Synthesis, Corticotropin-Releasing Factor Receptor Binding Affinity, and Pharmacokinetic Properties of Triazolo-, Imidazo-, and Pyrrolopyrimidines and -pyridines

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The synthesis and CRF receptor binding affinities of several new series of *N*-aryltriazolo- and -imidazopyrimidines and -pyridines are described. These cyclized systems were prepared from appropriately substituted diaminopyrimidines or -pyridines by nitrous acid, orthoester, or acyl halide treatment. Variations of amino (ether) pendants and aromatic substituents have defined the structure−activity relationships of these series and resulted in the identification of a variety of high-affinity agents (K_i's < 10 nM). On the basis of this property and lipophilicity differences, six of these compounds (**4d,i,n,x, 8k, 9a**) were initially chosen for rat pharmacokinetic (PK) studies. Good oral bioavailability, high plasma levels, and duration of four of these compounds (**4d,i,n,x**) prompted further PK studies in the dog following both iv and oral routes of administration. Results from this work indicated **4i**,**x** had properties we believe necessary for a potential therapeutic agent, and **4i**[1] has been selected for further pharmacological studies that will be reported in due course.

Introduction

Corticotropin-releasing factor (CRF), a 41-amino acid peptide, is the primary physiological regulator of the hypothalamic−pituitary−adrenal (HPA) axis by its action as a secretagogue of adrenocorticotropic hormone (ACTH), β-endorphin, and other pro-opiomelanocortin (POMC)-derived peptides from the anterior pituitary gland.[2,3] In addition to its endocrine role at the pituitary, immunohistochemical localization of CRF has demonstrated that the peptide has broad extrahypothalamic distribution in the central nervous system and produces a wide spectrum of autonomic, electrophysiological, and behavioral effects consistent with a neurotransmitter or neuromodulator role in the brain.[4−7] Evidence demonstrating that CRF may play a significant role in integrating response of the immune system to physiological, psychological, and immunological stressors also exists.[8,9]

Preclinical studies in rats and nonhuman primates provide some pharmacological support for the hypothesis that hypersecretion of CRF may be involved in symptoms seen in human depression and related disorders.[10] Clinical evidence supporting the hypothesis that CRF may indeed play a role in psychiatric disorders and neurological diseases, including depression and anxiety-related diseases, is considerable and compelling and is reviewed in the preceding paper.[11]

A role for CRF has also been postulated in the etiology of anxiety-related disorders. Centrally administered CRF produces effects in animals, including increased arousal in familiar and agitation to unfamiliar surroundings, consistent with the manifestations of anxiety.[12,13] Interactions between both benzodiazepine and non-benzodiazepine anxiolytics and CRF have been demonstrated in a variety of behavioral anxiety models.[14,15] Preliminary studies using the putative CRF receptor antagonist α-helical ovine CRF_{9-41} in a variety of behavioral paradigms show that the antagonist produces "anxiolytic-like" effects that are qualitatively similar to those of benzodiazepines.[12,16−18] Neurochemical, endocrine, and receptor binding studies have all demonstrated interactions between CRF and benzodiazepine anxiolytics providing further evidence for involvement of CRF in these disorders. Chlordiazepoxide has been shown to attenuate the "anxiogenic" effects of CRF in both the conflict[14] and the acoustic startle tests[19] in rats. The benzodiazepine receptor antagonist (Ro15-1788), which was without behavioral activity alone in the operant conflict test, reversed effects of CRF in a dose-dependent manner; the benzodiazepine inverse agonist (FG7142) enhanced the actions of CRF.[20]

The effects of centrally administered CRF are largely independent of the activation of ACTH and corticosteroids,[17] and the primary event through which CRF initiates its stressor responses is by interaction with

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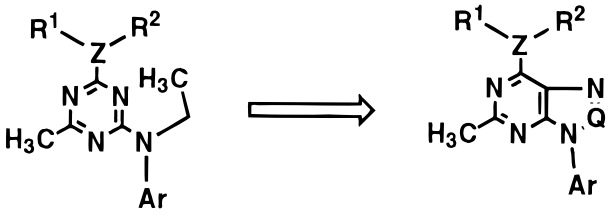
cell-surface proteins on brain target cells. The recent cloning of CRF_1 and CRF_2 receptor subtypes, also reviewed in the previous paper,[11] provides opportunity to differentiate some of the intricacies of CRF function by allowing study of its action as a hormone and a neurotransmitter.[21] However, to fully comprehend the physiological significance of these receptor subtypes and their associated responses, subtype-selective compounds must be identified.

Thus, the search for small-molecule receptor subtype-specific ligands is being carried out to help clarify the role of these various receptors in mediating physiological response. More importantly, however, based on the substantial body of pharmacological and clinical evidence, there is high anticipation that a small-molecule CRF receptor antagonist should indeed provide beneficial therapy for affective disorders or anxiety-related conditions due to CRF's role in orchestrating the body's response to stress.

Chemistry

Our initial chemistry effort in this area focused on optimization of the binding affinity of a non-peptide lead, identified by high-throughput screening of our in-house compound library.[11] After this lead identification, our Computer Assisted Drug Design (CADD) group determined conformational preferences and barriers to rotation by semiempirical methods and established global energy minima for certain of these compounds.[22] X-ray determinations and variable-temperature NMR spectroscopy provided physical evidence to support these calculated conformations. This work determined that a cyclized version of these diarylamines, wherein the alkyl group of the central nitrogen atom of **i** was rigidly held in position through attachment with the heterocyclic ring, should provide a constrained bicyclic ligand **ii** with potential high affinity for this receptor. This prediction



i ii

was indeed validated by the binding affinities of the first members of this series that were synthesized which were comparable to some of the best members of the noncyclized series as described in the accompanying paper.[23] A recent Pfizer report[24] also described high CRF receptor binding affinity for a similar bicyclic system during the course of this work. We then proceeded to optimize the activity of these bicyclics by preparing a variety of related molecules for evaluation of their binding affinity, as well as absorption, distribution, and pharmacokinetic properties of selected agents that might be considered for further evaluation as a potential therapeutic.

Triazolo- and Imidazopyrimidines 4 and 8, Respectively (Scheme 1). Treatment of dichloroaminopyrimidine **1**[25] with 2-bromo-4-isopropylaniline in ethoxyethanol at elevated temperatures afforded diarylamino adduct **2**, a key intermediate for the construction of the annelated heterocyclic systems. Cyclization of **2** to triazolopyrimidines **3** was accomplished using sodium nitrite in aqueous acid with methylene chloride as a cosolvent that considerably improved the yield of this conversion. Treatment of bicyclic chlorides **3** with primary or secondary amines in protic solvents such as ethanol in the presence of triethylamine produced the aminated triazolopyrimidines **4**. Triazolopyrimidine ring desmethyl analogues **11** and **12** were prepared from commercially available 5-amino-4,6-dichloropyrimidine using the reactions described to prepare **4** from **2**.

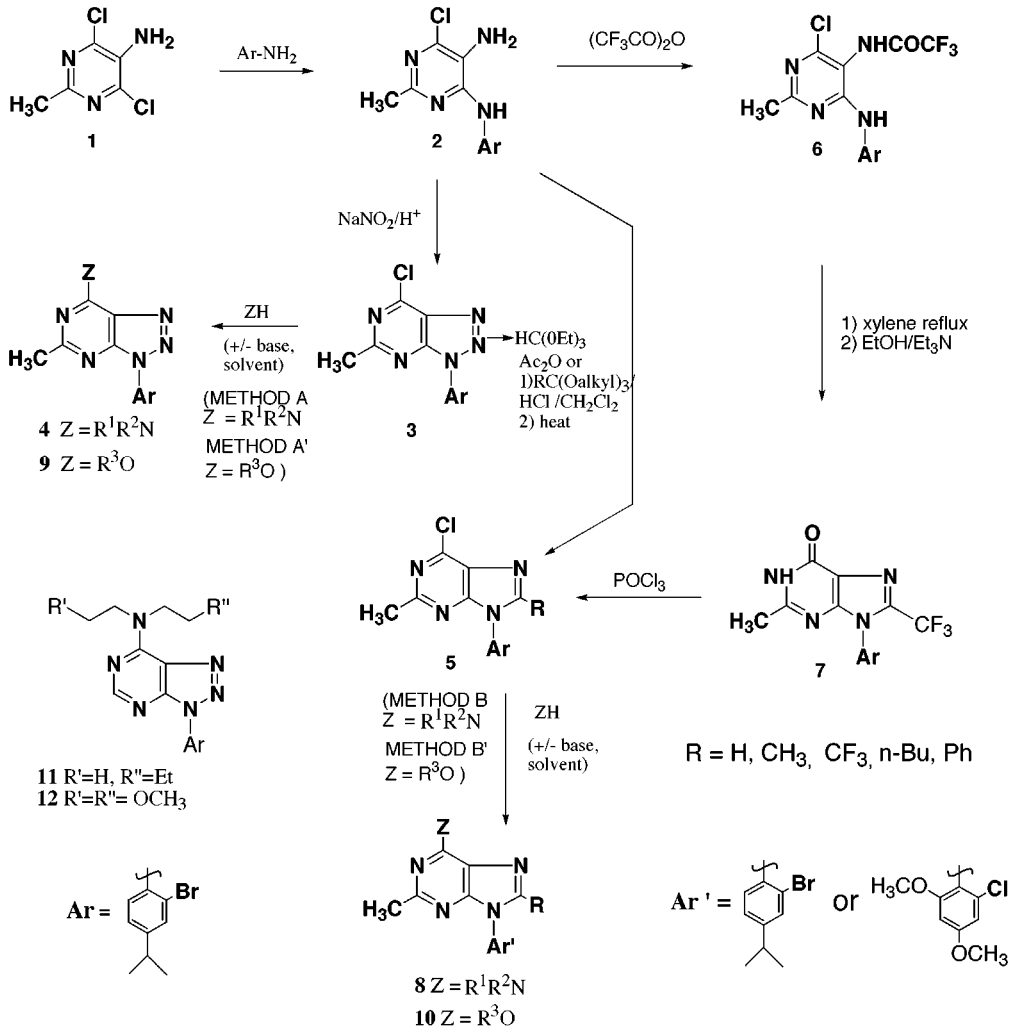
Alternatively, the diaminopyrimidine **2** was converted to imidazopyrimidines **5** (R = H) by heating with triethyl orthoformate in acetic anhydride. When other orthoesters were used under these reaction conditions, cyclization to the 8-substituted imidazopyrimidine was slow and displacement of the chloro group was observed. In these cases treatment of **2** with excess orthoester in CH_2Cl_2 in the presence of acid at room temperature cleanly afforded iminoether intermediates that thermally cyclized in refluxing xylene to the imidazopyrimidines **5** (R = CH_3, *n*-Bu, Ph). In the case of the 8-(trifluoromethyl)imidazopyrimidine **8n, 2** was acylated with trifluoroacetic anhydride to give amide **6** that unfortunately underwent hydrolysis when thermal cyclization in xylene was attempted. The isolated pyrimidone amide was converted to **7** in reluxing EtOH in the presence of Et_3N, and subsequent treatment with $POCl_3$ provided **5** (R = CF_3). Condensation with appropriate amines as described above yielded the aminated imidazopyrimidines **8** (Ar = 2-bromo-4-(1-methylethyl)-phenyl, R = H, CH_3, CF_3) (Table 1) or **8** (Ar = 2-chloro-4,6-dimethoxyphenyl, R = H, CH_3, *n*-Bu, Ph) (Table 2).

While a considerable amount of steric bulk could be accommodated as amino group substituents on the six-membered heterocycle of the bicyclic systems, it was of interest to determine whether chirality at this region in space might influence binding affinity. Thus, in the case where the two enantiomers of a chiral primary amine adduct were studied to determine whether stereochemistry at this site might influence binding affinity, HPLC was used for isomer separation. A Chiralcel-OJ column (95% MeOH/H_2O/0.1% TEA) of **4x** afforded both enantiomers, **4y**,**z**, in greater than 98% ee.

Intermediates **3** and **5** were also used to prepare alkoxy analogues of both of the above bicyclic systems. Thus, by treating these chloro derivatives with alkoxide, generated from the appropriate alcohol and sodium hydride in aprotic solvent, aryl ether adducts **9** and **10**, respectively, were produced (Table 1).

Alternate Route to 4 and 8 (Scheme 2). Attempted incorporation of arylamines other than the 2-bromo-4-isopropylaniline into these bicyclic systems using **1** proceeded with great difficulty. Typically we observed little or no coupling products with the more-hindered 2,4,6-trisubstituted amines, as well as with other 2,4-disubstituted anilines. In these cases we found that the more chemically reactive dichloronitropyrimidine **13** was a necessary precursor to these bicyclic systems (Scheme 2). Thus, using this nitro compound, monoadducts **14** were produced in DMSO at room temperature in high yield, albeit in most cases with hydrolysis of the remaining chloro group, a transformation which may

Scheme 1



have prevented bis-arylamine addition. Interestingly, of all the arylamines used in this sequence (Table 2), only collidylamine afforded **15** directly from **13** under the mild reaction conditions. The hydrolysis products **14** were readily reconverted to the desired nitro chlorides **15** using $POCl_3$; subsequent reduction of the nitro group with Fe in acidic media provided **2**.

