, 2-chloro-4,6-dimethylpyrimidine (1.35 g, 9.45 mmol) was added. The reaction was then warmed to reflux temperature and stirred for 42 h. After the mixture was cooled to room temperature, water was carefully added to quench the reaction. Three extractions with EtOAc, drying the combined organic layers, filtration, and removal of solvent in vacuo gave a brown solid. Recrystallization from EtOAc−hexanes afforded *N*-(2-bromo-4-methoxyphenyl)-4,6-dimethylpyrimidin-2-amine (547 mg, 25% yield): mp 118−119 °C; ^{1}H NMR ($CDCl_3$) δ 8.40 (d, 1H, J = 9), 7.2 (br s, 1H), 7.1 (d, 1H, J = 2), 6.9 (dd, 1H, J = 9, 2), 6.5 (s, 1H), 3.8 (s, 3H), 2.35 (s, 6H); CI-HRMS m/z calcd 308.0398, found 308.0404 $(M + H)^+$.

Sodium hydride (60% in oil, 56 mg, 1.4 mmol) was washed with hexanes and decanted twice. Anhydrous DMF (10 mL) was added with stirring. The above intermediate (357 mg (1.2 mmol) was added, and the reaction mixture was stirred for 30 min. Iodoethane (0.11 mL, 1.4 mmol) was added; the mixture was then stirred at ambient temperature for 16 h. Water (10 mL) was carefully added. The resulting mixture was extracted three times with dichloromethane (20 mL). The combined organic layers were washed with water (20 mL) twice, dried, and filtered. Solvent was removed in vacuo. Column chromatography (EtOAc−hexanes, 1:4) gave the title product, a tan solid, after removal of solvent in vacuo (293 mg, 75% yield): ^{1}H NMR ($CDCl_3$) δ 7.20 (d, 1H, J = 2), 7.15 (d, 1H, J = 7), 6.9 (dd, 1H, J = 7, 2), 6.3 (s, 1H), 4.25−4.15 (m, 1H), 3.8 (s, 3H), 3.75−3.65 (m, 1H); CI-HRMS m/z calcd 336.1077, found 336.0711 $(M + H)^+$. Anal. ($C_{15}H_{18}BrN_3O$) C, H, Br, N.

***N*-(2-Bromo-4-isopropylphenyl)-*N*-ethyl-4-(diethylamino)-6-methylpyrimidin-2-amine, Hydrochloride Salt (15-32) (Scheme 3, Steps G, H).** A mixture of 6-methyl-2-(methylthio)-4(3*H*)-pyrimidinone (20 g, 128 mmol) and 2-bromo-4-isopropylaniline (36 g, 169 mmol) was heated at 190 °C for 7 h. Vacuum distillation afforded a semisolid residue. Recrystallization from ether provided 2-[(2-bromo-4-isopropylphenyl)-amino]-6-methyl-4(3*H*)-pyrimidinone, a colorless white solid (24.0 g, 48% yield): ^{1}H NMR ($CDCl_3$) δ 8.04 (d, 1H, J = 8), 7.43 (d, 1H, J = 2), 7.18 (dd, 1H, J = 8, 2), 5.91 (s, 1H), 2.87 (m, 1H, J = 7.0), 2.21 (s, 3H), 1.22 (d, 6H, J = 7).

A mixture of the above intermediate (23 g, 66.7 mmol) and $POCl_3$ (70 mL) was stirred at room temperature for 5 h. Concentration in vacuo gave a thick liquid which was poured onto ice. After the ice had melted, EtOAc (200 mL) was added, and the two-phase mixture was stirred for 15 min. The organic phase was separated and washed with water (50 mL), a saturated $NaHCO_3$ solution (100 mL), and brine (100 mL). Drying, filtration, and removal of solvent in vacuo provided 2-[(2-bromo-4-isopropylphenyl)amino]-4-chloro-6-methylpyrimidine, a thick amber oil (23 g, 95% yield): ^{1}H NMR ($CDCl_3$) δ 8.34 (d, 1H, J = 8.4), 7.50 (br s, 1H), 7.42 (d, 1H, J = 2), 7.20 (dd, 1H, J = 8, 2), 6.67 (s, 1H), 2.86 (m, 1H, J = 7), 2.41 (s, 3H), 1.24 (d, 6H, J = 7).

