Synthesis and Biological Evaluation of 1-Aryl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one Inhibitors of Cyclin-Dependent Kinases

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Received October 16, 2002

Using a high-throughput screening strategy, a series of 1-aryl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]-pyrimidin-4-ones was identified that inhibit the cyclin-dependent kinase (CDK) 4/cyclin D1 complex-mediated phosphorylation of a protein substrate with IC_{50}s in the low micromolar range. On the basis of preliminary structure−activity relationships (SAR), a model was proposed in which these inhibitors occupy the ATP-binding site of the enzyme, forming critical hydrogen bonds to the same residue (Val96) to which the amino group in ATP is presumed to bind. X-ray diffraction studies on a later derivative bound to CDK2 support this binding mode. Iterative cycles of synthesis and screening lead to a novel series of potent, CDK2-selective 6-(arylmethyl)-pyrazolopyrimidinones. Placement of a hydrogen-bond donor in the meta-position on the 6-arylmethyl group resulted in ∼100-fold increases in CDK4 affinity, giving ligands that were equipotent inhibitors of CDK4 and CDK2. These compounds exhibit antiproliferative effects in the NCI HCT116 and other cell lines. The potency of these antiproliferative effects is enhanced in anilide derivatives and translates into tumor growth inhibition in a mouse xenograft model.

Introduction

Controlling the cell cycle by inhibition of the proteins that regulate its progression is an attractive strategy for addressing cancer and other diseases associated with abnormal cellular proliferation.[1] One family of such proteins, the cyclin-dependent kinases (CDKs), is made up of at least nine highly homologous enzymes that in association with specific regulatory subunits (cyclins) control progression of the cell cycle.[2] CDK/cyclin activity oscillates with cyclin expression/degradation and is further regulated by the action of several families of protein kinases and phosphatases.[3,4] A series of checkpoints serves to ensure the viability of progeny cells by preventing progression of cells with damaged DNA, an inappropriate chromosome count, or for which necessary structural features or conditions of nourishment do not exist. Passage through an initial G1 restriction point occurs upon release by the retinoblastoma protein (pRb) of the transcription factor E2F.[5] This is triggered by phosphorylation of several S/T residues on pRb, the primary substrate of CDK4/cyclin D. The fate of cells that enter G1 but which do not progress through the G1 or subsequent checkpoints is to undergo apoptosis.[6] Since tumor cells have misregulated cell cycles, it has been postulated that they may be especially sensitive to agents that restore checkpoint control. The importance of these kinase pathways is highlighted by the fact that the genes encoding CDKs, their cyclin partners, or their endogenous peptide inhibitors (CKIs) are mutated in a large proportion of human tumors.[7] One family of CKIs, the CIP/KIP class, is relatively promiscuous, having affinity for CDK2, CDK3, CDK4, and CDK6.[8] CKIs in the INK4 class, however, are highly selective for the closely related CDKs 4 and 6, suggesting the possibility of selective inhibition by small molecule mimics. For these reasons we sought to discover small-molecule protein kinase inhibitors that were selective for CDK/cyclin complexes, specifically CDK4/cyclin D1.

How applicable this approach is to cancer chemotherapy is still an unresolved question. While a number of small-molecule CDK inhibitors have been disclosed, clinical experience is limited to the ATP-competitive agent flavopiridol.[9] Flavopiridol is relatively nonselective in its inhibition of the various CDKs; it also appears to inhibit other protein kinases. More recently, great strides have been made in the search for CDK-selective kinase inhibitors,[1] including the discovery of a highly selective CDK4/cyclin D inhibitor by optimization of a nonselective lead.[10]

High-throughput screening of a subset (∼160 000 compounds) of DuPont's compound library against CDK4/cyclin D1 was performed with a readout of percent inhibition of pRb phosphorylation at 20 μM. Discrete IC_{50} values were obtained for compounds that were active upon retest and appeared chemically attractive. This screening strategy identified several series of inhibitors with modest potency (IC_{50} < 50 μM).

One intriguing lead uncovered via this strategy was the 4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one **1** (Fig-

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10.1021/jm020455u CCC: $27.50
Published on Web 10/27/2004

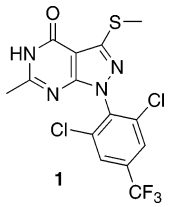

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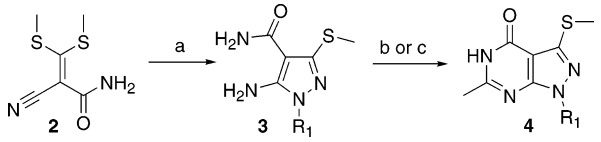
Figure 1. Lead structure from high-throughput screening against CDK4/cyclin D1.

Scheme 1[a]



[a] (a) R_1NHNH_2, MeOH, reflux; (b) CH_3CONH_2, Δ; (c) CH_3CO_2Et, NaOEt, EtOH, reflux.

ure 1). Although this ring system occurs in a number of biologically active agents, including coronary dilatating agents[11] and corticotropin-releasing factor (CRF) antagonists,[12] we were hopeful that this was coincidental and that a divergent SAR for CDK inhibition would emerge. To explore this possibility, compounds were prepared that had diverse groups at the substitutable positions of the pyrazolopyrimidine core.

Chemistry

The preparation of a series of 6-methyl-3-(methylthio)-pyrazolopyrimidin-4-ones was accomplished by a route analogous to those in the literature (Scheme 1).[13] Accordingly, hydrazines were allowed to react with dithioketeneacetal **2** in refluxing methanol to afford 3-aminopyrazole-4-carboxamides **3**.[14] These were converted to the target compounds **4** by heating at reflux with ethyl acetate and an alkali metal alkoxide in ethanol.[15]

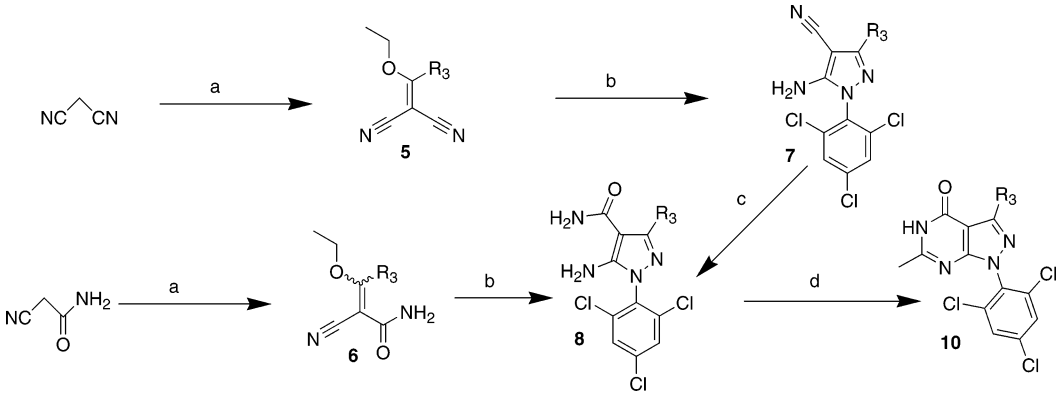
Aminopyrazole-4-carboxamides bearing other groups at the 3-position were prepared by the well-precedented routes shown in Schemes 2−4. Scheme 2 is analogous to Scheme 1, except that the methylthio leaving group is replaced by ethoxy in **5** and **6** and that the carboxamide in **2** may be replaced by a nitrile group. These react with 2,4,6-trichlorophenylhydrazine to give pyrazoles **7** and **8**, the former being hydrolyzed to the desired amides **8** by treatment with concentrated sulfuric acid. Hydrazones, available from condensation of hydrazines and aldehydes or aldehyde equivalents (Scheme 3), can be brominated to give bromohydrazones **9**. In what is probably the most versatile route, these react readily with salts of malononitrile and cyanoacetamide to afford **7** and **8**, respectively. Incorporation of an acetate equivalent to give 6-methyl-1-(2,4,6-trichlorophenyl)-pyrazolopyrimidin-4-ones **10** was accomplished as in Scheme 1. Scheme 4 illustrates the conversion of tetracyanoethylene to 3-cyano- and 3-carboxamidopyrazolopyrimidines.[16]

Standard alkylation conditions (Scheme 5) were used to convert **10i** to its *N*-methyl derivative **11**. The chloropyrimidine **12a**, prepared by heating **10i** in $POCl_3$ at reflux, underwent smooth addition−elimination reaction to give methoxypyrimidine **12b**. Aminopyrimidine **12c** was prepared from **7i** via the intermediacy of imidate **13**.

Condensation of trichlorophenylhydrazine with 3-oxovaleronitrile (Scheme 6) furnished the pyrazole **14**, which readily underwent electrophilic aromatic substitution with chlorosulfonic acid. Heating the resulting sulfonamide **15** with base and ethyl acetate in ethanol furnished cyclic sulfonamide **16**, albeit in low yield.

