Synthesis and Adenosine Receptor Affinity of a Series of Pyrazolo[3,4-*d*]pyrimidine Analogues of 1-Methylisoguanosine

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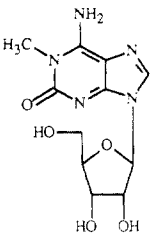
Pyrazolo[3,4-*d*]pyrimidines are pyrazolo analogues of purines. They have been shown to be a general class of compounds which exhibit A1 adenosine receptor affinity. Two series of pyrazolo[3,4-*d*]pyrimidine analogues of 1-methylisoguanosine have been synthesized. The first involved substitution of the N1-position while the second involved substitution of the N5-position. Both alkyl and aryl substituents were examined. All compounds were tested for A_1 adenosine receptor affinity by using a (*R*)-[^{3}H]-N^6-(phenylisopropyl)adenosine binding assay. The 3-chlorophenyl group showed the greatest activity in the N1-position and the butyl group produced the greatest activity in the N5-position. Combination of the best substituent in each of these positions enhanced the overall activity. The most potent compound was 4-amino-5-*N*-butyl-1-(3-chlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(5*H*)-one with an IC_{50} of 6.4×10^{-6} M. Selectivity at the receptor subclasses was examined by performing an A_2 adenosine receptor affinity assay with [^{3}H]CGS 21680. This series of compounds were slightly less potent at A_2 receptors. 4-Amino-5-*N*-butyl-1-(3-chlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(5*H*)-one was the most potent compound with an IC_{50} of 19.2×10^{-6} M.

1-Methylisoguanosine (1) is a pharmacologically active nucleoside that has been reported to occur in marine animals. We have reported its occurrence in the sponge *Tedania digitata*,[1,2] while it has also been reported to occur in the nudibranch *Anisidoris nobilis*[3] and the coral *Madracis mirabilis*.[4] This compound has potent muscle-relaxant activity,[5] lowers blood pressure,[5] and has antiinflammatory activity.[6] 1-Methylisoguanosine was originally prepared by the reaction of 5-amino-4-cyano-1β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)imidazole with methyl isocyanate to give 4-cyano-5-(3-methyl-1-uriedo)-1β-(2′,3′,5′-tri-*O*-acetyl-D-ribofuranosyl)imidazole which underwent cyclization and deprotection with methanolic ammonia.[1,2] An alternative synthesis involved the condensation of 5-amino-4-carbamoyl-1β-D-ribofuranosylimidazole with methyl isothiocyanate. The initially formed thiourea underwent a novel cyclodesulfurization with DCC to yield the same intermediate urea which was annulated with ethanolic ammonia to give the desired product.[7] A number of analogues of 1-methylisoguanosine have been reported.[4,6,8] A study of a number of 7- and 9-alkyl substituted 1-methylisoguanines were investigated in a benzodiazepine receptor binding assay.[4] In another study, structure–activity relationships were undertaken on a series of analogues with variation of substitution at N1, O2, C8, N9 and conversion of the N^6 amino to a keto analogues.[6] A third study reported the synthesis of 1-ethyl-3-methylisoguanosine in order to provide an analogue which must exist in the 6-imino form for spectroscopic studies.[8]



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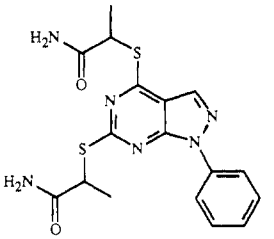

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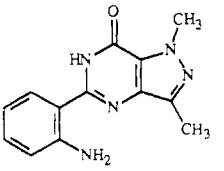

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These previous studies have retained the purine nucleus and examined the effects of substitution. We were interested to further explore the biological properties of this series of compounds by examining the role of the heterocyclic nucleus. Pyrazolo[3,4-*d*]pyrimidines have been shown to be a general class of compounds which exhibit adenosine receptor activity. Investigation of a series of 1-substituted 4,6-bis(alkylthio)-1*H*-pyrazolo[3,4-*d*]pyrimidines showed 4,6-bis[(2-carbamoylethyl)thio]-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (2) to have antagonist activity which was ca. 40 times greater than that of theophylline at the A_1 adenosine receptor.[9] A later investigation of a series of 5-substituted 1,3-dialkyl-1*H*-

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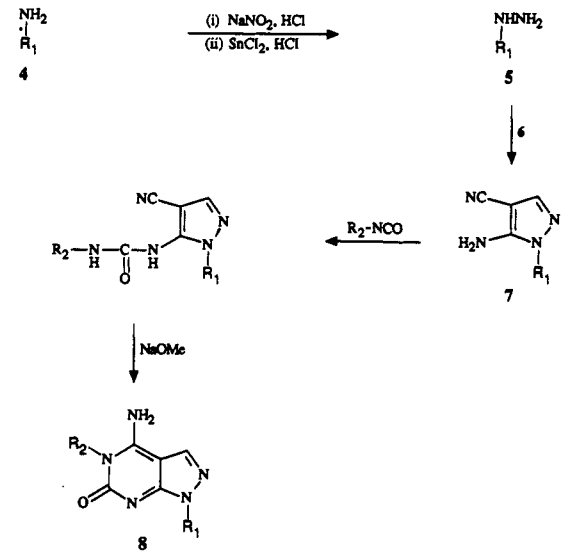
Scheme I



pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-ones showed 5-(2-aminophenyl)-1,3-dimethyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-(6*H*)-one (**3**) to have antagonist activity which was ca. 15 times greater than that of **2** at the A_1 adenosine receptor.[10] Other classes of non-xanthine adenosine antagonists including the 9-methyladenines and 7-deaza-9-phenyladenines have been reported recently.[11,12]

Coupling our interest in further unraveling the role of 1-methylisoguanosine and in investigating the effect of changes in the heterocyclic ring system, we now report the synthesis of several N1- and N5-substituted pyrazolo-[3,4-*d*]pyrimidines. The A_1 adenosine receptor affinity was measured with a (*R*)-[^{3}H]-N^6-(phenylisopropyl)adenosine (*R*[^{3}H]N^6-PIA) binding assay,[13] receptor selectivity was examined with a [^{3}H]CGS 21680 binding assay,[14] and the hydrophobicity index was measured by using a HPLC correlation method.[15]

Results and Discussion

Chemical Results. The general synthetic route to 1*H*-pyrazolo[3,4-*d*]pyrimidines (**8**) has been outlined in Scheme I. The appropriately substituted amine (**4**) was diazotized and reduced to yield a substituted hydrazine (**5**).[16-18] This hydrazine was reacted with (ethoxymethylene)malononitrile (**6**), providing a convenient synthesis of the corresponding 1-substituted 5-amino-4-cyanopyrazole (**7**).[19] An alternative synthesis which involved a one-pot reaction between the hydrazine, malononitrile, and triethyl orthoformate also produced the appropriate pyrazole (**7**).[20] The final and key step was the reaction of this pyrazole with a substituted isocyanate in the presence of base to give the desired 1*H*-pyrazolo-[3,4-*d*]pyrimidine (**8**). Two main series of compounds were synthesized. The first involved the variation of the substituent at the N1-position using different amines while the second involved the variation of the substituent at the N5-position using different isocyanates. Upon completion of this initial study the most biologically active substituent in each of these positions was optimized and combined in an attempt to enhance the overall activity.

The difficult step in this synthesis proved to be the isocyanate annulation. This reaction was optimized with the 5-amino-4-cyano-1-phenylpyrazole (**22**) and methyl isocyanate system. Initially this reaction was attempted by stirring the pyrazole with methyl isocyanate in pyridine for 12 h at 110 °C. This produced a highly insoluble white solid which proved difficult to purify and characterize. The disappearance of the nitrile functionality (at 2220 cm^{-1}) in the infrared spectrum indicated that this compound may be the desired pyrazolo[3,4-*d*]pyrimidine **41**. Further investigation suggested that this compound was a dimeric or polymeric molecule produced by the attack of one pyrazole amine on the nitrile of another. When the reaction was attempted by stirring pyrazole **22** with methyl isocyanate in DMF for 24 h at 80 °C, intermediate urea **40** was isolated. In this case the infrared spectrum showed that the nitrile had been retained and the ^{1}H NMR spectrum showed two exchangeable one proton singlets at δ 6.53 and 8.76 and a three proton doublet at δ 2.58 which corresponded to the NH and the $NHCH_3$ functionalities. This compound was produced by the attack of the pyrazole amine on the carbonyl moiety of methyl isocyanate. Upon treatment with ethanolic ammonia this compound cyclized to give pyrazolo[3,4-*d*]pyrimidine **41**. The overall yield of this two step process was 66%. The synthesis was achieved in one step by stirring pyrazole **22** and methyl isocyanate in the presence of sodium methoxide in DMF for 12 h at 60 °C. The yield of this one step methodology was 92%. The yield was further enhanced to 98% by stirring the starting materials with *n*-butyllithium in THF for 1 h at −70 °C and for 1 h at room temperature. The methyl isocyanate/sodium methoxide method was used to convert all of the other substituted pyrazoles to the corresponding pyrazolo[3,4-*d*]pyrimidines.

