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Anxiolytic activity of analogues of 4-benzylamino-2-methyl-7H-pyrrolo[2,3-*d*]pyrimidines

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Abstract – An extensive series of analogues of the lead anxiolytic 4-benzylamino-2-methylpyrrolo[2,3-*d*]pyrimidine **1** was synthesized and evaluated in the Geller–Seifter conflict test for anxiolytic activity to discover a less toxic derivative. Analysis of the SAR revealed that the most potent compounds were those with meta substituents on the benzylamino ring. In this group the most promising derivatives were 4-[bis(3,5-dimethylamino)]benzylamino-2-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine **12** and 4-(3,5-dimethylbenzylamino)-2-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine **24**. Potential metabolites of **12** were synthesized and checked for their anxiolytic activity. Less toxic analogues of the second lead **24** were prepared by extending the alkyl groups attached to the benzene ring moiety. The addition of a fluoro substituent to the benzene moiety in the extended alkyl chain analogue 4-(3,5-diethyl-2-fluorobenzylamino)-2-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine **34** resulted in a compound with a longer duration of activity relative to its analogue 4-(3,5-diethylbenzylamino)-2-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine **26**. © Elsevier, Paris

anxiolytic / pyrrolopyrimidine / Geller conflict test

1. Introduction

The most commonly used drugs in the treatment of generalized anxiety disorder are the benzodiazepines. These rapid-onset anxiolytics are very potent but are not curative. In severe cases of anxiety, patients may need lifelong therapy. The benzodiazepines exhibit numerous side effects, those most frequently encountered being sedation, alcohol potentiation, and withdrawal symptoms [1]. With the development of each successive generation of benzodiazepines, attempts to reduce these undesirable properties have met with some success. However, because of their common mechanism of action, interaction with the benzodiazepine binding site of the $GABA_A$ receptor complex, complete eradication of unwanted side effects seems unlikely to be attained. Hence the search for anxiolytics with different modes of action continues to be assiduously pursued in the pharmaceutical industry.

In a group of pyrrolo[2,3-*d*]pyrimidines originally synthesized in the Wellcome Research Laboratories as antipsychotics, we discovered potent anxiolytic activity with 4-benzylamino-2-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine **1** [2] (*figure 1*). The compound increased punished lever-pressing for food in a modified Geller–Seifter conflict test [3] in rats [4], an action similar to that produced by the standard benzodiazepine chlorodiazepoxide (Librium®) (*figure 1*) [4]. The anxiolytic activity of **1**, shown in *table I*, appeared to be operating by a different mode of action, because the compound did not show affinity in an array of receptor binding assays which included benzodiazepine–$GABA_A$–chloride channel complex, dopamine, α_1 adrenergic, α_2 adrenergic, β-adrenergic, δ-opiate and μ-opiate. The receptors commonly associated with anxiolysis are the benzodiazepine–$GABA_A$–chloride channel complex, and the $5\text{-}HT_{1A}$ subtypes. The Geller–Seifter test is sensitive to compounds that act at the former but relatively insensitive to compounds that act selectively at the latter [5]. However, the toxicity of **1** (LD_{50} in mice = 80 mg/kg, p.o.) precluded development of the compound for clinical studies.

Examination of the structure–activity relationship generated by testing derivatives of **1** with a broad spectrum of groups in positions 2, 4, 5, 6, and 7 of the molecule subsequently determined that all the substi-

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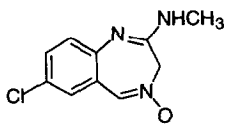
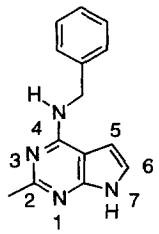


Chlorodiazepoxide
(Librium)



1

Figure 1.

tuents on the structure of **1** were essential for anxiolytic activity. The test data showed that, in position 2 of the molecule, the methyl substituent was preferred. Other alkyl, trifluoroalkyl, amino, phenyl and *N*-methyl piperazino substituents at this position likewise decreased anxiolytic potency. Tests on the small group of compounds with substituents at position 5, 6, or 7 of **1** did not encourage further exploration at these sites. Maintaining the relative position of the substituents and replacing the pyrrolo[2,3-*d*]pyrimidine moiety with other bicyclic heterocycles also abolished the anxiolytic activity [6].

Among the substituents at the 4-position of **1**, it appeared that the benzylamino moiety was the optimal one for anxiolytic activity. Our attention turned to the benzylamino group and substituents around the benzene ring. After examination of numerous substituted benzylamino analogues in the Geller–Seifter conflict test, we concluded that the most active compounds were those with meta substitution on the benzylamino moiety [6]. An examination of analogues of **1** based on this type of substitution is the focus of this report.

2. Chemistry

The substituted 4-benzylamino-2-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidines were prepared by displacement of the chloro group of 4-chloro-2-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine **2** [2] in refluxing water with the requisite benzylamine in the presence of the base K_2CO_3 (*figure 2*). When the benzylamines were not commercially available, they were obtained in almost all cases by reduction of the appropriately substituted benzonitriles using Pd on C in the presence of HCl or using $LiAlH_4$. The lone exception was for the preparation of the di-*tert*-butylbenzylamine **29**. In this case the benzylamine was prepared via hydrazinolysis of the corresponding pthalimidobenzyl intermediate (vide infra). The benzonitriles were prepared by multistep syntheses as illustrated in figures 3–8.

The preparations of 3-methylaminobenzonitrile **39** and the 3-dimethylaminobenzonitrile **40** are illustrated in *figure 3*. To prepare **39**, 3-aminobenzonitrile **37** was mono methylated by initial formation of the intermediate benzotriazolyl-methylamino adduct **38** and then reduction with $NaBH_4$ [7]. 3-Dimethylaminobenzonitrile **40** was prepared by reductive amination of **37** in the presence of excess formaldehyde [8].

Figure 4 illustrates the synthesis of the 3,5-bis(dimethylamino)benzonitrile **43** from 3,5-dinitrobenzonitrile **41**. The nitro groups of **41** were selectively reduced with iron. The amino groups of **42** were then alkylated by reductive amination in the presence of excess formaldehyde to provide **43** in 93% yield. The bis(3,5-methylamino)benzonitrile **45** and the 3-dimethylamino-5-methylaminobenzonitrile **46** were prepared from **42** as well. Treatment of **42** with benzotriazole and formaldehyde furnished the bis-benzotriazolylmethyl adduct **44** which was reduced with $NaBH_4$ to furnish the benzonitrile **45**. The 3-dimethyamino-5-methylaminobenzonitrile **46** was prepared in 44% yield by reductive amination of **45** with 1 equivalent of formaldehyde.

The 3,5-diisopropylbenzonitrile **48** was prepared from the corresponding bromo derivative **47** [9] by treating this material with CuCN in refluxing DMF [10] (96% yield, *figure 5*).

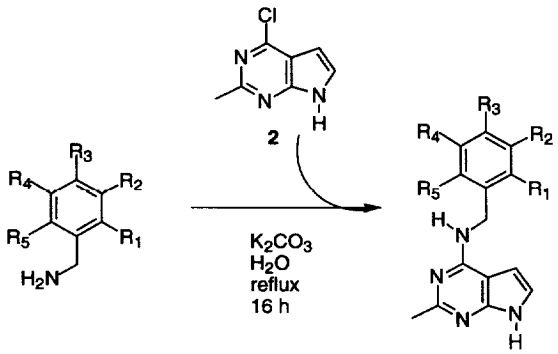
Figure 6 shows the synthesis of the precursor benzonitrile **52** used for the di-*n*-propyl benzylamine **31**. The dialdehyde **49** [11] was treated with EtMgBr to afford the dialcohol **50** in 99% yield as a mixture of stereoisomers. Dehydration with 85% H_3PO_4 afforded the divinyl compound **51** in 92% yield. Displacement of the bromine with CuCN as described previously gave nitrile **52** in 84% yield. The reduction of the double bonds of **52** was accomplished during the catalytic reduction of the nitrile to the benzylamine **31**.

3,5-Diethyl-2-fluorobenzonitrile **57** was prepared from 2-bromo-4,6-diethylaniline [12] (**53**) as shown in *figure 7*. Diazotization of **53** followed by treatment with HPF_6 [13] precipitated the diazonium salt **54** in 86% yield. This salt was refluxed in *p*-xylene to furnish an inseparable mixture of 1-bromo-3,5-diethyl-2-fluorobenzene **55** and the reduction product 1-bromo-3,5-diethylbenzene **56** in 70% crude yield. This mixture was treated with CuCN to furnish a mixture of the nitriles **57** and 3,5-diethylbenzonitrile **58** in a ~9:1 ratio as determined by ^{1}H-NMR. **57** was separated in 43% yield from the contaminating benzonitrile by silica gel chromatography. The same sequence, starting with 4-bromo-2,6-diethylaniline **59** [9], yielded the isomeric 3,5-diethyl-4-fluorobenzonitrile **62** (*figure 8*). In the preparation of this isomer, however, the diazonium salt **60** decomposed at a lower temperature than the corresponding analogue **54** and yielded **61** in 88% yield with no contamination from competing reduction of the diazonium salt.