Sodium hydride (100%, 240 mg, 10 mmol) was added to a solution of the above intermediate (2.5 g, 6.9 mmol) in anhydrous DMSO (10 mL). After 5 min, iodoethane (2.5 g, 16 mmol) was added, and the reaction mixture was stirred at ambient temperature for 1 h. The reaction mixture was partitioned between ethyl acetate and water, and the organic phase was washed with brine, dried, and evaporated to give *N*-(2-bromo-4-isopropylphenyl)-*N*-ethyl-4-chloro-6-methylpyrimidin-2-amine, a thick amber oil (2.05 g, 76.4% yield): ^{1}H NMR ($CDCl_3$) δ 7.51 (d, 1H, $J = 2$), 7.20 (m, 2H), 5.28 (s, 1H), 4.18 (m, 1H), 3.69 (m, 1H, $J = 7$), 2.92 (m, 1H, $J = 7$), 2.21 (br s, 3H), 1.28 (d, 6H, $J = 7$), 1.20 (t, 3H, $J = 7$); MS (CI) *m*/*z* 368, 370 $(M + H)^+$.

A solution of the above intermediate (900 mg, 2.31 mmol) in a 1:1 mixture of DMSO and diethylamine (5 mL) was stirred at reflux temperature for 4 h. Additional diethylamine (2.5 mL) was added at 0.5-h intervals. The cooled reaction mixture was poured onto brine and extracted twice with ethyl acetate. The combined extracts were dried and evaporated in vacuo. Column chromatography (EtOAc−hexanes, 15:85, then 30:70, then 1:1) gave the title compound, an oil (860 mg, 92% yield): ^{1}H NMR ($CDCl_3$) δ 7.48 (s, 1H), 7.15 (s, 2H), 5.65 (s, 1H), 4.15 (br s, 1H), 3.75 (br s, 1H), 3.21 (br s, 2H), 2.90 (m, 1H $J = 7$), 2.21 (br s, 3H), 1.25 (d, 6H, $J = 7$), 1.20 (t, 3H, $J = 7$), 0.97 (br s, 6H); MS (CI) *m*/*z* 405, 407 $(M + H)^+$. Anal. ($C_{20}H_{29}N_4Br$) C, H, N.

The HCl salt was prepared by treatment with a solution of HCl in ether (1 M, 3.0 mL, 3.0 mmol), followed by recrystallization from EtOAc−ether: mp 150−152 °C; CI-MS *m*/*z* 405, 407 $(M + H)^+$.

***N*-(2-Iodo-4-isopropylphenyl)-4-chloro-6-methylpyrimidin-2-amine (Scheme 3, Steps E, F).** A mixture of 2-iodo-4-isopropylphenylguanidine (12.0 g, 39.5 mmol), ethyl acetoacetate (15 mL, 118 mmol), and K_2CO_3 (2 g, 14.50 mmol) in ethanol (120 mL) was stirred at reflux temperature for 100 h. The reaction mixture was cooled to ambient temperature; the solvent was removed in vacuo. The residue was chromatographed (EtOAc−hexanes, 4:6) to give 2-*N*-[2-iodo-4-(1-methylethyl)phenyl]-4-hydroxy-6-methyl-2-pyrimidinamine (4.0 g, 27% yield): ^{1}H NMR ($CDCl_3$) δ 7.74 (d, 1H, $J = 8$), 7.70 (s, 1H), 7.23 (d, 1H, $J = 8$), 5.88 (s, 1H), 2.92−2.8 (m, 1H), 2.18 (s, 3H), 1.24 (d, 6H, $J = 7$); CI-MS *m*/*z* 370 $(M + H)^+$.