Biological Results. All of the pyrazolo[3,4-*d*]pyrimidines which were synthesized were screened for activity with an (*R*)-[^{3}H]-N^6-(phenylisopropyl)adenosine binding assay.[13] *R*[^{3}H]N^6-PIA has been shown to be a potent agonist in A_1 adenosine responsive cellular systems. The ability of the pyrazolo[3,4-*d*]pyrimidines to compete with *R*[^{3}H]N^6-PIA binding to the adenosine receptor was measured using rat brain membranes. As the *R*[^{3}H]N^6-PIA binding sites appear to be equivalent to A_1 adenosine receptor sites on the cell surface, this gives a measure of the affinity of the compound for the A_1 adenosine receptor.

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Table I. Adenosine Receptor Affinity and Hydrophobicity Index of the Pyrazolo[3,4-*d*]pyrimidines (8)

			R[^{3}H]N^6-PIA[a]		[^{3}H]CGS 21680[a]			
no.	R_1	R_2	% inhibn[c]	IC_{50},[d] μM	% inhibn[e]	IC_{50},[f] μM	A_2/A_1	k'[b]
41	Ph	Me	30 ± 2		7 ± 2			2.6
42	2-ClC_6H_4	Me	7 ± 1		0 ± 0			1.7
43	3-ClC_6H_4	Me	53 ± 5	16.5 ± 4.0	29 ± 4			14.2
44	4-ClC_6H_4	Me	37 ± 3		0 ± 0			13.6
45	2-BrC_6H_4	Me	6 ± 1		0 ± 0			1.8
46	3-BrC_6H_4	Me	40 ± 2		1 ± 2			17.5
47	4-BrC_6H_4	Me	34 ± 3		7 ± 1			18.9
48	2-FC_6H_4	Me	8 ± 1		0 ± 0			1.1
49	3-FC_6H_4	Me	44 ± 4		22 ± 4			5.8
50	4-FC_6H_4	Me	26 ± 2		0 ± 0			4.1
51	2-$NO_2C_6H_4$	Me	5 ± 1		7 ± 0			0.9
52	3-$NO_2C_6H_4$	Me	2 ± 0		1 ± 2			>36.5
53	4-$NO_2C_6H_4$	Me	5 ± 1		0 ± 0			>36.5
54	Me	Me	6 ± 2		0 ± 0			0.1
55	Pr	Me	5 ± 1		0 ± 0			0.8
45	2,4-$Cl_2C_6H_3$	Me	9 ± 3		0 ± 0			7.1
57	2,5-$Cl_2C_6H_3$	Me	1 ± 1		3 ± 3			4.8
58	3,5-$Cl_2C_6H_3$	Me	29 ± 4		0 ± 0			>36.5
59	Ph	Et	58 ± 6	20.1 ± 1.8	16 ± 3	81.8 ± 5.3	4.1	4.2
60	Ph	Pr	52 ± 6	50.7 ± 2.1	7 ± 1	50.2 ± 1.6	1.0	7.5
61	Ph	Bu	72 ± 5	9.6 ± 3.3	21 ± 3	24.8 ± 5.8	2.6	18.5
62	Ph	Ph	25 ± 2		0 ± 0			5.1
63	3-ClC_6H_4	Et	70 ± 5	19.8 ± 7.2	24 ± 4	23.5 ± 3.0	1.2	21.9
64	3-ClC_6H_4	Pr	68 ± 4	14.6 ± 5.0	35 ± 8	47.3 ± 3.0	3.2	>36.5
65	3-ClC_6H_4	Bu	58 ± 5	6.4 ± 0.1	38 ± 1	19.2 ± 1.2	3.0	>36.5
66	2-$NH_2C_6H_4$	Me	0 ± 1		0 ± 0			0.8
67	3-$NH_2C_6H_4$	Me	0 ± 1		0 ± 1			0.7
68	4-$NH_2C_6H_4$	Me	0 ± 0		0 ± 0			0.3

[a] Values represent means (±SEM) from three separate experiments. [b] Hydrophobicity index measured by retention time on a reverse phase HPLC column. [c] Percent inhibition of R[^{3}H]N^6-PIA binding by compounds at 20 μM to rat whole brain membranes. [d] Concentration required to inhibit 50% of R[^{3}H]N^6-PIA binding to rat whole brain membranes. [e] Percent inhibition of [^{3}H]CGS 21680 binding by compounds at 20 μM to rat striatal membranes. [f] Concentration required to inhibit 50% of [^{3}H]CGS 21680 binding to rat striatal membranes.

An initial screen was conducted with the compounds at 20 μM. IC_{50} values have been reported for all compounds which showed greater than 50% inhibition of R[^{3}H]N^6-PIA binding in the initial screen. The results of the biological evaluation are shown in Table I.

The substituted aromatic groups showed greater adenosine receptor affinity than the aliphatic groups in the N1-position. On examination of the compounds which contained monosubstituted aromatic groups, two main trends emerged. Firstly, substitution of the aromatic group with chlorine produced a greater response than bromine, fluorine, nitro, or amine substitution. Secondly, substitution of the aromatic group in the 3-position produced a greater response than 2- or 4-substitution. 3-Chlorophenyl compound **43** was most active with an IC_{50} of 16.5 × 10^{-6} M. For this reason a number of dichlorophenyl-substituted compounds including 3,5-dichlorophenyl compound **58** were synthesized, but the activity was not enhanced. The aliphatic groups showed greater adenosine receptor affinity than the aromatic group in the N5-position. Butyl compound **61** was most active with an IC_{50} of 9.6 × 10^{-6} M. In a previous study a number of N1-substituted isoguanosine analogues, which included methyl, ethyl, and butyl analogues, were analyzed for biological activity.[6] Skeletal muscle relaxant, hypothermic, cardiovascular, antiinflammatory, and antiallergic effects were all measured following oral or intravenous administration to mice. In each case methyl isoguanosine was found to produce the greatest response. In this series the ethyl (**59**), propyl (**60**), and butyl (**61**) substituted compounds were all found to be more active than the methyl-substituted compound (**41**). The most active N1- and N5-substituents were combined in order to optimize the overall activity. The combination of the 3-chlorophenyl group in the N1-position with the ethyl, propyl, or butyl group in the N5-position reduced the IC_{50}.

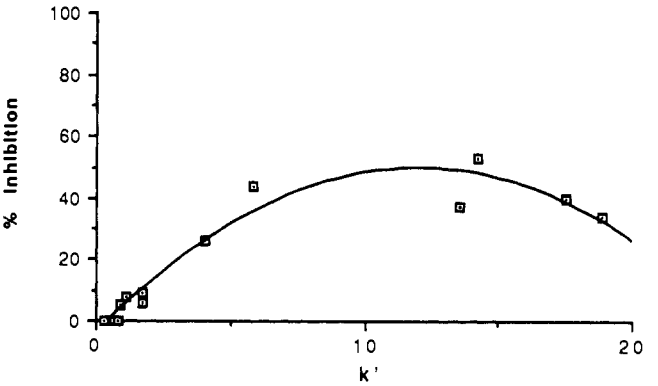

Figure 1. Relationship of the A1 adenosine receptor affinity (percent inhibition) and the hydrophobocity index (k') for the 1-substituted 4-amino-5-*N*-methylpyrazolo[3,4-*d*]pyrimidin-6-(5*H*)-ones (**42–53**).

The hydrophobicity index (k') of this series of pyrazolo[3,4-*d*]pyrimidines was measured by using a simple HPLC correlation method[15] and was used to analyze relationships with adenosine receptor binding. The hydrophobicity index has been previously used to relate protein binding in a series of N^6-alkyladenosine analogues.[21] Comparing the hydrophobicity index against adenosine receptor binding for all of the compounds tested, we failed to determine any relationships. However, when the compounds were broken up into various series, certain trends became apparent. The analogues with a monosubstituted aromatic group in the N1-position and a methyl group in

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the N5-position gave a distinct curve with a maximum biological activity corresponding to a k' value of 14.2 (Figure 1). Two of the compounds in this particular series, **52** and **53**, were found to have k' values greater than 36.5 and are not shown on the graph. The biological activity of these compounds was low, consistent with the downward trend in activity with high hydrophobicity.