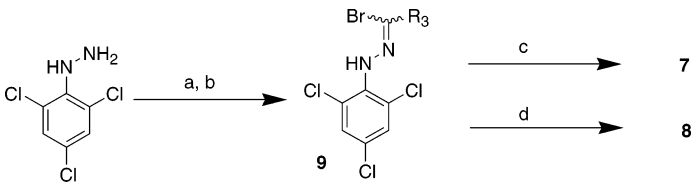
Scheme 7 illustrates the incorporation of diverse groups at the 6-position, a process accomplished by refluxing the R_6 carboxylic ester and sodium ethoxide with **3w** or **8i** in ethanol. This process affords direct

Scheme 2[a]



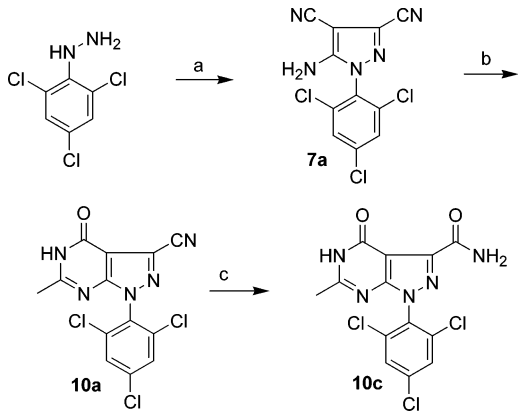
[a] (a) $R_3C(OEt)_3$, Ac_2O, Δ; (b) 2,4,6-trichlorophenylhydrazine, MeOH, reflux; (c) H_2SO_4; (d) CH_3CO_2Et, NaOEt, EtOH, reflux.

Scheme 3[a]



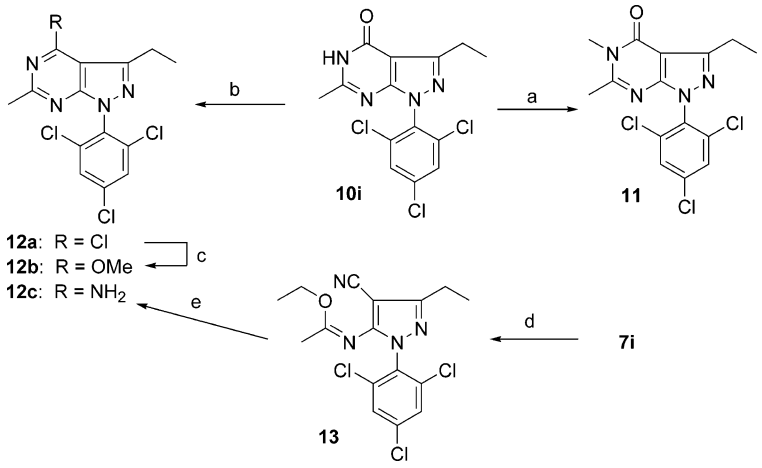
[a] (a) R_3CHO, EtOH; (b) *N*-bromosuccinimide, DMF, 0 °C; (c) malononitrile, NaOEt, EtOH, relux; (d) cyanoacetamide, NaOEt, EtOH, reflux.

Scheme 4[a]



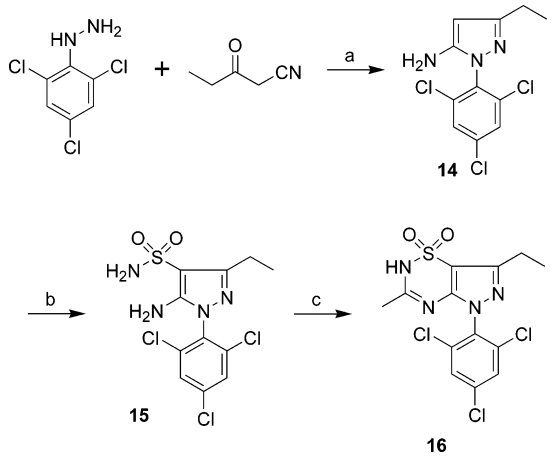
[a] Reagents: (a) tetracyanoethylene, EtOH, 69%; (b) (i) Ac_2O, (ii) AcOH, H_2O, 18%; (c) H_2SO_4, 52%.

Scheme 5[a]



[a] (a) TsOme, K_2CO_3, DMAC, 44%; (b) $POCl_3$, reflux; NaOMe, MeOH; (d) $CH_3C(OEt)_3$, Ac_2O, Δ; (e) NH_3, EtOH.

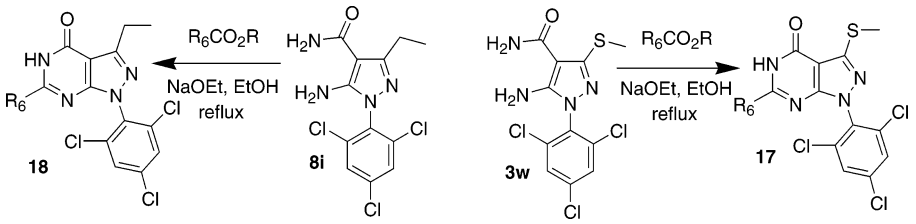
Scheme 6[a]



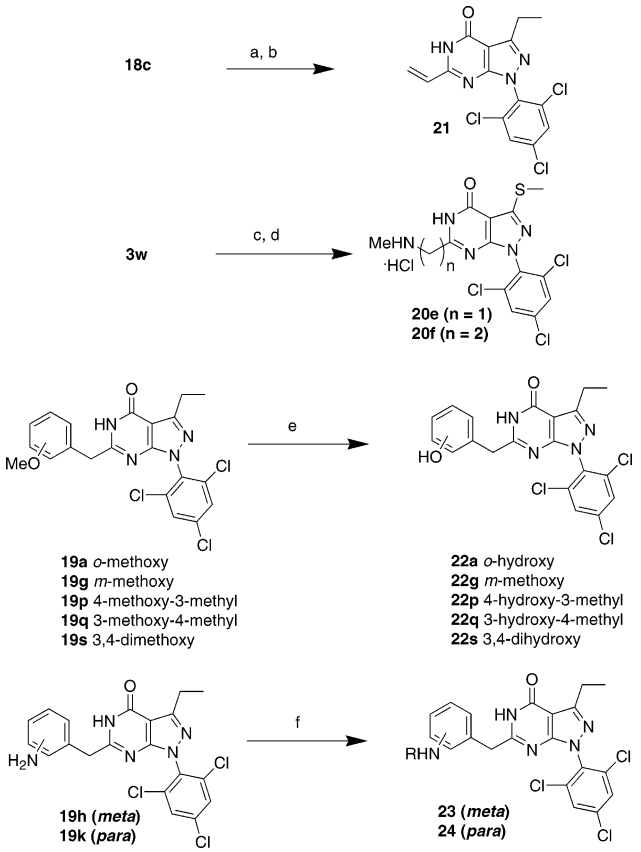
[a] Reagents: (a) (i) EtOH, reflux, (ii) HCl, EtOH, 48%. (b) (i) $ClSO_3H$, (ii) NH_3, THF, 75%, (c) EtOAc, NaOEt, EtOH, reflux, 7%.

access to products of high purity and tolerates a wide variety of functional groups. The pyrazolopyrimidines are isolated by quenching the reactions with aqueous acid, filtering, and rinsing the insoluble products to remove any excess reagents. Unsaturated esters did not react cleanly under these conditions, but they could be prepared by conventional elimination methods (**18c** to **21**). Scheme 8 illustrates this and other reactions to further derivatize 6-substituted pyrazolopyrimidines. Aliphatic amino esters were felt to be too nucleophilic to be used directly in the cyclization step, so the corresponding *tert*-butyl carbamates were instead employed. Removal of the *tert*-butoxycarbonyl protecting group with HCl in dioxane afforded **20e** and **20f**, respectively. Phenols could be used in the cyclization step without protection, or they could be prepared from the corresponding anisoles by treatment with BBr_3 in CH_2Cl_2 (**19** to **22**). Aminophenylacetic esters reacted well under these cyclization conditions, and the resulting anilines could be converted to anilide derivatives

Scheme 7



Scheme 8[a]



[a] (a) *m*-CPBA, CH_2Cl_2, DMF; (b) $PhCH_3$, Δ; (c) $BocN(Me)(CH_2)_nCO_2Et$, EtOH, reflux; (d) HCl, dioxane; (e) BBr_3; (f) coupling agent (see Experimental Section).

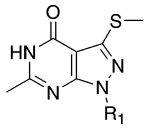
such as **23** or **24** by treatment with appropriate electrophiles in the presence of a base.

Results and Discussion

SAR trends for the pyrazolopyrimidine 1-position are summarized in Table 1.[17] Replacement of the substituted phenyl moiety with alkyl or unsubstituted aryl groups gave completely inactive compounds, as shown by entries **4a**−**f** in the table. Similarly, meta- or para-substituted phenyl R_1 groups did not prove to be satisfactory replacements. Of the monosubstitued R_1 phenyl groups surveyed, only the *o*-chloro appeared to be satisfactory (entry **4k**). Early analogues having disubstituted aryl groups (Entries **4l**−**r**) likewise exhibited only minimal potency. Only with the 2,6-dihalophenyl derivatives **4s** and **4t** were inhibitors comparable in potency to the 8 μM IC_{50} observed for **1** realized. The trisubstituted phenyl R_1 derivatives proved more satisfactory. While rearrangement of the phenyl substituents in **1** to give the 2,4-dichloro-6-(trifluoromethyl)phenyl derivative **4u** resulted in a loss of activity, entry **4v** demonstrated that replacement of one of the *o*-halogens with a small nonhalogen substituent was allowed. The best compound prepared in this series is trichlorophenyl derivative **4w**. The weak inhibition exhibited by **4u** and **4x** further highlights the delicate balance of steric and electronic factors that define acceptable R_1 substituents.