The above intermediate (2.5 g, 6.7 mmol) was dissolved in $POCl_3$ (20 mL), and the reaction mixture was stirred for 4 h. The mixture was then poured into ice water. The aqueous mix was stirred for 30 min and then extracted twice with EtOAc (200 mL). The combined organic extracts was washed with brine, dried, and concentrated in vacuo. The residue was chromatographed (EtOAc−hexanes, 1:4) to give 2-*N*-[2-iodo-4-(1-methylethyl)phenyl]-4-chloro-6-methyl-2-pyrimidinamine (1.64 g, 63% yield): ^{1}H NMR ($CDCl_3$) δ 8.17 (d, 1H, $J = 9$), 7.66 (d, 1H, $J = 2$), 7.27 (br s, 1H), 7.23 (dd, 1H, $J = 9$, 2), 2.89−2.90 (m, 1H), 2.40 (s, 3H), 1.23 (d, 6H, $J = 7$).

The above intermediate (1.6 g, 4.2 mmol) was reacted with NaH (60% in oil, prewashed with hexanes, 190 mg, 5.0 mmol) and iodoethane (1.0 g, 5.5 mmol) in DMF (25 mL) for 24 h. The reaction mixture was poured onto water (200 mL). The aqueous mix was extracted three times with EtOAc. The combined organic layers were dried, filtered, and concentrated in vacuo. Flash chromatography (EtOAc−hexanes, 1:6) afforded the title product (1.2 g, 70% yield): ^{1}H NMR($CDCl_3$) δ 7.77 (d, 1H, $J = 2$), 7.25 (dd, 1H, $J = 8$, 2), 7.09 (d, 1H, $J = 8$), 4.30−4.16 (m, 1H), 3.68−3.52 (m, 1H), 2.96−2.84 (m, 1H), 2.42−2.10 (m, 3H), 1.27 (d, 6H, $J = 7$), 1.22 (t, 3H, $J = 7$); CI-MS *m*/*z* 416, 418 $(M + H)^+$.

N-[2-(Methylthio)-4-carbethoxyphenyl]-*N*-ethyl-4,6-dimethyl-2-pyrimidinamine (Scheme 4, Steps I−L, Acid-Catalyzed Smiles Rearrangement). A solution of ethyl 4-aminobenzoate (5 g, 30.3 mmol) in CH_2Cl_2 (12 mL) was added to a solution of $NaHCO_3$ (4.2 g, 50 mmol) in water (12 mL). Iodine (9.25 g, 39.2 mmol) was added, and then the mixture was stirred at room temperature for 20 h. The reaction mixture was partitioned between EtOAc (100 mL) and a saturated Na_2SO_3 solution. The organic extract was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Column chromatography (20% EtOAc−hexanes) gave ethyl 4-amino-3-iodobenzoate (7.92 g, 90% yield): ^{1}H NMR ($CDCl_3$) δ 8.33 (d, 1H $J = 2$), 7.82 (dd, 1H, $J = 8$, 2), 6.70 (d, 1H, $J = 8$), 4.32 (q, 2H, $J = 7$), 4.50−4.00 (br s, 2H), 1.36 (t, 3H, $J = 7$).