Table I. Percent change produced by analogues **1–36** on punished lever pressing in rats.[a]

Analogue	Hours after injection	Dose[b]		Positive control (CDP)[c]	LD_{50}[d]
		12.5	25.0		
1	1	67[g] ± 7		131[g]	80
	2	48[g] ± 12			
	4	60[g] ± 18			
4	1	55[g] ± 15	85[g] ± 26		130
	2	35[g] ± 11	29[e] ± 16		
	4	32[g] ± 9	28[e] ± 29		
6	1	55[g] ± 11	47[g] ± 13		100
8	1	25[g] ± 3	34[g] ± 10	101[g]	> 100
	2		32[g] ± 10		i.p. mice
10	1	10 ± 10	62[g] ± 12	98[g]	< 250
	2	53[g] ± 8	28[g] ± 11		
12	1	43[g] ± 11	41[g] ± 3	60[g]	160
	2	35[g] ± 5	65[g] ± 7		i.p. mice
	4		45[g] ± 15		
14	1	17[g] ± 6	20 ± 9	89[g]	
	2	10 ± 4	22 ± 13		
16	1	47[g] ± 17	25[g] ± 10	71[g]	310
	2	28 ± 12	72[g] ± 17		i.p. mice
	4		30[g] ± 8		
18	1		12[e] ± 54	93[g]	
20	1	38[g] ± 14	29 ± 23		25
22	2		38[f]		
24	1	53[g] ± 15 @ 6.25 mg/kg		71[g]	
	2	90[g] ± 20 @ 6.25 mg/kg			
26	1	61[g] ± 13	55[g] ± 11	45[g]	190
	2	17 ± 18	25 ± 15		i.p. mice
28	1		16[g] ± 5	42[g]	
	2		15[e] ± 7		
30	1	27[g] ± 10	16 ± 12	44[g]	
	2		−1 ± 4		
32	1	12 ± 10	5 ± 4	67[g]	
34	1		41[g] ± 9	66[g]	
	2	15 ± 10	36 ± 19		
	4	28[g] ± 6	45[g] ± 16		
	24		47[f,g]		
36	1		19 ± 14	74[g]	
			17 ± 14		

[a]Relative to untreated baseline in the same subjects; $N = 6$ in most cases; [b]in mg/kg p.o.; [c]tested at 25 mg/kg 1 h after injection p.o.; [d]in mg/kg p.o. in rat except where otherwise indicated; [e]significant reduction in unpunished lever pressing; [f]standard error unavailable; [g]$p < 0.05$ by t-test.



3	R_2=OCH_3, R_1=R_3=R_4=R_5=H	4
5	R_2=R_4=OCH_3, R_1=R_3=R_5=H	6
7	R_2=$NHCH_3$, R_1=R_3=R_4=R_5=H	8
9	R_2=$N(CH_3)_2$, R_1=R_3=R_4=R_5=H	10
11	R_2=R_4=$N(CH_3)_2$, R_1=R_3=R_5=H	12
13	R_2=R_4=$NHCH_3$, R_1=R_3=R_5=H	14
15	R_2=$N(CH_3)_2$, R_4=$NHCH_3$, R_1=R_3=R_5=H	16
17	R_1=CH_3, R_2=R_3=R_4=R_5=H	18
19	R_2=CH_3, R_1=R_3=R_4=R_5=H	20
21	R_3=CH_3, R_1=R_2=R_4=R_5=H	22
23	R_2=R_4=CH_3, R_1=R_3=R_5=H	24
25	R_2=R_4=CH_2CH_3, R_1=R_3=R_5=H	26
27	R_2=R_4=*i*-Pr, R_1=R_3=R_5=H	28
29	R_2=R_4=*t*-Bu, R_1=R_3=R_5=H	30
31	R_2=R_4=*n*-Pr =R_3=R_5=H	32
33	R_1=F, R_2=R_4=CH_2CH_3, R_3=R_5=H	34
35	R_3=F, R_2=R_4=CH_2CH_3, R_1=R_5=H	36

Figure 2.

The synthesis of the di-*tert*-butyl benzylamine **29** is depicted in *figure 9*. Di-*tert*-butyl toluene **63** was treated with NBS in the presence of a radical initiator (benzoyl peroxide) affording the monobromo derivative **64** [14] in almost quantitative yield contaminated with minor amounts of the corresponding dibrominated toluene product and starting materials. Displacement of the benzylic bromine with potassium phthalimide afforded the phthaloyl derivative **65** in 82% yield. Hydrazinolysis of the phthaloyl group under standard conditions gave the final compound **29** in 80% yield.

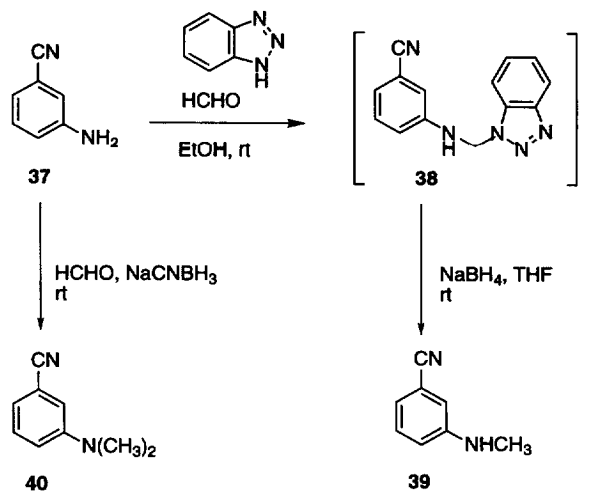


Figure 3.

3. Pharmacology

To determine the anxiolytic activity the pyrrolopyrimidine analogues **1–36** were tested in the Geller–Seifter conflict test [3, 4] in rats.

4. Results and discussion

Among the meta substituted series, the 3-methoxy and the 3,5-dimethoxybenzylamino analogues **4** and **6** had good anxiolytic activity in the Geller–Seifter conflict test (85% and 47% increase in punished lever pressing, respectively, after 1 h at 25 mg/kg, *table I*). However, compounds **4** and **6** possessed unacceptable toxicity as shown by their respective LD_{50}s of 130 and 100 mg/kg in rat. In an effort to obtain compounds that retained anxiolytic activity but were not toxic we investigated two types of structural modifications on the benzene ring. We examined the effect of replacement of the O-atom of the methoxyl group of **4** by preparation of the 3-methylamino and 3-dimethylamino analogues **8** and **10**, and we also explored the replacement of both O-atoms of the methoxyl groups of **6** by preparation of the bis(dimethylamino) analogue **12**. The 3-methylamino analog **8** is less

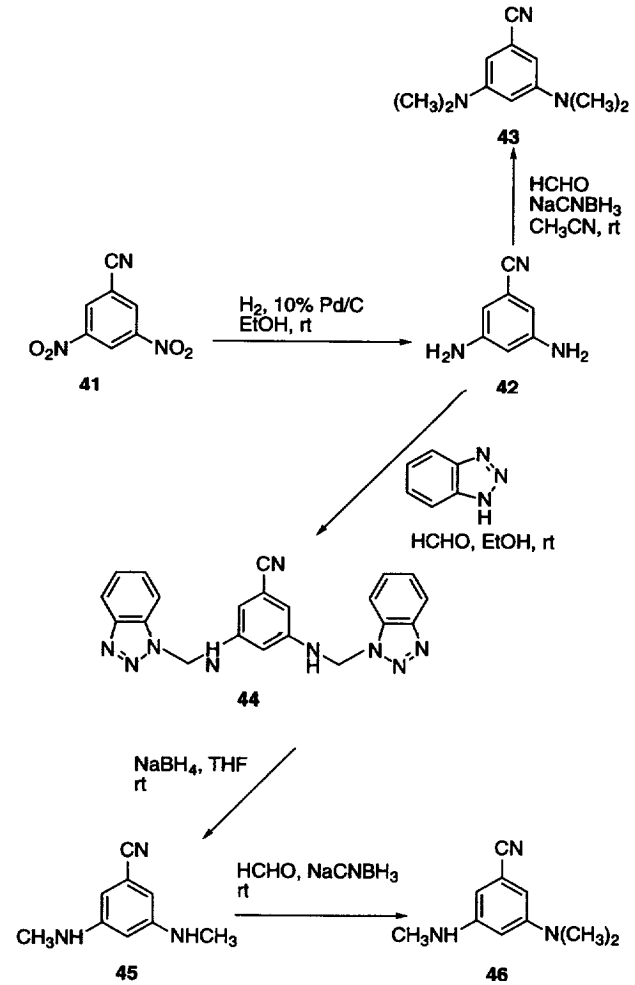


Figure 4.

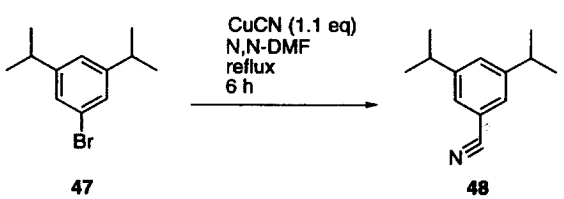


Figure 5.

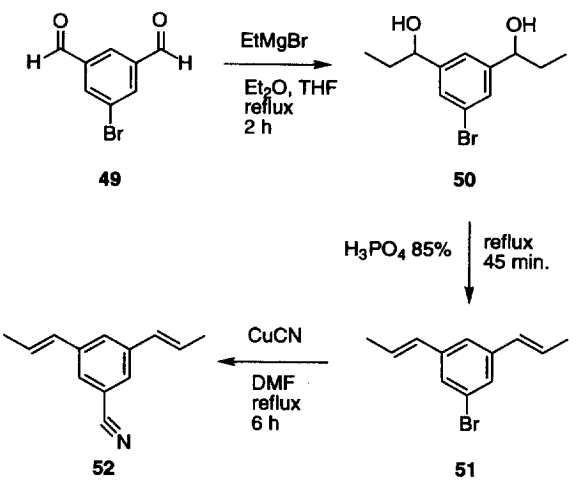


Figure 6.