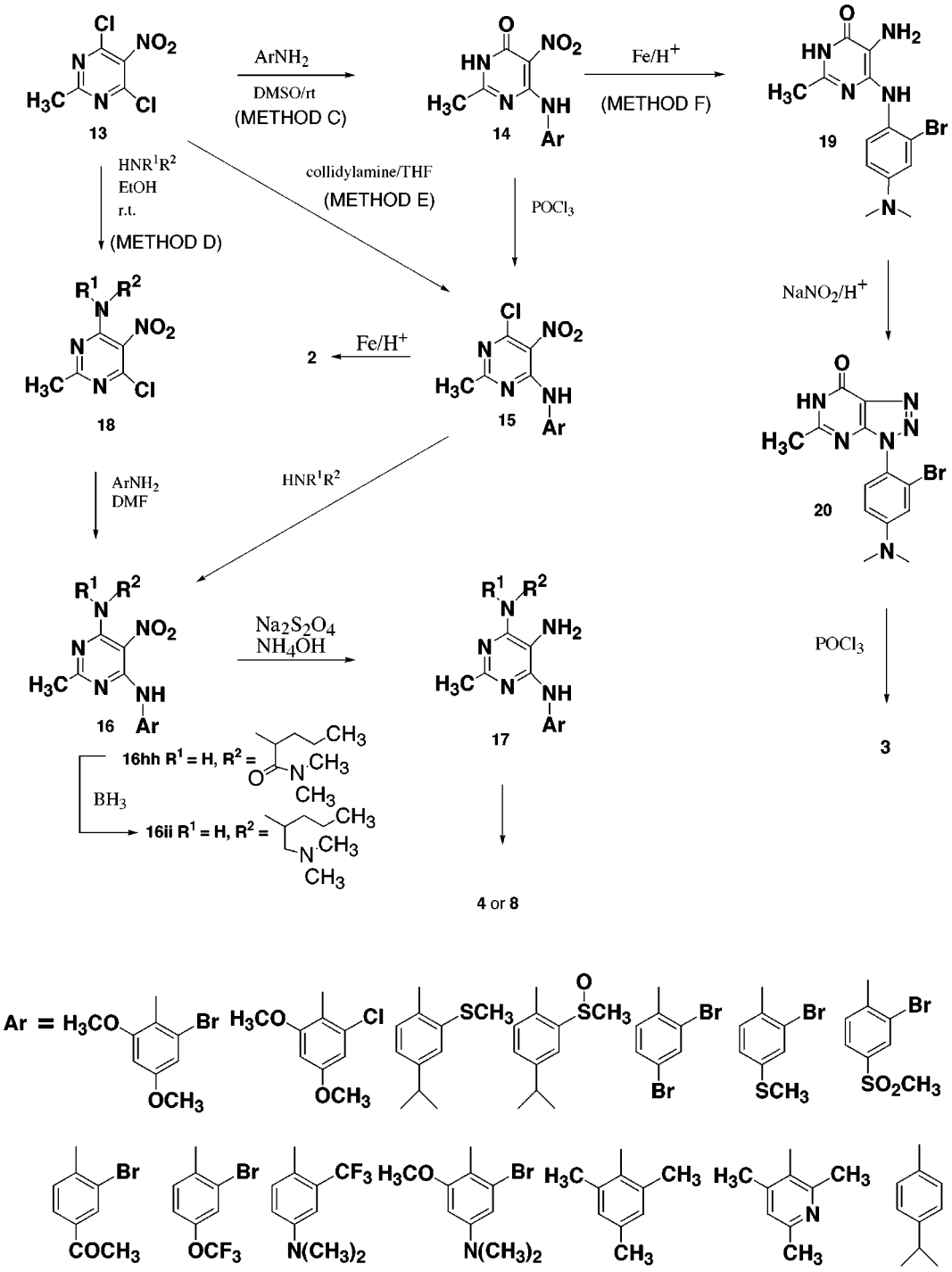
Intermediate **15** was also routinely used for the introduction of the dialkylamino substituent on the pyrimidine ring. The resultant diaminonitropyrimidines **16** were then reduced with sodium dithionite in ammonium hydroxide solution to produce the triaminopyrimidines **17,** precursors to **4** or **8.** Alternatively, dichloronitropyrimidine **13** was initially treated with secondary amines in ethanol in the presence of triethylamine at room temperature. In these cases there was generally no evidence of hydrolysis product. Upon subsequent treatment with arylamines in DMF, the nitro adduct **18** also provided **16**.

In certain cases we also reduced nitro adduct **14** to the diaminopyrimidone **19** which was then converted to bicyclic **20** under the usual conditions. This triazolopyrimidone readily chlorinated with $POCl_3$ providing yet another route to the bicyclic chlorides **3**. Thus, with these various pathways we were able to prepare an array of di- and trisubstituted aryl derivatives of these bicyclic systems.

An analogue of **4** with a norvaline-type side chain was also prepared by treatment of **13** with DL-norvaline dimethylamide to give **18**, which upon aniline condensation afforded the nitrodiamine **16** with the amide pendant. Borane reduction at room temperature yielded the reduced amide with the nitro group intact. Both of the norvaline-like pendants were converted to triazolopyrimidines **4hh**,**ii** after reduction with dithionite and cyclization with nitrous acid.

Triazolo- and Imidazopyridines 25 and 26, Respectively (Scheme 3). 2,4,-Dichloro-6-methyl-3-nitropyridine (**21**) readily prepared from commercially available 4-hydroxy-6-methyl-3-nitro-2(1*H*)-pyridone and $POCl_3$, was treated with a dialkylamine in refluxing ethanol to give a 3:1 mixture of regioisomers, **22** and **22′.** The position of the amine substituent was established by NOE experiments where enhancement of the butyl methylene protons adjacent to the nitrogen atom of the butylethylamino group was observed for the major isomer **22** upon irradiation of the ring proton. Coupling of the major product **22** with substituted anilines at elevated temperatures (120−140 °C) afforded adducts **23** which were reduced with $Na_2S_2O_4$ to the corresponding diaminopyridines **24**. Cyclization of these compounds with $NaNO_2$/HOAc or $CH_3C(OEt)_3$/HCl produced triazolopyridines **25** or imidazopyridines **26** (Table 3). In the case of the butylethylamino adduct, the minor

Scheme 2



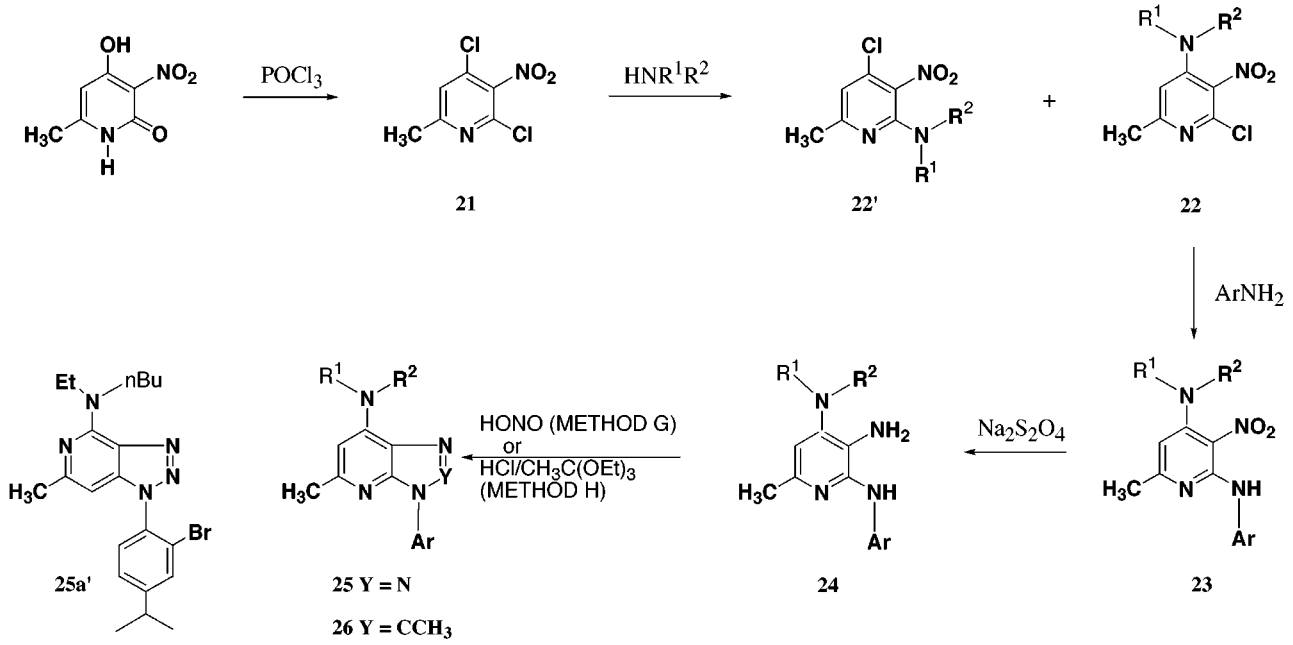
isomer **22′** was also isolated in sufficient quantity to allow synthesis of the isomeric triazolopyridine **25′**, using the same route and conditions described for the major isomer.

Pyrrolopyrimidines (Scheme 4). Selected representatives of this series **28** were synthesized from intermediates **27** described in a recent Pfizer patent[26] by condensation with primary and secondary alkylamines (Table 4).

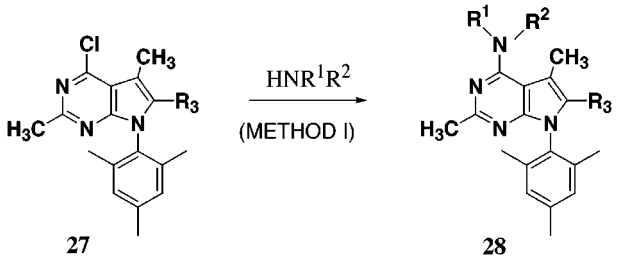
Results

SAR of Triazolo- and Imidazopyrimidines (Tables 1 and 2). Our initial investigations of the structure−activity relationships (SAR) in these series were on systems with the 2-bromo-4-isopropylphenyl substituent on the triazole or imidazole ring. Since the presence of the pyrimidine methyl group, as in the case of the anilinopyrimidines and -triazines,[11] was also found to be important for high binding affinity (cf. **4d** and **11**, K_i = 7.5 and 287 nM, respectively; **4i** and **12**, 3.7 and 203 nM, respectively), our study maintained this group as a constant. An early look at cyclic amines on the pyrimidine ring of these sytems (**4a,b**) also established that comparable open-chain substituents in general afforded compounds with better binding affinities. We also found that in the case of these noncyclic secondary

Scheme 3



Scheme 4



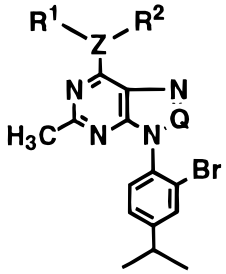
amine adducts, the presence of unsymmetrical alkyl substituents was preferable to identical chains. Using the high affinity of the *N*-ethylbutylamino compound **4d** as a template, we evaluated the effect of insertion of oxygen atoms within this side chain on receptor binding. Of these compounds bis(methoxyethyl)amino compound **4i** (3.7 nM) possessed binding affinity comparable to that of **4d** and slightly better than that of **4f** (10.5 nM). Lengthening one of these ether chains (**4j**, 66.9 nM) or converting ethers to hydroxyl groups (**4g**,**h**, 3460, 64 nM, respectively) diminished binding. When one methoxyethyl chain was maintained on the amino group, the other amino substituent could be varied (allyl, cyclopropyl, benzyl) with little change in the high binding (<10 nM) affinity (**4l**−**n**). However, the 3-picolyl analogue **4k** and secondary amines (**4o**−**q**) displayed poorer affinity.

We further explored primary amine adducts and determined that when the substituent on the nitrogen was a branched alkyl group, the K_i for these compounds was in most cases <10 nM (e.g., **4r**,**t**−**4v**). However, when these alkyl chains were tied back into a ring system (**4s**), the receptor affinity was considerably weaker (>100 nM). An alcohol group on this branched chain (**4w**) slightly diminished binding (25 nM) from the alkyl chains, whereas the ether homologue (**4x**) was comparable to the alkyl system. The presence of a chiral center in this latter compound allowed an evaluation of the effect of the stereochemistry of this side chain on binding affinity. In this case, however, racemate and enantiomers (**4x**−**z**) all possessed comparable affinities (<10 nM). Methylation of the secondary amine of one of these compounds (**4aa**) had an inconsequential effect on binding. The elimination of chirality by incorporating a symmetrical bis-ether pendant on the amine (**4bb**) also did not diminish binding from the 10 nM level.

Replacement of the amine proton of this symmetrical bis-ether with an alkyl group significantly affected binding when this group was larger than methyl (**4ee** vs **4dd**, 21 vs 123 nM). The presence of a *gem*-dimethyl group on the side-chain of the molecule with a single ether substituent (**4ee**) also afforded a compound with the poor binding affinity (ca. 200 nM) of the unsubstituted chain analogue (**4o**). Other variations in the branched chain of these primary amine adducts included benzyl **(4ff)**, ester **(4gg)**, amido **(4hh)**, and a second amino functionality **(4ii)**. The amide was the poorest binder (235 nM), whereas the corresponding amine had surprisingly good affinity (38.7 nM) in contrast to these types of diamino pendant analogues in the anilinopyrimidine and -triazine series.[11]

The imidazopyrimidines generally followed the same SAR as the triazolopyrimidines for amine substituents at this position of the pyrimidine ring. Compounds with acyclic alkyl-chain substituents on the amine with or without ether linkages (**8a**−**c**,**e**,**f**) possessed high binding affinity (ca. 10 nM); primary amine adducts also showed comparable binding affinity to the triazolo system. In the case of a molecule with a second amino group on an alkyl chain of a secondary amino adduct (**8d**), the binding affinity (2665 nM) was considerably weaker than that of the branched primary amino adduct (**4ii**) of the triazolo analogue (38.7 nM). The branched primary amine adducts (**8h**−**k**) also possessed the high affinity (<10 nM) of their analogues in the previous series. The presence of a methyl or trifluoro group at the 8-position of the imidazopyrimidine ring system (**8l**−**8n**) had little effect on binding affinity in these cases, indicating this site of the molecule has some steric tolerance. Replacement of the N atom linker of the

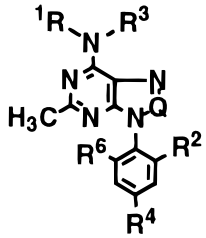
Table 1. CRH Receptor Binding Affinities of 2-Bromo-4-isopropylphenyltriazolo- and -imidazopyrimidines