CGS 21680 [2-[[*p*-(2-carboxyethyl)phenethyl]amino]-5'-(ethanecarboxamido)adenosine] has been shown to bind to adenosine A_2 receptors with both a high degree of selectivity (>170-fold) and high affinity. It is currently used as the standard radioligand for investigation of high affinity A_2 receptors, as it allows direct labeling of these sites without the need to block A_1 receptor binding.[14] None of the compounds showed greater than 50% inhibition of [^{3}H]CGS 21680 binding at 20 μM. IC_{50} values have been reported for the compounds which showed greater than 50% inhibition of R[^{3}H]N^6-PIA binding in the initial screen. Poor solubility of compound **43** precluded accurate determination of IC_{50}. The results of the biological evaluation are shown in Table I. 4-Amino-5-*N*-butyl-1-(3-chlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(5*H*)-one was the most potent compound at both receptors, with IC_{50s} of 6.4×10^{-6} M at A_1 receptors and 19.2×10^{-6} M at A_2 receptors, and was 3.5-fold A_1 selective.

Experimental Section

Melting points were determined on a Büchi melting point apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker WM-250 spectrometer at 250.1 and 62.8 MHz, respectively. The type of carbon atom was assigned by using the DEPT pulse sequence; q = methyl, t = methylene, d = methine, and s = quaternary. Unless otherwise stated, DMSO-d_6 was used as a solvent and TMS as an internal standard. IR spectra were recorded as KBr disks on a JASCO IR-810 spectrophotometer. (Ethoxymethylene)malononitrile,[22] phenylhydrazine,[16] and propylhydrazine[18] were prepared by literature methods. Ethanol was dried by reflux and distillation over magnesium turnings and a catalytic amount of iodine and was stored over 3-Å molecular sieves. Pyridine was dried by reflux and distillation over potassium hydroxide and sodium wire and was stored over 4-Å molecular sieves. DMF was dried by distillation under reduced pressure and storage over 4-Å molecular sieves. All other solvents were distilled prior to use. Merck silica gel (40–63 μm) was used for chromatography.

Substituted Phenylhydrazine Hydrochlorides (9–20). A suspension of 2-chloroaniline (10.0 g, 78 mmol) in concentrated hydrochloric acid (100 mL) was diazotized with sodium nitrite (5.4 g, 78 mmol) in water (50 mL) and reduced with stannous chloride (35.4 g, 156 mmol) in concentrated hydrochloric acid (35 mL) at 0 °C. The reaction was then stirred for 1 h at room temperature. The precipitate produced was collected and recrystallized from ethanol to give a white solid (**9**).

9: R_1 = 2-ClC_6H_4; yield 82%; mp 200–201 °C dec; ^{1}H NMR δ 6.92–7.41 (m, 4, CH_{arom}), 8.07 (br s, 1, NH), 10.49 (br s, 3, NH); ^{13}C NMR δ 115.0, 119.6, 122.4, 127.7, 129.5, 141.3; IR 2825 cm^{-1}.

10: R_1 = 3-ClC_6H_4; yield 79%; mp 240–243 °C dec; ^{1}H NMR δ 6.90–7.38 (m, 4, CH_{arom}), 8.62 (br s, 1, NH), 10.40 (br s, 3, NH); ^{13}C NMR δ 113.1, 114.0, 120.8, 130.6, 133.5, 147.3; IR 3030 cm^{-1}.

11: R_1 = 4-ClC_6H_4; yield 83%; mp 225–227 °C; ^{1}H NMR δ 6.97–7.34 (m, 4, CH_{arom}), 8.47 (br s, 1, NH), 10.34 (br s, 3, NH); ^{13}C NMR δ 116.2, 125.1, 128.7, 144.6; IR 2960 cm^{-1}.

12: R_1 = 2-BrC_6H_4; recrystallized from propan-2-ol; yield 49%; mp 189–201 °C dec; ^{1}H NMR δ 6.86–7.56 (m, 4, CH_{arom}), 7.90 (br s, 1, NH), 10.44 (br s, 3, NH); ^{13}C NMR δ 109.6, 114.9, 123.0, 128.4, 132.7, 142.4; IR 3000 cm^{-1}.

13: R_1 = 3-BrC_6H_4; recrystallized from propan-2-ol; yield 84%; mp 231–233 °C; ^{1}H NMR δ 6.94–7.25 (m, 4, CH_{arom}), 8.63 (br s, 1, NH), 10.40 (br s, 3, NH); ^{13}C NMR δ 113.5, 116.7, 121.9, 123.8, 130.8, 147.3; IR 3000 cm^{-1}.

14: R_1 = 4-BrC_6H_4; recrystallized from propan-2-ol; yield 63%; mp 209–211 °C; ^{1}H NMR δ 6.92–7.44 (m, 4, CH_{arom}), 8.50 (br s, 1, NH), 10.41 (br s, 3, NH); ^{13}C NMR δ 112.8, 116.6, 131.6, 145.1; IR 3000 cm^{-1}.

15–17: Substituted (fluorophenyl)hydrazines synthesized by this method were used directly in the next synthetic step to prevent decomposition upon standing.

18: R_1 = 2-$NO_2C_6H_4$; yield 31%; mp 194–195 °C; ^{1}H NMR δ 7.03–8.15 (m, 4, CH_{arom}), 9.21 (br s, 1, NH), 10.32 (br s, 3, NH); ^{13}C NMR δ 115.7, 120.3, 126.1, 133.9, 136.2, 141.3; IR 2950, 1522, 1330 cm^{-1}.

19: R_1 = 3-$NO_2C_6H_4$; yield 35%; mp 210–212 °C; ^{1}H NMR δ 7.39–7.65 (m, 4, CH_{arom}), 9.04 (br s, 1, NH), 10.66 (br s, 3, NH); ^{13}C NMR δ 107.9, 115.1, 120.3, 129.9, 146.7, 148.0; IR 3000, 1530, 1340 cm^{-1}.

20: R_1 = 4-$NO_2C_6H_4$; yield 45%; mp 210–212 °C; ^{1}H NMR δ 7.03–8.16 (m, 4, CH_{arom}), 9.58 (br s, 1, NH), 10.80 (br s, 3, NH); ^{13}C NMR δ 112.9, 125.3, 140.2, 151.6; IR 2880, 1518, 1322 cm^{-1}.

1-Substituted 5-Amino-4-cyanopyrazoles (22–39). Method A. Malononitrile (27.5 g, 0.42 mol), triethyl orthoformate (61.7 g, 0.42 mol), and phenylhydrazine (30.0 g, 0.28 mol) were refluxed in dry ethanol (100 mL) during which time the color darkened to brown. The reaction mixture was allowed to cool to room temperature, and the ethanol and unreacted triethyl orthoformate were removed under high vacuum to give a brown solid (37.0 g). The crude product was purified by flash chromatography on silica with methylene chloride as an eluent and recrystallization from ethyl acetate to give a white solid (**22**) (yield 58%).

Method B. (Ethoxymethylene)malononitrile (8.0 g, 66 mmol) and phenylhydrazine (7.1 g, 66 mmol) were refluxed in dry ethanol for 1 h. Upon cooling the crude solid precipitated and was purified by recrystallization from ethanol to give a white solid (**22**) (yield 71%).

22: R_1 = Ph; mp 138–139 °C; ^{1}H NMR δ 6.69 (br s, 2, NH_2), 7.41–7.57 (m, 5, CH_{arom}), 7.80 (s, 1, C_3H); ^{13}C NMR δ 73.5, 114.7, 124.1, 127.8, 129.4, 137.4, 141.6, 151.2; IR 3340, 3220, 3050, 2220 cm^{-1}.

23: R_1 = 2-ClC_6H_4; yield 60%; mp 139–140 °C; ^{1}H NMR δ 6.69 (br s, 2, NH_2), 7.49–7.69 (m, 4, CH_{arom}), 7.77 (s, 1, C_3H); ^{13}C NMR δ 71.7, 115.1, 128.5, 130.6, 131.6, 132.0, 134.5, 142.0, 152.7; IR 3360, 3190, 2220 cm^{-1}.

24: R_1 = 3-ClC_6H_4; yield 67%; mp 186–187 °C; ^{1}H NMR δ 6.66 (br s, 2, NH_2), 7.45–7.57 (m, 4, CH_{arom}), 7.81 (s, 1, C_3H); ^{13}C NMR 73.7, 114.6, 122.9, 124.1, 127.7, 131.2, 133.7, 138.6, 142.2, 151.7; IR 3460, 3320, 3225, 2240 cm^{-1}.

25: R_1 = 4-ClC_6H_4; yield 42%; mp 168.5–170 °C; ^{1}H NMR δ 6.77 (br s, 2, NH_2), 7.50–7.60 (m, 4, CH_{arom}), 7.80 (s, 1, C_3H); ^{13}C NMR δ 73.6, 114.5, 126.0, 129.4, 132.2, 136.4, 142.0, 151.5; IR 3450, 3300, 3190, 2225 cm^{-1}.

26: R_1 = 2-BrC_6H_4; yield 62%; mp 134–135 °C; ^{1}H NMR δ 6.64 (br s, 2, NH_2), 7.44–7.83 (m, 4, CH_{arom}), 7.75 (s, 1, C_3H); ^{13}C NMR δ 71.9, 114.7, 122.1, 128.9, 130.4, 131.6, 133.4, 136.0, 141.6, 152.4; IR 3360, 3190, 2220 cm^{-1}.