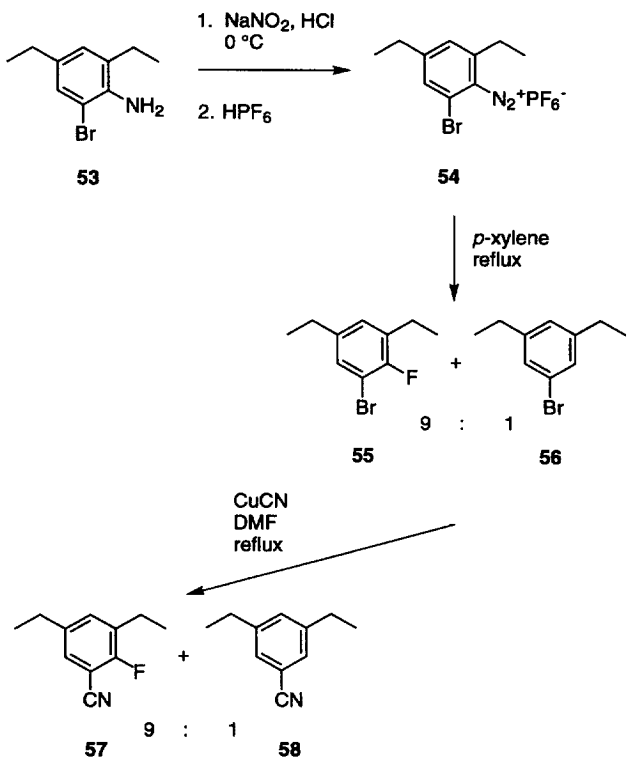


Figure 7.

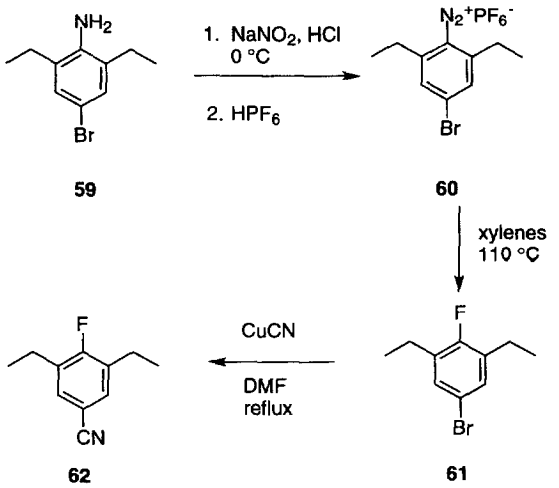


Figure 8.

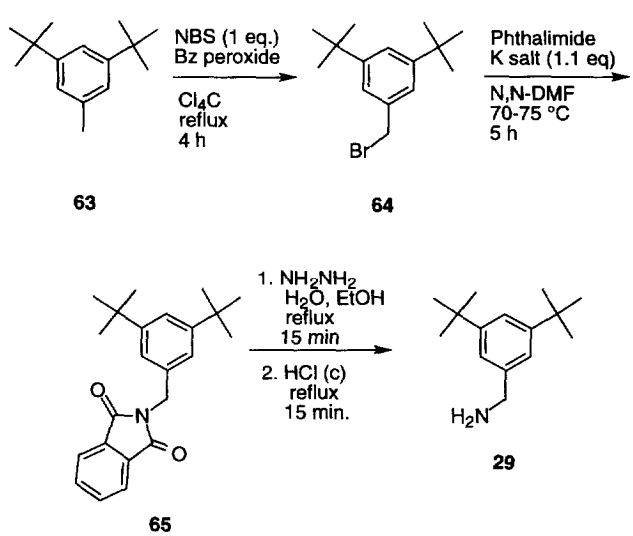


Figure 9.

active than **4** (34% vs 85% respectively, after 1 h at 25 mg/kg). Compound **10** retains most of the activity after 1 h (62%) but drops substantially after 2 h (28%). Compound **12** was promising because of its acceptable anxiolytic activity, which is retained even 4 h after injection (45%). Analysis of plasma and urine samples after oral administration of **12** revealed parent compound (31%) and two metabolites in amounts of 53% and 21% . To identify these components, **12** was incubated with S9 microsomal rat liver slices for 4 h at 37 °C, and the supernatant fraction was analyzed by GC-MS on a C-18 reversed phase column [15]. The results suggested that the two peaks observed were formed by the loss of one and two methyl groups from the parent molecule. Because the original benzylamino lead **1** did not produce any metabolites in a similar S9 experiment, we tentatively attributed the loss of methyl groups to the 3- and 5- dimethylamino moieties. We postulated the desmethylated compounds **14** and **16** as putative products and synthesized them unambiguously. Confirmation of these assignments was obtained by their identical co-elution with the metabolites found in rat liver fractions on HPLC assay. Evaluation in the Geller–Seifter conflict test showed that **16** (the major metabolite) was also an active anxiolytic (72% after 2 h at 25 mg/kg), while the other metabolite, 3,5-bis(dimethylamino)benzylamino **14**, was not (22%). However, further development of **12** was not pursued after discovery that it caused emesis in dogs at the doses that were therapeutic in rats (25 mg/kg p.o.). Also, although the LD_{50} in mice was > 500 mg/kg i.p., it was 160 mg/kg p.o. in mice.

In the second type of modification, we studied the effect of deletion of the O-atom of the substituent attached to the benzene moiety by preparation of the meta-methylbenzylamino analogue **20** (*figure 2* and *table I*), as well as its ortho **18** and para **22** congeners. All three compounds showed minimal increases in punished lever pressing as shown in *table I*. We also prepared the 3,5-dimethyl benzylamino compound **24** as a dideoxy analog of **6**. Compound **24** was a promising lead because of its potent anxiolytic activity (80% after 2 h at 12.5 mg/kg). Although **24** proved to be too toxic for use as an anxiolytic (it disrupted gross behavior at 12.5 mg/kg and killed 1 of 6 rats at 25 mg/kg), it nevertheless represented our most potent anxiolytic lead in this series to date. We sought to exploit the lead **24** by preparation and evaluation of higher alkyl homologs. *Table I* shows anxiolytic test results. Extension of the methyl group to produce the 3,5-di-*n*-propyl (**32**), di-isopropyl (**28**) and di-*tert*-butyl (**30**) analogues gave weakly active compounds. The 3,5-diethyl derivative **26** was potent; however, the duration of action was too brief, falling rapidly after the first hour (from 55% after 1 h to 25% after 2 h). Since this derivative had an encouragingly high LD_{50} in mice (190 mg/kg), it was used as the new lead compound. Seeking to find an explanation for its short duration of action, we submitted **26** for preliminary in vitro metabolism studies with S9 rat liver slices. GC-MS analysis of the supernatant fraction after incubation at 37 °C for 4 h revealed that the major metabolite had m/z = 311 (M + 1), which corresponded to a mass 16 units higher than the parent compound **26**. This observation was consistent with a monohydroxy-

lated metabolite of **26**. Because no such metabolite was observed in similar in vitro experiments with the unsubstituted compound **1**, we assumed that the site of the apparent hydroxylation was on the 3,5-diethylbenzylamino moiety. We presumed that the introduction of an electronegative fluorine atom onto the 3,5-diethylbenzylamino moiety may retard the oxidation of this moiety. If the short duration of action of **26** were due to its rapid metabolism, we suspected that a compound which was more metabolically stable would have the desired pharmacological effect. For ease of synthesis, the fluoro group was introduced on the benzene moiety at either the *ortho* (**34**) or *para* (**36**) sites.

Test results on the two fluorinated compounds indicated that the 2-F derivative **34** was very active at 25 mg/kg (41% after 1 h), with a long duration of action (47% after 24 h). The other isomer, the 4-F derivative **36**, had weaker activity (19% after 1 h). The addition of the fluoro atom in the structure of **34** ultimately had the desired longer duration of action. Whether this is due to added resistance to hydroxylation and enhanced metabolic stability is unclear.

None of the pyrrolopyrimidine analogues described above have shown receptor binding properties in standard assays for benzodiazepine, $GABA_A$, chloride channel, $5\text{-}HT_{1A}$ subtypes, dopamine, α_1-adrenergic, α_2-adrenergic, β-adrenergic, δ-opiate and μ-opiate receptors. Although the mechanism of action has not yet been elucidated, recent publications [16–18] on a series of pyrrolopyrimidines showing anxiolytic activity may suggest that these compounds act as selective corticotropin-releasing factor (CRF) receptor antagonists.