compd	Z	R_1	R_2	Q	% yield (method)[a]	mp (°C)	formula	anal.[b]	K_i (nM)[c]
4a	N	$-CH_2CH_2CH_2CH_2CH_2-$		N	80 (A)	amorph solid	$C_{19}H_{23}BrN_6$	C,H,N	46[d]
4b	N	$-CH_2CH_2CH_2CH_2-$		N	96 (A)	147.5−148	$C_{18}H_{21}BrN_6$	C,H,N	194
4c	N	Et	Et	N	89 (A)	92−93	$C_{18}H_{23}BrN_6$	C,H,N	25.3
4d	N	Et	*n*-Bu	N	97 (A)	84−86	$C_{20}H_{27}BrN_6$	C,H,N	7.5
11	N	Et	*n*-Bu	N	55 (A)	oil	$C_{19}H_{25}BrN_6$	C,H,N	287
4e	N	Et	$(CH_2)_2OCH_3$	N	64 (A)	oil	$C_{19}H_{25}BrN_6O$	C,H,N	10.5
4f	N	*n*-Bu	*n*-Bu	N	64 (A)	oil	$C_{22}H_{31}BrN_6$	C,H,N	32.5
4g	N	$(CH_2)_2OH$	$(CH_2)_2OH$	N	83 (A)	70−72	$C_{18}H_{23}BrN_6O_2$	C,H,N	3460
4h	N	$(CH_2)_2OCH_3$	$(CH_2)_2OH$	N	42 (A)	120−122	$C_{19}H_{25}BrN_6O_2$	C,H,N	64.0
4i	N	$(CH_2)_2OCH_3$	$(CH_2)_2OCH_3$	N	92 (A)	93−94	$C_{20}H_{27}BrN_6O_2$	C,H,N	3.7
12	N	$(CH_2)_2OCH_3$	$(CH_2)_2OCH_3$	N	38 (A)	oil	$C_{19}H_{25}BrN_6O_2$	C,H,N	203
4j	N	$(CH_2)_2OCH_3$	$(CH_2)_3OCH_3$	N	76 (A)	oil	$C_{21}H_{29}BrN_6O_2$	C,H,N	66.9
4k	N	$(CH_2)_2OCH_3$	3-picolyl	N	27 (A)	oil	$C_{23}H_{26}BrN_7O$	C,H,N	48.5
4l	N	$(CH_2)_2OCH_3$	allyl	N	80 (A)	oil	$C_{20}H_{25}BrN_6O$	C,H,N	4,3
4m	N	$(CH_2)_2OCH_3$	cyclopropylmethyl	N	67 (A)	oil	$C_{21}H_{27}BrN_6O$	C,H,N	4.6
4n	N	$(CH_2)_2OCH_3$	benzyl	N	69 (A)	121−122	$C_{24}H_{27}BrN_6O$	C,H,N	5.0
4o	N	$(CH_2)_2OCH_3$	H	N	91 (A)	134−136	$C_{17}H_{21}BrN_6O$	C,H,N	242
4p	N	$(CH_2)_3OCH_3$	H	N	29 (A)	109−110	$C_{18}H_{23}BrN_6O$	C,H,N	291
4q	N	*n*-Bu	H	N	95 (A)	149−151	$C_{18}H_{23}BrN_6$	C,H,N	69.6
4r	N	$CH(Et)_2$	H	N	86 (A)	171−172	$C_{19}H_{25}BrN_6$	C,H,N	5.2
4s	N	*c*-pentyl	H	N	99 (A)	120.5−122	$C_{19}H_{23}BrN_6$	C,H,N	120
4t	N	$CH(Et)(CH_2)_2CH_3$	H	N	90 (A)	154−155	$C_{20}H_{27}BrN_6$	C,H,N	2.8
4u	N	$CH(Et)(CH_2)_3CH_3$	H	N	48 (A)	137−138	$C_{21}H_{29}BrN_6$	C,H,N	3.1
4v	N	$CH[(CH_2)_3CH_3]_2$	H	N	83 (A)	162−163	$C_{21}H_{29}BrN_6$	C,H,N	4.5
4w	N	$CH(Et)CH_2OH$	H	N	76 (A)	157−159	$C_{18}H_{23}BrN_6O$	C,H,N	25.3
4x	N	$CH(Et)CH_2OCH_3$	H	N	73 (A)	133−134	$C_{19}H_{25}BrN_6O$	C,H,N	6.1
4y	N	(+)-$CH(Et)CH_2OCH_3$[d]	H	N	59 (A)	114−115	$C_{19}H_{25}BrN_6O$	C,H,N	7.5
4z	N	(−)-$CH(Et)CH_2OCH_3$[e]	H	N	29 (A)	114−115	$C_{19}H_{25}BrN_6O$	C,H,N	9.4
4aa	N	(+)-$CH(Et)CH_2OCH_3$	Me	N	59 (A)	oil	$C_{20}H_{27}BrN_6O$	C,H,N	11.2
4bb	N	$CH(CH_2OMe)_2$	H	N	98 (A)	156−158.5	$C_{19}H_{25}BrN_6O_2$	C,H,N	10.3
4cc	N	$CH(CH_2OMe)_2$	CH_3	N	81 (A)	oil	$C_{20}H_{27}BrN_6O_2$	C,H,N	21.3
4dd	N	$CH(CH_2OMe)_2$	Et	N	96 (A)	oil	$C_{21}H_{29}BrN_6O_2$	C,H,N	123
4ee	N	$C(CH_3)_2(CH_2OMe)$	H	N	82 (A)	129.5−130	$C_{19}H_{25}BrN_6O$	C,H,N	195
4ff	N	$CH(Bz)CH_2OCH_3$	H	N	27 (A)	67−69	$C_{24}H_{27}BrN_6O$	C,H,N	36.7
4gg	N	$CH(Et)COOCH_3$	H	N	67 (A)	67−69	$C_{19}H_{23}BrN_6O_2$	C,H,N	19.4
4hh	N	$CH(nPr)CON(CH_3)_2$	H	N	67 (D)	163−164	$C_{21}H_{28}BrN_7O$	C,H,N	235
4ii	N	$CH(nPr)CH_2N(CH_3)_2$	H	N	73 (D)	179−180 dec	$C_{21}H_{30}BrN_7H_2O$	C,H,N	38.7
8a	N	Et	Et	CH	48 (B)	oil	$C_{19}H_{24}BrN_5$	HRMS[f]	12.4
8b	N	Et	*n*-Bu	CH	56 (B)	oil	$C_{21}H_{28}BrN_5$	C,H,N	4.3
8c	N	Et	$(CH_2)_2OCH_3$	CH	49 (B)	oil	$C_{20}H_{26}BrN_5O$	C,H,N	9.2
8d	N	Et	$(CH_2)_2N(CH_3)_2$	CH	41 (B)	oil	$C_{21}H_{29}BrN_6$	C,H,N	2665
8e	N	*n*-Bu	*n*-Bu	CH	31 (B)	oil	$C_{23}H_{32}BrN_5$	C,H,N	6.1
8f	N	$(CH_2)_2OCH_3$	$(CH_2)_2OCH_3$	CH	96 (B)	oil	$C_{21}H_{28}BrN_5O_2$	C,H,N	5.9
8g	N	$(CH_2)_2OCH_3$	H	CH	79 (B)	125−126	$C_{18}H_{22}BrN_5O$	C,H,N	210
8h	N	$CH(Et)(CH_2)_2CH_3$	H	CH	81 (B)	amorph solid	$C_{21}H_{28}BrN_5$	C,H,N	4.4
8i	N	$CH(Et)(CH_2)_3CH_3$	H	CH	62 (B)	oil	$C_{22}H_{30}BrN_5$	C,H,N	5.1
8j	N	$CH[(CH_2)_2CH_3]_2$	H	CH	87 (B)	87−88	$C_{24}H_{34}BrN_5$	C,H,N	3.6
8k	N	$CH(Et)(CH_2)_2OCH_3$	H	CH	55 (B)	105−106	$C_{20}H_{26}BrN_5O$	C,H,N	8.0
8l	N	Et	*n*-Bu	CMe	46 (B)	oil	$C_{22}H_{30}BrN_5$	C,H,N	0.9
8m	N	$(CH_2)_2OCH_3$	$(CH_2)_2OCH_3$	CMe	85 (B)	oil	$C_{22}H_{30}BrN_5O_2$	C,H,N	6.7
8n	N	Et	*n*-Bu	CCF_3	52 (B)	69−70	$C_{22}H_{27}BrF_3N_5$	C,H,N	1.4
9a	O	$CH(Et)CH_2OMe$		N	62 (A′)	75−76	$C_{19}H_{24}BrN_5O_2$	C,H,N	1.9
10a	O	$CH(Et)CH_2OMe$		CH	39 (B′)	oil	$C_{20}H_{25}BrN_4O_2$	C,H,N	9.7
9b	O	$CH(Et)_2$		N	21 (A′)	oil	$C_{19}H_{24}BrN_5O$	C,H,N	1.9
10b	O	$CH(Et)_2$		CH	29 (B′)	oil	$C_{20}H_{25}BrN_4O$	C,H,N	3.5
9c	O	$CH(Et)(CH_2)_3Me$		N	39 (A′)	oil	$C_{21}H_{28}BrN_5O$	C,H,N	1.5
10c	O	$CH(Et)(CH_2)_3Me$		CH	11 (B′)	oil	$C_{22}H_{29}BrN_4O$	HRMS	2.9
9d	O	$CH(CH_2OMe)_2$		N	66 (A′)	oil	$C_{19}H_{24}BrN_5O_3$	HRMS	7.1
9e	O	$CH(CH_2OEt)_2$		N	63 (A′)	112−114	$C_{21}H_{28}BrN_5O_3$	C,H,N	39.7

[a] Methods are described in Schemes 1−4; yields reported are for the last step in the sequence. [b] All compounds gave acceptable combustion analysis results (±0.4%) unless otherwise indicated. [c] Receptor binding affinities are for the human cloned CRH recetor ($hCRF_1$) expressed in 293EBNA cells. K_i values < 50 nM are the result of at least two separate test results; K_i values > 50 nM are single measurements. [d] Prepared from the enatiomerically pure chiral amine (+35.5°, $c = 0.2$, $CHCl_3$). [e] Isolated from the racemate by HPLC using a chiral column (−32.5°, $c = 0.2$, $CHCl_3$). [f] High-resolution mass spectrometry.

Table 2. CRH Receptor Binding Affinities of *N*-Aryltriazolo- and -imidazolopyrimidines



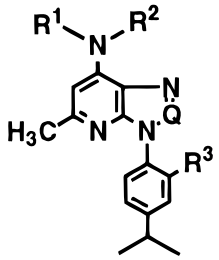
compd	R_2	R_4	R_6	Q	R_1	R_3	% yield (method)[a]	mp (°C)	formula	anal.[b]	K_i (nM)[c]
4i	Br	$CH(Me)_2$	H	N	CH_2CH_2OMe	CH_2CH_2OMe	92 (A)	105−109S	$C_{20}H_{27}N_6BrO_2$	C,H,N	3.7
4jj	H	$CH(Me)_2$	H	N	CH_2CH_2OMe	CH_2CH_2OMe	12[e]	142−143	$C_{20}H_{28}N_6O_2$	C,H,N	3918
4kk	SMe	$CH(Me)_2$	H	N	CH_2CH_2OMe	CH_2CH_2OMe	74 (D)	oil	$C_{21}H_{30}N_6O_2S$	C,H,N	15.3
4ll	SO_2Me	$CH(Me)_2$	H	N	CH_2CH_2OMe	CH_2CH_2OMe	68 (D)	143−145	$C_{21}H_{30}N_6O_4S$	C,H,N	1026
4mm	Br	OMe	OMe	N	CH_2CH_3	$CH_2CH_2CH_2Me$	83 (C)	oil	$C_{19}H_{25}BrN_6O_2$·HCl	C,H,N	3.0
4nn	Br	OMe	OMe	N	CH_2CH_2OMe	CH_2CH_2OMe	46 (D)	124−126	$C_{19}H_{25}BrN_6O_4$	C,H,N	28.3
4oo	Br	OMe	$N(Me)_2$	N	CH_2CH_2OMe	CH_2CH_2OMe	45 (C)	141.5−143.5	$C_{20}H_{28}BrN_7O_3$	C,H,N	48.2
4pp	Cl	OMe	OMe	N	CH_2CH_3	$CH_2CH_2CH_2Me$	80 (C)	112.5−114	$C_{19}H_{25}ClN_6O_2$	C,H,N	4.4
4qq	Cl	OMe	OMe	N	CH_2CH_2OMe	CH_2CH_2OMe	69 (C)	156−157.5	$C_{19}H_{25}ClN_6O_4$	C,H,N	21.0
4rr	Br	Br	H	N	CH_2CH_2OMe	CH_2CH_2OMe	98 (D)	oil	$C_{17}H_{20}Br_2N_6O_2$	C,H,N	7.4
4ss	Br	Br	H	N	CH_2CH_3	$CH_2CH_2CH_2Me$	83 (D)	oil	$C_{17}H_{20}Br_2N_6$	C,H,N	20.4
4tt	Br	SMe	H	N	CH_2CH_3	$CH_2CH_2CH_2Me$	39 (D)	oil	$C_{18}H_{23}BrN_6S$	C,H,N	10.6
4uu	Br	SO_2Me	H	N	CH_2CH_3	$CH_2CH_2CH_2Me$	89 (D)	141−143	$C_{18}H_{23}BrN_6SO_2$	C,H,N	100
4vv	Br	COMe	H	N	CH_2CH_3	$CH_2CH_2CH_2Me$	14 (D)	oil	$C_{19}H_{23}BrN_6O$·1/2H_2O	C,H,N	33.7
4ww	Br	$N(Me)_2$	H	N	CH_2CH_2OMe	CH_2CH_2OMe	95 (F,A)	108−109	$C_{19}H_{26}BrN_7O_2$	C,H,N	10.5
4xx	Br	OCF_3	H	N	CH_2CH_2OMe	CH_2CH_2OMe	55 (C)	oil	$C_{18}H_{20}BrF_3N_6O_3$	C,H,N	33.2
4yy	CF_3	$N(Me)_2$	H	N	CH_2CH_2OMe	CH_2CH_2OMe	88 (C)	105−109	$C_{20}H_{26}N_7F_3O_2$	C,H,N	23.5
4zz	CF_3	$N(Me)_2$	H	N	CH_2CH_3	$CH_2CH_2CH_2Me$	91 (C)	142−143	$C_{20}H_{26}N_7F_3$	C,H,N	15.4
4ab	CH_3	CH_3	CH_3	N	CH_2CH_2OMe	CH_2CH_2OMe	98 (C)	83.5−85	$C_{20}H_{28}N_6O_2$	C,H,N	24.1
4ac	CH_3	CH_3	CH_3	N	CH_2CH_3	$CH_2CH_2CH_2Me$	97 (C)	oil	$C_{19}H_{27}N_6$	C,H,N	27.5
4ad[d]	CH_3	CH_3	CH_3	N	CH_2CH_2OMe	CH_2CH_2OMe	92 (E)	59−60.5	$C_{19}H_{27}N_7O_2$	C,H,N	416
4ae[d]	CH_3	CH_3	CH_3	N	CH_2CH_3	$CH_2CH_2CH_2Me$	86 (E)	oil	$C_{19}H_{27}N_7$	C,H,N	65
4af	CH_3	CH_3	CH3	N	CH_2CH_2OMe	H	97 (C)	141.5−143.5	$C_{16}H_{22}N_6O$	C,H,N	1204
4ag	CH_3	CH_3	CH_3	N	CH_2CH_2OMe	CH_2Me	86 (C)	oil	$C_{18}H_{26}N_6O$	HRMS	20.7
4ah	CH_3	CH_3	CH_3	N	$CH(Et)CH_2OMe$	H	75 (C)	156−157.5	$C_{19}H_{26}N_6O$	C,H,N	59.4
4ai	CH_3	CH_3	CH_3	N	$CH(Et)_2$	H	79 (C)	184.5−186.5	$C_{19}H_{26}N_6$	C,H,N	5.7
4aj	CH_3	CH_3	CH_3	N	CH(Et)*n*-Bu	H	73 (C)	141.5−142.5	$C_{21}H_{30}N_6$	C,H,N	7.6
4ak	CH_3	CH_3	CH_3	N	CH(Me)*n*-Pr	H	86 (C)	151−152	$C_{19}H_{26}N_6$	C,H,N	7.1
4al	CH_3	CH_3	CH_3	N	$CH(n\text{-}Pr)_2$	H	72 (C)	145−146.5	$C_{21}H_{30}N_6$	C,H,N	4.0
4am	CH_3	CH_3	CH_3	N	$CH(Bn)CH_2OMe$	H	75 (C)	oil	$C_{24}H_{28}N_6O$	HRMS	27.7
4an	Br	Br	H	N	$CH(n\text{-}Pr)_2$	H	83 (C)	128−129	$C_{18}H_{21}Br_2N_6$	C,H,N	0.8
8f	Br	$CH(Me)_2$	H	CH	CH_2CH_2OMe	CH_2CH_2OMe	96 (B)	oil	$C_{21}H_{28}BrN_5O_2$	C,H,N	5.9
8o	Br	OMe	OMe	CH	CH_2CH_3	$CH_2CH_2CH_2Me$	79 (C)	125.5−126	$C_{20}H_{26}BrN_5O_2$	C,H,N	3.5
8p	Br	OMe	OMe	CH	CH_2CH_2OMe	CH_2CH_2OMe	10 (D)	137−138	$C_{20}H_{26}BrN_5O_4$	C,H,N	31.3
8q	Cl	OMe	OMe	CH	CH_2CH_3	$CH_2CH_2CH_2Me$	94 (C)	126.5−127	$C_{20}H_{26}ClN_5O_2$	C,H,N	5.2
8r	Cl	OMe	OMe	CH	$CH_2CH_2CH_2OMe$	CH_2CH_2OMe	82 (C)	141.5−142	$C_{21}H_{28}ClN_5O_4$	C,H,N	23.8
8s	CH_3	CH_3	CH_3	CH	CH_2CH_2OMe	CH_2CH_2OMe	86 (C)	117−120	$C_{21}H_{29}N_5O_2$	C,H,N	20.7
8t	CH_3	CH_3	CH_3	CH	CH_2CH_3	$CH_2CH_2CH_2Me$	87 (C)	86.5−88.5	$C_{21}H_{29}N_5$	C,H,N	25.0
8u	Cl	OMe	OMe	CMe	CH_2CH_3	$CH_2CH_2CH_2Me$	97 (C)	106.5−108.5	$C_{21}H_{28}ClN_5O_2$	C,H,N	3.5
8v	Cl	OMe	OMe	C-*n*-Bu	CH_2CH_3	$CH_2CH_2CH_2Me$	86 (C)	oil	$C_{24}H_{34}ClN_5O_2$	C,H,N	22
8w	Cl	OMe	OMe	CC_6H_5	CH_2CH_3	$CH_2CH_2CH_2Me$	99 (C)	oil	$C_{26}H_{30}ClN_5O_2$	C,H,N	133

[a−c] See Table 1 for explanation. [d] 3-Pyridinyl rather than phenyl. [e] Obtained as a side product from a stannylation reaction of the 2-bromo compound; details will be reported at a later date.

primary amine adducts with an oxygen resulted in a series of ethers that also had high binding affinity (<10 nM), comparable to that of the nitrogen-linked analogues, in either of these systems (**9a**−**d**, **10a**−**c**). Extension of the terminal ether groups (**9e**) did, however, adversely affect binding (39.7 nM), a result not observed with **4v** where all atoms of this extended chain were carbon (4.5 nM).