27: R_1 = 3-BrC_6H_4; yield 68%; mp 191–192 °C; ^{1}H NMR δ 6.86 (br s, 2, NH_2), 7.43–7.70 (m, 4, CH_{arom}), 7.81 (s, 1, C_3H); ^{13}C NMR δ 73.7, 114.5, 121.9, 123.2, 126.6, 130.7, 131.3, 138.9, 142.2, 151.6; IR 3460, 3300, 2240 cm^{-1}.

28: R_1 = 4-BrC_6H_4; yield 67%; mp 178–180 °C; ^{1}H NMR δ 6.79 (br s, 2, NH_2), 7.44–7.73 (m, 4, CH_{arom}), 7.80 (s, 1, C_3H); ^{13}C NMR δ 73.7, 114.6, 120.7, 126.3, 132.4, 136.8, 142.0, 151.5; IR 3360, 3210, 2220 cm^{-1}.

29: R_1 = 2-FC_6H_4; yield 40%; mp 149–151 °C; ^{1}H NMR δ 6.77 (br s, 2, NH_2), 7.31–7.61 (m, 4, CH_{arom}), 7.78 (s, 1, C_3H); ^{13}C NMR δ 72.1, 114.8, 117.0, 124.8, 125.3, 129.5, 131.5, 142.3, 152.8, 157.0; IR 3370, 3240, 2245 cm^{-1}.

30: R_1 = 3-FC_6H_4; yield 68%; mp 163–165 °C; ^{1}H NMR δ 6.85 (br s, 2, NH_2), 7.23–7.60 (m, 4, CH_{arom}), 7.81 (s, 1, C_3H); ^{13}C NMR δ 73.7, 111.5, 114.7, 114.8, 120.1, 131.2, 138.9, 142.1, 151.6, 162.3; IR 3460, 3310, 3220, 2230 cm^{-1}.

31: R_1 = 4-FC_6H_4; yield 56%; mp 183–185 °C; ^{1}H NMR δ 6.71 (br s, 2, NH_2), 7.31–7.55 (m, 4, CH_{arom}), 7.77 (s, 1, C_3H); ^{13}C NMR δ 73.3, 114.7, 116.3, 126.9, 133.9, 141.7, 151.6, 161.3; IR 3430, 3330, 3240, 2230 cm^{-1}.

32: R_1 = 2-$NO_2C_6H_4$; yield 33%; mp 179–180.5 °C; ^{1}H NMR δ 6.93 (br s, 2, NH_2), 7.69–8.16 (m, 4, CH_{arom}), 7.78 (s, 1, C_3H); ^{13}C NMR δ 72.7, 114.6, 125.5, 129.6, 130.0, 130.6, 134.5, 142.7, 145.7,

(22) Jones, R. G. Reactions of Orthoesters with Active Methylene Compounds. *J. Am. Chem. Soc.* **1952**, *74*, 4889–4891.

152.9; IR 3350, 3190, 2220, 1522, 1340 cm^{-1}.

33: R_1 = 3-$NO_2C_6H_4$; yield 68%; mp 154–156 °C; ^1H NMR δ 7.01 (br s, 2, NH_2), 7.77–8.30 (m, 4, CH_{arom}), 7.87 (s, 1, C_3H); ^{13}C NMR δ 74.0, 114.4, 118.8, 122.3, 130.3, 131.0, 138.3, 142.6, 148.3, 151.9; IR 3350, 3200, 2220, 1535, 1345 cm^{-1}.

34: R_1 = 4-$NO_2C_6H_4$; yield 37%; mp 225–226.5 °C; ^1H NMR δ 7.06 (br s, 2, NH_2), 7.80–8.38 (m, 4, CH_{arom}), 7.91 (s, 1, C_3H); ^{13}C NMR δ 74.5, 114.2, 124.2, 124.9, 142.8, 143.0, 145.9, 152.0; IR 3450, 3320, 2230, 1518, 1350 cm^{-1}.

35: R_1 = Me; yield 78%; mp 222.5–223.5 °C; ^1H NMR δ 3.34 (s, 3, CH_3), 6.54 (br s, 2, NH_2), 7.49 (s, 1, C_3H); ^{13}C NMR δ 34.4, 72.2, 115.2, 139.8, 151.6; IR 3375, 3315, 3140, 2200 cm^{-1}.

36: R_1 = Pr; yield 47%; mp 163–164.5 °C; ^1H NMR δ 0.80 (t, 3, J = 7.4 Hz, CH_3), 1.63 (m, 2, J = 7.1, 7.4 Hz, $CH_3CH_2CH_2$), 3.81 (t, 2, J = 7.1 Hz, $CH_2CH_2CH_3$), 6.55 (br s, 2, NH_2), 7.51 (s, 1, C_3H); ^{13}C NMR δ 10.6, 21.7, 48.0, 72.0, 115.3, 139.9, 151.3; IR 3400, 3330, 3160, 2200 cm^{-1}.

37: R_1 = 2,4-$Cl_2C_6H_3$; yield 64%; mp 141–142 °C; ^1H NMR δ 6.77 (br s, 2, NH_2), 7.52–7.88 (m, 3, CH_{arom}), 7.78 (s, 1, C_3H); ^{13}C NMR δ 71.8, 114.7, 128.6, 130.0, 131.7, 133.2, 133.6, 135.2, 142.1, 152.8; IR 3350, 3200, 2220 cm^{-1}.

38: R_1 = 2,5-$Cl_2C_6H_3$; yield 82%; mp 201–204 °C; ^1H NMR δ 6.81 (br s, 2, NH_2), 7.62–7.73 (m, 3, CH_{arom}), 7.78 (s, 1, C_3H); ^{13}C NMR δ 71.8, 114.8, 130.5, 131.0, 131.5, 131.8, 132.3, 135.6, 142.3, 152.9; IR 3340, 3160, 2230 cm^{-1}.

39: R_1 = 3,5-$Cl_2C_6H_3$; yield 69%; mp 166–168 °C; ^1H NMR δ 6.99 (br s, 2, NH_2), 7.57–7.68 (m, 3, CH_{arom}), 7.83 (s, 1, C_3H); ^{13}C NMR δ 73.9, 114.4, 123.0, 127.4, 134.7, 139.5, 142.6, 151.9; IR 3420, 3340, 3240, 2220 cm^{-1}.

4-Cyano-5-(3-methyl-1-ureido)-1-phenylpyrazole (40). 5-Amino-4-cyano-1-phenylpyrazole (1.0 g, 5.4 mmol) was dissolved in DMF (10 mL). Methyl isocyanate (0.40 mL, 6.5 mmol) was added and the reaction was stirred for 24 h at 80 °C. The solvent was evaporated under vacuum, yielding a yellow oily solid. This solid was washed with ethyl acetate and recrystallized from ethyl acetate and methanol to give a white solid (0.96 g, 73%); mp 255–257 °C; ^1H NMR δ 2.58 (d, 3, J = 4.6 Hz, CH_3), 6.53 (br s, 1, NH), 7.45–7.58 (m, 5, CH_{arom}), 8.17 (s, 1, C_3H), 8.80 (br s, 1, NH); ^{13}C NMR δ 26.4 (q, CH_3), 88.4 (s, C_4), 113.4 (s, CN), 124.4 (d, $C_{2'}$, $C_{6'}$), 128.8 (d, $C_{4'}$), 129.4 (d, $C_{3'}$, $C_{5'}$), 137.5 (s, $C_{1'}$), 142.3 (d, C_3), 142.5 (s, C_5), 154.4 (s, C=O); IR 3325, 2220, 1640 cm^{-1}. Anal. ($C_{12}H_{11}N_5O$) C, H, N.

1-Substituted 4-Amino-5-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(5*H*)-ones (41–58). Method A. 5-(3-Methyl-1-uriedo)-4-cyano-1-phenylpyrazole (0.2 g, 0.8 mmol) and 30% ammonium hydroxide (3 mL) were dissolved in DMF (5 mL) and stirred for 24 h at room temperature. The reaction mixture was neutralized with 1 M hydrochloric acid and the solvent was evaporated under vacuum to give a white solid. The solid was recrystallized from methanol and ethyl acetate to give pure **41** (yield 90%).

Method B. 5-Amino-4-cyano-1-phenylpyrazole (1.0 g, 5.4 mmol) and sodium methoxide (0.59 g, 10.8 mmol) were dissolved in DMF (10 mL). Methyl isocyanate (0.40 mL, 6.5 mmol) was added and the reaction was stirred for 12 h at 60 °C. The reaction mixture was neutralized with 1 M hydrochloric acid and the solvent was evaporated under vacuum to give a white solid. The solid was washed with ethyl acetate, boiled in water, and recrystallized from methanol and ethyl acetate to give pure **41** (yield 92%). Method B was employed to synthesize compounds **42–58**.