Our enthusiasm over having modestly potent, low molecular weight CDK4 inhibitors was tempered somewhat by our desire to have inhibitors that did not bind in the ATP pocket. We were concerned that ATP antagonists would exhibit poor selectivity toward other kinases and potentially toward other classes of enzymes having nucleoside binding pockets.[18] We were thus gratified to find that while **4w** exhibited little selectivity for any particular CDK (Table 2), it did not appear to inhibit the serine/threonine kinases protein kinase A (PKA) or C (PKC). Further, **4w** showed little affinity for the tyrosine kinase c-Abl. A modest level of cellular potency (IC_{50} = 2.5 μM) was observed for **4w** when assayed for growth inhibition of the HCT116 line (vide infra). This growth inhibition compares favorably with an IC_{50} value of 9.2 μM against the Ag1523 line of

Table 1. CDK4/Cyclin D1 Inhibition of 6-Methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones[17b]



no.	R_1	step 1 yield, %	step 2 method[a] (yield, %)	K4/D1 IC_{50}, μM
1	2,6-dichloro-4-(CF_3)phenyl			8
4a	benzyl	15	B (9)	>150
4b	*n*-butyl	33	B (32)	>200
4c	*tert*-butyl	34	B (29)	>100
4d	2-hydroxyethyl	50	B (43)	>210
4e	phenyl	*b*	B (90)	>180
4f	2-pyridyl	92	B (77)	>180
4g	3-chlorophenyl	94	B (9)	>160
4h	4-chlorophenyl	74	B (62)	>160
4i	4-isopropylphenyl	83	B (16)	>160
4j	2-methoxyphenyl	88	B (35)	>83
4k	2-chlorophenyl	66	B (16)	19
4l	4-chloro-2-methylphenyl	87	B (51)	74
4m	2,3-dichlorophenyl	94	B (21)	50
4n	2,4-dimethylphenyl	84	B (72)	48
4o	2,4-dichlorophenyl	69	B (28)	37
4p	2,5-dimethylphenyl	76	B (34)	25
4q	2,5-dichlorophenyl	64	B (53)	23
4r	2-chloro-5-(CF_3)phenyl	59	B (56)	15
4s	2-chloro-6-fluorophenyl	65	B (67)	6.8
4t	2,6-dichlorophenyl	73	B (42)	5.3
4u	2,4-dichloro-6-(CF_3)phenyl	44	B (57)	29
4v	2,4-dichloro-6-methylphenyl	64	B (76)	11
4w	2,4,6-trichlorophenyl	88	A (90)	2.1
4x	2,4,6-trimethylphenyl	76	B (66)	37

[a] Method A: Scheme 1, conditions b. Method B: Scheme 1, conditions c. [b] Prepared in 59% yield by treatment of 5-amino-4-cyano-3-(methylthio)-4-phenylpyrazole (Maybridge) with concentrated sulfuric acid.

human fibroblasts. The NCI HCT116 line was chosen for routine cellular assays, because it expresses high levels of CDK/cyclin proteins,[19] therefore posing a challenge to this class of kinase inhibitors. In addition, the HCT 116 line can be grown as a xenograft in nude mice. For comparison, the IC_{50s} of staurosporine and racemic flavopiridol in the above assays are listed in the table.

With these data in hand we sought improved CDK inhibitors through modification of the 3-(methylthio) substituent. Table 3 details the SAR trends realized through these modifications. Of the electron-withdrawing groups sampled at this position, only the trifluoromethyl analogue **10d** inhibits CDK4/cyclin D1 to a significant degree. Compounds **10e**−**k** comprise a brief steric series that appears to define a very narrow range of allowable substitution for this position. While ethyl appears to be slightly better than methylthio, smaller or larger linear alkyl groups are significantly worse. Finally, while placement of a hydroxymethyl group at R_3 results in a modest loss of potency versus the same-sized alkyl analogue **10i**, the hydroxyethyl homologue **10m** was devoid of activity. A plausible explanation for this is found upon comparison of the ^{1}H NMR spectrum of **10m** with those of other pyrazolopyrimidinones prepared thus far. The occurrence of the hydroxyl proton in **10m** as a sharp triplet at 4.81 ppm is indicative of intramolecular hydrogen bonding. Further, the pyrimidinone NH, which normally resonates at 12−13 ppm (DMSO-d_6) is shifted to 10.5 ppm. This implies a profound distortion of the electronics of the pyrimidinone ring.

The data presented in Table 4 provide a persuasive argument for the pyrimidinone motif as the preferred tautomer in these compounds. The lack of activity observed for the *N*- and *O*-methyl derivatives **11** and **12b** suggests that the presence of a hydrogen-bond donor is critical. That it must be donated from the ring nitrogen is established by the lack of activity of **12c**, which, like ATP, is in all probability present in solution as the aminopyrimidine tautomer. The sulfonamide **16** was also shown to be inactive, presumably because the tetrahedral sulfonyl group cannot attain the appropriate geometry to act as a hydrogen-bond acceptor. Taken together these observations suggest that the pyrimidinone forms a bidentate hydrogen bond.

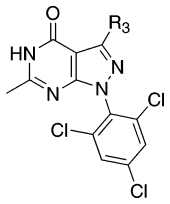
We now turned to the preparation of a series of R_6 analogues. The availability of a broader range of kinase assays for routine compound screening now allowed us to better evaluate issues of selectivity. As the previous iteration of screening (Table 3) had identified **10i**, a compound with a 2-fold improvement in potency (over **4w**) toward CDK4/cyclin D1 but 4-fold improvement toward CDK2/cyclinE, we prepared to address the possibility that this series was intrinsically CDK2-selective.

Just as at R_3, it initially appeared that only a very narrow range of substituents would be tolerated at R_6 (Table 5). In our steric series (first 8 entries), only the ethyl analogue **18b** showed comparable potency to **10i** toward CDK 4/cyclin D1. α-Branching or placement of polar functional groups close to the pyrazolopyrimidine core proved detrimental to activity. While compounds bearing aryl groups at this position (entries **17n**, **18e**) bound poorly to CDK4/cyclin D1, those having an intervening methylene or ethylene tether had reasonable IC_{50}s. The improvement in CDK2/cyclin E inhibition realized by tethering an aryl group at this position is even more profound, as demonstrated by **18i**. At 24 nM, this compound is 10-fold selective for CDK2/cyclin E vs CDK1/cyclin B and >100-fold selective for CDK2/cyclin E over CDK4/cyclin D. The benzyl-substituted pyrimidinone **18i** also showed improved cellular potency (Table 6) over **4w**. Placing a benzyl group at the R_6 position in the R_3 = SMe series also improves CDK2/cyclin E inhibition (**17p**). Comparison of the enzyme inhibitory data for **18i** and **17p** supports the conclusion drawn from Table 4 that the R_3 = ethyl series has slightly greater potency against CDKs than the SMe series.

Table 2. Spectrum of Activity of Pyrazolopyrimidin-4-one **4w**[17b]

	enzyme and cellular IC_{50}s (mM)							
no.	PKA	PKC	c-Abl	K4/D1	K2/E	K1/B	HCT116	Ag1523
4w	>500	>500	420	2.1	0.36	6.2	2.5	9.2
(±)-flavopiridol	>500	110	160	0.090	0.95	0.52	0.49	6.7
staurosporine	0.08	0.033	1.1	–	0.029	0.032	0.006	

Table 3. CDK4/Cyclin D1 Inhibition of 6-Methyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]-pyrimidin-4-ones[17b]

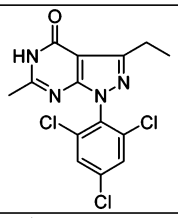
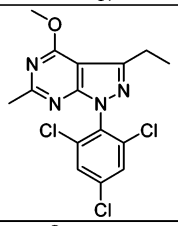
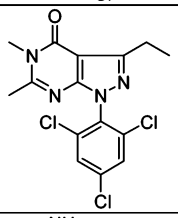
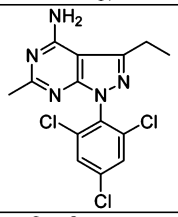
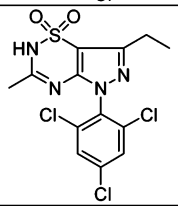


no.	R_3	step 1 method[a] (% yield)	step 2 yield, %	K4/D1 IC_{50}, μM
4w	methylthio	2.1		
10a	cyano	E (69)	(18)[b]	>28
10b	sulfonylmethyl	(60)[c]	14	
10c	carboxamido	(52)[b]	12	
10d	trifluoromethyl	D (31)	43	3.1
10e	phenyl	A (49)	64	>120
10f	benzyl	D (15)	81	>48
10g	*n*-butyl	A (68)	75	>6.5
10h	*n*-propyl	B (34)	13	5.7
10i	ethyl	A (70)	78	1.1
10j	methyl	A (67)	81	3.5
10k	H	F (43)	47	12
10l	hydroxymethyl	D (40)[d]	58	7.0
10m	2-hydroxyethyl	C (44)[d]	66	>140

[a] Method A: Scheme 2, conditions a−c. Method B: Scheme 2, conditions a, b. Method C: Scheme 3, conditions a−c, e. Method D: Scheme 3, conditions a, b, d. Method E: Scheme 4. Method F: Scheme 2, conditions b, c. [b] See Scheme 4. [c] Prepared from **4w** by oxidation with excess *m*-CPBA. [d] In this sequence, the alcohol group was protected as a *tert*-butyldiphenylsilyl ether.