The influence on binding affinity of substituents on the phenyl ring and its replacement with an appropriately substituted pyridine were also studied (Table 2). Since the bis(methoxyethyl)amino and the *N*-ethylbutylamino groups afforded high-affinity agents in the 2-bromo-4-isopropylphenyl series, the effect of changes in phenyl substitution was primarily determined on triazolo- and imidazopyrimidines with these two amino groups. As in the case of the anilinopyrimidines and -triazines,[11] there was a need for at least one bulky ortho-substituent on the phenyl ring for high binding affinity (**4i**,**jj**, 4.5 and 3918 nM, respectively), conferring orthogonality between the planes of the phenyl and bicyclic ring systems.[23] Replacement of bromo with methylthio (**4kk**) was without significant effect, whereas the methyl sulfone (**4ll**) was a much weaker binder (1026 nM). Compounds with a 2-bromo substituent and a replacement for the 4-isopropyl group (**4rr**−**ww**) tended to be slightly weaker binders than the isopropyl analogue, with the electron-withdrawing acetyl (**4vv**) and methylsulfonyl **(4uu)** groups showing the greatest decrease in binding affinity (33.7 and 100 nM, respectively). This 4-position of the phenyl group seems to favor the presence of an alkyl substituent as long as it does not exceed certain steric requirements as delineated in the previous paper. Other disubstituted phenyl groups also afforded no improvement in the binding of the bromoisopropyl pairing (**4xx**−**zz**), except when the

Table 3. CRH Receptor Binding Affinities of N-Substituted Phenyltriazolo- and -imdazolopyridines



compd	R_1	R_2	R_3	Q	% yield (method)[a]	mp (°C)	formula	anal.[b]	K_i (nM)[c]
25a	Et	*n*-Bu	Br	N	70 (G)	168−171	$C_{21}H_{28}BrN_5 \cdot HCl$	C,H,N,Br	3.2
26a	Et	*n*-Bu	Br	CCH_3	68 (H)	oil	$C_{23}H_{31}BrN_4 \cdot HCl \cdot H_2O$	C,H,N	3.9
25b	$(CH_2)_2OCH_3$	$(CH_2)_2OCH_3$	Br	N	45 (G)	87−89.5	$C_{21}H_{28}BrN_5O_2 \cdot HCl \cdot H_2O$	C,H,N	4.7
26b	$(CH_2)_2OCH_3$	$(CH_2)_2OCH_3$	Br	CCH_3	90 (H)	oil	$C_{23}H_{31}BrN_4O_2 \cdot HCl \cdot H_2O$	C,H,N	9.8
25c	Et	*n*-Bu	SMe	N	81 (G)	oil	$C_{22}H_{31}N_5S$	C,H,N	2.2
25d	Et	*n*-Bu	SO_2Me	N	68 (G)	151−153	$C_{22}H_{31}N_5O_2S$	C,H,N,S	83
25e	$(CH_2)_2OCH_3$	$(CH_2)_2OCH_3$	SMe	N	74 (G)	oil	$C_{22}H_{31}N_5O_2S$	C,H,N,S	4.7
25f	$(CH_2)_2OCH_3$	$(CH_2)_2OCH_3$	SO_2Me	N	68 (G)	143−145	$C_{22}H_{31}N_5O_4S$	C,H,N,S	476
25a′	Et	*n*-Bu	Br	N	70 (G)	oil	$C_{21}H_{28}N_5Br$	C,H,N	>10000

[a−c] See Table 1 for explanation.

2,4-dibromophenyl group was paired with the 4-heptylamino adduct in the triazolopyrimidine series (**4an**) providing a compound with about 1 nM affinity.

Certain compounds with a 2,4,6-trisubstituted phenyl group were also prepared. In the case of the 2-halo-4,6-dimethoxy compounds the butylethylamino compounds (**4mm**,**pp**) were comparable in binding affinity to **4i** and 5−9 times more potent the bis-ether analogues (**4nn**,**qq**, 28 and 21 nM, respectively). Thus, substituents on the phenyl ring do indeed influence the SAR of the bicyclic system. Replacement of the 6-methoxy group with dimethylamino also diminished affinity about 2-fold in the bromo series. Compounds with a 2,4,6-trimethylphenyl group (**4ab**,**ac**,**af**−**ah**) tended to be poorer binders by a factor of 2−fold, compared to 2-bromo-4-isopropylphenyl compounds that possessed the same amino substituents. However, when the 2,4,6-trimethylphenyl series was explored more fully, primary amino adducts with branched alkyl chains (**4ai**−**al**) had comparable binding affinity (<10 nM) to **4i**; an amino group with a 2-(1-methoxy-3-phenylpropyl) chain (**4am**) was a weaker binder (28 nM). The 3-collidyl derivatives of both the bis-ether and dialkylamino compounds (**4ad**,**ae**) were not as potent (416 and 65 nM) as their mesityl counterparts (**4ab**,**ac**) with K_i's of about 25 nM. There was no significant difference between the binding affinities of the trisubstituted phenyl compounds in the triazolo and imidazo series (**8o**−**t vs 4mm**,**nn**,**pp**,**qq**,**ab**,**ac**). A further look at the effect of substituents on the carbon atom of the imidazo ring in the 2-chloro-4,6-dimethoxyphenyl series indicated that methyl was again equivalent (3.5 nM) to hydrogen (**8u**), but a butyl chain diminished binding by about 6-fold (**8v**) and a phenyl group by about 40-fold (**8w**).

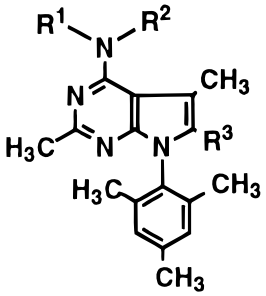
SAR of Triazolo- and Imidazopyridines (Table 3). In the annelated pyridine series, representatives with both the bis(methoxyethyl)amino and the *N*-ethylbutylamino groups were prepared. In the cases with the 2-bromo-4-isopropylphenyl substituent, triazolo- and 8-methylimidazopyridines (**25a**,**b**, **26a**,**b**) possessed high affinity (<10 nM) that was comparable to that of the pyrimidine analogues. As in the case of the annelated pyrimidines, replacement of 2-bromo for 2-thiomethyl maintained this high affinity (**25c**,**e**), whereas the corresponding sulfones diminished binding 20−100-fold (**25d**,**f**). Surprisingly, **25a′**, the isomeric pyridine analogue of **25a**, showed no affinity for the CRF receptor up to 10 μM, demonstrating the importance of a nitrogen heteroatom at the 4-position of this bicyclic system for optimal receptor interaction of these molecules.

SAR of Pyrrolopyrimidines (Table 4). Selected members of these compounds were synthesized to determine the importance of the five-membered ring N atoms on binding, in light of the profound effect observed for the pyrimidine nitrogens (vide supra). In the course of this study, a Pfizer patent on this series of molecules[27] disclosed their utility as CRF receptor antagonists. The compounds we prepared are included for comparison with the other bicyclic systems. This series showed trends comparable to the other *N*-ethylbutylamino compounds with **28a**,**d** possessing high binding (<2 nM), which in this case was slightly better than the bis-ether analogue **28f** (7 nM). The morpholinyl derivative (**28e**) also showed the diminished binding (201 nM) of other cyclic secondary amines found in the earlier described series. The presence of an alcohol on the side chain (**28b**) or an unbranched primary amine adduct **(28c)** showed decreased binding affinity (about 30 nM) as was also seen in the other bicyclic series.

Discussion and Pharmacokinetic Studies

The evaluation of amino groups on the pyrimidine ring established early on that acyclic alkyl groups were preferred substituents for maximum CRF receptor binding affinity in these bicyclic systems. The interruption of this alkyl chain with oxygen atoms generally maintained this high afffinity while decreasing the lipophilicity of these molecules. Lipohilicity has long been recognized as an important property for enabling molecules to penetrate the blood−brain barrier (BBB). The identification of agents with appropriate hydrophobic/hydrophilic balance has been shown to increase the probability of finding potential centrally acting thera-

Table 4. CRH Receptor Binding Affinities of *N*-(2,4,6-Trimethylphenyl)pyrrolopyrimidines



compd	R_1	R_2	R_3	% yield (method)[a]	mp (°C)	formula	anal.[b]	K_i (nM)[c]
28a	Et	*n*-Bu	CH_3	79 (I)	>325	$C_{24}H_{34}N_4$·HCl	C,H,N	0.84
28b	H	$HOCH_2CHEt$	CH_3	79 (I)	199−200	$C_{22}H_{30}N_4O$·HCl	C,H,N	27.3
28c	H	*n*-Bu	CH_3	72 (I)	219−220	$C_{23}H_{30}N_4$	C,H,N	29.0[d]
28d	Et	*n*-Bu	H	89 (I)	171−172	$C_{23}H_{32}N_4$	C,H,N	1.61[d]
28e	$-CH_2CH_2OCH_2CH_2-$		CH_3	78 (I)	157.5−15	$C_{22}H_{28}N_4O$	HRMS	201[d]
28f	$(CH_2)_2OCH_3$	$(CH_2)_2OCH_3$	CH_3	34 (I)	oil	$C_{24}H_{34}N_4O_2$	HRMS	7.1[d]

[a−c] See Table 1 for explanation. [d] Single test results.

peutic agents,[28,29] the goal of this work. Indeed, when the log *P* of certain of these molecules was calculated,[30] we routinely found values >6 suggesting that these lipophilic agents might possess unacceptable absorption, distribution, or pharmacokinetic properties. Thus, the investigation of secondary amines in these series afforded a way of decreasing this property through removal of one of these chains. However, it was found in these cases that a branched chain alkyl group of five or six carbon atoms was necessary for optimal binding (**4r**,**t**−**w**) diminishing the advantage of removing one of the alkyl chains; the ether analogues of these chains, then, provided the means of reducing the hydrophobicity of these substituents. In the case of **4x**, a branched alkyl ether chain analogue, enantiomers **4y**,**z** were also evaluated for binding affinity and found to be equivalent suggesting that while a hydrophobic binding pocket with definite steric limits was present at the site of interaction, discrete spatial orientation of the group occupying this location was not necessary. This same high binding affinity was also observed in the pyrimidinyl ethers for these branched chain analogues though no significant lipophilicity advantage was attained (**9a**−**c**, **10a**−**c**). The introduction of various functionality on these side chains that we felt might favorably affect lipophilicity or solubility generally diminished binding affinity (**4gg**−**ii**).

A number of di- and trisubstituted phenyl analogues in the triazolopyrimidine series maintained the high binding affinity of *N*-ethylbutylamino and bis(methoxyethyl)amino compounds **4d**,**i**, respectively (Table 2). In some cases where groups such as the methanesulfonyl (**4ll**,**uu)** were introduced to decrease lipophilicity, binding affinity was adversely affected; diminished binding was also observed with an acetyl group (**4vv**). A larger number of analogues were prepared in the 2,4,6-trimethylphenyl series of both the triazolo- and imidazopyrimidines, and in general the SAR followed that of the 2-bromo-4-isopropylphenyl series. The trimethylated pyridyl analogues **4ad**,**ae** both showed diminished affinity compared to their phenyl counterparts.

The SAR of the active regioisomer in the triazolo- or imidazopyridines was also comparable to the SAR of the pyrimidine series. Introduction of the lipophilicity-diminishing methanesulfonyl group had the same detrimental effect on binding as indicated earlier. While two of the pyrrolopyrimidines (**28a**,**d**) were among the compounds with the highest binding affinity in this group, their high lipophilicity (calculated log *P* > 6) and extreme insolubility made them difficult to work with and evaluate in secondary pharmacological paradigms. The in vivo activity of **28d** as a selective CRF receptor antagonist in the learned-helplessness procedure, a putative model of depression, has recently been reported.[31] The high doses (10−32 mg/kg) of this compound needed to show activity via intraperitoneal administration are suggestive of suboptimal pharmacokinetic properties.

Of the compounds in these various series, we selected high-affinity representatives with various amine substituents and the 2-bromo-4-isopropylphenyl group on the triazole or imidazole ring for further study. These included a dialkylamino (**4d**), a bis(alkoxyalkyl)amino (**4i**), a methoxyethylbenzylamino (**4n**), two racemic methoxypentylamino (**4x**, **8k**), and a branched chain alkoxyalkyl ether (**9a**) compound. Our initial work[32] evaluated the pharmacokinetic (PK) parameters of these compounds in rats after po dosing (1 mg/kg, methocel suspension). We felt that compounds with good bioavailability, blood plasma levels, and duration would provide reliable indicators of the biological activity of this new type of potential therapeutic agent. As shown in Table 5 several of these compounds (**4d**,**i**,**n**,**x**) have reasonably good oral bioavailability (16−27%); moreover, maximum plasma levels (C_{max}) 3−10 times the K_i value of the compound and long plasma duration, where the plasma concentration persisted above the K_i level of the compound for 8−16 h, were also observed. Since these compounds were expected to mediate their in vivo responses through interaction with receptors in the CNS, steady-state brain-to-plasma ratios for these compounds were also determined in the rat. Of these compounds, **4i**,**x** showed a favorable brain-to-plasma ratio of ca. 0.6.