5. Experimental protocols

5.1. Chemistry

Melting points were determined in open glass capillaries by use of a Thomas–Hoover apparatus, and are uncorrected. ^{1}H-NMR spectra were recorded at 300 MHz with a Varian XL-300 spectrometer or at 200 MHz with a Gemini 200 spectrometer. CIMS were recorded with a platform mass spectrometer (Fisons Instrument) operated in a APcI (Atmospheric pressure chemical ionization) mode. IR spectra were taken with a Mattson FTIR. Evaporations were performed under diminished pressure in a Bücchi rotatory evaporator at 40 °C under water aspirator pressure unless otherwise indicated. Solutions were dried over anhydrous Na_2SO_4 or $MgSO_4$. TLC was performed on precoated glass plates (0.25 mm) with Silica Gel $60F_{254}$ (E. Merck, Darmstadt). Flash column chromatography was performed with Silica Gel 60 (230–400 mesh, E. Merck, Darmstadt). Elemental analyses were determined by Atlantic Microlab (Atlanta, GA).

*5.1.1. General procedure for the catalytic reduction of substituted benzonitriles to benzylamine hydrochlorides **7, 9, 11, 13, 15, 23, 31, 33 and 35***

A solution of the benzonitrile in EtOH was treated with conc HCl (1.1 equiv. plus one equivalent for each basic nitrogen on the phenyl moiety) and then with a slurry of 10% Pd on carbon in EtOH. The resulting suspension was hydrogenated at 50 psi on a Parr reactor until the calculated pressure drop was observed. The catalyst was removed by filtration and the filtrate was evaporated to a solid. The solids were either washed with EtOH/Et_2O solutions or recrystallized from isopropanol or EtOH to yield the pure benzylamine hydrochloride salts.

*3-Methylaminobenzylamine dihydrochloride **7**:* 21%; m.p. 185–190 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.57 (br s, 3 H, NH_3), 7.46–7.28 (m, 5 H, Ph H, $NHCH_3$), 4.00 (m, 2 H, CH_2), 2.83 (s, 3 H, CH_3). Anal. ($C_8H_{12}N_3$•2 HCl) C, H, N.

*3-Dimethylaminobenzylamine dihydrochloride **9**:* 57%: m.p. 206–208 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 8.65 (br s, 3 H, NH_3), 7.77–7.25 (m, 4 H, Ph H), 4.04 (q, J = 5.8 Hz, 2 H, CH_2NH_3), 3.06 (s, 6 H, CH_3). Anal. ($C_9H_{14}N_2$•2 HCl) C, H, N.

*3,5-Bis(dimethylamino)benzylamine trihydrochloride **11**:* 82%; m.p. 245–250 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 8.71 (br s, 3 H), 7.18 (s, 3 H), 3.97 (q, J = 5.7 Hz, 2 H). Anal. ($C_{11}H_{19}N_3$•3HCl•H_2O) C, H, N, Cl.

*3,5-Bis(methylamino)benzylamine trihydrochloride **13**:* 80%; m.p. 248–252 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.55 (br s, 6 H), 6.80 (s, 2 H, Ph H), 3.91 (q, J = 5.5 Hz, 2 H, CH_2NH_3), 2.76 (s, 6 H, CH_3). Anal. ($C_9H_{15}N_3$•3 HCl) C, H, N, Cl.

*3-Dimethylamino-5-methylaminobenzylamine trihydrochloride **15**:* 68%; m.p. 239–243 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 9.50 (br s, 3 H), 8.61 (br s, 3 H), 7.10 (s, 1 H, Ph H), 6.93 (s, 1 H, Ph H), 6.87 (s, 1 H Ph H), 3.96 (q, 2 H, J = 5.7 Hz, CH_2NH_3), 3.01 (s, 6 H, CH_3NCH_3), 2.82 (s, 3 H, CH_3NH). Anal. ($C_{10}H_{17}N_3$•3 HCl) C, H, N, Cl.

*3,5-Dimethylbenzylamine hydrochloride **23**:* 80%; m.p. 251.5–253.5 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.39 (s, 3 H, $PhCH_2NH_3$), 7.11 (s, 2 H, Ph H-2 and Ph H-6), 7.03 (s, 1 H, Ph H-4), 3.93 (s, 2 H, $PhCH_2NH_3$), 2.29 (s, 6 H, CH_3). Anal. ($C_9H_{13}N$•HCl) C, H, N, Cl.

*3,5-Di-n-propylbenzylamine hydrochloride **31**:* 68%; m.p. 160–162 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.58 (br s, 3 H, $PhCH_2NH_3$), 7.07 (s, 2 H, Ph H-2 and Ph H-6), 6.95 (s, 1 H, Ph H-4), 3.93 (s, 2 H, $PhCH_2NH_3$), 2.52 (q, 4 H, $CH_2CH_2CH_3$), 1.60 (m, 4 H, $CH_2CH_2CH_3$), 0.90 (t, J = 7.2, 6 H, $CH_2CH_2CH_3$); CIMS m/z 192 (M + 1 free base)$^+$. Anal. ($C_{13}H_{21}N$•HCl) C, H, N, Cl.

*3,5-Diethyl-2-fluorobenzylamine hydrochloride **33**:* 88%; m.p. 224–226 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.37 (br s, 3 H, $PhCH_2NH_3$), 7.23 (dd, J = 2.1 Hz, J = 6.9 Hz, 1 H, Ph H), 7.14 (dd, J = 2.1 Hz, J = 7.0 Hz, 1 H, Ph H), 3.98 (s, 2 H, $PhCH_2NH_3$), 2.63–2.51 (m, 4 H, CH_2CH_3), 1.15 (m, 6 H, CH_2CH_3). Anal. ($C_{11}H_{16}NF$•HCl) C, H, N, Cl.

*3,5-Diethyl-4-fluorobenzylamine hydrochloride **35**:* 65%; m.p. 234–235 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.46 (br s, 3 H, $PhCH_2NH_3$), 7.27 (d, J = 7.9 Hz, 2 H, Ph H), 3.91 (s, 2 H, $PhCH_2NH_3$), 2.58 (q, J = 7.6 Hz, 4 H, CH_2CH_3), 1.15 (t, J = 7.6 Hz, 6 H, CH_2CH_3); CIMS m/z 165 (M – NH_2)$^+$. Anal. ($C_{11}H_{16}NF$•HCl) C, H, N, Cl.

*5.1.2. General procedure for the $LiAlH_4$ reduction of substituted benzonitriles to benzylamine hydrochlorides **25** and **27***

*3,5-Diethylbenzylamine **25**:* To a mechanically stirred suspension of $LiAlH_4$ (0.36 g, 9.4 mmol) in dry Et_2O (9.4 mL), a solution of 3,5-diethylbenzonitrile [12] (1.43 g, 9.0 mmol) in dry Et_2O (28 mL) was added over a period of 15 min. After stirring at room temperature for 30 min it was cooled to 0–5 °C in an ice/water bath. The reaction mixture was first quenched by slow addition of EtOAc (12 mL) followed by H_2O (19 mL). The ice/water bath was then removed and the suspension stirred for additional 15 min at room temperature and then diluted with more H_2O (100 mL). The mixture was extracted

with CH_2Cl_2 (3 x 200 mL). The combined organic solution was washed with H_2O (200 mL), dried, filtered and evaporated to afford compound **25** (1.35 g, 92%) as a yellow oil. This material was obtained analytically pure as its HCl salt by first dissolving compound **25** in EtOH saturated with HCl and then precipitating the HCl salt with Et_2O. The white solid was filtered and washed with Et_2O: m.p. 228–230 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.60 (br s, 3 H, $PhCH_2NH_3$), 7.26 (s, 2 H, Ph H-2 and Ph H-6), 6.99 (s, 1 H, Ph H-4), 3.97 (s, 2 H, $PhCH_2NH_3$), 2.59 (q, J = 7.5 Hz, 4 H, CH_2CH_3), 1.20 (t, J = 7.5 Hz, 6 H, CH_2CH_3); CIMS *m/z* 164 (M + 1 free base)$^+$. Anal. ($C_{11}H_{17}N$•HCl) C, H, N, Cl.

3,5-Di-isopropylbenzylamine hydrochloride ***27****:* From **48**, using the same reduction method for preparation of **25**; 85%; m.p. 204–207 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.65 (br s, 3 H, $PhCH_2NH_3$); 7.14 (s, 2 H, Ph H-2 and Ph H-6), 7.04 (s, 1 H, Ph H-4), 4.00 (s, 2 H, $PhCH_2NH_3$), 2.86 (m, 2 H, $CH(CH_3)_2$), 1.22 (d, J = 7.6 Hz, 12 H, $CH(CH_3)_2$), CIMS *m/z* 192 (M + 1 free base)$^+$. Anal. ($C_{13}H_{21}N$•HCl) C, H, N, Cl.

5.1.3. General procedure for the synthesis of 2-methyl-4-(substitutedbenzyl)amino-7H- pyrrolo[2,3-d]pyrimidines ***4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, and 36***

A suspension of 2-methyl-4-chloro-7*H* -pyrrolo[2,3-*d*]pyrimidine **1** (134 mg, 0.8 mmol), the appropriately substituted benzylamine (1.0 mmol) and K_2CO_3 (138 mg, 1.0 mmol) in H_2O (4 mL) was refluxed overnight. After cooling to room temperature, the solids were filtered, washed with H_2O, and dried with vacuum at 60 °C. After crystallization from the appropriate solvent, the final compounds were obtained as white solids. When the HCl salt of the amine was used instead of the free base, one additional equivalent of base was used.