Method C. 5-Amino-4-cyano-1-phenylpyrazole (0.50 g, 2.7 mmol) was dissolved in THF (15 mL) and was then cooled to −70 °C on a methanol/dry ice bath. *n*-Butyllithium (2.0 mL, 1.6 M, 6.5 mmol) and methyl isocyanate (0.40 mL, 6.5 mmol) were added, and the reaction was stirred for 1 h at −70 °C and 1 h at room temperature. Water (5 mL) was added to quench the reaction and the solvent was evaporated under vacuum to give a white solid. The solid was washed in ethyl acetate, boiled in water, and recrystallized from methanol and ethyl acetate to give pure **41** (yield 98%).

41: R_1 = Ph, R_2 = Me; mp 308–310 °C; ^1H NMR δ 3.37 (s, 3, CH_3), 7.21–8.16 (m, 5, CH_{arom}), 8.16 (s, 1, C_3H), 8.26 (br s, 1, NH), 8.94 (br s, 1, NH); ^{13}C NMR δ 29.4 (q, CH_3), 93.1 (s, C_{3a}), 119.9 (d, $C_{2'}$, $C_{6'}$), 125.0 (d, $C_{4'}$), 128.7 (d, $C_{3'}$, $C_{5'}$), 135.3 (d, C_3), 139.5 (s, $C_{1'}$), 153.7 (s, C_4), 154.7 (s, C_{7a}), 155.6 (s, C_6); IR 3300, 1640 cm^{-1}. Anal. ($C_{12}H_{11}N_5O$) C, H, N.

42: R_1 = 2-ClC_6H_4, R_2 = Me; recrystallized from DMSO and water; yield 81%; mp 261–262 °C; ^1H NMR δ 3.35 (s, 3, CH_3), 7.46–7.66 (m, 4, CH_{arom}), 8.15 (s, 1, C_3H), 8.26 (br s, 1, NH), 8.99 (br s, 1, NH); ^{13}C NMR δ 29.6 (q, CH_3), 91.2 (s, C_{3a}), 127.8 (d, $C_{6'}$), 130.1 (d, $C_{4'}$), 130.3 (d, $C_{5'}$), 130.5 (d, $C_{3'}$), 131.5 (s, $C_{2'}$), 135.4 (d, C_3), 135.6 (s, $C_{1'}$), 153.4 (s, C_4), 154.4 (s, C_{7a}), 156.7 (s, C_6); IR 3410, 3050, 1690 cm^{-1}. Anal. ($C_{12}H_{10}N_5OCl$) C, H, N.

43: R_1 = 3-ClC_6H_4, R_2 = Me; recrystallized from DMSO and water; yield 67%; mp 296–299 °C; ^1H NMR δ 3.38 (s, 3, CH_3), 7.26–8.36 (m, 4, CH_{arom}), 8.19 (s, 1, C_3H), 8.29 (br s, 1, NH), 9.00 (br s, 1, NH); ^{13}C NMR δ 29.7 (q, CH_3), 92.6 (s, C_{3a}), 117.9 (d, $C_{6'}$), 119.0 (d, $C_{2'}$), 124.8 (d, $C_{4'}$), 130.6 (d, $C_{5'}$), 133.2 (s, $C_{3'}$), 135.7 (d, C_3), 140.6 (s, $C_{1'}$), 153.3 (s, C_4), 154.2 (s, C_{7a}), 156.1 (s, C_6); IR 3340, 2960, 1690 cm^{-1}. Anal. ($C_{12}H_{10}N_5OCl$) C, H, N.

44: R_1 = 4-ClC_6H_4, R_2 = Me; recrystallized from DMSO and water; yield 69%; mp 298–301 °C; ^1H NMR δ 3.37 (s, 3, CH_3), 7.51–8.24 (m, 4, CH_{arom}), 8.18 (s, 1, C_3H), 8.24 (br s, 1, NH), 8.98 (br s, 1 NH), ^{13}C NMR δ 29.7 (q, CH_3), 92.6 (s, C_{3a}), 121.2 (d, $C_{2'}$, $C_{6'}$), 128.8 (d, $C_{3'}$, $C_{5'}$), 129.1 (s, $C_{4'}$), 135.7 (d, C_3), 138.3 (s, $C_{1'}$), 153.3 (s, C_4), 154.2 (s, C_{7a}), 155.8 (s, C_6); IR 3465, 3090, 1685 cm^{-1}. Anal. ($C_{12}H_{10}N_5Cl$) C, H, N.

45: R_1 = 2-BrC_6H_4, R_2 = Me; recrystallized from DMSO and methanol; yield 47%; mp 362–364 °C; ^1H NMR δ 3.33 (s, 3, C_3H), 7.40–7.82 (m, 4, CH_{arom}), 8.12 (s, 1, C_3H), δ 8.23 (br s, 1, NH), 8.90 (br s, 1, NH); ^{13}C NMR δ 29.5 (q, CH_3), 91.1 (s, C_{3a}), 121.8 (s, $C_{2'}$), 128.4 (d, $C_{6'}$), 130.5 (d, $C_{4'}$), 130.8 (d, $C_{5'}$), 133.2 (d, $C_{3'}$), 135.1 (d, C_3), 137.3 (s, $C_{1'}$), 153.4 (s, C_4), 154.3 (s, C_{7a}), 156.6 (s, C_6); IR 3400, 3050, 1685 cm^{-1}. Anal. ($C_{12}H_{10}N_5OBr$), C, H, N.

46: R_1 = 3-BrC_6H_4, R_2 = Me; recrystallized from DMSO and methanol; yield 44%; mp 318–320 °C; ^1H NMR δ 3.37 (s, 3, CH_3), 7.42–8.49 (m, 4, CH_{arom}), 8.19 (s, 1, C_3H), 8.64 (br s, 2, NH_2); ^{13}C NMR δ 29.6 (q, CH_3), 92.7 (s, C_{3a}), 118.3 (d, $C_{6'}$), 121.6 (s, $C_{3'}$), 121.8 (d, $C_{2'}$), 127.7 (d, $C_{4'}$), 130.9 (d, $C_{5'}$), 135.9 (d, C_3), 140.7 (s, $C_{1'}$), 153.4 (s, C_4), 154.2 (s, C_{7a}), 156.0 (s, C_6); IR 3325, 2940, 1690 cm^{-1}. Anal. ($C_{12}H_{10}N_5OBr$) C, H, N.

47: R_1 = 4-BrC_6H_4, R_2 = Me; recrystallized from DMSO and methanol; yield 48%; mp 350–355 °C; ^1H NMR δ 3.37 (s, 3, CH_3), 7.63–8.20 (m, 4, CH_{arom}), 8.18 (s, 1, C_3H), 8.26 (br s, 1, NH), 8.97 (br s, 1, NH); ^{13}C NMR δ 29.6 (q, CH_3), 92.6 (s, C_{3a}), 117.3 (s, $C_{4'}$), 121.5 (d, $C_{2'}$, $C_{6'}$), 131.7 (d, $C_{3'}$, $C_{5'}$), 135.7 (d, C_3), 138.7 (s, $C_{1'}$), 153.3 (s, C_4), 154.1 (s, C_{7a}), 155.9 (s, C_6); IR 3450, 3070, 1680. Anal. ($C_{12}H_{10}N_5OBr$).

48: R_1 = 2-FC_6H_4, R_2 = Me; recrystallized from methanol; yield 64%; mp 334–337 °C; ^1H NMR δ 3.38 (s, 3, CH_3), 7.32–7.59 (m, 4, CH_{arom}), 8.36 (s, 1, C_3H), 8.76 (br s, 1, NH), 9.63 (br s, 1, NH); ^{13}C NMR δ 29.9 (q, CH_3), 92.1 (s, C_{3a}), 116.7 (d, $C_{3'}$), 124.8 (d, $C_{6'}$), 125.1 (s, $C_{1'}$), 128.8 (d, $C_{5'}$), 130.7 (d, $C_{4'}$), 136.5 (d, C_3), 152.4 (s, C_4), 152.7 (s, C_{7a}), 153.5 (s, C_6), 156.5 (s, $C_{2'}$); IR 3320, 3145, 1735 cm^{-1}. Anal. ($C_{12}H_{10}N_5OF$) C, H, N.