Subsequently, we performed further in vivo evaluations of the four compounds with good rat oral bioavailability in the dog at 1 mg/kg by both iv (ethanol/PEG

Table 5. Summary of Pharmacokinetic Parameters of Selected Compounds in Rats after 1 mg/kg po Dose

compd	K_i[a] (nM)	plasma C_{max}[b] (nM)	plasma AUCT[c] (nM·h)	%F[d]	plasma C_{max}/K_i[e]	B/P[f]	cLog P[g]
4d (soln)[k]	7.5	49.2[i]	471[i]	26.5[i]	7[i]	ND[h]	7.4
4i (susp)[l]	3.7	30[j]	98[j]	17[j]	8[j]	0.6[m]	5.04
4n (susp)[l]	5.8	12.5[i]	137[i]	15.5[i]	2[i]	0.2[j,n]	6.23
4x (susp)[l]	6.1	30.7[i]	146[i]	21[i]	5[i]	0.6[o]	4.93
8k (susp)[l]	8.0	19.9[j]	30[j]	7[j]	2[j]	ND	4.85
9a (susp)[l]	1.9	5.9[j]	21[j]	6[j]	4[j]	ND	5.20

[a] Receptor binding affinities are for the cloned CRF receptor ($hCRF_1$) expressed in 293EBNA cells. [b] Peak plasma level of compound in nM. [c] Plasma area under the compound concentration−time curve. [d] Oral bioavailability. [e] The ratio of the peak plasma concentration (nM) over the K_i of the compound. [f] Brain concentration to plasma concentration. [g] Calculated log P of these compounds. [h] Not determined. [i] Average of two determinations. [j] Single determination. [k] Compounds were administered in EtOH/PG (20:80). [l] Compound administered in methocel. [m] Infusion of 0.67 mg/kg/h in EtOH/PG (20:80). [n] Infusion of 0.025 and 0.25 mg/kg/h in EtOH/PG/PEG/H2O (10:30:30:30). [o] Infusion of 0.033 and 0.33 mg/kg/h in EtOH/PG/PEG (20:40:40).

Table 6. Pharmacokinetic Parameters of Selected Compounds after 1 mg/kg iv[a] Administration in Dogs

compd	n	Cl[b] (L/h/kg)	V_{ss}[c] (L/kg)	$t_{1/2}$[d] (h)
4d	4	0.555 ± 0.130	7.54 ± 2.68	26.0 ± 10.5
4i	6	0.478 ± 0.078	15.55 ± 3.90	33.2 ± 9.7
4n	5	0.523 ± 0.098	24.72 ± 14.79	46.7 ± 26.3
4x	5	0.443 ± 0.119	10.10 ± 4.36	19.0 ± 11.1

[a] iv formulation: ethanol/PEG 400/water (5:65:30) for **4d**,**n**,**x**; ethanol/PG/PEG 400/water (10:30:30:30) for **4i**. [b] Clearance. [c] Volume of distribution at steady state. [d] Elimination half-life.

400/H_2O) and po (methocel with or without Tween 80/H_2O) routes of administration. In the iv study (Table 6) clearance, volume of distribution, and elimination half-lives were determined. Of particular significance in these measurements were the longer elimination half-lives generally observed in the dog than in the rat. In the oral dog study (Table 7) C_{max}, t_{max} (the time to reach maximum plasma levels), area under the curve (AUCT) (a measure of the duration of the parent molecule over time), and bioavailability (%F) were determined. Of these compounds **4i**,**x** showed the best pharmacokinetic profiles: high oral bioavailabilities (30% and 53%, respectively) and plasma levels (307 and 546 nM, respectively); rapid absorption to reach peak plasma levels (0.67 and 0.5 h, respectively); and long elimination half-lives (33 and 19 h, respectively). Thus, in this species these two compounds again showed PK properties that were sought for a pharmacological agent with therapeutic potential. We favored **4i** for additional evaluation since it possessed favorable in vivo pharmacological properties which along with that of other related compounds will be reported in due course.

Conclusion

We have described the synthesis and CRF receptor binding affinities of several series of *N*-aryl polyazaindoles. Variations of amino (ether) pendants and aromatic substituents have defined the structure−activity relationships of these series and resulted in the identification of a variety of high-affinity agents. On the basis of this property and lipophilicty differences, six of these compounds (**4d**,**i**,**n**,**x**, **8k**, **9a**) were initially chosen for rat pharmacokinetic (pk) studies. Good oral bioavailability and high plasma levels and duration of four of these compounds (**4d**,**i**,**n**,**x**) prompted further pharmacokinetic studies in the dog following both iv and oral routes of administration. Results from this work indicated **4i**,**x** had properties we believe to be important for a potential therapeutic agent, and **4i** has been selected for further pharmacological studies that will be reported in subsequent manuscripts.

Experimental Section

Proton NMR spectra were obtained on VXR or Unity 300- or 400-MHz instruments (Varian Instruments, Palo Alto, CA); chemical shifts were recorded in ppm (δ) from an internal TMS standard in deuteriochloroform or deuteriodimethyl sulfoxide as specified. Coupling constants were measured in hertz (Hz). Mass spectra were measured with a Hewlett-Packard 5988A or a Finnigan MAT 8230 mass spectrometer with a particle beam interface using NH_3 for chemical ionization. High-resolution mass spectra (HRMS) were obtained on a VG 70-VSE instrument with NH_3 as a carrier gas for chemical ionization. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Elemental analyses were performed by Quantitative Technologies, Inc., Whitehouse, NJ. Solvents and reagents were obtained from commercial vendors and used without further purification unless otherwise indicated. Melting points were obtained on a Thomas-Hoover capillary apparatus and are uncorrected.

4-*N*-[2-Bromo-4-(1-methylethyl)phenyl]-6-chloro-2-methylpyrimidine-4,5-diamine (2). 5-Amino-4,6-dichloro-2-methylpyrimidine (**3**)[25] (28.5 g, 0.16 mol) and 2-bromo-4-isopropylaniline (34.24 g, 0.16 mol) in 2-ethoxyethanol (100 mL) were refluxed at 135 °C for 30 h. After the reaction mixture was cooled, the solvent was removed in vacuo, the residue taken up into dichloromethane, and the organic phase washed with water, dried over anhydrous magnesium sulfate, and filtered. Solvent removal gave an oil that was purified by flash chromatography (silica gel) using methanol/CH_2Cl_2 (1:100) to yield the desired product as a cream-colored solid (32.1 g, 56%): mp 144.5−146 °C.

Method A: General Procedures for the Synthesis of 2-Bromo-4-isopropylphenyltriazolopyrimidines 4. 3-[2-Bromo-4-(1-methylethyl)phenyl]-7-chloro-5-methyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (3). To **2** (12.5 g, 0.035 mol) in dichloromethane (125 mL) and 50% aqueous acetic acid (125 mL) was added sodium nitrite (2.55 g, 0.037 mol) in water (10 mL) dropwise at room temperature. After addition, the reaction was stirred for an additional 15 min. The organic layer was then separated, washed with water, and dried with anhydrous magnesium sulfate. Solvent removal gave a residue that was purified by flash chromatography (silica gel) using CH_2Cl_2 to afford a light brown oil. Crystallization from 1:1 hexane/pentane (150 mL) yielded **3** as an off-white solid (12.1 g, 94%): mp 72−74 °C.

7-[Bis(2-methoxyethyl)amino]-3-[2-bromo-4-(1-methylethyl)phenyl]-5-methyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (4i). To **3** (3.1 g, 8.45 mmol) in ethanol (50 mL) was added bis(2-methoxyethyl)amine (1.35 g, 10.1 mmol), followed by triethylamine (1.02 g, 10.1 mmol) and the reaction mixture was refluxed for 3 h. Solvent removal in vacuo gave a residue that was partitioned between ethyl acetate and water. The organic layer was then washed with brine, dried with anhydrous magnesium sulfate, and stripped down to a pale yellow liquid that was purified by a flash chromatography (silica gel) using CH_2Cl_2. Recrystallization of the isolated product from hexane gave **4i** as a white crystalline solid (3.62 g, 92%): mp 93−94 °C; ^{1}H NMR ($CDCl_3$) δ 1.25 (d, 6H, $CH(CH_3)_2$), 2.58 (s, 3H, C-5 CH_3), 3.0 (m, 1H, $CH(CH_3)_2$), 3.39−3.4 (2s, 6H, 2 OCH_3), 3.7−3.85 (2t, 4H, 2 N−CH_2), 4.1−4.6 (2t, 4H, 2 −CH_2−O−CH_3),7.4−7.6 (2m, 3H, Ar); MS (CI) $M^+ = 463.2$, $M + 2 = 465.2$.

Method B: General Procedures for the Synthesis of 2-Bromo-4-isopropylphenylimidazopyrimidines 8 and Other Aryl 8-Substituted Imidazopyridines. 9-[2-Bromo-

Table 7. Pharmacokinetic Parameters of Selected Compounds after 1 mg/kg po Administration in Dogs

compd[a]	*n*	C_{max}[b] (nM)	T_{max}[c] (h)	AUC[e] (nM·h)	%*F*[e]	K_i (nM)[f]
4d	4	141.3 ± 55.1	1.25 ± 0.5	824.3 ± 89.3	18.4 ± 7.1	5.0
4i	6	307.6 ± 131.6	0.67 ± 0.26	914.4 ± 212.7[g]	30.7[h]	4.7
4n	5	39.04 ± 15.49	0.8 ± 0.3	365.7 ± 191.8	7.3 ± 4.1	5.8
4x	5	545.8 ± 123.9	0.50 ± 0.31	3144.9 ± 958.7	53.1 ± 10.5	7.3

[a] po formulation: 0.25% methocel (with 0.1% Tween 80 for **4d**,**n**). [b] Peak plasma concentration of compound after po administration. [c] Time to achieve maximum plasma levels. [d] Area under the compound concentration−time curve. [e] Oral bioavailability. [f] Binding affinity constant. [g] Value determined from 0 to 24 h. [h] Dogs were given iv or po dose.

4-(1-methylethyl)phenyl]-6-chloro-2-methyl-9*H*-imidazo-[4,5-*d*]pyrimidine (5, R = H). Compound **2** (12.2 g, 0.034 mol) in triethyl orthoformate (90 mL) and acetic anhydride (90 mL) was heated at 120 °C for 5 h. The solvent was stripped off in vacuo, the residue was partitioned between chloroform and water, and the pH of the aqueous phase was adjusted to 8. After extracting with additional chloroform, the extracts were washed with brine, dried with anhydrous magnesium sulfate, and stripped down to a brown oil. The crude oil was purified by flash chromatography (silica gel) using CH_2Cl_2, followed by recrystallization from petroleum ether to give **5** (R = H) as an off-white crystalline solid (4.9 g, 40%): mp 90−91 °C; ^{1}H NMR ($CDCl_3$) δ 1.25 {d, 6H, $CH(CH_3)_2$}, 2.8 (s, 3H, C-2 CH_3), 3.0 {m, 1H, $CH(CH_3)_2$}, 7.2 (m, 2H, Ar), 7.45 (s, 1H, Ar), 8.18 (s, 1H, C-8 CH).

5 (R = CH₃, *n*-Bu, Ph; Ar = 2-chloro-4,6-dimethoxyphenyl). To 1 mmol of **2** in 10 mL of CH_2Cl_2 was added 10 mmol of ortho ester and 5 mmol of anhydrous HCl/dioxane solution, and the reaction mixture was stirred for 1−2 h (R = *n*-Bu, CH_3) on 30 h (R = Ph) at room temperature. Partitioning the reaction mixture with saturated $NaHCO_3$ solution and $CHCl_3$ and the usual workup gave a residue that was taken up into xylenes and refluxed for 13−16 h (R = *n*-Bu, CH_3) or 26 h (R = Ph). The solvent was removed in vacuo to give a residue that was crystallized from ether (R = CH_3) or hexane (R = *n*-Bu) or chromatographed (R = Ph) to afford **5** (Ar = 2-chloro-4,6-dimethoxyphenyl; R = CH_3, *n*-Bu, Ph).

4-[2-Bromo-4-(1-methylethyl)phenylamino]-6-chloro-2-methyl-5-(trifluoroacetylamino)pyrimidine (6). A solution of 2.5 g (7 mmol) of **2** in 15 mL of trifluoroacetic anhydride was refluxed under nitrogen overnight. Solvent removal in vacuo gave a homogeneous oil [TLC, silica: 1:50 MeOH/CH_2Cl_2; R_f (SM) 0.41; R_f (prod) 0.65] that was used without further purification for the cyclization process.

6-Hydroxy-9-[2-bromo-4-(1-methylethyl)phenyl]-2-methyl-9*H*-imidazo[4,5-*d*]pyrimidine (7). The above residue was taken up into 15 mL of *p*-xylene and reluxed overnight. Solvent removal gave, after crytallization from EtOH, 2.4 g of white solid that turned out to be the pyrimidone analogue of **6**: mp >265 °C; MS: M^+ 433. To 1.65 g (0.0038 mol) of this pyrimidone amide in 35 mL of EtOH was added 1.5 mL of Et_3N, and the solution was refluxed overnight. The solvent was removed in vacuo and the residue taken up into CH_2Cl_2, washed with water, and dried. Solvent removal gave 1.55 g (99%) of **7** which was converted to the chloride **5** without further purification.

Conversion of 7 to 5 (R = CF_3). To 1.85 g (4.5 mmol) of 7 in 20 mL of $POCl_3$ was added 0.8 g of *N*,*N*-diethylaniline dropwise over a 10-min period, and the reaction mixture was refluxed for 3 h. After solvent removal in vacuo, the brown residue was treated with ice−water, and the mixture was stirred for 1 h and then extracted with ether. The combined extracts were washed with water and dried with $MgSO_4$. Solvent removal gave a viscous residue that was chromatographed (CH_2Cl_2) to afford 1.1 g of oil that crystallized upon trituration with pentanes to give 1.05 g (54%) of white solid **5** (R = CF_3): mp 132−133 °C.