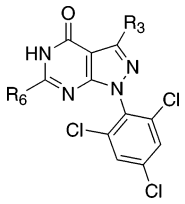
These observations prompted us to prepare the series of 6-(arylmethyl)pyrazolopyrimidin-4-ones shown in Table 6. Substitution of the ortho-position to give the anisole derivative **19a** proved detrimental to activity. Interestingly, the hydroxymethyl isostere **19b** showed little change from the unsubstituted parent compound. While π-donating substituents were not favorable in the ortho-position, the 2-pyridyl analog **19c** proved to be even worse. Placement of a single substituent at the meta-position generally resulted in moderately potent and CDK2/cyclin E-selective compounds with generally unimpressive cellular potency. Para-substituted compounds, on the other hand, were generally better enzyme inhibitors than **18i** and were usually potent in cells. No real gains accrued from making 3,4-disubstituted analogues until 3,4-dimethoxybenzyl derivative **19s** was deprotected to give the key catechol **22s**. While the CDK2/cyclin E inhibition displayed by this compound is unremarkable, against CDK4/cyclin D1 **22s** has an IC_{50} of 44 nM and is 102 and 68 times more potent than **19s** and the unsubstituted analogue **18i**, respectively. Examination of the ratio of CDK4/cyclin D1 inhibition for several other anisole/phenol pairs shows that this observation is not anomalous. The *m*-phenols **22q**, **22g**, and **19u** are 24, 74, and 190 times more potent against CDK4/cyclin D1 than the corresponding anisoles. The data for compound **23** imply that this phenomenon is associated with the acidity of the meta-substituent. While aniline **19h** shows modest CDK4/cyclin D1 potency, the more acidic sulfonamide **23** is fairly potent and balanced in its inhibition of CDK4/cyclin D1 and CDK2/cyclin E. The improvements in CDK inhibition detailed above do not appear to have come at the expense of selectivity over the other protein kinases in our panel. The IC_{50}s for **22s** against PKA, PKC, and c-Abl are >100 μm.

Table 4. CDK/Cyclin Inhibition of 3-Ethyl-6-methyl-1-(2,4,6-trichlorophenyl)pyrazolo[3,4-*d*]pyrimidin-4-one (10i) and Isosteres 17b

no.	Structure	Prepared in:	K4/D1 IC_{50} (μM)	K_2/E IC_{50} (μM)
10i		Scheme 2	1.1	0.14
12b		Scheme 5	>27	2.5
11		Scheme 5	>27	>2.7
12c		Scheme 5	>140	
16		Scheme 6	>51	>13

The *p*-aniline derivative **19k** offered an attractive handle for pursuing the effects of larger groups on CDK SAR. Accordingly, the anilide derivatives shown in Table 7 were prepared. While only subtle changes in kinase inhibition were realized with amides **24**, they are profoundly different from the parent aniline in their effects upon transformed cell lines. Relatively large substituents, including the protected glycinamide **24b** are accommodated at the para-position. Charged groups are well-tolerated at this position, suggesting that they may be solvent-exposed. We felt that placement of a basic amine in this position would result in compounds having higher aqueous solubility while maintaining enzyme affinity and cellular potency. Glycinamide **24c** (log P = 3.3), while an excellent enzyme inhibitor, showed only modest activity in the HCT116 growth inhibition assay. Homologation of **24c** to give the *N,N*-dimethylglycinamide **24d** resulted in increased lipophilicity and a reduced number of hydrogen-bond donors. While **24d** had comparable in vitro properties to the

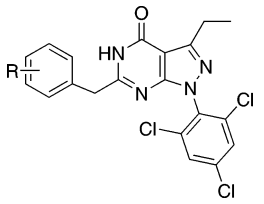
Table 5. CDK4/Cyclin D1 and CDK2/Cyclin E Inhibition of 1-(2,4,6-Trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]-pyrimidin-4-ones[17b]



no.	R_3	R_6	% yield	IC_{50} (μM) K4/D1	IC_{50} (μM) K2/E
4w	SMe	methyl	90	2.1	0.36
10i	Et	methyl	78	1.1	0.14
17a	SMe	H	27	95	2.7
17b	SMe	isopropyl	59	10	
17c	SMe	*n*-propyl	52	9.1	
18a	Et	isobutyl	36	>6.3	0.11
17d	SMe	cyclopropyl	35	4.3	0.40
18b	Et	ethyl	84	0.94	0.096
21	Et	vinyl	(67)[a]	2.9	0.31
17e	SMe	$BocN(Me)CH_2$	100	>50	
20e	SMe	$MeNHCH_2HCl$	100	>57	
17f	SMe	$BocN(Me)CH_2CH_2$	69	>48	19
20f	SMe	$MeNHCH_2CH_2{\cdot}HCl$	100	28	6.6
17g	SMe	$CH_3CH(OH)$	66	27	
17h	SMe	CH_3SCH_2	52	15	0.31
18c	Et	$CH_3SCH_2CH_2$	80	6.5	
17i	SMe	MeO_2C	20	240	
17j	SMe	$HO(CH_2)_3$	81	13	0.76
17k	SMe	$HO(CH_2)_5$	80	10	0.42
18d	Et	$HO(CH_2)_4$	87	2.4	0.25
17l	SMe	CH_2F	94	44	1.6
17m	SMe	CF_3	100	>116	
17n	SMe	2-furyl	55	36	
18e	Et	3,4-dimethoxyphenyl	14	>21	0.58
17o	SMe	CH_2-2-thienyl	83	20	
18g	Et	CH_2CH_2-phenyl	89	7.1	1.8
18h	Et	CH_2CH_2-imidazol-4-yl	55	3.6	0.62
18i	Et	benzyl	88	3.0	0.024
17p	SMe	benzyl	84	11	0.044

[a] Prepared by oxidation and thermal elimination of **18c**.

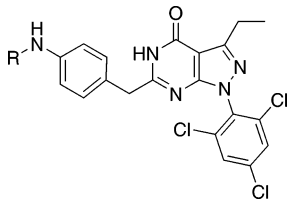
Table 6. CDK/Cyclin Inhibition of 6-(Arylmethyl)-1-(2,4,6-trichlorophenyl)-3-ethyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]-pyrimidin-4-ones[17b]



no.	R	% yield	IC_{50} (μM) K4/D1	IC_{50} (μM) K2/E	IC_{50} (μM) K1/B	IC_{50} (μM) HCT116
18i	H		3.0	0.024	0.26	0.60
19a	2-methoxy	91	>22	0.10		3.4
22a	2-hydroxy	91[a]	>11	0.12		5.5
19b	2-(hydroxy methyl)	88	2.9	0.027	>0.27	0.49
19c	2-aza	87	9.7	0.23		3.7
19d	3-amino-2-methyl	89	>2.2	0.019	>0.27	0.19
19e	3-methyl	95	>2.2	0.039	>0.56	2.1
19f	3-ethoxycarbonylmethyl	42	>1.9	0.15		
19g	3-methoxy	76	5.0	0.032	0.41	1.7
19h	3-amino	69	2.9	0.040	0.27	0.47
19i	3-aza	97	1.3	0.017	0.22	0.30
19j	4-aza	92	3.0	0.038	0.49	0.55
19k	4-amino	89	2.7	0.015	0.15	0.42
19l	4-methoxy	81	2.2	0.016	0.15	0.60
19m	4-hydroxy	58	2.0	0.016	0.10	0.099
19n	4-bromo	96	>1.2	0.058		2.3
19o	4-dimethylamino	80	>2.1	0.023	>0.26	0.34
19p	4-methoxy-3-methyl	89	>2.1	0.067	0.87	2.1
22p	4-hydroxy-3-methyl	91[a]	>2.2	0.027	>0.27	0.50
19q	3-methoxy-4-methyl	97	2.1	0.11		9.5
19r	4-hydroxy-3-methoxy	88	6.6	0.076	0.86	0.32
19s	3,4-dimethoxy	57	4.5	0.11	>0.51	0.61
22s	3,4-dihydroxy	93[a]	0.044	0.020	0.24	0.72
19t	3,5-dihydroxy	50	0.059	0.022		0.30
19u	3-hydroxy-4-methoxy	65	0.024	0.019	0.20	0.13
22q	3-hydroxy-4-methyl	90[a]	0.088	0.023	0.52	0.67
22g	3-hydroxy	90[a]	0.068	0.014	0.19	0.40
23	3-methylsulfonamido	83[b]	0.16	0.26		0.47

[a] Prepared by demethylation of the corresponding anisole with BBr_3. [b] Prepared by treatment of **19h** with methanesulfonyl chloride and pyridine in CH_2Cl_2.

Table 7. CDK/Cyclin Inhibition of 6-(4-Acylamino)benzyl)-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]-pyrimidin-4-ones[17b]



no.	R	IC_{50} (μM) K4/D	IC_{50} (μM) K2/E	IC_{50} (μM) K1/B	IC_{50} (μM) HCT	log *P*
19k	H	2.7	0.015	0.15	0.42	3.8
24a	CH_3CO	1.3	0.009	0.038	0.029	3.9
24b	$(CH_3)_3COCONHCH_2CO$	1.7	0.016	0.18	0.16	4.9
24c	$H_2NCH_3CO{\cdot}HCl$	0.46	0.013	0.07	0.12	3.3
24d	$(CH_3)_2NCH_2CO{\cdot}HCl$	0.59	0.018	0.10	0.034	4.2

parent glycinamide, further refinement of its physical parameters led to a significant increase in cellular potency. To assess its selectivity against other protein kinases, dimethylglycinamide **24d** was assayed against PKA and c-Abl, and no significant inhibition (IC_{50}s > 150 μM) was observed. Compound **24d** has an IC_{50} of 0.19 μM against the Ag1523 line of human fibroblasts. Expressed as a multiple of the HCT116 IC_{50}, this yields a ratio of 5.6:1, a number little different from the ratio obtained for racemic flavopiridol and **4w**. The data in Table 8 demonstrate that **24d** inhibits growth in Rb^+, Rb^-, $p53^+$, and $p53^-$ cell lines. The latter point is of importance for a series of CDK inhibitors, since the p53 pathway is misregulated in a large number of cancers.[20] While an inhibitor selective for the cyclin D-associated CDKs would be expected to inhibit growth preferentially in the Rb^+ lines,[10b] the broad spectrum of antiproliferative activity observed for **24d** is unsurprising in light of its modest selectivity for CDK2.