49: R_1 = 3-FC_6H_4, R_2 = Me; recrystallized from methanol; yield 61%; mp 306–307.5 °C; ^1H NMR δ 3.38 (s, 3, CH_3), 7.03–8.15 (m, 4, CH_{arom}), 8.19 (s, 1, C_3H), 8.29 (br s, 1, NH), 9.01 (br s, 1, NH); ^{13}C NMR δ 29.7 (q, CH_3), 92.7 (s, C_{3a}), 106.5 (d, $C_{2'}$), 111.6 (d, $C_{4'}$), 115.3 (d, $C_{6'}$), 130.7 (d, $C_{5'}$), 135.8 (d, C_3), 140.8 (s, $C_{1'}$), 153.3 (s, C_4), 154.2 (s, C_{7a}), 156.1 (s, C_6), 162.2 (s, $C_{3'}$); IR 3485, 2920, 1695 cm^{-1}. Anal. ($C_{12}H_{10}N_5OF$) C, H, N.

50: R_1 = 4-FC_6H_4, R_2 = Me; recrystallized from methanol; yield 59%; mp 291–292 °C; ^1H NMR δ 3.37 (s, 3, CH_3), 7.27–8.20 (m, 4, CH_{arom}), 8.16 (s, 1, C_3H), 8.29 (br s, 1, NH), 8.93 (br s, 1, NH); ^{13}C NMR δ 29.6 (q, CH_3), 92.4 (s, C_{3a}), 115.5 (d, $C_{3'}$, $C_{5'}$), 121.8 (d, $C_{2'}$, $C_{6'}$), 135.3 (d, C_3), 135.8 (s, $C_{1'}$), 153.3 (s, C_4), 154.2 (s, C_{7a}), 155.5 (s, C_6), 159.5 (s, $C_{4'}$); IR 3475, 2920, 1680 cm^{-1}. Anal. ($C_{12}H_{10}N_5OF$) C, H, N.

51: R_1 = 2-$NO_2C_6H_4$, R_2 = Me; recrystallized from DMSO and water; yield 46%; mp 344–346 °C; ^1H NMR δ 3.34 (s, 3, CH_3), 7.60–8.07 (m, 4, CH_{arom}), 8.16 (s, 1, C_3H), 8.35 (br s, 1, NH), 9.02 (br s, 1, NH); ^{13}C NMR δ 29.6 (q, CH_3), 91.5 (s, C_{3a}), 124.9 (d, $C_{6'}$), 127.7 (d, $C_{3'}$), 128.4 (d, $C_{4'}$), 130.5 (s, $C_{1'}$), 133.5 (d, $C_{5'}$), 136.4 (d, C_3), 144.2 (s, $C_{2'}$), 153.4 (s, C_4), 154.1 (s, C_{7a}), 156.2 (s, C_6); IR 3455, 3060, 1700, 1540, 1370 cm^{-1}. Anal. ($C_{12}H_{10}N_6O_3$) C, H, N.

52: R_1 = 3-$NO_2C_6H_4$, R_2 = Me; recrystallized from DMSO; yield 40%; mp 396–398 °C; ^1H NMR δ 3.38 (s, 3, CH_3), 7.75–9.12 (m, 4, CH_{arom}), 8.25 (s, 1, C_3H), 8.35 (br s, 1, NH), 9.08 (br s, 1, NH); ^{13}C NMR δ 29.7 (q, CH_3), 92.7 (s, C_{3a}), 113.6 (d, $C_{2'}$), 119.4 (d, $C_{4'}$), 125.2 (d, $C_{6'}$), 130.5 (d, $C_{5'}$), 136.5 (d, C_3), 140.2 (s, $C_{1'}$), 146.2 (s, $C_{3'}$), 153.4 (s, C_4), 154.2 (s, C_{7a}), 156.5 (s, C_6); IR 3440, 3100, 1700,

1560, 1325 cm^{-1}. Anal. ($C_{12}H_{10}N_6O_3$) C, H, N.

53: R_1 = 4-$NO_2C_6H_4$, R_2 = Me; recrystallized from DMSO; yield 49%; mp 340–366 °C; ^{1}H NMR δ 3.37 (s, 3, CH_3), 8.27 (s, 1, C_3H), 8.36–8.58 (m, 4, CH_{arom}), 8.36 (br s, 1, NH), 8.96 (br s, 1, NH); IR 3445, 3100, 1685, 1560, 1350. Anal. ($C_{12}H_{10}N_6O_3$) C, H, N.

54: R_1 = Me, R_2 = Me; recrystallized from water; yield 72%; mp 326–328 °C; ^{1}H NMR δ 3.32 (s, 3, CH_3), 3.57 (s, 3, CH_3), 7.87 (s, 1, C_3H), 8.10 (br s, 1, NH), 8.76 (br s, 1, NH); ^{13}C NMR δ 29.4 (q, CH_3), 32.4 (q, CH_3), 91.1 (s, C_{3a}), 133.1 (d, C_3), 153.2 (s, C_4), 154.0 (s, C_{7a}), 155.1 (s, C_6); IR 3410, 3100, 1700 cm^{-1}. Anal. ($C_7H_9N_5O$) C, H, N.

55: R_1 = Pr, R_2 = Me; recrystallized from methanol; yield 74%; mp 253–256 °C; ^{1}H NMR δ 0.78 (t, 3, J = 7.4 Hz, CH_3), 1.69 (m, 2, J = 7.0, 7.4 Hz, $CH_2CH_2CH_3$), 3.32 (s, 3, CH_3), 3.90 (t, 2, J = 7.0 Hz, $CH_2CH_2CH_3$), 7.97 (s, 1, C_3H), 8.41 (br s, 2, NH_2); ^{13}C NMR δ 10.8 (q, CH_3), 22.0 (t, $CH_2CH_2CH_3$), 29.5 (q, CH_3), 46.6 (t, $CH_2CH_2CH_3$), 91.1 (s, C_{3a}), 133.4 (d, C_3), 153.2 (s, C_4), 154.2 (s, C_{7a}), 155.0 (s, C_6); IR 3410, 2970, 1640 cm^{-1}. Anal. ($C_9H_{13}N_5O$) C, H, N.

56: R_1 = 2,4-$Cl_2C_6H_3$, R_2 = Me; yield 73%; mp 266.5–267.5 °C; ^{1}H NMR δ 3.34 (s, 3, CH_3), 7.50–7.86 (m, 3, CH_{arom}), 8.15 (s, 1, C_3H), 8.37 (br s, 1, NH), 8.87 (br s, 1, NH); ^{13}C NMR δ 29.6 (q, CH_3), 91.1 (s, C_{3a}), 128.0 (d, $C_{6'}$), 129.7 (d, $C_{5'}$), 131.3 (d, $C_{3'}$), 132.5 (s, $C_{2'}$), 134.0 (s, $C_{4'}$), 134.7 (s, $C_{1'}$), 136.0 (d, C_3), 153.4 (s, C_4), 154.3 (s, C_{7a}), 156.8 (s, C_6); IR 3310, 2940, 1700 cm^{-1}. Anal. ($C_{12}H_9N_5OCl_2$) C, H, N.

57: R_1 = 2,5-$Cl_2C_6H_3$, R_2 = Me; yield 77%; mp 373–375 °C; ^{1}H NMR δ 3.34 (s, 3, CH_3), 7.59–7.73 (m, 3, CH_{arom}), 8.15 (s, 1, C_3H), 8.38 (br s, 1, NH), 8.85 (br s, 1, NH); ^{13}C NMR δ 29.5 (q, CH_3), 91.0 (s, C_{3a}), 129.8 (d, $C_{6'}$), 130.2 (s, $C_{2'}$), 130.2 (d, $C_{4'}$), 131.6 (d, $C_{3'}$), 131.7 (s, $C_{5'}$), 135.7 (d, C_3), 136.6 (s, $C_{1'}$), 153.4 (s, C_4), 154.2 (s, C_{7a}), 156.9 (s, C_6); IR 3250, 2960, 1700 cm^{-1}. Anal. ($C_{12}H_9N_5OCl_2$), C, H, N.

58: R_1 = 3,5-$Cl_2C_6H_3$, R_2 = Me; recrystallized from DMSO and methanol; yield 65%; mp 354–356 °C; ^{1}H NMR δ 3.34 (s, 3, CH_3), 7.48 (t, 1, J = 1.9 Hz, $C_{4'}H$), 8.21 (s, 1, C_3H), 8.32 (d, 2, J = 1.9 Hz, $C_{2'}H$, $C_{6'}H$), 8.43 (br s, 1, NH), 9.02 (br s, 1, NH); ^{13}C NMR δ 29.7 (s, CH_3), 92.7 (s, C_{3a}), 117.3 (d, $C_{2'}$, $C_{6'}$), 124.2 (d, $C_{4'}$), 134.4 (s, $C_{3'}$, $C_{5'}$), 136.5 (d, C_3), 141.2 (s, $C_{1'}$), 153.3 (s, C_4), 154.1 (s, C_{7a}), 156.5 (s, C_6); IR 3350, 2960, 1685 cm^{-1}. Anal. ($C_{12}H_9N_5OCl_2$) C, H, N.

5-*N*-Substituted 4-Amino-1-aryl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(5*H*)-ones (59–65). The appropriately substituted isocyanate (ethyl, propyl, butyl, or phenyl isocyanate) was used as in method B for the 1-substituted compounds.