6-(*N*-Ethylbutylamino)-9-[2-bromo-4-(1-methylethyl)-phenyl]-2-methyl-9*H*-imidazo[4,5-*d*]pyrimidine (8b). Compound **5** (1.1 g, 3 mmol) in *N*-ethylbutylamine (5.0 g) was heated at reflux for 1 h. The excess amine was removed under vacuum, and the residue was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried with anhydrous magnesium sulfate, and stripped down to a pale yellow liquid. The crude residue was purified by a flash chromatography (silica gel) using CH_2Cl_2 to give **8b** as a light brown oil (0.73 g, 56%): ^{1}H NMR ($CDCl_3$) δ 1.0−1.2 (2t, 6H, 2 CH_3), 1.25 {d, 6H, $CH(CH_3)_2$}, 1.4−1.6 (2m, 4H, 2 CH_2) 2.58 (s, 3H, C-2 CH_3), 3.0 (m, 1H, $CH(CH_3)_2$), 3.5 (q, 2H, $-N-CH_2-CH_3$), 4.0 (br, 2H, $-N-CH_2$), 7.25−7.4 (m, 2H, Ar), 7.6 (s, 1H, Ar), 7.8 (s, 1H, C-8 CH); MS M^+ = 430.1, M + 2 = 432.1

Methods A′ and B′: General Procedure for the Synthesis of 3-[2-Bromo-4-(1-methylethyl)phenyl]-7-alkoxy-5-methyl-3*H*-1,2,3-triazolo[4,5-*d*]- or -9*H*-imidazo[4,5-*d*]-pyrimidines 9 and 10. 3-[2-Bromo-4-(1-methylethyl)phenyl]-7-(1-methoxymethylpropoxy)-5-methyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (9a). To 1-methoxy-2-butanol (0.26 g, 2.4 mmol) in toluene (20 mL) was added 60% NaH (mineral oil) (0.12 g, 2.4 mmol), and the mixture was stirred at room temperature for 10 min. Compound **3** (0.74 g, 2.0 mmol) was then added, and the reaction mixture was refluxed for 1 h, cooled to room temperature, and quenched with water (10 mL). The organic layer was separated, washed with brine, dried with anhydrous magnesium sulfate, and stripped down to a pale yellow liquid that was purified by a flash chromatography (silica gel) using CH_2Cl_2 as an eluent to afford **9a** as a colorless oil (0.54 g, 62%): ^{1}H NMR ($CDCl_3$) δ 1.05 (t, 3H, CH_3), 1.35 (d, 6H, $CH(CH_3)_2$), 1.95 (q, 2H, CH_2), 2.78 (s, 3H, C-5 CH_3), 3.0 (m, 1H, $CH(CH_3)_2$), 3.4 (s, 3H, OCH_3), 3.6−3.8 (m, 2H, $O-CH_2$), 5.85 (m, H, O−CH), 7.4 (m, 2H, Ar), 7.6 (s, 1H, Ar); MS M^+ = 434.2, M + 2 = 436.2

Method C: General Procedures for the Synthesis of 15. 4-*N*-(2,4,6-Trimethylphenyl)-6-hydroxy-2-methyl-5-nitropyrimidin-4-amine (14, Ar = 2,4,6-trimethylphenyl). To 4,6-dichloro-2-methyl-5-nitropyrimidine (**13**) (12.58 g, 60.48 mmol) dissolved in DMSO (200 mL) was added 2,4,6-trimethylaniline (7.43 mL, 52.8 mmol) dropwise via syringe over 1 h. The reaction was stirred at room temperature for 18 h, then poured onto water (1.6 L), and allowed to stir overnight. The resultant precipitated pyrimidone was filtered and dried to constant weight affording **12** (8.02 g, 51%) as a light yellow solid: mp >225 °C; ^{1}H NMR ($CDCl_3$) δ 12.23 (bs, 1H), 10.60 (s, 1H), 6.95 (s, 2H), 2.34 (s, 3H), 2.33 (s, 3H), 2.16 (s, 6H). Anal. ($C_{14}H_{16}N_4O_3$) C, H, N.

In a similar manner the following compounds were prepared in 40−80% yield: **14**, Ar = 2-bromo-4-(trifluoromethoxyl)-phenyl; **14**, Ar = 2-(trifluoromethyl)-4-(dimethylamino)phenyl; **14**, Ar = 2-bromo-4,6-dimethoxyphenyl; **14**, Ar = 2-chloro-4,6-dimethoxyphenyl; **14**, Ar = 2-bromo-4-(dimethylamino)-6-methoxyphenyl.

4-*N*-(2,4,6-Trimethylphenyl)-6-chloro-2-methyl-5-nitropyrimidin-4-amine (15, Ar = 2,4,6-trimethylphenyl). Product **14** from part A (3.1 g, 11 mmol) was suspended in phosphorus oxychloride (25 mL) and heated to just under reflux for 1 h, to give a dark solution. The reaction was pipetted slowly and cautiously onto 700 mL of ice/water, stirred 30 min at room temperature, partitioned with methylene chloride (200 mL), and transferred to a separatory funnel. The aqueous layer was separated and extracted with methylene chloride (3 × 50 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to constant weight to afford (3.18 g, 97%) **15** as a bright yellow solid: mp 128−130 °C; ^{1}H NMR ($CDCl_3$) δ 8.79 (bs, 1H), 6.96 (s, 2H), 2.42 (s, 3H), 2.33 (s, 3H), 2.15 (s, 6H).

In a similar manner the following compounds were prepared in 40−80% yield: **15**, Ar = 2-bromo-4-(trifluoromethoxy)-phenyl; **15**, Ar = 2-(trifluoromethyl)-4-(dimethylamino)phenyl;

15, Ar = 2-bromo-4,6-dimethoxyphenyl; **15**, Ar = 2-chloro-4,6-dimethoxyphenyl; **15**, Ar = 2-bromo-4-(dimethylamino)-6-methoxyphenyl.

General Procedure for the Conversion of 15 to 16. 4-*N*-(2,4,6-Trimethylphenyl)-6-(*N*-*n*-butylethylamino)-2-methyl-5-nitropyrimidin-4-amine (16, Ar = 2,4,6-trimethylphenyl; R^1 = Et, R^2 = *n*-Bu). To the chloronitropyrimidine **15** (Ar = 2,4,6-trimethylphenyl) (0.2 g, 0.7 mmol) in 5 mL of acetone were added 87 mg (87 mmol) of Et_3N and 88 mg (88 mmol) of *N*-*n*-butylethylamine via syringe causing a precipitate to form in the bright yellow-orange solution. After stirring at room temperature for 1−2 h, the solvent was removed in vacuo and the residue chromatographed (SiO_2, 10% EtOAc/hexanes) to give 262 mg (98%) of bright yellow crystalline solid **16** (Ar = 2,4,6-trimethylphenyl; R^1 = Et, R^2 = *n*-Bu): mp 76−77.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.16 (s, 6H), 2.20 (s, 3H), 2.31 (s, 3H), 3.47 (m, 4H), 6.93 (s, 2H).

4-*N*-[2-Bromo-4-(dimethylamino)phenyl]-5-amino-6-hydroxy-2-methylpyrimidin-4-amine (19). To **14** (Ar = 2-bromo-4-(dimethylamino)phenyl) (3.8 g, 11.2 mmol) in 50 mL of MeOH containing 220 mL of 1 N HCl solution was added 1.2 g of powdered Fe. After stirring the mixture at room temperature overnight, the solution was then basified to pH 10 with 1 N NaOH solution and filtered through a cake of Celite which was washed with several portions of EtOAc and then MeOH. The layers were separated, and the aqueous phase was extracted with additional EtOAc. The combined extracts were washed with water, dried over $MgSO_4$, and filtered. Solvent removal gave a residue that was chromatographed (SiO_2, 10% MeOH/$CHCl_3$) to give 1.35 g (63%) of gray solid **19**: ^{1}H NMR (CD_3OD) δ 2.20 (s, 3H), 2.90 (s, 6H), 6.78 (dd, *J* = 9.1, 2.9 Hz, 1H), 6.95 (d, *J* = 2.9 Hz, 1H), 7.38 (d, J+9.1 Hz, 1 Hz); MS M + 1 338.

3-[2-Bromo-4-(dimethylamino)phenyl]-7-hydroxy-5-methyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (20). To **19** (1.35 g, 14 mmol) in 17 mL of HOAc and 11 mL of CH_2Cl_2 was added $NaNO_2$ (4.3 mmol), and the reaction mixture was stirred at room temperature for 4 h. Water was then added, the mixture was extracted with several portions of CH_2Cl_2, and the combined extracts were washed with water, dried over $MgSO_4$, and filtered. Solvent removal gave a residue that was chromatographed (SiO_2, 10% MeOH/$CHCl_3$) to give 0.91 g (65%) of brown solid **20**: MS M + 1 349.

3-[2-Bromo-4-(dimethylamino)phenyl]-7-chloro-5-methyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (3, Ar = 2-bromo-4-(dimethylamino)phenyl). This intermediate was prepared by the same procedure as is descibed for the conversion of **14** to **15**. The crude product was purified by chromatography (SiO_2, 10% MeOH/$CHCl_3$) to give the title compound (50%): MS M + 1 367.

Method D: General Procedures for the Synthesis 17 from 13 via 18. 4-*N*-Bis(2-methoxyethyl)-6-chloro-2-methyl-5-nitropyrimidin-4-amine (18, R^1, R^2 = MeOCH$_2$CH$_2$). To **13** (4.16 g, 20 mmol) in ethanol (50 mL) was added triethylamine (2.02 g, 20.0 mmol) followed by dropwise addition of bis(2-methoxyethyl)amine (2.7 g, 20.0 mmol) in ethanol (10.0 mL) over 30 min at room temperature. After stirring the reaction mixture at room temperature for 1 additional h, solvent removal in vacuo gave a residue that was partitioned between ethyl acetate and water. The organic layer was washed with brine, dried with anhydrous magnesium sulfate, and stripped down to a residue that was purified by a flash chromatography (silica gel, CH_2Cl_2) to afford 5.9 g (97%) of an orange-yellow liquid (**18**, R^1, R^2 = $MeOCH_2CH_2$): ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H, C-2 CH_3), 3.35 (s, 6H, 2 −OCH_3's), 3.55 (t, 4H, 2 −N−CH_2's), 3.75 (t, 4H, 2 −O−CH_2's).

In a similar manner **18** (R^1 = Et, R^2 = Bu) was also prepared in 80% yield. **4-*N*-Bis(2-methoxyethyl)-6-*N*-(2-bromo-4,6-dimethoxyphenyl)-2-methyl-5-nitropyrimidine-4,6-diamine (16, R^1, R^2 = MeOCH$_2$CH$_2$; Ar = 2-bromo-4,6-dimethoxyphenyl).** To **18** (R^1, R^2 = $MeOCH_2CH_2$) (3.85 g, 12.6 mmol) in anhydrous DMF (30.0 mL) was added 2-bromo-4,6-dimethoxyaniline (3.07 g; 13.3 mmol), and the mixture was heated at 60 °C for 6 days. Solvent removal in vacuo gave a residue that was partitioned between ethyl acetate and water. The organic layer was then washed with brine, dried with anhydrous magnesium sulfate, and stripped down to a residue that was purified by a flash chromatography (silica gel, 1:50 MeOH/CH_2Cl_2) to afford **16** (R^1, R^2 = $MeOCH_2CH_2$; Ar = 2-bromo-4,6-dimethoxyphenyl) (4.03 g, 64%) as a yellow crystalline solid: mp 95−96 °C; ^{1}H NMR ($CDCl_3$) d 2.15 (s, 3H, CH_3), 3.4 (s, 6H, 2 O−CH_3's), 3.6−3.75 (m, 8H, 4 CH_2's), 3.8−3.83 (2 s, 6H, 2 Ar−OCH_3's), 6.45 (s, 1H, Ar−H), 6.8 (s, 1H, Ar−H), 9.15 (s, 1H, NH); HRMS calcd for M + H ($C_{19}H_{27}N_5O_6Br_1$) 500.114470, found 500.114616.

In a similar manner **16** (R^1= Et, R^2 = Bu) was also prepared in comparable yield. These intermediates were prepared with the following aryl groups: **16**, Ar = 2-(methylthio)-4-isopropylphenyl; **16**, Ar = 2-(methylsulfonyl)-4-isopropylphenyl; **16**, Ar = 2,4-dibromophenyl; **16**, Ar = 2-bromo-4-(methylthio)-phenyl; **16**, Ar = 2-bromo-4-(methylsulfonyl)phenyl; **16**, Ar = 2-bromo-4-acetylphenyl.

4-*N*-Bis(2-methoxyethyl)-6-*N*-(2-bromo-4,6-dimethoxyphenyl)-2-methylpyrimidine-4,5,6-triamine (17, R^1, R^2 = MeOCH$_2$CH$_2$; Ar = 2-bromo-4,6-dimethoxyphenyl). Compound **16** was reduced according to the method described in the preparation of **24** from **23** (Scheme 3) to afford **17** as a viscous oil (72%): ^{1}H NMR ($CDCl_3$) δ 2.35 (s, 3H, CH_3), 3.4 (s, 6H, 2 O−CH_3), 3.5−3.6 (2 t, 8H, 4 CH_2), 3.75−3.80 (2 s, 6H, 2 Ar−OCH_3), 6.25 (bs, 1H, NH), 6.45 (s, 1H, Ar−H), 6.75 (s, 1H, Ar−H).

7-*N*-Bis(2-methoxyethyl)-3-(2-bromo-4,6-dimethoxyphenyl)-5-methyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine (4nn). Compound **17** was cyclized according to the method used in the conversion of **2** to **3** to afford **4nn** as a white crystalline solid (46%): mp 124−126 °C; ^{1}H NMR ($CDCl_3$) δ 2.55 (s, 3H, CH_3), 3.4 (s, 6H, 2 O−CH_3's), 3.65 (s, 3H, Ar−OCH_3), 3.75−3.85 (2 t, 4H, 2 CH_2's), 3.9 (s, 3H, Ar−OCH_3), 4.1 (t, 2H, CH_2), 4.55 (t, 2H, CH_2), 6.55 (s, 1H, Ar−H), 6.85 (s, 1H, Ar−H); Mass M^+ = 481.1, M + 2 = 483.1.

Synthesis of 4hh,ii. DL-Norvaline Dimethylamide. A solution of *N*-CBz-DL-norvaline (10.0 g, 39.8 mmol) in THF (100 mL) was treated with 1-hydroxybenzotriazole hydrate (6.45 g, 47.7 mmol) in portions over 30 min. Dimethylamine hydrochloride (4.22 g, 51.7 mmol), triethylamine (8.00 mL, 57.4 mmol), and dicyclohexylcarbodiimide (9.84 g, 47.7 mmol) were then added in sequence. The resulting mixture was allowed to stir for 12 h at room temperature, then diluted with ethyl acetate (100 mL), and filtered through Celite. The filtrate was washed with water (150 mL) and brine (100 mL), and the aqueous layers were back-extracted in sequence with ethyl acetate (100 mL). The organic extracts were combined, dried over $MgSO_4$, filtered, and evaporated. The residual oil was separated by flash chromatography (silica gel, 30:70 ethyl acetate/hexane) to afford *N*-CBz-DL-norvaline dimethylamide (7.52 g, 27.0 mmol, 68%): TLC R_f 0.10 (30:70 ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 7.36−7.27 (5H, m), 5.68 (1H, br d, *J* = 8.4 Hz), 5.09 (2H, s), 4.67 (1H, dt, *J* = 8.2, 5.1 Hz), 3.08 (3H, s), 2.96 (3H, s), 1.71−1.30 (4H, m), 0.93 (3H, t, *J* = 7.3 Hz); MS (ESI) *m/e* 281 (1), 280 (15), 279 (100).

A solution of *N*-CBz-DL-norvaline dimethylamide (6.82 g, 24.5 mmol) in methanol (50 mL) was treated with 5% Pd on carbon (1 g) and subjected to hydrogen atmosphere (50 psi) in a Paar apparatus for 14 h. The mixture was filtered through Celite and evaporated to afford the title product as an oil (2.72 g, 18.9 mmol, 77%): TLC R_f 0.06 (30:70 ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 3.77−3.72 (1H, m), 3.04 (3H, s), 2.98 (3H, s), 2.53 (2H, br s), 1.62−1.32 (4H, m), 0.94 (3H, t, *J* = 7.0 Hz); MS (H_2O-GC/MS) *m/e* 145 (100).