Our modeling experiments started from the assumption that the pyrimidinone is forming a bidentate hydrogen bond in the region where the aminopyrimidine moiety of ATP binds.[21] Using the Modeller program[22] a series of cyclin-bound CDK4 homology models was generated based on crystallographic data from CDK2/cyclin A.[23] The Whatif[24] program was used to select the best model, and compounds were hand-docked into the active site using InsightII v97.0 (MSI, San Diego, CA). The preferred binding mode for **10i** (blue) is presented in Figure 2 with ATP shown in magenta and the proposed bidentate hydrogen bond to Val96 indicated by dashed lines.

To obtain the binding mode of these inhibitors, the crystal structure of **22s** complexed to CDK2 was determined (1.85 Å resolution). The entire molecule is well

Table 8. Antiproliferative Effects of *N,N*-Dimethylglycinamide **24d** against Selected Cell Lines

cell line	IC_{50} (nM)	PRb function	p53 function
HCT116	34	+	+
MDA MB468	12	-	-
NCI H460	16	+	+
A498	57	?	+
T47D	16	+	-
MCF7	14	+	+
DU145	15	-	-
COLO205	13	+	-

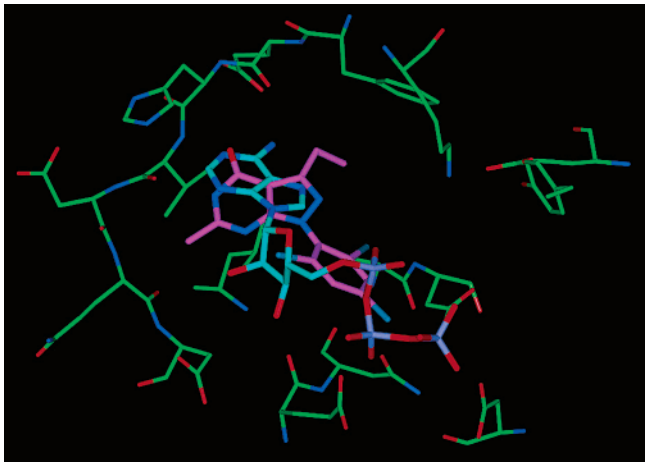


Figure 2. Homology model of CDK4 with **10i** (magenta) and ATP (light blue) bound. Hydrogen bonds between the pyrimidinone N5 and Val96 carbonyl and the pyrimidinone carbonyl and Val96 N are shown by dashed lines.

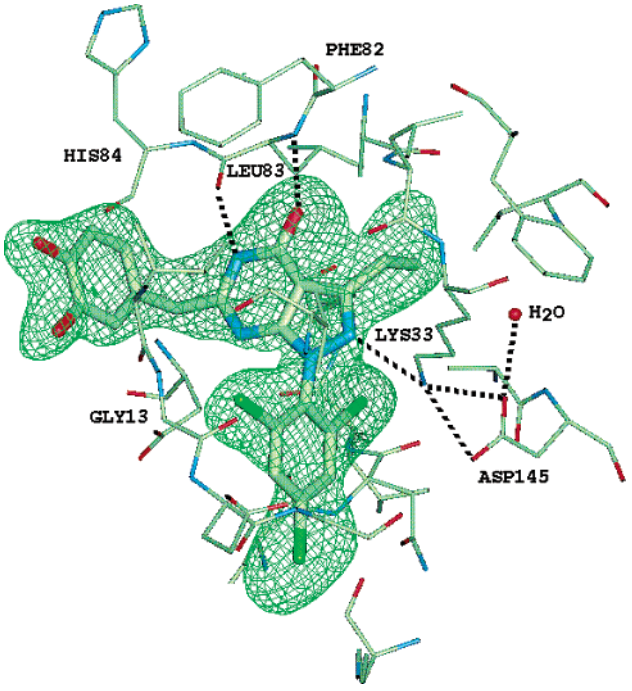


Figure 3. Crystal structure of **22s** bound to CDK2. Hydrogen bonds between the pyrimidinone N5 and Leu83 carbonyl and the pyrimidinone carbonyl and Leu 83 N are shown by dashed lines.

defined as shown in Figure 3 with the final electron density. The inhibitor molecule binds as ATP and other reported inhibitors,[25] in the deep cleft between the *N*-terminal and *C*-terminal domains. There are three hydrogen bonds between the protein and **22s**: pyrimidine N5 to Leu83 carbonyl oxygen (2.67 Å), pyrimidinone carbonyl oxygen to backbone nitrogen of the same residue (3.04 Å), and pyrazole N2 to Nϵ of Lys33 (2.90 Å). The observation of these first two interactions validates our homology model, which predicts a bidentate hydrogen bond to the corresponding residue in CDK4 (Val96). The C3 ethyl group from the pyrazole ring is pointing deeper into the pocket and has hydrophobic interactions with the Val 64 side chain. Near the ethyl group (3.40 Å) there is a water molecule that forms hydrogen bonds with the side chain and backbone nitrogen of Asp145 (2.70, 3.02 Å, respectively). The trichlorophenyl group provides a hydrophobic interaction with either the glycine-rich loop or the *C*-terminal domain—one of the *o*-chlorines with Ile10 and Gly11 and the other with Gln131 and Ala144—while the *p*-chlorine chlorine is directed toward Thr14 and Lys129. These interactions allow the trichlorophenyl group to bridge the *N*-terminal and *C*-terminal domains. The R_6 aryl group is projected out of the enzyme, with the phenol moiety on the opposite side of the phenyl ring from Phe82 (His95 in CDK4). If the same binding mode applies to CDK4, rotating the aryl ring 180° would place the *m*-hydroxyl group within hydrogen-bonding distance of His95, possibly explaining the improvement in CDK4/cyclinD potency of *m*-phenols over compound not able to donate a hydrogen bond from this position. Other residues within 3.5 Å of ST550 are Gly13, Val18, Ala 31, Phe82, His84, Lys129, and Asn132.

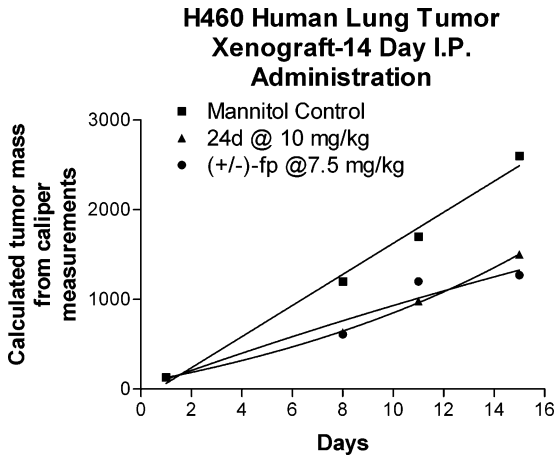


Figure 4. Caliper-estimated tumor mass plots for **24d** (●), racemic flavopiridol [(±)-fp, ▲], and vehicle (■). Compounds were dosed ip, q2d × 7 in female nude mice bearing NCI H460 tumors (see Experimental Section).

To assess the viability of this series of compounds in vivo we dosed **24d** in nude mice harboring xenografts from the NCI H460 cell line. Like HCT116, this line expresses high levels of CDK/cyclin proteins. The response curves for an ip, q2d × 7 dosing schedule are shown in Figure 4. Tumor masses were estimated by caliper measurement. The percentage tumor growth inhibition (TGI) values were obtained by comparing the tumor volumes from animals receiving compound with those receiving vehicle (4% aqueous mannitol) only. TGIs of 41% and 49% were observed for **24d** (10 mg/kg) and racemic flavopiridol (7.5 mg/kg), respectively. In a study with HCT116 xenografts,[26] mice dosed ip (q1d × 19) with 10 mg/kg of **24d** and 7.5 mg/kg of racemic flavopiridol exhibited TGIs of 46 and 44%,

respectively (based on measured tumor masses upon termination of the study at day 19).

Conclusion

Optimization of the CDK4/cyclin D1 screening hit **1** has led to a series of potent 6-arylmethylpyrazolopyrimidin-4-one inhibitors that show modest selectivity for CDK2/cyclin E over other CDKs. This series appears to be selective for CDKs over several other protein kinases. The best CDK4/cyclin D1 inhibitors in this series are 50−330 times more potent than the screening hit and are characterized by having a relatively acidic hydrogen-bond donor in the meta-position of the pyrazolopyrimidine R_6 benzyl. The best antiproliferative activity is seen in those compounds having anilide groups at the para-position of the pyrazolopyrimidine R_6 benzyl. Modeling and X-ray crystallography experiments indicate that groups at this position are projected out of the enzyme and into solvent, offering opportunities to modulate physical properties (solubility, partition coefficient, etc.) without altering enzyme inhibition. The *N,N*-dimethylglycinamide **24d** is highly potent in a range of antiproliferative assays and shows activity in mouse xenograft models.