4-(3-Methoxybenzylamino)-2-methyl-7H-pyrrolo[2,3-d]pyrimidine ***4****:* 25%; m.p. 173–174 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 11.25 (s, 1 H, N*H*-7), 7.73 (t, J = 6.0 Hz, 1 H, $PhCH_2NH$), 7.21 (t, J = 8.1 Hz, 1 H, Ph H), 6.97–6.75 (m, 5 H, H-6, Ph H), 6.49 (dd, J = 1.9 Hz, J = 3.3 Hz, H-5), 4.66 (d, J = 6.0 Hz, 2 H, $PhCH_2NH$), 3.70 (s, 3 H, OCH_3), 2.35 (s, 3 H, CH_3-2); CIMS *m/z* 269 (M + 1)$^+$. Anal. ($C_{15}H_{16}N_4O$) C, H, N.

4-(3,5-Dimethoxybenzylamino)-2-methyl-7H-pyrrolo[2,3-d]-pyrimidine ***6****:* 41%; m.p. 195–197 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 11.25 (s, 1 H, N*H*-7), 7.70 (t, J = 6.0 Hz, 1 H, $PhCH_2NH$), 6.96 (dd, J = 2.4 Hz, J = 3.3 Hz, 1 H, H-6), 6.51 (m, 3 H, Ph H), 6.35 (t, J = 2.2 Hz, H-5), 4.62 (d, J = 6.0 Hz, 2 H, $PhCH_2NH$), 3.69 (s, 6 H, OCH_3), 2.35 (s, 3 H, CH_3-2); CIMS *m/z* 299 (M + 1)$^+$. Anal. ($C_{16}H_{18}N_4O_2$) C, H, N.

4-(3-Methylaminobenzylamino)-2-methyl-7H-pyrrolo[2,3-d]-pyrimidine ***8****:* 73%; m.p. 208–211 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 11.26 (s, 1 H, N*H*-7), 7.68 (t, J = 6.0 Hz, 1H, *H*NCH_2), 7.07–6.95 (m, 2 H, Ph H, H-6), 6.56–6.37 (m, 4 H, Ph H, H-5), 5.59 (q, J = 5.0 Hz, 1 H, N*H*CH_3), 4.61 (d, J = 6.2 Hz, 2 H, $PhCH_2NH$), 2.64 (d, J = 5.2 Hz, 3 H, CH_3N), 2.38 (s, 3 H, CH_3-2). Anal. ($C_{15}H_{17}N_5$) C, H, N.

4-(3-Dimethylaminobenzylamino)-2-methyl-7H-pyrrolo[2,3-d]-pyrimidine ***10****:* 71%; m.p. 192–196 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 11.28 (s, 1 H, N*H*-7), 7.71 (t, J = 6.2 Hz, 1H, $PhCH_2NH$), 7.18–6.53 (m, 6 H, aromatic Hs), 4.65 (d, J = 5.8 Hz, 2 H, $PhCH_2NH$), 2.87 (s, 3 H, CH_3N), 2.39 (s, 3 H, CH_3-2). Anal. ($C_{16}H_{19}N_5$) C, H, N.

4-[(3,5-Bis(dimethylamino)benzyl)amino]-2-methyl-7H-pyrrolo[2,3-d]pyrimidine ***12****:* 68%; m.p. 224–226 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.21 (s, 1 H, N*H*-7), 7.60 (t, J = 5.6 Hz, 1 H, $PhCH_2NH$), 6.95 (t, 1 H, J = 2.1 Hz), 6.52 (s, 1 H, H-5), 6.19 (s, 2 H, *o*-Ph H), 5.90 (t, 1 H, *p*-Ph H), 4.55 (d, J = 5.8 Hz, 2 H, $PhCH_2NH$), 2.83 (s, 12 H, NCH_3), 2.38 (s, 3 H, CH_3-2). Anal.($C_{18}H_{24}N_6$) C, H, N.

4-[3,5-Bis(methylamino)benzylamino]-2-methyl-7H-pyrrolo-[2,3-d]pyrimidine ***14****:* 32%; m.p. 214–216 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 11.23 (s, 1 H, N*H*-7), 7.58 (t, J = 5.1 Hz, 1 H, $PhCH_2NH$), 6.95 (dd, J = 2.3 Hz, J = 3.3 Hz, 1 H, H-6), 6.54 (m, 1 H, H-5), 5.85 (d, J = 1.9 Hz, 2 H, *o*-Ph H), 5.62 (t, J = 1.8 Hz, 1 H, *p*-Ph *H*), 5.28 (q, J = 4.9 Hz, 2 H, $NHCH_3$), 4.51 (d, J = 5.8 Hz, 2 H, $PhCH_2NH$), 2.61 (d, J = 5.1 Hz, 6 H, CH_3NH), 2.38 (s, 3 H, CH_3-2). Anal. ($C_{16}H_{20}N_6$) C, H, N.

4-(3-Dimethylamino-5-methylaminobenzylamino)-2-methyl-7H-pyrrolo[2,3-d]pyrimidine ***16****:* 69%; m.p. > 195 °C (dec.); ^{1}H-NMR (200 MHz, DMSO-d_6) δ 11.24 (s, 1 H, N*H*-7), 7.60 (t, J = 5.7 Hz, 1 H, N*H*-4), 6.96 (t, J = 2.7 Hz, 1 H, H-6), 6.54 (s, 1 H, H-5), 6.07 (s, 1 H Ph *H*), 5.98 (s, 1 H, Ph *H*), 5.78 (s, 1 H Ph *H*), 5.36 (q, J = 5.3 Hz, 1 H, *H*NCH_3), 4.54 (d, J = 5.9 Hz, 2 H, CH_2Ph), 2.82 (s, 6 H, $C_{18}H_3NCH_3$), 2.63 (d, J = 5.1 Hz, 3 H, CH_3NH), 2.38 (s, 3 H, CH_3-2). Anal. ($C_{17}H_{22}N_6$) C, H, N.

4-(2-Methylbenzylamino)-2-methyl-7H-pyrrolo[2,3-d]pyrimidine ***18****:* 80%; m.p. 210–212 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.25 (s, 1 H, N*H*-7), 7.63 (t, J = 5.8 Hz, 1 H, $PhCH_2NH$), 7.30–7.10 (m, 4 H, Ph H), 6.95 (m, 1 H, H-6), 6.47 (s, 1 H, H-5), 4.64 (d, J = 5.8 Hz, 2 H, $PhCH_2NH$), 2.33 (s, 6 H, CH_3-2 and CH_3Ph); CIMS *m/z* 253.0 (M + 1)$^+$. Anal. ($C_{15}H_{16}N_4$) C, H, N.

4-(3-Methylbenzylamino)-2-methyl-7H-pyrrolo[2,3-d]pyrimidine ***20****:* 86%; m.p. 184–185 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.28 (s, 1 H, N*H*-7), 7.14 (t, J = 6.0 Hz, 1 H, $PhCH_2NH$), 7.25–6.98 (m, 4 H, Ph H), 6.95 (m, 1 H, H-6), 6.50 (d, J = 1.0 Hz, 1 H, H-5), 4.65 (d, J = 6.5 Hz, 2 H, $PhCH_2NH$), 2.36 (s, 3 H, CH_3-2), 2.25 (s, 3 H, CH_3Ph); CIMS *m/z* 253.0 (M + 1)$^+$. Anal. ($C_{15}H_{16}N_4$) C, H, N.

4-(4-Methylbenzylamino)-2-methyl-7H-pyrrolo[2,3-d]pyrimidine ***22****:* 50%; m.p. 208–211 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.24 (s, 1 H, N*H*-7), 7.70 (t, J = 6.0 Hz, 1 H, $PhCH_2NH$), 7.22 (d, J = 7.9 Hz, 2 H, Ph H), 7.09 (d, J = 8.1 Hz, 2 H, Ph H), 6.94 (m, 1 H, H-6), 6.47 (s, 1 H, H-5), 4.63 (d, J = 5.8 Hz, 2 H, $PhCH_2NH$), 2.34 (s, 3 H, CH_3-2), 2.24 (s, 3 H, CH_3Ph); CIMS *m/z* 253.0 (M + 1)$^+$. Anal. ($C_{15}H_{16}N_4$) C, H, N.

4-(3,5-Dimethylbenzylamino)-2-methyl-7H-pyrrolo[2,3-d]-pyrimidine ***24****:* 73%; m.p. 193–196 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.28 (s, 1 H, N*H*-7), 7.71 (t, J = 5.8 Hz, 1 H, $PhCH_2NH$), 6.98–6.83 (m, 4 H, Ph *H*, H-6), 6.52 (d, J = 1.7 Hz, 1 H, H-5), 4.65 (d, J = 5.8 Hz, 2 H, $PhCH_2NH$), 2.38 (s, 3 H, CH_3-2), 2.25 (s, 6 H, $PhCH_3$). Anal. ($C_{16}H_{18}N_4$•0.3 H_2O) C, H, N.

4-(3,5-Diethylbenzylamino)-2-methyl-7H-pyrrolo[2,3-d]-pyrimidine ***26****:* 60%; m.p. 172–174 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.24 (br s, 1 H, N*H*-7); 7.69 (t, J = 6.0 Hz, 1 H, $PhCH_2NH$), 7.00 (s, 2 H, Ph H-2 and Ph H-6), 6.93 (d, J = 1.2 Hz, 1 H, H-6), 6.89 (s, 1 H, Ph H-4), 6.49 (d, J = 1.2 Hz, 1 H, H-5), 4.62 (d, J = 6.0 Hz, 2 H, $PhCH_2NH$), 2.51 (q, J = 7.5 Hz, 4 H, CH_2CH_3), 2.35 (s, 3 H, CH_3-2), 1.12 (t, J = 7.5 Hz, 6 H, CH_2CH_3); CIMS *m/z* 295 (M + 1)$^+$. Anal. ($C_{18}H_{22}N_4$) C, H, N.