Ethyl 4-amino-3-iodobenzoate (4 g, 13.7 mmol), 4,6-dimethyl-2-mercaptopyrimidine (2.32 g, 16.5 mmol), K_2CO_3 (2.37 g, 17.00 mmol), and Cu powder (0.52 g, 8.46 mmol) were mixed in DMF (30 mL) and stirred at reflux temperature for 30 min. After being cooled to ambient temperature, the mixture was diluted with EtOAc (60 mL) and the solids were collected by filtration. The filtrate was partitioned between EtOAc (100 mL) and water (100 mL). The organic extract was washed twice with water (100 mL). The combined aqueous layers were extracted with EtOAc (50 mL). The combined organic extracts were washed with brine (50 mL), dried, and concentrated in vacuo. The crude product, *S*-(2-amino-5-carbethoxyphenyl)-4,6-dimethyl-2-thiopyrimidine (3.95 g, 95%), was used without further purification: ^{1}H NMR ($CDCl_3$) δ 8.19 (d, 1H, $J = 2$), 7.92 (dd, 1H, $J = 8$, 2), 6.77 (d, 1H, $J = 8$), 6.70 (s, 1H), 4.70 (br s, 2H), 4.31 (q, 2H, $J = 7$), 2.33 (s, 3H), 1.36 (t, 3H, $J = 7$).

The above intermediate (2.95 g, 7.72 mmol) was dissolved in MeOH (40 mL) and treated with a solution of HCl in ether (1 M, 10 mL, 10 mmol) at room temperature for 60 h. The solvent was concentrated in vacuo. The residue was dissolved in EtOH (70 mL), and the resulting solution was cooled to 0 °C. $NaBH_4$ (603 mg, 16 mmol) was added in portions, and the mixture was allowed to warm to room temperature with stirring over 1 h. Iodomethane (0.6 mL, 9.6 mmol) was added; then the reaction mixture was stirred for 16 h. EtOAc (50 mL) was added, and the precipitate was removed by filtration. The filtrate was concentrated in vacuo. Column chromatography (20% EtOAc−hexanes) gave *N*-[2-(methylthio)-4-carbethoxyphenyl]-4,6-dimethyl-2-pyrimidinamine (1.45 g, 45% overall yield): ^{1}H NMR($CDCl_3$) δ 8.80 (d, 1H, $J = 9$ Hz), 8.47 (br s, 1H), 8.22 (d, 1H, $J = 2$), 8.00 (dd, 1H, $J = 9$, 2), 6.60 (s, 1H), 4.36 (q, 2H, $J = 7$), 2.42 (s, 3H), 2.41 (s, 3H), 1.39 (t, 3H, $J = 7$).

The above intermediate (1.2 g, 3.8 mmol) was dissolved in dry DMF (12 mL) and treated with NaH (60% in oil, 278 mg, 6.88 mmol) at room temperature for 15 min. EtI (0.64 mL, 8 mmol) was added, the reaction mixture was stirred for 16 h, and then it was partitioned between EtOAc (100 mL) and water (30 mL). The organic extract was washed twice with water (30 mL) and then brine (30 mL), dried, filtered, and concentrated in vacuo. Column chromatography (20% EtOAc−hexanes) provided the title product (1.2 g, 92% yield): mp 99−100 °C; ^{1}H NMR ($CDCl_3$) δ 7.96 (d, 1H, $J = 2$), 7.85 (dd, 1H, $J = 8$, 2), 7.21 (d, 1H, $J = 8$), 4.40 (q, 2H, $J = 7$), 4.05−3.90 (br s, 2H), 2.42 (s, 3H), 2.22 (s, 6H), 1.41 (t, 3H, $J = 7$), 1.19 (t, 3H, $J = 7$). Anal. ($C_{18}H_{23}N_3O_2S$) C, H, N, S.