4-*N*-[2-Bromo-4-(1-methylethyl)phenyl]-6-[1-(*N*,*N*-dimethylcarboxamido)butylamino]-2-methyl-5-nitropyrimidin-4-amine (16, Ar = 2-bromo-4-(1-methylethyl)phenyl; R^1 = H, R^2 = DL-norvaline dimethylamide). The general methods used to convert **13** to **18** to **16** were employed (65% over both steps): mp 139−141 °C; TLC R_f 0.17 (30:70 ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 11.37 (1H, br s), 9.67 (1H, br d, *J* = 6.6 Hz), 8.20 (1H, d, *J* = 8.4 Hz), 7.47 (1H, d, *J* = 1.8 Hz), 7.20 (1H, dd, *J* = 8.4, 1.8 Hz), 5.37 (1H, q, *J* = 5.5 Hz),

3.21 (3H, s), 3.01 (3H, s), 2.89 (1H, heptet, J = 7.0 Hz), 2.36 (3H, s), 1.90−1.79 (2H, m), 1.50−1.39 (2H, m), 1.26 (6H, d, J = 7.0 Hz), 0.97 (3H, t, J = 7.3 Hz); MS (ESI) *m/e* 496 (23), 495 (100), 494 (22), 493 (99).

3-[2-Bromo-4-(1-methylethyl)phenyl]-7-[1-(*N*,*N*-dimethylcarboxamido)butylamino]-5-methyl-3*H*-1,2,3-triazolo-[4,5-*d*]pyrimidine (4hh). The general method used to convert **16** to **17** was employed to prepare 5-amino-4-[2-bromo-4-(1-methylethyl)phenyl]-6-[1-(*N*,*N*-dimethylcarboxamido)butylamino]-2-methylpyrimidine (96%). The general method used to convert **2** to **3** was used to prepare the title product (67%): mp 163−164 °C (ether); TLC R_f 0.16 (50:50 ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 7.64 (0.314H, d, J = 1.8 Hz), 7.63 (0.686H, d, J = 1.8 Hz), 7.41−7.30 (2H, m), 6.97 (0.686H, br d, J = 8.0 Hz), 6.66 (0.314H, br d, J = 8 Hz), 6.20−6.12 (0.314H, m), 5.54−5.44 (0.686H, m), 3.27 (0.942H, s), 3.25 (2.058H, s), 3.02 (3H, s), 2.97 (1H, heptet, J = 7 Hz), 2.57 (2.058H, s), 2.55 (0.942H, s), 1.99−1.78 (2H, m), 1.58−1.41 (2H, m), 1.31 (1.884H, d, J = 7 Hz), 1.30 (4.116H, d, J = 7 Hz), 0.99 (2.058H, t, J = 7 Hz), 0.97 (0.942H, t, J = 7 Hz); MS (NH_3−CI) *m/e* 478 (3), 477 (25), 476 (100), 475 (31), 474 (100).

4-*N*-[2-Bromo-4-(1-methylethyl)phenyl]-6-[1-(*N*,*N*-dimethylamino)-2-pentylamino]-2-methyl-5-nitropyrimidin-4-amine (16, Ar = 2-bromo-4-(1-methylethyl)phenyl; R^1 = H, R^2 = 1-(*N*,*N*-dimethylamino)-2-pentylamino). A solution of 4-[2-bromo-4-(1-methylethyl)phenyl]-6-[1-(*N*,*N*-dimethylcarboxamido)butylamino]-2-methyl-5-nitropyrimidin-4-amine (650 mg, 1.32 mmol) in THF (5 mL) was treated with a solution of borane−THF complex (3.00 mL, 1.0 M, 3.0 mmol) at ambient temperature. After being allowed to stir for 14 h, the reaction mixture was delivered slowly to an equal volume of 1 N aqueous $NaHCO_3$ solution with stirring. The resulting mixture was extracted with dichloromethane (2 × 50 mL), and the extracts were combined, dried over Na_2SO_4, filtered, and evaporated. The residue was separated by flash chromatography (silica gel, 20:80 ethyl acetate/hexane) to afford first the title product (390 mg, 0.813 mmol, 62%) and then recovered unreacted starting material (180 mg, 28%). For the product (an oil): TLC R_f 0.47 (30:70 ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 11.39 (1H, br s), 9.27 (1H, br d, J = 8.8 Hz), 8.24 (1H, d, J = 8.4 Hz), 7.49 (1H, d, J = 1.8 Hz), 7.22 (1H, dd, J = 8.4, 1.8 Hz), 5.20−5.09 (1H, m), 3.10 (1H, dd, J = 13.9, 2.6 Hz), 3.00 (1H, dd, J = 13.9, 7.7 Hz), 2.90 (1H, heptet, J = 7.0 Hz), 2.69 (3H, s), 2.65 (3H, s), 2.40 (3H, s), 1.79−1.50 (2H, m), 1.46−1.34 (2H, m), 1.26 (6H, d, J = 7.0 Hz), 0.96 (3H, t, J = 7.3 Hz); MS (NH_3-CI) *m/e* 483 (3), 482 (25), 481 (100), 480 (29), 479 (99).

4-*N*-[2-Bromo-4-(1-methylethyl)phenyl]-6-[1-(*N*,*N*-dimethylamino)-2-pentylamino]-2-methylpyrimidine-4,5-diamine (17, Ar = 2-bromo-4-(1-methylethyl)phenyl; R^1 = H, R^2 = 1-(*N*,*N*-dimethylamino)-2-pentylamino). The general procedure used to prepare **17** from **16** was employed (100%): ^{1}H NMR ($CDCl_3$) δ 8.27 (1H, d, J = 8.4 Hz), 7.49 (1H, br s), 7.38 (1H, d, J = 1.8 Hz), 7.16 (1H, dd, J = 8.4, 1.8 Hz), 4.99 (1H, d, J = 8.8 Hz), 4.73 (1H, br), 3.02 (2H, d, J = 4.4 Hz), 2.84 (1H, heptet, J = 6.6 Hz), 2.75 (3H, s), 2.62 (3H, s), 2.41 (3H, s), 2.15 (2H, s), 1.65−1.55 (2H, m), 1.42−1.32 (2H, m), 1.23 (6H, d, J = 6.6 Hz), 0.93 (3H, t, J = 7.1 Hz); MS (NH_3-CI) *m/e* 453 (3), 452 (25), 451 (100), 450 (27), 449 (99).

3-[2-Bromo-4-(1-methylethyl)phenyl]-7-[1-(*N*,*N*-dimethylamino)-2-pentylamino]-5-methyl-3*H*-1,2,3-triazolo[4,5-*d*]-pyrimidine (4ii). The general method used to convert **2** to **3** was employed and upon workup with chromatography afforded first the title product (73%), followed by the isomeric product 7-[2-bromo-4-(1-methylethyl)phenyl]-3-[1-(*N*,*N*-dimethylamino)-2-pentylamino]-5-methyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine (13%). For **4ii**: mp 179−180 °C dec; ^{1}H NMR ($CDCl_3$) δ 7.66 (1H, d, J = 1.5 Hz), 7.39 (1H, d, J = 8.4 Hz), 7.36 (1H, dd, J = 8.4, 1.5 Hz), 6.20 (1H, d, J = 8 Hz), 5.11 (1H, br), 3.13 (2H, d, J = 5.5 Hz), 3.00 (1H, heptet, J = 7.0 Hz), 2.76 (3H, s), 2.68 (3H, s), 2.60 (3H, s), 1.80−1.65 (2H, m), 1.52−1.42 (2H, m), 1.31 (6H, d, J = 7.0 Hz), 0.97 (3H, t, J = 7.3 Hz); MS (NH_3-CI) *m/e* 464 (3), 463 (25), 462 (99), 461 (28), 460 (100). For the isomer: TLC R_f 0.05 (30:70 ethyl acetate/hexane); ^{1}H NMR ($CDCl_3$) δ 8.61 (1H, d, J = 8.4 Hz), 8.20 (1H, br s), 7.48 (1H, d, J = 1.8 Hz), 7.26 (1H, dd, J = 8.4, 1.8 Hz), 5.08−4.98 (1H, m), 3.27 (1H, dd, J = 12.6, 9.7 Hz), 2.91 (1H, heptet, J = 7.0 Hz), 2.68 (3H, s), 2.67 (1H, dd, J = 12.6 Hz), 2.22 (6H, s), 2.21−2.11 (1H, m), 1.99−1.89 (1H, m), 1.29−1.19 (1H, m), 1.27 (6H, d, J = 7.0 Hz), 1.16−1.05 (1H, m), 0.88 (3H, t, J = 7.1 Hz); MS (NH_3-CI) *m/e* 464 (3), 463 (25), 462 (100), 461 (29), 460 (98).

Method E (Ar = collidyl): Alternate Method for the Synthesis of 4-Amino-6-chloro-2-methyl-5-nitropyrimidines 15 (Ar = collidyl). 3-Amino-2,4,6-trimethylpyridine. 3-Nitro-2,4,6-trimethylpyridine (14.89 g, 89.70 mmol) in methanol (250 mL) containing 10% palladium/carbon (1.5 g) was hydrogenated at 55 psi for 2 h. The reaction mixture was filtered through wet Celite, and the Celite filter rinsed with methanol (5 × 30 mL). The filtrate was concentrated in vacuo to dryness and the residue purified by chromatography (silica gel; methylene chloride/methanol, 95/5) to 3-amino-2,4,6-trimethylpyridine (12.42 g, 100%) as a viscous oil.

4-*N*-(2,4,6-Trimethylpyridyl)-6-chloro-2-methyl-5-nitropyrimidin-4-amine (15, Ar = 2,4,6-trimethylpyridyl). To 4,6-dichloro-2-methyl-5-nitropyrimidine (**13**) (10.10 g, 48.60 mmol) in anhydrous tetrahydrofuran (200 mL) were added triethylamine (6.8 mL, 48.60 mmol) and 3-amino-2,4,6-trimethylpyridine (3.30 g, 24.3 mmol), and the reaction was stirred for 72 h at room temperature. The solution was diluted with water (1 L) and extracted with ethyl acetate (4 × 200 mL). The combined organic extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated to dryness in vacuo. Chromatography of the crude product (silica gel; ethyl acetate/hexanes, 1/1) gave **15** (Ar = collidyl, 4.8 g, 64%) as a faint yellow solid: mp 134−136 °C; ^{1}H NMR ($CDCl_3$) δ 8.78 (bs, 1H), 6.97 (s, 1H), 2.43 (s, 3H), 2.39 (s, 3H), 2.16 (s, 3H), 2.05 (s, 3H).

4-*N*-(2,4,6-Trimethylpyridyl)-6-chloro-2-methylpyrimidine-4,5-diamine (2, Ar = collidyl). To **15** (Ar = collidyl) (4.8 g, 15.6 mmol) in acetic acid (6 mL) was added powdered iron (4.36 g, 78.0 mmol), and the heterogeneous reaction was stirred for 5 min at 0 °C, then refluxed for 3 h, cooled, and filtered through Celite. The Celite pad was washed with ethyl acetate (500 mL), and the dark filtrate was concentrated in vacuo to near dryness. The residue was redissolved in ethyl acetate/water, and the layers were separated. The aqueous layer was extracted several times with ethyl acetate, and the combined extracts were dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. Chromatography of the crude product (silica gel; methylene chloride/methanol, 95/5) gave **2** (Ar = collidyl) (3.1 g, 72%): ^{1}H NMR ($CDCl_3$) δ 6.94 (s, 1H), 6.26 (bs, 1H), 3.36 (bs, 2H), 2.52 (s, 3H), 2.40 (s, 3H), 2.34 (s, 3H), 2.16 (s, 3H).

2,4-Dichloro-6-methyl-3-nitropyridine (21). 4-Hydroxy-6-methyl-3-nitropyridone (18.67 g, 0.11 mol) and diethylaniline (19 mL, 0.12 mol) were heated at reflux in $POCl_3$ (85 mL) for 3 h. After cooling the solution was poured into ice/water (800 mL), and after stirring for 2.5 h the mixture was extracted with EtOAc (3 × 400 mL). The combined organic extracts were washed with $NaHCO_3$ (200 mL) and brine (200 mL), dried ($MgSO_4$), and stripped in vacuo. The residue was dissolved in EtOAc (100 mL) and passed through a glass funnel packed with 1 in. of silica gel and 1 in. of Celite. The filtrate was stripped in vacuo to give **21** which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 7.30 (s, 1H), 2.61 (s, 3H).

2-Chloro-4-(*N*-butyl-*N*-ethylamino)-6-methyl-3-nitropyridine (22, R^1 = Et, R^2 = Bu). 2,4-Dichloro-6-methyl-3-nitropyridine (**21**) (1 g, 4.83 mmol), *N*-ethylbutylamine (0.75 mL, 5.55 mmol), and *N*,*N*-diisopropylethylamine (1 mL, 6 mmol) were stirred at 25 °C for 24 h and at reflux for 5 h. The mixture was stripped in vacuo, and the residue was partitioned between EtOAc (75 mL) and water (50 mL). The organic layer was washed with water (30 mL) and brine (30 mL), dried ($MgSO_4$), and stripped in vacuo. The residue was chromatographed (silica gel; 10% EtOAc/hexanes) to give regiomer **22′** (270 mg, 21%): ^{1}H NMR ($CDCl_3$) δ 6.56 (s, 1H), 3.30−3.42 (m, 4H), 2.38 (s, 3H), 1.49−1.59 (m, 2H), 1.23−1.35 (m, 2H),1.15

(t, 3H, J = 7.3 Hz), 0.92 (t, 3H, J = 7.0 Hz). And regiomer **22** (840 mg, 64%): ^{1}H NMR ($CDCl_3$) δ 6.52 (s, 1H), 3.28 (q, 2H, J = 7.0), 3.18 (dd, 2H, J_1 = 7.3 Hz, J_2 = 8.1 Hz), 2.44 (s, 3H), 1.49−1.60 (m, 2H), 1.24−1.37 (m, 2H), 1.17 (t, 3H, J = 7.3 Hz), 0.94 (t 3H, J = 7.3 Hz). The major regioisomer was characterized as the 4-adduct **22** by NOE NMR experiments.

2-[*N*-(2-Bromo-4-(1-methylethyl)phenyl)amino]-4-(*N*-butyl-*N*-ethylamino)-6-methyl-3-nitropyridine (23a). 2-Chloro-4-(*N*-butyl-*N*-ethylamino)-6-methyl-3-nitropyridine (**22**) (R^1 = Et, R^2 = Bu) (1.088 g, 4 mmol) and 2-bromo-4-isopropylaniline (1.712 g, 8 mmol) were heated at 140 °C for 4.5 h. The cooled mixture was then partitioned between EtOAc (11 mL) and 0.5 N NaOH solution (30 mL). The EtOAc solution was washed with brine (30 mL), dried ($MgSO_4$), and stripped in vacuo, and the residue was chromatographed (silica gel; 5% EtOAc/hexanes) to give **23a** (920 mg, 51%): ^{1}H NMR ($CDCl_3$) δ 9.54 (s, 1 H), 8.33 (d, 1H, J = 8.8 Hz), 7.41 (d, 1H, J = 1.8 Hz), 7.14 (dd 1H, J_1 = 8.8 Hz, J_2 = 1.8 Hz), 6.24 (s, 1H), 3.18−3.32 (m, 4H), 2.80−2.90 (m, 1H), 2.36 (s, 3H), 1.54−1.65 (m, 2H), 1.18−1.40 (m, 11H), 0.93 (t, 3H, J = 7.0 Hz).