4-(3,5-Di-isopropylbenzylamino)-2-methyl-7H-pyrrolo[2,3-d]-pyrimidine ***28****:* 70%; m.p. 216–218 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.24 (br s, 1 H, N*H*-7), 7.70 (t, J = 6.0 Hz, 1 H, $PhCH_2NH$), 7.01 (s, 2 H, Ph H-2 and Ph H-6), 6.98 (d, J = 3.0 Hz, 1H, H-6), 6.94 (s, 1 H, Ph H-4), 6.49 (d, J = 3.0 Hz, 1 H, H-5), 4.62 (d, J = 6.0 Hz, 2 H, $PhCH_2NH$), 2.78 (m, J = 7.2 Hz, 2 H, $CH(CH_3)_2$), 2.36 (s, 3 H, CH_3-2), 1.14 (d, J = 7.2 Hz, 12 H, $CH(CH_3)_2$); CIMS *m/z* 323 (M + 1)$^+$. Anal. ($C_{20}H_{26}N_4$•0.2 H_2O) C, H, N.

4-(3,5-Di-tert-butybenzylamino)-2-methyl-7H-pyrrolo[2,3-d]-pyrimidine ***30****:* 85%; m.p. 260–262 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.23 (br s, 1 H, N*H*-7), 7.71 (t, *J* = 6.0 Hz, 1 H, $PhCH_2NH$), 7.25 (s, 3 H, Ph H-2, Ph H-4, and Ph H-6), 6.93 (br s, 1 H, H-6), 6.49 (br s, 1 H, H-5), 4.62 (d, *J* = 6.0 Hz, 2 H, $PhCH_2NH$), 2.37 (s, 3 H, CH_3-2), 1.23 (s, 18 H, $C(CH_3)_3$); CIMS *m/z* 351 $(M + 1)^+$. Anal. ($C_{22}H_{30}N_4$) C, H, N.

4-(3,5-Di-n-propybenzylamino)-2-methyl-7H-pyrrolo[2,3-d]-pyrimidine ***32****:* 33%; m.p. 183–185 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.21 (br s, 1 H, N*H*-7), 7.67 (t, *J* = 6.0 Hz, 1 H, $PhCH_2NH$), 6.97 (s, 2 H, Ph H-2 and Ph H-6), 6.93 (s, 1 H, Ph H-4), 6.84 (br s, 1 H, H-6), 6.49 (br s, 1 H, H-5), 4.62 (d, *J* = 6.0 Hz, 2 H, $PhCH_2NH$), 2.48 (m, 4 H, $CH_2CH_2CH_3$), 2.35 (s, 3 H, CH_3-2), 1.54 (m, 4 H, $CH_2CH_2CH_3$), 0.85 (t, *J* = 7.4, 6 H, $CH_2CH_2CH_3$); CIMS *m/z* 323 $(M + 1)^+$. Anal. ($C_{20}H_{26}N_4$) C, H, N.

4-(3,5-Diethyl-2-fluorobenzylamino)-2-methyl-7H-pyrrolo-[2,3-d]pyrimidine ***34****:* 59%; m.p. 157–159 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.26 (s, 1 H, N*H*-7), 7.67 (t, *J* = 5.8 Hz, 1H, $PhCH_2NH$), 7.08 (dd, *J* = 2.1 Hz, *J* = 6.9 Hz, 1 H, Ph *H*), 6.99–6.94 (m, 3 H, Ph H, H-6), 6.50 (dd, *J* = 1.6 Hz, *J* = 3.1 Hz, 1 H, H-5), 4.66 (d, *J* = 5.7 Hz, 2 H, $PhCH_2NH$), 2.58 (q, *J* = 7.6 Hz, H, CH_2CH_3), 2.49 (m, 2 H, CH_2CH_3), 2.35 (s, 3 H, CH_3-2), 1.12 (m, 6 H, CH_2CH_3); CIMS *m/z* 313.2 $(M + 1)^+$. Anal. ($C_{18}H_{21}N_4F$) C, H, N.

4-(3,5-Diethyl-4-fluorobenzylamino)-2-methyl-7H-pyrrolo-[2,3-d]pyrimidine ***36****:* 45%; m.p. 191–193 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 11.25 (s, 1 H, N*H*-7), 7.70 (t, *J* = 6.0 Hz, 1 H, $PhCH_2NH$), 7.10 (d, *J* = 7.1 Hz, 1 H, Ph H), 6.94 (m, 1 H, H-6), 6.47 (m, 1 H, H-5), 4.59 (d, *J* = 5.8 Hz, 2 H, $PhCH_2NH$), 2.56 (q, *J* = 7.6 Hz, 4 H, CH_2CH_3), 2.36 (s, 3 H, CH_3-2), 1.12 (t, *J* = 7.6 Hz, 6 H, CH_2CH_3); CIMS *m/z* 313.0 $(M + 1)^+$. Anal. ($C_{18}H_{21}N_4F$) C, H, N.

5.1.4. 3-Methylaminobenzonitrile ***39***

A mixture of 3-aminobenzonitrile **37** (10.00 g, 84.6 mmol), benzotriazole (10.40 g, 84.6 mmol), and 37.9% formaldehyde (6.2. mL) in EtOH (225 mL) was stirred at room temperature for 23 h. A white solid was collected by filtration to provide 16.28 g (77%) of the intermediate **38**. To a suspension of the intermediate **38** (8.00 g, 32 mmol) in THF was added $NaBH_4$ (2.67 g, 70.6 mmol) in three portions over the course of 30 min. The resulting mixture was allowed to stir at room temperature for 4 days and then it was evaporated to a syrup. The syrup was taken up in H_2O (200 mL) and this mixture was extracted with hexanes (3 x 200 mL). The combined hexane layers were dried over $MgSO_4$ and then evaporated to 3.51 g (83%) of the colorless oil, **39**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.22 (m, 2 H, Ph H, N*H*), 7.02 (m, 1 H, Ph H), 6.86 (s, 3 H, CH_3). Anal. ($C_8H_8N_2$) C, H, N.

5.1.5. 3-Dimethylaminobenzonitrile ***40***

A mixture of **37** (3.00 g, 25.4 mmol), 37.9% aqueous formaldehyde (19 mL, 10 equiv.), and $NaCNBH_3$ (5.04 g, 3 equiv.) in CH_3CN (100 mL) at room temperature was treated with glacial HOAc dropwise over the course of 20 min. The reaction mixture was allowed to stir at room temperature for 2 h at which point another portion of glacial acetic acid (2.5 mL) was added. The resulting suspension was allowed to stir for an additional 30 min and then the mixture was poured into Et_2O. This mixture was washed with 1 N KOH (3 x 150 mL) and then brine (1 x 200 mL). The Et_2O layer was dried over K_2CO_3 and then evaporated to an oil. The oil was chromatographed on silica gel to funish 3.47 g (94%) of **40** as an oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.28 (m, 1 H Ph *H*), 6.91 (m, 3 H, Ph *H*), 2.98 (s, 6 H, NCH_3); IR (NaCl) 2225 cm^{-1} (CN); Anal. ($C_9H_{10}N_2$) C, H, N.

5.1.6. 3,5-Diaminobenzonitrile ***42***

A suspension of 3,5-dinitrobenzonitrile **41** (5.00 g, 25.9 mmol), iron filings (15 g), and ferrous sulphate (1.67 g) in H_2O (80 mL) was stirred and heated at reflux for 2 h. The suspension was cooled to room temperature and then the suspension was filtered. The solids were suspended in MeOH (200 mL) at room temperature overnight and then filtered. The precipitate was washed with additional portions of hot MeOH (3 x 100 mL). The combined methanolic filtrates were evaporated to yield 2.33 g (68%) of **42** as a beige crystalline solid: m.p. 188–192 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 6.07 (s, 3 H), 5.24 (s, 4 H); IR (nujol) 2224 cm^{-1}. Anal. ($C_7H_7N_3$) C, H, N.

5.1.7. 3,5-Bis(dimethylamino)benzonitrile ***43***

A mixture of 3,5-diaminobenzonitrile **42** (7.00 g, 53 mmol), 37.9% aqueous formaldehyde (63 mL, 15 equiv.) in CH_3CN was treated with $NaCNBH_3$ (17.53 g, 5 equiv.) in two portions over the course of 10 min. Sufficient glacial acetic acid was added to adjust the pH to 7 (litmus paper). The resulting reaction mixture was stirred at room temperature for 2 h and then evaporated to ~150 mL. The concentrate was taken up in 1 N KOH solution (200 mL) and and this mixture was extracted with Et_2O (2 x 300 mL). The Et_2O layers were combined, dried over K_2CO_3, and evaporated to an oil. The oil was chromatographed on silica gel eluting with hexanes/EtOAc 9:1 to afford 9.31 g (93%) of **43** as a solid: m.p. 75–78 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 6.37 (s, 2 H), 6.13 (s, 1 H), 2.91 (s, 12 H); IR (CH_2Cl_2) 2227.9 cm^{-1}. Anal. ($C_{11}H_{15}N_3$) C, H, N.