***N*-(4-Acetyl-2-bromo-6-methoxyphenyl)-*N*-ethyl-4-(diethylamino)-6-methylpyrimidinamine (15-57).** A mixture of *N*-(2,4-dibromo-6-methoxyphenyl)-*N*-ethyl-4-(diethylamino)-6-methylpyrimidinamine (2.0 g, 4 mmol), ethoxyvinyltri-*n*-butyltin (1.4 mL, 4.15 mmol), $Pd(PPh_3)_2Cl_2$ (70 mg, 0.1 mmol), and $Pd(PPh_3)_4$ (100 mg, 0.1 mmol) in dry toluene (15 mL) was heated at reflux temperature for 20 h. After being cooled to room temperature, the reaction mixture was concentrated in vacuo and the residue was taken up in a 10% HCl solution (75 mL) and stirred for 1 h. The aqueous solution was washed twice with ether (30 mL) and then basified with a 50% NaOH solution. The basic solution was extracted twice with EtOAc (50 mL), and the combined organic extracts were washed with brine (30 mL), dried, filtered, and concentrated in vacuo. The residue was chromatographed (20% EtOAc−hexanes) to give the title product (890 mg, 51% yield): mp 91−94 °C; ^{1}H NMR ($CDCl_3$) δ 7.80 (d, 1H, $J = 2$), 7.51−7.46 (m, 1H), 5.72−5.65 (m, 1H), 4.05−3.80 (m, 2H), 3.81 (s, 3H), 3.55−3.45 and 3.15−3.05 (2m, 4H), 2.61 (s, 3H), 2.29, 2.02 (2s, 3H), 1.10−1.24 and 0.82−0.94 (2m, 6H), 1.16 (t, 3H, $J = 7$). Anal. ($C_{20}H_{27}BrN_4O_2$) C, H, N, Br.

2-*N*-[4-Methoxy-2-(methylthio)phenyl]-*N*-ethyl-4,6-dimethyl-2-pyrimidinamine (15-17). A mixture of 2-*N*-[4-

bromo-2-(methylthio)phenyl]-*N*-ethyl-4,6-dimethyl-2-pyrimidinamine (352 mg, 1 mmol), CuBr (14.3 mg, 0.1 mmol), a solution of MeONa in MeOH (25% w/w, 0.5 mL, 2.5 mmol), and dry DMF (5 mL) was heated to reflux temperature and stirred for 1.5 h. After being cooled to ambient temperature, the reaction mixture was partitioned between EtOAc (100 mL) and water (30 mL). The organic layer was washed twice with water (30 mL) and once with brine, dried, and concentrated in vacuo. The residue was chromatographed (20% EtOAc−hexanes) to give the title product (210 mg, 69% yield): mp 128−130 °C; ^{1}H NMR ($CDCl_3$) δ 7.04 (d, 1H, J = 9), 6.80 (d, 1H, J = 3), 7.70 (dd, 1H, J = 9, 3), 6.28 (s, 1H), 4.35−4.20 (m, 1H), 3.85 (s, 3H), 3.65−3.50 (m, 1H), 2.35 (s, 3H), 2.22 (s, 6H), 1.18 (t, 3H, J = 7). Anal. ($C_{16}H_{21}N_3OS\cdot0.25H_2O$) C, H, N, S.

***N*-[2-Bromo-4-(dimethylamino)phenyl]-*N*-ethyl-4-(diethylamino)-6-methyl-1,3,5-triazin-2-amine (28-1) (Scheme 5, Steps W−Z).** A solution of methylmagnesium bromide in ether (3 M, 100 mL, 300 mmol) was added dropwise to a stirred solution of cyanuric chloride (13.0 g, 70.5 mmol) in anhydrous dichloromethane (300 mL) at −20 °C. After the addition was complete, the reaction mixture was stirred at −20 °C for 4 h, after which time water (38 mL, 2.1 mol) was added dropwise at a rate such that the temperature of the reaction stayed below 10 °C. After warming to room temperature, the reaction mixture was diluted with additional water and methylene chloride and passed through a pad of filter-aid. The organic layer was dried and evaporated to give 2,4-dichloro-6-methyl-1,3,5-triazine, a yellow solid (9.0 g, 77.8%): mp 170 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.71 (3H, s).

A mixture of 2,4-dichloro-6-methyltriazine (1.78 g, 10.8 mmol), 2-bromo-4-(dimethylamino)aniline (2.33 g, 10.8 mmol), diisopropylethylamine (4.19 g, 32.5 mmol), and dioxane (20 mL) was stirred at room temperature for 16 h; then it was poured onto water (200 mL). Three extractions with EtOAc (75 mL), two washings of the combined organic layers with water (50 mL), drying the combined organic layers, filtration, and removal of solvent in vacuo provided a solid.