Experimental Section

General Procedures. Melting points were determined on a Thomas-Hoover Uni-melt apparatus and are uncorrected. ^{1}H NMR spectra were recorded on dilute solutions in $CDCl_3$ or DMSO-d_6 at 300 MHz on Varian Unity instruments. Chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane. Low-resolution mass spectral analyses were performed on HP5988A (NH_3−CI) and Micromass Platform II (ESI) instruments. High-resolution mass spectra were obtained on VG70-VSE (NH_3-DCI) and Finnigan MAT95S (ESI) instruments. Combustion analyses were performed by Quantitative Technologies Inc., Whitehouse, NJ, and are within ±0.4% of the theoretical values. Reactions were performed under an atmosphere of dry nitrogen or argon in flame-dried glassware and were monitored for completeness by thin-layer chromatography (TLC) using silica gel 60 F-254 (0.25 mm) plates. Visualization of TLC plates was accomplished by I_2 vapor, phosphomolybdic acid in ethanol, ceric ammonium molybdate in aqueous methanol, or UV light absorption at 254 nm. Flash column chromatography was performed by the method of Still[27] using 230−400 mesh silica gel (E Merck). Tetrahydrofuran was distilled from potassium/benzophenone ketyl immediately prior to use. Other solvents and reagents were purchased from commercial sources and were used without further purification.

Compounds **1, 2,** and **4w** were prepared according to the procedures disclosed by Chen.[12]

Representative Procedure for the Preparation of 5-Amino-3-(methylthio)pyrazole-4-carboxamides (3). 5-Amino-4-carboxamido-1-(2,4-dimethylphenyl)-3-(methylthio)pyrazole (3n). To a stirred solution of 621 mg (2.94 mmol) of **2** in 10 mL of MeOH was added 609 mg (3.53 mmol) of 2,4-dimethylphenylhydrazine hydrochloride followed by 610 μL (4.41 mmol) of Et_3N. The solution was stirred overnight at reflux, cooled to ambient temperature, and treated with 30 mL of water. The resulting solid was filtered and rinsed with water. Recrystallization from EtOAc−hexanes afforded 681 mg (84%) of **3n** as an off-white solid: mp 148−149 °C; ^{1}H NMR (DMSO-d_6) δ 7.18 (s, 1H), 7.12 (s, 2H), 6.78 (br s, 2H), 6.13 (d, 2H, J = 6.6 Hz), 2.41 (s, 3H), 2.31 (s, 3H), 1.99 (s, 3H); MS (*m/z*) 277 $(M + H)^+$.

5-Amino-1-benzyl-4-carboxamido-3-(methylthio)pyrazole (3a): mp 118−120 °C; ^{1}H NMR ($CDCl_3$) δ 7.18−7.36 (m, 5H), 5.21 (s, 2H), 5.16 (s, 2H), 2.54 (s, 3H); MS (*m/z*) 245 $(M + H - H_2O)^+$.

5-Amino-1-*n*-butyl-4-carboxamido-3-(methylthio)pyrazole (3b): mp 114−116 °C; ^{1}H NMR (DMSO-d_6) δ 6.77 (br s, 2H), 6.38 (s, 2H), 3.85 (t, 2H, J = 7.3 Hz), 2.42 (s, 3H), 1.57−1.67 (m, 2H), 1.19−1.31 (m, 2H), 0.89 (t, 3H, J = 7.3 Hz). MS (*m/z*) 229 $(M + H)^+$.

5-Amino-1-*tert*-butyl-4-carboxamido-3-(methylthio)pyrazole (3c): mp 137−139 °C; ^{1}H NMR (DMSO-d_6) δ 6.81 (br s, 2H), 6.33 (d, 2H, J = 8.0 Hz), 2.39 (s, 3H), 1.48 (s, 9H); MS (*m/z*) 227 $(M - H)^-$.

5-Amino-4-carboxamido-1-(2-hydroxyethyl)-3-(methylthio)pyrazole (3d): ^{1}H NMR (DMSO-d_6) δ 6.74 (br s, 2H), 6.24 (s, 2H), 4.91 (t, 1H, J = 5.1 Hz), 3.89 (t, 2H, J = 5.7 Hz), 3.59−3.71 (m, 2H), 2.38 (s, 3H).

5-Amino-4-carboxamido-3-(methylthio)-1-phenylpyrazole (3e): mp 147−148 °C; ^{1}H NMR ($CDCl_3$) δ 7.45−7.99 (m, 4H), 7.37−7.42 (m, 1H), 5.73 (s, 2H), 2.59 (s, 3H); MS (*m/z*) 249 $(M + H)^+$.

5-Amino-4-carboxamido-3-(methylthio)-1-(pyrid-2-yl)pyrazole (3f): mp 192−193 °C; ^{1}H NMR (DMSO-d_6) δ 8.39−8.42 (m, 1H), 7.92−7.99 (m, 1H), 7.81 (dd, 1H, J = 8.4, 0.9 Hz), 7.70 (s, 2H), 7.24−7.28 (m, 1H), 6.74 (br s, 2H), 2.54 (s, 3H); MS (*m/z*) 250 $(M + H)^+$.

5-Amino-4-carboxamido-1-(3-chlorophenyl)-3-(methylthio)pyrazole (3g): mp 138−140 °C; ^{1}H NMR ($CDCl_3$) δ 7.45 (t, 1H, J = 1.8 Hz), 7.25−7.37 (m, 2H), 7.18 (dd, 1H, J = 6.2, 1.7 Hz), 5.93 (s, 2H), 2.41 (s, 3H); MS (*m/z*) 283 $(M + H)^+$.

5-Amino-4-carboxamido-1-(4-chlorophenyl)-3-(methylthio)pyrazole (3h): mp 168−169 °C; ^{1}H NMR ($CDCl_3$) δ 7.46−7.53 (m, 4H), 5.70 (s, 2H), 2.58 (s, 3H); MS (*m/z*) 283 $(M + H)^+$.

5-Amino-4-carboxamido-1-(4-isopropylphenyl)-3-(methylthio)pyrazole (3i): mp 86−87 °C; ^{1}H NMR ($CDCl_3$) δ 7.44 (d, 2H, J = 8.1), 7.35 (d, 2H, J = 7.8 Hz), 5.66 (s, 2H), 2.96 (m, 1H), 2.58 (s, 3H), 1.27 (d, 6H, J = 6.9 Hz); MS (*m/z*) 291 $(M + H)^+$.

5-Amino-4-carboxamido-1-(2-methoxyphenyl)-3-(methylthio)pyrazole (3j): mp 220−221 °C; ^{1}H NMR ($CDCl_3$) δ 7.38−7.45 (m, 2H), 7.05−7.12 (m, 2H), 5.63 (s, 2H), 3.88 (s, 3H), 2.58 (s, 3H); MS (*m/z*) 279 $(M + H)^+$.

5-Amino-4-carboxamido-1-(2-chlorophenyl)-3-(methylthio)pyrazole (3k): mp 164−165 °C; ^{1}H NMR (DMSO-d_6) δ 7.65 (d, 1H, J = 7.9 Hz), 7.47−7.56 (m, 3H), 6.76 (br s, 2H), 6.34 (d, 2H, J = 5.5 Hz), 2.41 (s, 3H); MS (*m/z*) 283 $(M + H)^+$.

5-Amino-4-carboxamido-1-(4-chloro-2-methylphenyl)-3-(methylthio)pyrazole (3l): mp 148−149 °C; ^{1}H NMR (DMSO-d_6) δ 7.36 (s, 1H), 7.24−7.33 (m, 2H), 5.39 (s, 2H), 2.57 (s, 3H), 2.16 (s, 3H); MS (*m/z*) 298 $(M + H)^+$.

5-Amino-4-carboxamido-1-(2,3-dichlorophenyl)-3-(methylthio)pyrazole (3m): ^{1}H NMR ($CDCl_3$) δ 7.77−7.83 (m, 1H), 7.45−7.53 (m, 2H), 6.77 (br s, 2H), 6.45 (s, 2H), 2.40 (s, 3H); MS (*m/z*) 315 $(M - H)^-$.

5-Amino-4-carboxamido-1-(2,4-dichlorophenyl)-3-(methylthio)pyrazole (3o): mp 168−169 °C; ^{1}H NMR ($CDCl_3$) δ 7.59 (s, 1H), 7.42 (s, 2H), 5.51 (br s, 2H), 2.58 (s, 3H); MS (*m/z*) 315 $(M - H)^-$.

5-Amino-4-carboxamido-1-(2,5-dimethylphenyl)-3-(methylthio)pyrazole (3p): ^{1}H NMR (DMSO-d_6) δ 7.25 (d, 1H, J = 7.5 Hz), 7.19 (dd, 1H, J = 8.1, 0.9 Hz), 7.07 (s, 1H), 6.77 (br s, 2H), 6.15 (d, 2H, J = 6.0 Hz), 2.41 (s, 3H), 2.29 (s, 3H), 1.98 (s, 3H); MS (*m/z*) 275 $(M - H)^-$.

5-Amino-4-carboxamido-1-(2,5-dichlorophenyl)-3-(methylthio)pyrazole (3q): mp 196−197 °C; ^{1}H NMR (DMSO-d_6) δ 7.68 (d, 1H, J = 8.7 Hz), 7.67 (d, 1H, J = 1.9 Hz), 7.61 (dd, 1H, J = 8.4, 2.6 Hz), 6.77 (br s, 2H), 6.48 (s, 1H), 6.46 (s, 1H), 2.41 (s, 3H); MS (*m/z*) 317 $(M + H)^+$.