5.1.8. 3,5-Bis(1H-benzotriazol-1ylmethylamino)benzonitrile ***44***

A suspension of **42** (1.04 g, 7.8 mmol) in EtOH (50 mL) was treated with benzotriazole (1.92 g, 2.0 equiv.) and then 37.9% formaldehyde (1.33 g, 2.0 equiv.). The resulting suspension was stirred at room temperature for 17 h and then filtered to collect 2.53 g (82%) of **44** as an off-white solid: m.p. 190–193 °C; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.05 (m, 4 H, Ph *H*), 7.64–7.36 (m, 6 H), 6.55 (s, 3 H), 6.08 (d, *J* = 7.1 Hz, 4 H); IR (NaCl) 2225 cm^{-1} (CN). Anal. ($C_{21}H_{17}N_9$) C, H, N.

5.1.9. 3,5-Bis(methylamino)benzonitrile ***45***

A suspension of **44** (22.73 g, 57.5 mmol) in THF (500 mL) was treated with $NaBH_4$ (8.88 g, 4 equiv.) in two portions over the course of 10 min. The resulting suspension was stirred at room temperature under N_2 for 2 days and then concentrated in vacuo to a gum. The gum was taken up in H_2O (200 mL, Caution, significant foaming occurred!) and this mixture was extracted with Et_2O (1 x 300 mL). The aqueous layer was reextracted with CH_2Cl_2 (1 x 200 mL). The organic layers were combined, dried over K_2CO_3, and evaporated to a yellow solid. The solid was chromatographed on silica gel eluting with CH_2Cl_2 to provide 6.31 g (68%) of **45** as a yellow solid: m.p. 128.5–130 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 6.10 (d, *J* = 2.2 Hz, 2 H, Ph H), 5.96 (t, *J* = 2.0 Hz, 1 H, Ph H), 5.86 (q, *J* = 4.5 Hz, 2 H, $NHCH_3$), 2.65 (d, *J* = 5.0 Hz, 6 H, CH_3); IR (NaCl) 2221.1 cm^{-1} (CN). Anal. ($C_9H_{11}N_3$) C, H, N.

5.1.10. 3-Dimethylamino-5-methylaminobenzonitrile ***46***

A solution of **45** (2.40 g, 14.9 mmol) in CH_3CN (50 mL) was treated with 37.9% paraformaldehyde solution (1.1 mL, 1.0 equiv.) followed by $NaCNBH_3$ (1.48 g, 1.5 equiv.). The pH of the resulting suspension was adjusted to pH = 5 by the dropwise addition of glacial acetic acid. The resulting suspension was stirred at 20–30 °C for 3 h and then evaporated. The concentrate was taken up in 1 N NaOH solution (50 mL) and this mixture was extracted with Et_2O (2 x 75 mL). The Et_2O

layers were combined, dried over K_2CO_3, and evaporated to an oil. The oil was chromatographed on silica gel eluting with hexanes/EtOAc 9:1, then 5:1, and finally, 1:1 to afford 1.14 g (44%) of **46** as a yellow solid: m.p. 54.5–57.5 °C; ^{1}H-NMR (200 MHz, DMSO-d_6) δ 6.30 (s, 1 H, Ph *H*), 6.20 (s, 1 H, Ph *H*), 6.09 (t, *J* = 2.0 Hz, 1 H, Ph *H*), 5.91 (q, *J* = 4.9 Hz, 1 H, *H*NCH$_3$), 2.89 (s, 6 H, C*H*$_3$NC*H*$_3$), 2.68 (d, *J* = 5.1 Hz, 3 H, C*H*$_3$NH); IR (NaCl) 2205 cm^{-1} (CN). Anal. ($C_{10}H_{13}N_3$) C, H, N.

5.1.11. 3,5-Di-isopropylbenzonitrile ***48***

A suspension of 3,5-diisopropyl bromobenzene **47** [9] (1.2 g, 5.0 mmol) and CuCN (0.52 g, 5.75 mmol) in dry DMF (1.50 mL) was refluxed for 6 h. After cooling to 70–90 °C, the brown suspension was poured into a well stirred solution of ethylenediamine (4 mL) in H_2O (12 mL). The dark blue solution was extracted with CH_2Cl_2 (2 x 50 mL). The combined organic solution was successively washed with 10% aqueous NaCN (20 mL) and H_2O (2 x 50 mL). The resulting organic layer was dried, filtered, and evaporated to afford **48** (0.90 g, 96%) as a brown liquid that was used in the next step without any further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.32 (s, 2 H, Ph H-2 and Ph H-6), 7.29 (s, 1 H, Ph H-4), 2.92 (m, 2 H, C*H*(CH$_3$)$_2$), 1.25 (d, *J* = 7.5 Hz, 12 H, CH(C*H*$_3$)$_2$); CIMS *m/z* 188 (M + 1)$^+$.

5.1.12. 3,5-Di-(1-hydroxypropyl)-bromobenzene ***50***

To a well-stirred solution of 3.0 M EtMgBr in Et_2O (13.2 mL, 40 mmol) at 0 °C, was slowly added a solution of 5-bromoisophthalaldehyde **49** [11] (2.13 g, 10 mmol) in dry THF (16 mL). The green slurry was refluxed for 2 h. After cooling to room temperature, the heavy dark suspension was poured into a cold (0–5 °C) stirred, saturated solution of NH_4Cl. After stirring for additional 10 min at room temperature, the organic layer was separated and the aqueous phase washed with Et_2O (2 x 100 mL). The combined organic solution was dried, filtered and evaporated to afford **50** (2.8 g, 99%) as a yellow syrup that solidifies upon standing. An analytically pure sample was obtained as a mixture of stereoisomers after purification by flash column chromatography (hexanes/EtOAc 2:1): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.20–7.40 (m, 3 H, Ph H), 4.56 (m, 2 H, C*H*(OH)CH$_2$CH$_3$), 2.18 (br s, 2 H, OH), 1.78 (m, 4 H, CH(OH)C*H*$_2$CH$_3$), 0.89 (m, 6 H, CH(OH)CH$_2$C*H*$_3$); CIMS *m/z* 273 (M + 1)$^+$ and 275 (M + 3)$^+$. Anal. ($C_{12}H_{17}BrO_2$) C, H, Br.

5.1.13. 3,5-Di-(1-propenyl)-bromobenzene ***51***

A suspension of **50** (1.4 g, 5 mmol), in 85% H_3PO_4 (20 mL) was refluxed for 45 min. After cooling to room temperature, it was diluted by slow addition of H_2O (50 mL) and then extracted with CH_2Cl_2 (3 x 40 mL). The organic solution was washed with H_2O (2 x 50 mL), dried, filtered and evaporated to afford **51** (1.10 g, 92%) as a syrup. An analytically pure sample was obtained after purification by flash column chromatography (hexanes): ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.88 (m, 6 H, C*H*$_3$), 6.25 (m, 4 H, vinyl-H), 7.15–7.30 (m, 3 H, Ar H); CIMS *m/z* 237 (M + 1)$^+$ and 239 (M + 3)$^+$. Anal. ($C_{12}H_{13}Br$) C, H, Br.

5.1.14. 3,5-Di-(1-propenyl)-benzonitrile ***52***

Compound **52** was obtained in 84% yield from **51** following the same procedure previously described for the synthesis of **48**. An analytically pure sample was obtained after purification by flash column chromatography (hexanes/EtOAc/95:5). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.20–7.40 (m, 3 H, Ph H), 6.18 (m, 4 H, vinyl-H), 1.90 (m, 6 H, C*H*$_3$); CIMS *m/z* 184 (M + 1)$^+$. Anal ($C_{13}H_{13}N$) C, H, N.

5.1.15. 2-Bromo-4,6-diethyldiazonium hexafluorophosphorate ***54***

A suspension of 2-bromo-4,6-diethylaniline **53** [12] (6.68 g, 29.3 mmol), (c) HCl (14.5 mL), and H_2O (55 mL) was treated with a solution of $NaNO_2$ (2.92 g, 42.3 mmol) in H_2O (7 mL) dropwise at –7 °C over the course of 10 min. After the addition was complete, the mixture was allowed to stir at –5 °C for and additional 20 min. HPF_6 (60% wt solution, 8.5 mL, 35.2 mmol) was then added in one portion to the cold mixture. The resulting suspension was allowed to warm to room temperature, and then a light pink solid was collected by filtration and washed with H_2O and EtOH. The solid was dried overnight in vacuo (oil pump, 25 °C) over (c) H_2SO_4 to yield **54** (9.70 g, 86%) as an off-white solid: m.p. 158–162 °C (dec.). Anal. ($C_{10}H_{12}N_2PF_6Br$) C, H, N, Br.

5.1.16. 1-Bromo-3,5-diethyl-2-fluorobenzene ***55***

A suspension of **54** (8.39 g, 21.8 mmol) in *p*-xylene (75 mL) was heated at reflux under a nitrogen atmosphere for 3 h. The volume of the solution was reduced to 1/2 by distillation in vacuo in the fume hood (to prevent exposure to the poisonous PF_5 (g). Complete distillation of the xylene was carried by rotary evaporation in vacuo to give a brown liquid. The liquid was purified by flash column chromatography (hexanes) to provide **55** (3.70 g) as a colorless liquid. It was not possible to determine the ratio of the desired compound to the contaminating 1-bromo-3,5-diethylbenzene **56** from the ^{1}H-NMR spectrum or GC chromatogram. The liquid was used in the next step without further purification.