Column chromatography (EtOAc−hexanes, 1:4) gave one fraction after removal of solvent in vacuo: *N*-[2-bromo-4-(dimethylamino)phenyl]-4-chloro-6-methyltriazin-2-amine (2.13 g, 57% yield): mp 183−184 °C; NMR ($CDCl_3$) δ 7.90−7.80 (m, 1H), 7.45−7.28 (m, 1H), 6.90 (d, 1H, J = 3), 6.70 (dd, 1H, J = 9, 3), 2.48 (s, 3H), 2.96 (s, 6H).

Sodium hydride (60% in oil, 200 mg, 5.0 mmol) was washed with hexanes and decanted twice. Anhydrous DMF (7 mL) was added, followed by a solution of the above intermediate (1.63 g, 4.8 mmol) in DMF (6 mL). The reaction mixture was stirred until gas evolution stopped. Iodoethane (780 mg, 5.00 mmol) was added. The reaction mixture was stirred for 60 h at room temperature; then it was poured onto water (100 mL). Three extractions with EtOAc (75 mL), two washings of the combined organic layers with water (50 mL), drying the combined organic layers, filtration, and removal of solvent in vacuo provided a solid. Column chromatography (EtOAc−hexanes, 1:4) gave *N*-[2-bromo-4-(dimethylamino)phenyl]-*N*-ethyl-4-chloro-6-methyltriazin-2-amine, a white solid (R_f = 0.3, 1.45 g, 82% yield), after removal of solvent in vacuo: ^{1}H NMR ($CDCl_3$) δ 7.00 (d, 1H, J = 9), 6.95 (d, 1H, J = 3), 6.69−6.64 (m, 1H), 4.26−4.12 (m, 1H), 3.69−3.61 (m, 1H), 3.00, 2.99 (2s, 6H), 2.49, 2.28 (2s, 3H), 1.21 (t, 3H, J = 7).

A mixture of the above intermediate (400 mg, 1.1 mmol), diethylamine (788 mg, 10.8 mmol), and diisopropylethylamine (0.42 g, 3.24 mmol) was stirred at room temperature for 16 h. The reaction mixture was diluted with water (10 mL) and extracted three times with EtOAc (10 mL). The combined organic layers were dried, filtered, and concentrated in vacuo. Flash chromatography (CH_2Cl_2−MeOH, 98.5:1.5) afforded the title product, a solid (R_f = 0.35, 440 mg, 100% yield), after removal of solvent in vacuo: mp 76−78 °C; ^{1}H NMR ($CDCl_3$) δ 7.05−7.03 (m, 1H), 6.97 (d, 1H, J = 3), 6.67 (d, 1H, J = 9), 4.30−4.15 (m, 1H), 3.80−3.25 (m, 4H), 3.25−3.00 (m, 1H), 2.97 (s, 6H), 2.35, 2.15 (2s, 3H), 1.25−1.00 (m, 8H), 0.95−0.8 (br s, 1H); CI-HRMS m/z calcd for $C_{18}H_{27}BrN_6$ 407.1559, found 407.1578 $(M + H)^+$. Anal. ($C_{18}H_{27}BrN_6$) C, H, N.