5-Amino-4-carboxamido-1-(2-chloro-5-(trifluoromethyl)phenyl)-3-(methylthio)pyrazole (3r): mp 196−197 °C; ^{1}H NMR ($CDCl_3$) δ 7.78 (s, 1H), 7.71 (s, 2H), 7.61 (dd, 1H, J = 8.4, 2.6 Hz), 5.58 (br s, 2H), 2.59 (s, 3H); MS (*m/z*) 351 $(M + H)^+$.

5-Amino-4-carboxamido-1-(2-chloro-6-fluorophenyl)-3-(methylthio)pyrazole (3s): mp 169−170 °C; ^{1}H NMR ($CDCl_3$) δ 7.37−7.50 (m, 2H), 7.21(td, 1H, J = 8.2, 1.6 Hz), 5.48 (br s, 2H), 2.58 (s, 3H); MS (*m/z*) 301 $(M + H)^+$.

5-Amino-4-carboxamido-1-(2,6-dichlorophenyl)-3-(methylthio)pyrazole (3t): mp 156−159 °C; ^{1}H NMR ($CDCl_3$) δ 7.65 (d, 1H, J = 8.8 Hz), 7.65 (d, 1H, J = 7.3 Hz), 7.71 (dd, 1H, J = 9.3, 6.8 Hz), 6.77 (br s, 2H), 6.50 (s, 2H), 2.39 (s, 3H); MS (*m/z*) 317 $(M + H)^+$.

5-Amino-4-carboxamido-1-(2,4-dichloro-6-(trifluoromethyl)phenyl)-3-(methylthio)pyrazole (3u): mp 180−181°C; ^{1}H NMR (DMSO-d_6) δ 8.28 (d, 1H, J = 2.6 Hz), 8.03 (d, 1H, J = 2.3 Hz), 6.75 (br s, 2H), 6.57 (d, 2H, J = 3.3 Hz), 2.37 (s, 3H); MS (*m/z*) 385 $(M + H)^+$.

5-Amino-4-carboxamido-1-(2,4-dichloro-6-methylphenyl)-3-(methylthio)pyrazole (3v): mp 180−182 °C; ^{1}H NMR ($CDCl_3$) δ 7.41 (d, 1H, J = 2.2 Hz), 7.28 (d, 1H, J = 2.2 Hz), 5.37 (br s, 2H), 2.57 (s, 3H), 2.13 (s, 3H); MS (*m/z*) 331 $(M + H)^+$.

5-Amino-4-carboxamido-3-(methylthio)-1-(2,4,6-trimethylphenyl)pyrazole (3x): mp 163−164 °C; ^{1}H NMR (DMSO-d_6) δ 8.28 (d, 1H, J = 2.6 Hz), 8.03 (d, 1H, J = 2.3 Hz), 6.75 (br s, 2H), 6.57 (d, 2H, J = 3.3 Hz), 2.37 (s, 3H); MS (*m/z*) 289 $(M - H)^-$.

Representative Procedure for the Preparation of 6-Methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]-pyrimidin-4-ones (4) from 5-Amino-4-carboxamido-3-(methylthio)pyrazoles (3): 1-(2,4-Dimethylphenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4n). To a stirred solution of 160 mg (0.58 mmol) of **3n** in 5 mL of EtOH was added 0.46 mL (4.6 mmol) of EtOAc followed by 1.7 mL (4.6 mmol) of a 2.66 M solution of NaOEt in EtOH. The solution was stirred for 6 h at reflux, cooled to ambient temperature, and treated with 30 mL of 3% aqueous HOAc. The resulting solid was filtered and rinsed with water. Recrystallization from EtOAc−hexanes afforded 126 mg (72%) of **4n** as an off-white solid: mp 246−249 °C; ^{1}H NMR (DMSO-d_6) δ 12.15 (br s, 1H), 7.17−7.21 (m, 2H), 7.15 (d, 1H, J = 8.1), 2.49 (s, 3H), 2.32 (s, 3H), 2.24 (s, 3H), 1.99 (s, 3H); MS (*m/z*) 299 $(M - H)^-$. Anal. ($C_{15}H_{16}N_4OS$) C, H, N, S.

1-Benzyl-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4a): yield 9%; mp 262−264 °C; ^{1}H NMR ($CDCl_3$) δ 10.50 (br s, 1H), 7.27−7.35 (m, 5H), 5.43 (s, 2H), 2.62 (s, 3H, CH_3), 2.51 (s, 3H, CH_3); MS m/z calcd for $C_{14}H_{14}N_4SO$ (M^+) 286.0888, found 286.0947.

1-*n*-Butyl-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4b): yield 32%; mp 211−213 °C; ^{1}H NMR (DMSO-d_6) δ 12.02 (br s, 1H), 4.17 (t, 2H, J = 7.1 Hz), 2.53 (s, 3H), 2.35 (s, 3H), 1.69−1.80 (m, 2H), 1.20−1.32 (m, 2H), 0.89 (t, 3H, J = 7.4 Hz); MS (*m/z*) 251 $(M - H)^-$. Anal. ($C_{11}H_{16}N_4OS$) C, H, N, S.

1-*tert*-Butyl-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4c): yield 29%; ^{1}H NMR (DMSO-d_6) δ 11.99 (br s, not integrated), 2.48 (s, 3H), 2.30 (s, 3H), 1.62 (t, 9H); MS (*m/z*) 251 $(M - H)^-$.

1-(2-Hydroxyethyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4d): yield 43%; mp 251−253 °C; ^{1}H NMR (DMSO-d_6) δ 11.97 (br s, 1H), 4.81 (t, 1H, J = 5.3 Hz), 4.17 (t, 2H, J = 5.9 Hz), 3.68−3.75 (m, 2H), 2.49 (s, 3H), 2.30 (s, 3H); MS (*m/z*) 239 $(M - H)^-$. Anal. ($C_9H_{12}N_4O_2S \cdot 0.1H_2O$) C, H, N, S.

6-Methyl-3-(methylthio)-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4e) was prepared as example **4n**, except that precipitation from the reaction with water gave the sodium salt: yield 90%; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 8.08 (d, 2H, J = 8.1 Hz), 7.48 (t, 2H, J = 8.1 Hz), 7.27 (t, 1H, J = 8.0 Hz), 2.56 (s, 3H), 2.32 (s, 3H); MS m/z calcd for $C_{13}H_{13}N_4SO$ $(M + H)^+$ 273.0810, found 273.0805. Anal. ($C_{13}H_{11}N_4OSNa \cdot 0.25EtOAc$) C, H, N, S.

6-Methyl-3-(methylthio)-1-(pyrid-2-yl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4f): yield 77%; mp 297−299 °C; ^{1}H NMR (DMSO-d_6) δ 8.54 (dd, 1H, J = 4.7, 0.8 Hz), 7.91−8.02 (m, 2H), 7.37−7.42 (m, 1H), 2.55 (s, 3H), 2.32 (s, 3H); MS m/z calcd for $C_{12}H_{12}N_5SO$ $(M + H)^+$ 274.0762, found 274.0759.

1-(3-Chlorophenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4g): yield 9%; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.40 (br s, 1H), 8.16 (t, 1H, J = 1.0 Hz), 8.05−8.09 (m, 1H), 7.53 (t, 1H, J = 7.7 Hz), 7.36−7.41 (m, 1H), 2.59 (s, 3H), 2.40 (s, 3H); MS m/z calcd for $C_{13}H_{11}N_4SOCl$ $(M)^+$ 306.0342, found 306.0340.

1-(4-Chlorophenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4h): yield 62%; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.40 (br s, not integrated), 8.16 (d, 2H, J = 7.6 Hz), 7.61 (d, 2H, J = 7.6 Hz), 2.81 (s, 3H), 2.41 (s, 3H); MS m/z calcd for $C_{13}H_{12}N_4SOCl$ $(M + H)^+$ 307.0418, found 307.0420. Anal. ($C_{13}H_{11}N_4OSCl \cdot {}^1/_4H_2O$) C, H, N, S, Cl.

1-(4-Isopropylphenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4i): yield 16%; mp 271−273 °C; ^{1}H NMR ($CDCl_3$) δ 11.10 (br s, not integrated), 7.91 (d, 2H, J = 7.6 Hz), 7.33 (d, 2H, J = 7.6 Hz), 2.91−3.01 (m, 1H), 2.70 (s, 3H), 2.57 (s, 3H), 1.30 (d, 6H, J = 7.7 Hz); MS m/z calcd for $C_{16}H_{18}N_4SO$ (M^+) 314.1201, found 314.1200.

1-(2-Methoxyphenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4j): yield 35%; mp 274−275 °C; ^{1}H NMR ($CDCl_3$) δ 10.58 (br s, 1H), 7.39−7.48 (m, 2H), 7.08−7.12 (m, 2H), 3.80 (s, 3H), 2.69 (s, 3H), 2.49 (s, 3H); MS m/z calcd for $C_{14}H_{15}N_4SO_2$ $(M + H)^+$ 303.0916, found 303.0895. Anal. ($C_{14}H_{14}N_4O_2S \cdot {}^1/_4MeOH$) C, H, N, S.