4-Isopropyl-2-(thiomethyl)aniline In a dried flask, under N_2 a mixture of 2-iodo-4-isopropylaniline (6.0 g, 23.0 mmol), sodium thiomethoxide (1.9 g, 26.4 mmol), and copper powder (0.70 g, 11.0 mmol) in anhydrous DMF (50 mL) was refluxed for l h. The cooled reaction mixture was filtered through Celite and the filtrate partitioned between H_2O and EtOAc. The aqueous layer was extracted with EtOAc (2×), and the combined extracts were washed with H_2O (3×) followed by brine and dried over $MgSO_4$. The filtered solution was concentrated in vacuo to give crude product (5.38 g.) that was chromatographed (silica gel; 5% EtOAc/hexane) to give the aniline as a brown liquid (2.10 g, 50%): ^{1}H NMR ($CDCl_3$) δ 7.22 (d, 1H, J = 2.1 Hz), 6.97 (dd, 1H, J_1= 8 Hz, J_2 = 2.2 Hz), 6.68 (d, 1H, J = 8 Hz), 4.15 (br s, 2H), 2.75−2.82 (m, 1H), 2.36 (s, 3H), 1.20 (d, 6H, J = 7 Hz).

2-Chloro-4-(*N*,*N*-bis(methoxyethyl)amino)-6-methyl-3-nitropyridine (22, R^1, R^2 = bis(methoxyethyl)). 2,4-Dichloro-6-methyl-3-nitropyridine (4 g, 19.32 mmol) was treated with bis(methoxyethyl)amine (3.5 mL, 23.66 mmol) in the presence of *N*,*N*-diisopropylethylamine in ethanol (30 mL) at 25 °C for 60 h and at reflux for 7 h. The product was purified by chromatography (silica gel; 20%EtOAc/hexanes followed by 40%EtOAc/hexanes) to give **22** (R^1, R^2 = methoxyethyl) (4 g, 68%).

2-[*N*-(2-Bromo-4-(1-methylethyl)phenyl)amino]-4-(*N*,*N*-bis(methoxyethyl)amino)-6-methyl-3-nitropyridine (23b, R^1, R^2 = methoxyethyl, Ar = 4-(1-methylethyl)phenyl). Heating 2-chloro-4-(*N*,*N*-bis(methoxyethyl)amino)-6-methyl-3-nitropyridine (1.87 g, 6.15 mmol) with 2-bromo-4-isopropylaniline (2.63 g, 12.3 mmol) at 140 °C for 6 h afforded a residue that was purified by chromatography (silica gel; 25%EtOAc/hexanes) to give **23b** (1.3 g, 44%).

2-[*N*-(2-(Thiomethyl)-4-(1-methylethyl)phenyl)amino]-4-(*N*-butyl-*N*-ethylamino)-6-methyl-3-nitropyridine (23c). Using the method above, the title compound was produced as an oil in 65% yield after purification by chromatography (silica gel; 95% hexane/3%EtOAc/CH_2Cl_2).

3-Amino-2-[*N*-(2-bromo-4-(1-methylethyl)phenyl)amino]-4-(*N*-butyl-*N*-ethylamino)-6-methylpyridine (24a). To 2-[*N*-(2-bromo-4-(1-methylethyl)phenyl)amino]-4-(*N*-butyl-*N*-ethylamino)-6-methyl-3-nitropyridine (1.17 g, 2.6 mmol) in dioxane (60 mL) and water (60 mL) containing concentrated NH_4OH (2 mL) was added $Na_2S_2O_4$ (3.63 g, 20.8 mmol), and the mixture was stirred at 25 °C for 2 h. The reaction was extracted with EtOAc (160 mL), and the organic extract was washed with water (3 × 50 mL) and brine (50 mL), dried ($MgSO_4$), and stripped in vacuo. The residue was chromatographed (silica gel; 5% EtOAc/hexanes) to give **24a** (960 mg, 88%): ^{1}H NMR ($CDCl_3$) δ 7.59 (d, 1H, J = 8.4 Hz), 7.36 (d, 1H, J = 2.2 Hz), 7.06 (dd, 1H, J_1= 8 Hz, J_2= 2.2 Hz), 6.54 (s, 1H), 6.51 (brs, 1H), 3.59 (brs, 2H), 2.94−3.06 (m, 4H), 2.77−2.81 (m, 1H), 2.39 (s, 3H), 1.35−1.50 (m, 2H), 1.25−1.34 (m, 2H), 1.21 (d, 6H, J = 7.0 Hz), 1.03 (t, 3H, J = 7.3 Hz), 0.90 (t, 3H, J = 7.3 Hz).

3-Amino-2-*N*-[2-bromo-4-(1-methylethyl)phenyl)amino]-4-(*N*,*N*-bis(methoxyethyl)amino)-6-methylpyridine (24b, R^1, R^2 = methoxyethyl; Ar = 4-(1-methylethyl)phenyl). Synthesized by reducing the corresponding 3-nitro analogue **21** as described earlier and purified by chromatography (silica gel; 20%EtOAc/hexanes) to give **24b** (96%).

3-Amino-2-[*N*-(2-(thiomethyl)-4-(1-methylethyl)phenyl)amino]-4-(*N*-butyl-*N*-ethylamino)-6-methylpyridine (24c). Purified by chromatography (silica gel; 10% EtOAc/CH_2Cl_2) to give **22c** as an oil (62%).

3-Amino-2-[*N*-(2-(thiomethyl)-4-(1-methylethyl)phenyl)amino]-4-[*N*,*N*-bis(methoxyethyl)amino]-6-methylpyridine (22e). Purified by chromatography (silica gel; 30% EtOAc/hexane) to give **22**e as an oil (68%).

6-(*N*-Ethylbutylamino)-9-[2-bromo-4-(1-methylethyl)-phenyl]-2-methyl-9*H*-triazolo[4,5-*d*]pyridine (25a) (Method G). To a two-phase mixture containing 3-amino-2-[*N*-(2-bromo-4-(1-methylethyl)phenyl)amino]-4-(*N*-butyl-*N*-ethyl amino)-6-methylpyridine (0.5 g, 1.19 mmol) dissolved in CH_2Cl_2 and 50% aqueous AcOH (4 mL) was added sodium nitrite (87.5 mg, 1.27 mmol) in small portions. The reaction mixture was stirred at 25 °C for 2 h and partitioned between EtOAc (80 mL) and water (30 mL). The organic extract was washed with brine (30 mL), dried ($MgSO_4$), and stripped in vacuo. The residue was chromatographed on silica gel (10% EtOAc/hexanes eluent) to give **25a** (360 mg, 70%): ^{1}H NMR ($CDCl_3$) δ 7.63 (d, 1H, J = 1.5 Hz), 7.44 (d, 1H, J = 8.1 Hz), 7.33 (dd, 1H, J_1 = 8.1 Hz, J_2 = 1.5 Hz), 6.10 (s, 1H), 3.8−4.0 (br m, 4 H), 2.90−3.05 (m, 1H), 2.49 (s, 3H), 1.70−1.82 (m, 2H), 1.40−1.55 (m, 2H), 1.35 (t, 3H, J = 6.9 Hz), 1.30 (d, 6H, J = 7.0 Hz), 1.01 (t, 3H, J = 7.0 Hz). The free base was converted to the hydrochloride salt using anhydrous HCl in ether.

6-(*N*-Ethylbutylamino)-9-[2-bromo-4-(1-methylethyl)-phenyl]-2-methyl-9*H*-triazolo[4,5-*d*]pyridine (25a′). The title compound was prepared from pyridine regioisomer **22′** (Scheme 3, method G) via the methodology described for the preparation of **25a** to give **25a′** in comparable yield as an opaque oil: ^{1}H NMR ($CDCl_3$) δ 7.65 (s, 1H), 7.35 (s, 2H), 6.19 (s, 1H), 4.05 (m, 4H), 3.01 (quintet, 1H, J = 7 Hz), 2.40 (s, 3H), 1.75 (m, 2H), 1.45 (m, 2H), 1.33 (t, 3H, J = 7.3 Hz), 1.32 (d, 6H, J = 7 Hz), 0.99 (t, 3H, J = 7.3 Hz).

6-(*N*-Ethylbutylamino)-9-[2-(thiomethyl)-4-(1-methylethyl)phenyl]-2-methyl-9*H*-triazolo[4,5-*d*]pyridine (25c). Purified by chromatography (silica gel; 20% EtOAc/hexane) to give **25c** as an oil (81% yield).

6-(*N*,*N*-Bis(methoxyethyl)amino)-9-[2-(thiomethyl)-4-(1-methylethyl)phenyl]-2-methyl-9*H*-triazolo[4,5-*d*]pyridine (25e). Purified by chromatography (silica gel; 30% EtOAc/hexane) to give **25e** as an oil (68% yield).

6-(*N*-Ethylbutylamino)-9-[2-(methylsulfonyl)-4-(1-methylethyl)phenyl]-2-methyl-9*H*-triazolo[4,5-*d*]pyridine (25d). To **25c** (250 mg, 0.63 mmol) in methanol (5 mL) and H_2O (2.5 mL) at room temperature was added sodium periodate (200 mg, 0.95 mmol), and the reaction mixture was stirred overnight. After H_2O and EtOAc were added, the aqueous phase extracted with additional EtOAc (2×). The combined extracts were washed with H_2O followed by brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a foam (0.24 g) which was taken up into CH_2Cl_2 (5 mL) and H_2O (5 mL). Benzyltriethylammonium chloride (132 mg, 0.58 mmol) followed by $KMnO_4$ (275 mg, 1.74 mmol) were added, and the reaction mixture was stirred at room temperature overnight. After partitioning between H_2O and EtOAc, the layers were separated and the aqueous layer was extracted with EtOAc (2 × 10 mL). The combined extracts were washed with H_2O followed by brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give an oil (0.26 g) that was chromatographed (silica gel; gradient from 20% to 30% EtOAc/hexane) to give **25d** (0.17 g, 68%): mp 151−153 °C; ^{1}H NMR ($CDCl_3$) δ 7.39 (d, 1H, J = 8 Hz), 7.32 (d, 1H, J = 1.8 Hz), 7.19 (dd, 1H, J_1= 8 Hz, J_2= 1.8 Hz), 6.09 (s, 1H), 3.9−4.0 (m, 2H), 3.8−3.9 (m, 2H), 2.95−3.04 (m, 1H), 2.49 (s, 3H), 2.38 (s, 3H), 1.7−1.8 (m,

2H), 1.4−1.53 (m, 2H), 1.34 (t, 3H, J = 7.0 Hz), 1.31 (d, 6H, J = 6.6 Hz), 1.00 (t, 3H, J = 7.3).

6-(*N*,*N*-Bis(methoxyethyl)amino)-9-[2-(methylsulfonyl)-4-(1-methylethyl)phenyl]-2-methyl-9*H*-triazolo[4,5-*d*]pyridine (25f). Prepared using the same sequence as described for **25d** and purified by chromatography (silica gel; 50% EtOAc/hexane) to give **25f** as a white solid (68%).

6-(*N*-Ethylbutylamino)-9-[2-bromo-4-(1-methylethyl)-phenyl]-2,8-dimethyl-9*H*-imidazo[4,5-*d*]pyridine (26a) (Method H). To a solution of 3-amino-2-[*N*-(2-bromo-4-(1-methylethyl)phenyl)amino]-4-(*N*-butyl-*N*-ethylamino)-6-methylpyridine (460 mg, 1.1 mmol) in triethyl orthoacetate (5 mL) was added 0.5 mL of concentrated HCl, and the reaction was stirred at 25 °C for 2 h. The mixture was partitioned between EtOAc (75 mL) and $NaHCO_3$ (50 mL), and the organic extract was washed with brine (30 mL), dried ($MgSO_4$), and stripped in vacuo. The residue was chromatographed (silica gel; 20% EtOAc/hexanes) to give **26a** (330 mg, 68%): ^{1}H NMR ($CDCl_3$) δ 7.59 (s, 1H), 7.32 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.8 Hz), 7.28 (d, 1H, J = 8.4 Hz), 6.13 (s, 1H), 3.6−4.0 (m, 4H), 2.9−3.03 (m, 1H), 2.41 (s, 3H), 2.29 (s, 3H), 1.61−1.75 (m, 2H), 1.35−1.5 (m, 2H). The free base was converted to the hydrochloride salt using anhydrous HCl in ether.

6-(*N*,*N*-Dimethoxyethylamino)-9-[2-bromo-4-(1-methylethyl)phenyl]-2-methyl-9*H*-triazolo[4,5-*d*]pyridine (25b). Synthesized by cyclization of the 3-aminopyridine **24** with $NaNO_2$ in AcOH. The product was purified by chromatography (silica gel; 2% MeOH/CH_2Cl_2), followed by HCl salt formation and crystallization fron EtOAc/ether/hexanes to give **25b** (45%): ^{1}H NMR ($CDCl_3$) δ 7.68 (s, 1H), 7.55 (d, 1H J = 8.0 Hz), 7.45 (d, 1H J = 8 Hz), 6.42 (s, 1H), 4.6−4.7 (m, 1H).

6-(*N*,*N*-Bis(methoxyethyl)amino)-9-[2-bromo-4-(1-methylethyl)phenyl]-2,8-dimethyl-9*H*-imidazo[4,5-*d*]pyridine (26b). Synthesized by cyclization of 3-amino-2-[*N*-(2-bromo-4-(1-methylethyl)phenyl)amino]-4-(*N*,*N*-bis(methoxyethyl)amino)-6-methylpyridine with triethyl orthoacetate under HCl catalysis as described earlier. The product was purified by chromatography (silica gel; 25%EtOAc/hexanes) to give **26b** (90%); ^{1}H NMR ($CDCl_3$) δ 8.12 (d, 1H, J = 2.2 Hz), 7.69 (dd, 1H, J_1= 8 Hz, J_2= 2.2 Hz), 7.55 (d, 1H, J = 8 Hz), 6.23 (s, 1H), 4.1−4.22 (m, 4H), 3.75 (t, 4H J = 5.5 Hz), 3.39 (s, 6H), 3.37 (s, 3H), 3.05−3.15 (m, 1H), 2.48 (s, 3H), 1.36 (d, 6H, J = 7.0 Hz).

Biology. 1. CRF-R1 Receptor 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.

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]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, which provides K_i values for inhibition which are then used to assess biological activity.

2. Pharmacokinetic Studies. Pharmacokinetic parameters were determined in rats after an oral dose of 1 mg/kg prepared in a 0.25% methylcellulose suspension. At 30 min and 1, 2, 4, 8, and 16 h after dosing, rats were sacrificed and blood samples were collected into tubes containing EDTA. Dogs (n = 4) were given 1 mg/kg of compound iv in a cosolvent vehicle or 1 mg/kg po in 0.25% methylcellulose suspension (with 0.1% Tween 80 in some cases). 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. Steady-state brain-to-plasma ratios in rats were determined approximately 76 h after infusion. Blood and brain tissue were collected at the end of the infusion. Plasma was separated by centrifugation and stored frozen until analysis. Compounds were extracted from plasma by simple liquid−liquid extraction. LC/MS/MS analysis was performed on a Sciex (Thornhill, Ontario) model APIIII triple quadrapole mass spectrometer interfaced with a turbo ion spray ionization source. 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).

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