***N*-(2-Bromo-4-isopropylphenyl)-*N*-ethyl-4-(dipropylamino)-6-methyl-1,3,5-triazin-4-amine (28-11) (Scheme 5, Steps Y′, Z).** A solution of 2,4-dichloro-6-methyl-1,3,5-triazine (1.3 g, 7.9 mmol), diisopropylamine (3.0 mL, 17.4 mmol), and *N*-ethyl-2-bromo-4-isopropylaniline (1.92 g, 7.9 mmol) in THF (65 mL) was heated at reflux temperature for 2 h. The cooled reaction mixture was poured onto water, and the aqueous mixture was extracted twice with EtOAc. The combined extracts were washed with brine, dried, and evaporated in vacuo. Column chromatography (EtOAc−hexanes, 1:19, then 1:9) provided *N*-(2-bromo-4-isopropylphenyl)-*N*-ethyl-2-chloro-6-methyl-1,3,5-triazin-4-amine, an oil (1.96 g, 67% yield): ^{1}H NMR ($CDCl_3$) (this spectrum is consistent for approximately equal populations of two rotamers) δ 7.53 (s, 1H), 7.25 (d, 1H, J = 8), 7.10 (d, 1H, J = 8), 4.19 (m, 1H), 3.67 (m, 1H), 2.94 (m, 1H), 2.51 (s, 1.5H), 2.28 (s, 1.5H), 1.29 (d, 3H, J = 7), 1.28 (d, 3H, J = 7), 1.23 (t, 1.5H, J = 7), 1.22 (t, 1.5H, J = 7).

A solution of the above intermediate (1.2 g, 3.2 mmol), dipropylamine (2.6 g, 3.5 mL, 25.2 mmol), and diisopropylethylamine (0.65 mL, 3.7 mmol) in dry DMSO (20 mL) was heated at 150 °C for 1 h. The cooled mixture was poured onto water (150 mL) and extracted twice with ethyl acetate (75 mL). The combined extracts were washed with brine, dried, and evaporated in vacuo. Column chromatography (CH_2Cl_2−MeOH, 98:2) afforded the title product, an oil (1.16 g, 94% yield). Anal. ($C_{17}H_{32}N_5Br$) C, H, N, Br.

2-*N*-[4-Acetyl-2-(methylthio)phenyl]-*N*-ethyl-4,6-dimethyl-2-pyrimidinamine (15-18). 2-*N*-[4-Cyano-2-(methylthio)phenyl]-*N*-ethyl-4,6-dimethyl-2-pyrimidinamine (0.5 g, 1.68 mmol) was dissolved in dry C_6H_6 (10 mL). A solution of CH_3MgI in ether (3 M, 1.1 mL, 3.3 mmol) was added. The mixture was stirred for 2 h and at reflux temperature for 1 h. After the mixture cooled to room temperature, water (5 mL) and a 10% HCl solution (5 mL) were added. Stirring was continued for 15 min. A 1 M NaOH solution was added until the reaction mixture was alkaline. Three extractions with EtOAc, drying the combined organic layers, filtration, and removal of solvent in vacuo afforded a residue. Column chromatography (20% EtOAc−hexanes) provided the title product (370 mg, 70% yield): mp 125−126 °C; ^{1}H NMR ($CDCl_3$) δ 7.89 (d, 1H, J = 2), 7.75 (dd, 1H, J = 8, 2), 7.25 (d, 1H, J = 8), 6.33 (s, 1H), 4.05−3.90 (m, 2H), 2.64 (s, 3H), 2.43 (s, 3H), 2.23 (s, 6H), 1.20 (t, 3H, J = 7). Anal. ($C_{17}H_{21}N_3OS$) C, H, N, S.

Biology. Rat CRH Receptor Binding Assay: This assay was performed essentially as described in the literature[53] with minor modifications. Mean K_i values from two determinations each are reported.

Cloned Human CRH_1 Binding Assay: The following is a description of the isolation of cell membranes containing cloned human CRF-R1 receptors for use in the standard binding assay as well as a description of the assay itself.

Messenger RNA was isolated from human hippocampus. The mRNA was reverse-transcribed using oligo (dt) 12−18, and the coding region was amplified by PCR from start to stop codons. The resulting PCR fragment was cloned into the *Eco*RV site of pGEMV, from whence the insert was reclaimed using *Xho*I + *Xba*I and cloned into the *Xho*I + *Xba*I sites of vector pm3ar (which contains a CMV promoter, the SV40 't′ splice and early poly(A) signals, an Epstein−Barr viral origin of replication, and a hygromycin selectable marker). The resulting expression vector, called phchCRFR, was transfected in 293EBNA cells, and cells retaining the episome were selected in the presence of 400 μM hygromycin. Cells surviving 4 weeks of selection in hygromycin were pooled, adapted to growth in suspension, and used to generate membranes for the binding assay described below. Individual aliquots containing approximately 1×10^8 of the suspended cells were then centrifuged to form a pellet and frozen.