59: R_1 = Ph, R_2 = Et; recrystallized from DMSO and methanol; yield 65%; mp 306–308 °C; ^{1}H NMR δ 1.14 (t, 3, J = 7.0 Hz, CH_3), 4.00 (q, 2, J = 7.0 Hz, CH_2), 7.19–8.16 (m, 5, CH_{arom}), 8.19 (s, 1, C_3H), 8.38 (br s, 1, NH), 8.87 (br s, 1, NH); ^{13}C NMR δ 12.3 (q, CH_3), 37.1 (t, CH_2), 92.6 (s, C_{3a}), 120.0 (d, $C_{2'}$, $C_{6'}$), 125.2 (d, $C_{4'}$), 128.8 (d, $C_{3'}$, $C_{5'}$), 135.4 (d, C_3), 139.3 (s, $C_{1'}$), 152.6 (s, C_4), 153.9 (s, C_{7a}), 155.7 (s, C_6); IR 3400, 2930, 1680 cm^{-1}. Anal. ($C_{13}H_{13}N_5O$) C, H, N.

60: R_1 = Ph, R_2 = Pr; recrystallized from DMSO and methanol; yield 80%; mp 232–234 °C; ^{1}H NMR δ 0.90 (t, 3, J = 7.3 Hz, CH_3), 1.56 (m, 2, J = 7.3, 7.7 Hz, $CH_2CH_2CH_3$), 3.88 (t, 2, J = 7.7 Hz, $CH_2CH_2CH_3$), 7.20–8.15 (m, 5, CH_{arom}), 8.19 (s, 1, C_3H), 8.29 (br s, 1, NH), 8.85 (br s, 1, NH); ^{13}C NMR δ 10.7 (q, CH_3), 19.9 (t, $CH_2CH_2CH_3$), 43.3 (t, $CH_2CH_2CH_3$), 92.6 (s, C_{3a}), 120.0 (d, $C_{2'}$, $C_{6'}$), 125.2 (d, $C_{4'}$), 128.8 (d, $C_{3'}$, $C_{5'}$), 135.4 (d, C_3), 139.3 (s, $C_{1'}$), 152.7 (s, C_4), 154.0 (s, C_{7a}), 155.7 (s, C_6); IR 3610, 3345, 3025, 1700 cm^{-1}. Anal. ($C_{14}H_{15}N_5O$) C, H, N.

61: R_1 = Ph, R_2 = Bu; recrystallized from DMSO and methanol; yield 68%, mp 224–226 °C; ^{1}H NMR δ 0.89 (t, 3, J = 7.2, Hz, CH_3), 1.33 (m, 2, J = 7.0, 7.2 Hz, $CH_2CH_2CH_2CH_3$), 1.50 (m, 2, J = 7.0, 7.3 Hz, $CH_2CH_2CH_2CH_3$), 3.93 (t, 2, J = 7.3 Hz, $CH_2CH_2CH_2CH_3$), 7.20–8.15 (m, 5, CH_{arom}), 8.19 (s, 1, C_3H), 8.29 (br s, 1, NH), 8.53 (br s, 1, NH); ^{13}C NMR δ 13.6 (q, CH_3), 19.3 (t, $CH_2CH_2CH_2CH_3$), 28.8 (t, $CH_2CH_2CH_3$), 41.8 (t, $CH_2CH_2CH_2CH_3$), 92.6 (s, C_{3a}), 120.0 (d, $C_{2'}$, $C_{6'}$), 125.2 (d, $C_{4'}$), 128.8 (d, $C_{3'}$, $C_{5'}$), 135.5 (d, C_3), 139.3 (s, $C_{1'}$), 152.7 (s, C_4), 154.1 (s, C_{7a}), 155.7 (s, C_6); IR 3320, 3080, 1700 cm^{-1}. Anal. ($C_{15}H_{17}N_5O$) C, H, N.

62: R_1 = Ph, R_2 = Ph; recrystallized from DMSO and methanol; yield 55%; mp 249–250 °C; ^{1}H NMR δ 7.12 (br s, 1, NH), 7.26–8.20 (m, 10, CH_{arom}), 8.29 (s, 1, C_3H), 9.04 (br s, 1, NH), ^{13}C NMR δ 93.8 (s, C_{3a}), 120.1 (d, $C_{2'}$, $C_{6'}$), 125.1 (d, $C_{2'}$, $C_{6'}$), 128.4 (d, $C_{4'}$), 128.8 (d, $C_{4'}$), 129.1 (d, $C_{3'}$, $C_{5'}$), 129.8 (d, $C_{3'}$, $C_{5'}$), 136.0 (d, C_3), 137.0 (s, $C_{1'}$), 139.7 (s, $C_{1'}$), 153.9 (s, C_4), 154.9 (s, C_{7a}), 156.3 (s, C_6); IR 3460, 3090, 1695 cm^{-1}. Anal. ($C_{17}H_{13}N_5O$) C, H, N.

63: R_1 = 3-ClC_6H_4, R_2 = Et; recrystallized from DMSO and water; yield 71%; mp 278–280 °C; ^{1}H NMR δ 1.14 (t, 3, J = 7.0 Hz, CH_3), 3.98 (q, 2, J = 7.0 Hz, CH_2), 7.27–8.36 (m, 4, CH_{arom}), 8.20 (s, 1, C_3H), 8.37 (br s, 1, NH), 8.94 (br s, 1, NH); ^{13}C NMR δ 12.2 (q, CH_3), 37.2 (t, CH_2), 92.7 (s, C_{3a}), 117.9 (d, $C_{6'}$), 119.0 (d, $C_{2'}$), 124.8 (d, $C_{4'}$), 130.6 (d, $C_{5'}$), 133.3 (s, $C_{3'}$), 136.0 (d, C_3), 140.6 (d, $C_{1'}$), 152.6 (s, C_4), 153.9 (s, C_{7a}), 156.1 (s, C_6). IR 3350, 2945, 1680 cm^{-1}. Anal. ($C_{13}H_{12}N_5OCl$) C, H, N.

64: R_1 = 3-ClC_6H_4, R_2 = Pr; recrystallized from DMSO and methanol; yield 62%; mp 233–235 °C; ^{1}H NMR δ 0.90 (t, 3, J = 7.3 Hz, CH_3), 1.56 (m, 2, J = 7.3, 7.7 Hz, $CH_2CH_2CH_3$), 3.88 (t, 2, J = 7.7 Hz, $CH_2CH_2CH_3$), 7.26–8.36 (m, 4, CH_{arom}), 8.20 (s, 1, C_3H), 8.41 (br s, 1, NH), 8.88 (br s, 1, NH); ^{13}C NMR δ 10.7 (q, CH_3), 19.8 (t, $CH_2CH_2CH_3$), 43.4 (t, $CH_2CH_2CH_3$), 92.7 (s, C_{3a}), 117.9 (d, $C_{6'}$), 119.0 (d, $C_{2'}$), 124.8 (d, $C_{4'}$), 130.6 (d, $C_{5'}$), 133.2 (s, $C_{3'}$), 136.0 (d, C_3), 140.5 (s, $C_{1'}$), 152.8 (s, C_4), 154.0 (s, C_{7a}), 156.1 (s, C_6); IR 3650, 3310, 3030, 1700 cm^{-1}. Anal. ($C_{14}H_{14}N_5OCl$) C, H, N.

65: R_1 = 3-ClC_6H_4, R_2 = Bu; recrystallized from DMSO and water; yield 72%; mp 230–232 °C; ^{1}H NMR δ 0.85 (t, 3, J = 7.2 Hz, CH_3), 1.32 (m, 2, J = 7.2, 7.2 Hz, $CH_2CH_2CH_2CH_3$), 1.51 (m, 2, J = 7.2, 7.4 Hz, $CH_2CH_2CH_2CH_3$), 3.92 (t, 2, J = 7.4 Hz, $CH_2CH_2CH_2CH_3$), 7.26–8.36 (m, 4, CH_{arom}), 8.20 (s, 1, C_3H), 8.35 (br s, 1, NH), 8.91 (br s, 1, NH); ^{13}C NMR δ 13.6 (q, CH_3), 19.3 (t, $CH_2CH_2CH_2CH_2$), 28.8 (t, $CH_2CH_2CH_3$), 41.8 (t, $CH_2CH_2CH_2CH_3$), 92.7 (s, C_{3a}), 117.9 (d, $C_{6'}$), 119.0 (d, $C_{2'}$), 124.8 (d, $C_{4'}$), 130.6 (d, $C_{5'}$), 133.3 (s, $C_{3'}$), 136.1 (d, C_3), 140.6, (s, $C_{1'}$), 152.7 (s, C_4), 154.0 (s, C_{7a}), 156.1 (s, C_6); IR 3350, 3100, 2950, 1700 cm^{-1}. Anal. ($C_{15}H_{16}N_5OCl$) C, H, N.