5.1.17. 3,5-Diethyl-2-fluorobenzonitrile ***57***

A suspension of **55** (3.53 g, 15.3 mmol), CuCN (1.37 g, 15.3 mmol) and DMF (8 mL) was heated at reflux under a nitrogen atmosphere for 4 h. The reaction mixture was poured into a solution of ethylene diamine/H_2O 1:2 (60 mL), and the resulting mixture was extracted with CH_2Cl_2 (2 x 60 mL). The CH_2Cl_2 layers were combined, washed with 10% aqueous NaCN solution (1 x 60 mL) and H_2O (1 x 60 mL), dried and evaporated to a yellow oil. The oil was purified by flash column chromatography (hexanes/CH_2Cl_2 1:1) to yield a yellow oil. This oil was further chromatographed (hexanes/toluene 4:1) to provide **57** (1.16 g, 43%) as a colorless liquid: ^{1}H-NMR (300 MHz, DMSO-d_6) d 7.26 (m, 2 H, Ph H), 2.66 (m, 4 H, C*H*$_2$CH$_3$), 1.23 (m 6 H, CH$_2$C*H*$_3$). Anal. ($C_{11}H_{12}NF$) C, H, N.

5.1.18. 4-Bromo-2,6-diethyldiazonium hexafluorophosphorate ***60***

A suspension of 4-bromo-2,6-diethylaniline **59** [9] (5.00 g, 21.9 mmol), conc. HCl (12 mL), and H_2O (41 mL) was treated with a solution of $NaNO_2$ (2.03 g, 29.4 mmol) in H_2O (6 mL) dropwise at –7 °C over the course of 15 min. After the addition was complete, the mixture was allowed to stir at –5 °C for and additional 30 min. HPF_6 (60% wt solution, 6.4 g, 26.3 mmol) was then added in one portion to the cold mixture. The resulting suspension was allowed to warm to room temperature, and then a solid was collected by filtration and washed with H_2O and EtOH. The solid was dried overnight in vacuo (oil pump, 25 °C) over (c) H_2SO_4 to yield **60** (7.40 g, 88%) as a light purple solid, which slowly decomposed upon standing at room temperature: m.p. 115–120 °C (dec.).

5.1.19. 1-Bromo-3,5-diethyl-4-fluorobenzene ***61***

A suspension of **60** (12.00 g, 31.2 mmol) in *p*-xylene (100 mL) was slowly heated to 115 °C under a nitrogen atmosphere and then maintained at this temperature for an additional 30 min. The xylenes were removed by distillation in vacuo (water aspirator) in the fume hood (to prevent exposure to any

residual poisonous PF_5 (g)). The concentrate was purified by flash column chromatography (hexanes) to provide **61** (6.53 g, 91%) as a colorless liquid: ^{1}H-NMR (200 MHz, DMSO-d_6) δ 7.15 (br s, J = 6.2 Hz, 2 H, Ph H), 2.63 (q, J = 7.2 Hz, 4 H, CH_2CH_3), 1.21 (t, J = 7.6 Hz, 6 H, CH_2CH_3). The liquid was used in the next step without further purification.

5.1.20. 3,5-Diethyl-4-fluorobenzonitrile ***62***

A suspension of **61** (3.00 g, 13.0 mmol) and CuCN (1.34 g, 15.0 mmol) in DMF (6 mL) was heated at reflux under a nitrogen atmosphere for 5 h. The reaction mixture was then poured into a solution of ethylene diamine/H_2O 1:2 (50 mL). This mixture was extracted with CH_2Cl_2 (2 x 50 mL). The CH_2Cl_2 layers were combined and washed with 10% NaCN solution (1 x 65 mL) then H_2O (1 x 50 mL). The CH_2Cl_2 layer was dried and then evaporated to an amber oil. The oil was purified by flash column chromatography (hexanes/CH_2Cl_2 1:1) to give **62** (1.87 g, 81%) as a colorless oil: ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.67 (br s, J = 6.6 Hz, 2 H, Ph H), 2.63 (q, J = 7.1 Hz, 4 H, CH_2CH_3), 1.15 (t, J = 7.6 Hz, 6 H, CH_2CH_3). Anal. ($C_{11}H_{12}NF$) C, H, N.

5.1.21. 3,5-Di-tert-butylbromobenzene ***64***

A solution of 3,5-di-*tert*-butyl toluene **63** (4.56 g, 5.30 mL, 22 mmol), benzoyl peroxide (20 mg, 0.08 mmol) and NBS (3.9 g, 22 mmol) in CCl_4 (12 mL) was refluxed for 4 h. After cooling to room temperature, the solids were filtered and the filtrate was evaporated to afford **64** [14] (6.4 g, 99%) as a colorless syrup. ^{1}H-NMR shows a 5:1:1 mixture of **64**, the dibrominated and starting materials. The crude mixture was used in the next step without any further purification. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.23–7.26 (m, 3 H, Ph H), 4.52 (s, 2 H, $PhCH_2Br$), 1.33 (s, 18 H, $C(CH_3)_3$); CIMS m/z 282 $(M + 1)^+$ and 284 $(M + 3)^+$.

5.1.22. N-(3,5-di-tert-butyl benzyl)phthalimide ***65***

A suspension of **64** (6.0 g, 15 mmol) and potassium phthalimide (3.33 g, 18 mmol) in dry DMF (70 mL) was heated at 70–75 °C in an oil bath for 5 h. The reaction mixture was cooled to room temperature and H_2O (170 mL) was added. A yellow oil separated and was extracted with CH_2Cl_2 (2 x 150 mL). The combined organic solution was dried, filtered, and evaporated to a yellow syrup that was crystallized from MeOH to afford **65** (4.3 g, 82%) as a white solid. An analytically pure sample was obtained by recrystallization from MeOH: mp 141–143 °C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.84 (q, J = 2.0 Hz and J = 5.5 Hz, 2 H, Phth-H), 1.31 (s, 18 H, $C(CH_3)_3$), 7.69 (q, J = 2.0 Hz and J = 5.5 Hz, 2 H, Phth-H), 7.35 (s, 3 H, Ph H), 4.83 (s, 2 H, $PhCH_2N$); CIMS m/z 350 $(M + 1)^+$. Anal. ($C_{23}H_{27}NO_2$) C, H, N.

5.1.23. 3,5-Di-tert-butylbenzylamine ***29***

A suspension of **65** (3.5 g, 10 mmol) and hydrazine monohydrate (2 mL) in EtOH (65 mL) was refluxed for 15 min. A white gelatinous precipitate was formed. The suspension was cooled to room temperature and HCl (c) (5 mL) was added. The suspension was refluxed for additional 15 min. After cooling to room temperature, H_2O (70 mL) was added and the precipitate filtered and washed with H_2O. Most of the EtOH from the filtrate was evaporated with vacuum. The white precipitate was filtered and dried with vacuum at 60 °C to afford the HCl salt of **29** (2.0 g, 80%). This material was used in the next step without any further purification. An analytically pure sample was obtained by crystallizing from EtOH–Et_2O: m.p. 284–287 °C; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.59 (br s, 3 H, $PhCH_2NH_3$), 7.37 (s, 1 H, Ph H-4), 7.31 (s, 2 H, Ph H-2 and Ph H-6), 4.02 (s, 2 H, $PhCH_2NH_3$), 1.29 (s, 18 H, $C(CH_3)_3$); CIMS m/z 220 (M + 1 free base)$^+$. Anal. ($C_{15}H_{25}N$•HCl) C, H, N, Cl.

5.2. Pharmacological evaluation

Geller and Seifter designed the first operant procedure to be widely applied as a screening method for putative anxiolytics [3]. Food-deprived rats were trained to press a lever for sweetened milk on a multiple schedule of reinforcement in daily sessions. During parts of the session, a tone discriminative stimulus signaled that each lever press would produce milk but would be punished by a foot shock, which reduced lever presses during the tone. Rapid-onset anxiolytics such as barbiturates and benzodiazepines reversed the punishment-induced reduction in lever presses. Pollard and Howard [4] modified the basic Geller–Seifter design by using an incremental rather than constant-level shock (along with a light discriminative stimulus and a food pellet reward), which is the version employed in this study. Specifically, ovariectomized Long–Evans rats from Charles River Laboratories (Raleigh, NC) were trained to press a lever for 45 mg pellets in daily 1 h sessions 6 days a week (Sunday–Friday) in a Coulbourn operant chamber. They were given 1 h access to food post-session and 2 h on Saturday. A daily session consisted of four periods of reinforcement on a variable-interval 2 min schedule (a pellet was delivered for a lever-press that occurred on the average 2 min after the previous pellet) and four 3-min periods of reinforcement on a fixed-ratio 1 schedule (a pellet was delivered for every lever press). Under fixed ratio 1, a cue light was illuminated, and each lever-press in the 3-min period was accompanied by a 500 msec 60 Hz foot-shock that began at 0.00 mA and increased by 0.05 mA with each press (the 'conflict' or 'punishment' period). Mondays and Thursdays were baseline days; Tuesdays and Fridays were compound test days, on which compound was administered by gavage (p.o.) at 1, 2, or 4 h before session. Before the experimental compounds were tested, the rats had had extensive experience with the behavioral task and had received repeated tests with the standard positive control benzodiazepine chlorodiazepoxide to ensure that they consistently showed increased punished lever-pressing under the influence of a standard anxiolytic.

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