For the binding assay a frozen pellet described above containing 293EBNA cells transfected with hCRFR1 receptors was homogenized in 10 mL of ice-cold tissue buffer (50 mM HEPES buffer, pH 7.0, containing 10 mM $MgCl_2$, 2 mM EGTA, 1 μg/L aprotinin, 1 μg/mL leupeptin, and 1 μg/mL pepstatin).

The homogenate was centrifuged at 40000*g* for 12 min and the resulting pellet rehomogenized in 10 mL of tissue buffer. After another centrifugation at 40000*g* for 12 min, the pellet was resuspended to a protein concentration of 360 μg/mL to be used in the assay. Protein determinations were performed according to the method of Lowry et al.[59] using bovine serum albumin as a standard.

Binding assays were performed in 96-well plates, each well having a 300-μL capacity. To each well was added 50 μL of test drug dilutions (final concentration of drugs ranged from 10^{-10} to 10^{-5} M), 100 μL of [^{125}I]ovine-CRF ([^{125}I]o-CRF) (final concentration 150 pM), and 150 μL of the cell homogenate described above. Plates were then allowed to incubate at room temperature for 2 h before filtering the incubate over GF/F filters (presoaked with 0.3% poly(ethylenimine)) using an appropriate cell harvester. Filters were rinsed two times with ice-cold assay buffer before removing individual filters and assessing them for radioactivity on a gamma counter.

Curves of the inhibition of [^{125}I]o-CRF binding to cell membranes at various dilutions of test drug were analyzed by the iterative curve-fitting program LIGAND,[54] which provides K_i values for inhibition which are then used to assess biological activity. K_i values are the average of at least two determinations unless otherwise noted (>100 or >1000).

Pharmacokinetic Studies: Pharmacokinetic parameters were determined in rats (n = 4) at the doses and vehicles specified in the text. At 15 min (iv only), 30 min, and 1, 2, 4, 8, and 16 h after dosing, rats were sacrificed at the specified times and trunk blood samples were collected into tubes containing EDTA. Dogs (n = 3) were given 5 mg/kg of compound iv or 5 mg/kg po in 0.5% methylcellulose suspension. Blood samples were collected from jugular veins at predose, 5, 15, and 30 min, and 1, 2, 4, 8, 10, 12, 16, 24, 32, 48, 56, and 72 h after dosing. Compounds were extracted from plasma by simple liquid−liquid extraction. The liquid chromatography consisted of a Perkin-Elmer series 200 solvent delivery system (Norwalk, CT), a Perkin-Elmer ISS 200 autoinjector, and a Waters Symmetry octyl minibore column (2.1 × 50 mm). Concentrations were determined from UV absorption data relative to appropriate internal standards.

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Supporting Information Available: Synthetic procedures and spectral and physical data for the following intermediates: *N*-ethyl-*N*-(2-bromo-4-isopropyl)aniline, 2-bromo-4-(dimethylamino)aniline, 2-bromo-4-methoxyaniline, 2,6-dibromo-4-methoxyaniline, 4-amino-3-bromobenzonitrile, 4-(dimethylamino)-2-methylaniline, 4-(dimethylamino)-2-(trifluoromethyl)aniline, 2-iodo-4-(1-methylethyl)aniline, 2-bromo-4-(2-hydroxyethyl)aniline, 2-bromo-6-iodo-4-isopropylaniline, and 2-bromo-4-isopropyl-6-(methylthio)aniline. This information is available free of charge via the Internet at http://pubs.acs.org.

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