4-Amino-1-(Aminophenyl)-5-*N*-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(5*H*)-ones (66–68). 4-Amino-5-*N*-methyl-1-(2-nitrophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6(5*H*)-one (1.0 g, 3.5 mmol) was finely ground and suspended in acetic acid (20 mL). Palladium on carbon (0.4 g) was added and the reaction was placed under a 50 kPa atmosphere of hydrogen for 24 h. The reaction mixture was filtered and the acetic acid was removed under high vacuum to produce a pale brown solid. This solid was purified by recrystallization from DMSO and water to give a pure solid (66).

66: R_1 = 2-$NH_2C_6H_4$, R_2 = Me; yield 89%; mp 283–290 °C; ^{1}H NMR δ 3.36 (s, 3, CH_3), 5.16 (br s, 2, NH_2), 6.61–7.19 (m, 4, CH_{arom}), 8.15 (s, 1, C_3H), 8.21 (br s, 1, NH), 8.94 (br s, 1 NH): ^{13}C NMR δ 29.5 (q, CH_3), 91.5 (s, C_{3a}), 116.2 (d, $C_{3'}$), 116.7 (d, $C_{5'}$), 124.1 (s, $C_{1'}$), 127.0 (d, $C_{6'}$), 128.4 (d, $C_{4'}$), 135.1 (d, C_3), 143.3 (s, $C_{2'}$), 153.4 (s, C_4), 154.1 (s, C_{7a}), 155.5 (s, C_6); IR 3325, 2945, 1705. Anal. ($C_{12}H_{12}N_6O$) C, H, N.

67: R_1 = 3-$NH_2C_6H_4$, R_2 = Me; recrystallized from methanol; yield 66%; mp 233–235 °C; ^{1}H NMR δ 3.37 (s, 3, CH_3), 5.31 (br s, 2, NH_2), 6.41–7.34 (m, 4, CH_{arom}), 8.13 (s, 1, C_3H), 8.24 (br s, 1, NH), 8.90 (br s, 1, NH); ^{13}C NMR δ 29.7 (q, CH_3), 92.5 (s, C_{3a}), 105.9 (d, $C_{2'}$), 108.2 (d, $C_{6'}$), 111.3 (d, $C_{4'}$), 129.0 (d, $C_{5'}$), 134.7 (d, C_3), 140.1 (s, $C_{1'}$), 149.2 (s, $C_{3'}$), 153.3 (s, C_4), 154.3 (s, C_{7a}), 155.4 (s, C_6); IR 3440, 3330, 3100, 1680. Anal. ($C_{12}H_{12}N_6O$) C, N, H.

68: R_1 = 4-$NH_2C_6H_4$, R_2 = Me; recrystallized from DMSO and methanol; yield 75%; mp 290–294 °C; ^{1}H NMR δ 3.38 (s, 3, CH_3), 5.16 (br s, 2, NH_2), 6.59–7.63 (m, 4, CH_{arom}), 8.06 (s, 1, C_3H), 8.19 (br s, 1, NH), 8.81 (br s, 1 NH); ^{13}C NMR δ 29.6 (q, CH_3), 92.1 (s, C_{3a}), 113.5 (d, $C_{3'}$, $C_{5'}$), 122.2 (d, $C_{2'}$, $C_{6'}$), 128.6 (s, $C_{1'}$), 134.0 (d, C_3), 146.8 (s, $C_{4'}$), 153.3 (s, C_4), 154.2 (s, C_{7a}), 154.5 (s, C_6); IR 3340, 3100, 1665 cm^{-1}. Anal. ($C_{12}H_{12}N_6O$) C, H, N.

A_1 Adenosine Receptor Binding Assay. Compounds were assessed for their ability to inhibit the binding of the selective A_1 agonist radioligand (*R*)-[^{3}H]N^6-(phenylisopropyl)adenosine to whole rat brain synaptosomal membranes.[13] Male Wistar Rats (300–400 g) were killed by exposure to hyperbaric carbon dioxide and decapitated. Whole rat brains were quickly excised and homogenized in 8 volumes of ice-cold 0.32 M sucrose with a prechilled glass homogenizer fitted with a Teflon pestle operating at 800 rpm (Potter–Elvejham homogenizer). The homogenate was centrifuged at 1000*g* for 10 min at 4 °C and the pellet discarded. The supernatant was recentrifuged at 14500*g* for 20 min at 4 °C. The resulting pellet was washed three times by resuspending in 8 volumes of ice-cold incubation buffer (50 mM

Tris-HCl, pH 7.4 and 1 mM $MgCl_2$) and recentrifuged at 14500g. The final pellet was then resuspended in incubation buffer to a final protein concentration of ca. 1 mg/mL and stored at −30 °C for up to several months without significant loss of activity. Protein concentration of the synaptosomal membrane was determined via a modified Lowry protein assay.[23] Before assaying, the required aliquot of synaptosomal membrane was thawed and incubated with adenosine deaminase (2 IU/mL, Sigma Type VI) at 37 °C for 30 min in order to remove endogenous adenosine. The assay mixture contained an aliquot of synaptosomal membrane (100–200 μg/mL), 2 nM *R*[^{3}H]N^6-PIA (New England Nuclear 36 Ci/mmol; Amersham 45.9 Ci/mmol) test compound (5 μL) and incubation buffer in a final volume of 1 mL. This was then incubated for 60 min at 37 °C. All test compounds were dissolved in dimethyl sulfoxide (Aldrich AR grade) and added to the incubation mixture in a volume of 5 μL. This volume of DMSO was found to decrease control binding by 10–15%. Nonspecific binding was defined in the presence of 100 μM *R*-N^6-PIA. The reaction was terminated by the addition of 4 mL of ice-cold incubation buffer and filtered through Whatman GF/B filters pre-soaked in incubation buffer, followed by rapid washing with 2 volumes (4 mL) of incubation buffer. The filters were then placed in vials containing 10 mL of aqueous counting scintillant (Amersham) and left overnight before being counted in a liquid scintillation counter (United Technologies/Packard Tricarb 2000CA liquid scintillation analyzer). All compounds were initially screened at a concentration of 20 μM. Any compound which was found to inhibit control binding by greater than 50% was then subjected to a full concentration–inhibition study to determine the IC_{50}. All assays were performed a minimum of three times in triplicate with similar results. Control data included beginning to end controls for both total binding and nonspecific binding as well as complete characterization of the assay, including detailed IC_{50}s of known compounds and saturation and kinetic experiments. Data from screening assays were expressed as percent inhibition with respect to the control incubation. Results from concentration–inhibition studies were analyzed with a nonlinear-least-squares curve-fitting program.[24]

A_2 Adenosine Receptor Binding Assay. Compounds were assayed for their ability to inhibit binding of the selective adenosine A_2 agonist radioligand [^{3}H]CGS 21680 [2-[[*p*-(2-carboxyethyl)phenethyl]amino]-5′-(ethanecarboxamido)adenosine] to rat striatal synaptosomal membranes.[14] Striata were removed from male Wistar rats (300–400 g) and homogenized in 20 volumes of ice-cold incubation buffer (50 mM Tris-HCl, pH 7.4, and 10 mM $MgCl_2$). The homogenate was then centrifuged at 48000g at 4 °C. The pellet was then resuspended in incubation buffer and recentrifuged as above. The resulting pellet was then resuspended in incubation buffer to a final protein concentration of 1–5 mg/mL, determined as for the A_1 tissue. The membrane suspension was also frozen at −30 °C for several months without significant loss of binding activity. The binding assay was similar to that used for A_1 with the following alterations. The assay mixture contained incubation buffer (50 mM Tris-HCL, pH 7.4, and 10 mM $MgCl_2$), 5 nM [^{3}H]CGS 21680 (New England Nuclear 48.6 Ci/mmol), and an aliquot of striatal membrane (100–200 μg/mL). Nonspecific binding was defined in the presence of 20 μM 2-chloroadenosine (Sigma). Incubation time was 90 min at 23 °C. All other aspects were identical with the A_1 assay. Initial 20 μM screens were followed by IC_{50}s for selected compounds.

Hydrophobicity Index. An index of the hydrophobicity was provided by the retention time of the pyrazolo[3,4-*d*]pyrimidines on a reverse-phase HPLC column. An ETPKortec K35M system fitted with a Dynamax 60A 0.45 × 20 cm ODS silica column was used. This system was isocratically eluted with 0.05 M NaH_2PO_4/Na_2HPO_4 (pH 7.4)–CH_3OH (6:4) which had been adjusted to pH 7.4 with 1 M phosphoric acid. The hydrophobicity index (k') was calculated for each compound with the formula $k' = (t_R - t_0)/t_0$ where t_R is the retention time of the compound and t_0 is the retention time of the solvent front.

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