1-(2-Chlorophenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4k): yield 16%; mp 278−279 °C; ^{1}H NMR ($CDCl_3$) δ 10.58 (br s, 1H), 7.41−7.60 (m, 4H), 2.66 (s, 3H), 2.49 (s, 3H); MS m/z calcd for $C_{13}H_{11}N_4SOCl$ (M^+) 306.0342, found 306.0320. Anal. ($C_{13}H_{11}N_4OSCl \cdot {}^1/_4H_2O$) C, H, N, S, Cl.

1-(4-Chloro-2-methylphenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4l): yield 51%; mp 268−269 °C; ^{1}H NMR (DMSO-d_6) δ 12.24 (br s, not integrated), 7.43 (s, 1H), 7.34 (s, 2H), 2.57 (s, 3H), 2.31 (s, 3H), 2.17 (s, 3H); MS m/z calcd for $C_{14}H_{14}N_4SOCl$ $(M + H)^+$ 321.0577, found 321.0575. Anal. ($C_{14}H_{13}N_4OSCl \cdot {}^1/_4H_2O$) C, H, N, S, Cl.

1-(2,3-Dichlorophenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4m): yield 21%; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.29 (br s, 1H), 7.86 (dd, 1H, J = 8.4, 2.2 Hz), 7.50−7.65 (m, 2H), 2.51 (s, 3H), 2.26 (s, 3H); MS m/z calcd for $C_{13}H_{11}N_4SOCl_2$ $(M + H)^+$ 341.0031, found 341.0029.

1-(2,4-Dichlorophenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4o): yield 28%; mp 298−299 °C; ^{1}H NMR ($CDCl_3$) δ 11.83 (br s, 1H), 7.24 (d, 1H, J = 2.1 Hz), 7.13 (d, 1H, J = 8.3 Hz), 7.07 (dd, 1H, J = 8.1, 2.1 Hz), 2.26 (s, 3H), 2.02 (s, 3H); MS m/z calcd for $C_{13}H_{10}N_4SOCl_2$ (M^+) 339.9952, found 339.9968. Anal. ($C_{13}H_{10}N_4OSCl_2$) C, H, N, S, Cl.

1-(2,5-Dimethylphenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4p): yield 34%; mp 257−258 °C; ^{1}H NMR (DMSO-d_6) δ 12.16 (br s, 1H), 7.12−7.28 (m, 3H), 2.49 (s, 3H), 2.29 (s, 3H), 2.25 (s, 3H), 1.97 (s, 3H); MS (*m/z*) 299 $(M - H)^-$. Anal. ($C_{15}H_{16}N_4OS$) C, H, N, S.

1-(2,5-Dichlorophenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4q): yield 53%; mp 263−264 °C; ^{1}H NMR (DMSO-d_6) δ 12.03 (br s, not integrated), 7.36−7.32 (m, 2H), 7.30 (dd, 1H, J = 8.4, 2.4 Hz), 2.50 (s, 3H), 2.27 (s, 3H); MS m/z calcd for $C_{13}H_{10}N_4SOCl_2$ (M^+) 339.9952, found 339.9947. Anal. ($C_{13}H_{10}N_4OSCl_2$) C, H, N, S, Cl.

1-(2-Chloro-5-trifluoromethylphenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4r): yield 56%; mp 259−260 °C; ^{1}H NMR ($CDCl_3$) δ 11.47 (br s, 1H), 7.81 (s, 1H), 7.67−7.75 (m, 2H), 2.66 (s, 3H), 2.53 (s, 3H); MS m/z calcd for $C_{14}H_{11}N_4SF_3OCl$ (M^+) 375.0294, found 375.0298.

1-(2-Chloro-6-fluorophenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4s): yield 67%; mp 276−277 °C; ^{1}H NMR ($CDCl_3$) δ 11.39 (br s, 1H), 7.39−7.47 (m, 2H), 7.18−7.23 (m, 1H), 2.65 (s, 3H), 2.51 (s, 3H); MS m/z calcd for $C_{13}H_{11}N_4SFOCl$ (M^+) 325.0326, found 325.0302.

1-(2,6-Dichlorophenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4t): yield 42%; mp 306−307 °C; ^{1}H NMR ($CDCl_3$) δ 11.42 (br s, not integrated), 7.50 (d, 2H, J = 8.1 Hz), 7.41 (dd, 1H, J = 9.0, 6.0 Hz), 2.66 (s, 3H), 2.51 (s, 3H); MS m/z calcd for $C_{13}H_{10}N_4SOCl_2$ (M^+) 339.9952, found 339.9954. Anal. ($C_{13}H_{10}N_4OSCl_2$) C, H, N, S, Cl.

1-(2,4-Dichloro-6-trifluoromethenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4u): yield 57%; mp 250−253 °C; ^{1}H NMR (DMSO-d_6) δ 12.41 (br s, 1H), 8.38 (d, 1H, J = 2 Hz), 8.17 (d, 1H, J = 2 Hz), 2.49 (s, 3H), 2.30 (s, 3H).

1-(2,4-Dichloro-6-methylphenyl)-6-methyl-3-(methylthio)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4v): yield 76%; mp 287−289 °C; ^{1}H NMR (DMSO-d_6) δ 12.29 (br s, 1H), 7.71 (d, 1H), 7.55 (s, 1H), 2.48 (s, 3H), 2.25 (s, 3H), 1.97 (s, 3H); MS m/z calcd for $C_{14}H_{12}N_4SOCl_2$ (M^+) 355.0187, found 355.0174. Anal. ($C_{14}H_{12}N_4OSCl_2$) C, H, N, S, Cl.

6-Methyl-3-(methylthio)-1-(2,4,6-trimethylphenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (4x): yield 66%. ^{1}H NMR (DMSO-d_6) δ 12.15 (br s, 1H, NH), 7.17−7.21 (m, 2H, Ph), 7.00 (s, 2H, Ph), 2.48 (s, 3H, SCH_3), 2.28 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 1.84 (s, 6H, CH_3); MS (*m/z*) 313 ($M - H)^-$.

Representative Procedure for the Preparation Alkoxyalkylidenemalononitriles (5) from Ortho Esters and Malononitrile: 2-Cyano-3-methoxyhept-2-enenitrile (5g). To a stirred solution of 2.64 g (40 mmol) of malononitrile in 9 mL of acetic anhydride was added 6.49 g (40 mmol) of trimethyl orthovalerate. The solution was stirred for 15 h at reflux, cooled, and poured into water. The mixture was extracted with ether, and the organic extract was washed with aqueous $NaHCO_3$ and then brine. The solution was dried ($MgSO_4$) and concentrated under reduced pressure to afford 5.9 g (90%) of **5g** as a pale amber oil: ^{1}H NMR ($CDCl_3$) δ 4.15 (s, 3H), 2.65 (t, 2H, J = 7.3 Hz), 1.40−1.69 (m, 4H), 0.98 (t, 3H, J = 7.2 Hz).

2-Cyano-3-methoxycinnamonitrile (5e): yield 81%; ^{1}H NMR ($CDCl_3$) δ 7.40−7.68 (m, 5H), 3.94 (s, 3H); MS (*m/z*) 202 ($M + NH_4)^+$.

2-Carboxamido-3-methoxyhex-2-enenitrile (6h). To a stirred solution of 3.36 g (40 mmol) of cyanoacetamide in 9 mL of acetic anhydride was added 6.72 mL (42 mmol) of trimethyl orthobutyrate. The solution was stirred for 16 h at reflux, cooled, and diluted with ether. The resulting solid was filtered and rinsed with ether to afford 5.9 g (42%) of **5h** as an off-white solid: mp 102−103 °C; ^{1}H NMR (DMSO-d_6) δ 7.47 (br s, 1H), 7.24 (br s, 1H), 3.99 (s, 3H), 2.63 (t, 2H, J = 6.8 Hz), 1.54−1.66 (m, 2H), 0.98 (t, 3H, J = 7.5 Hz).

5-Amino-3,4-dicyano-1-(2,4,6-trichlorophenyl)pyrazole (7a). To a stirred solution of 25.0 g (118 mmol) of 2,4,6-trichlorophenyl hydrazine in 250 mL of absolute ethanol at 0 °C was added 15.1 g (118 mmol) of tetracyanoethylene (**CAUTION:** Evolves HCN!). The solution was stirred for 10 min and was warmed to ambient temperature. After stirring for 1 h, the solid was filtered and washed with ethanol to afford 25.4 g (69%) of **7a** as a white powder: ^{1}H NMR (DMSO-d_6) δ 8.00 (s, 2H), 7.65 (s, 2H); MS (*m/z*) 310 ($M - H)^-$; IR (KBr) ν 3390, 2252, 2236, 1652 cm^{-1}.

Representative Procedure for the Preparation of 5-Amino-4-cyano-1-(2,4,6-trichlorophenyl)pyrazoles (7) from Alkoxyalkylidenemalononitriles (5): 5-Amino-3-*n*-butyl-4-cyano-1-(2,4,6-trichlorophenyl)pyrazole (7g). To a stirred solution of 4.10 g (25 mmol) of 2-cyano-3-methoxyhept-2-enenitrile in 60 mL of MeOH was added 5.92 g (28 mmol) of 2,4,6-trichlorophenylhydrazine. The solution was stirred for 2 h at reflux, after which time it was cooled to ambient temperature and poured into 1 N aqueous HCl. The mixture was extracted with EtOAc, and the organic extract was washed with aqueous $NaHCO_3$ and then brine. The solution was dried ($MgSO_4$) and concentrated under reduced pressure. Chromatography on silica gel (elution with 1:1 THF−hexanes, then THF) afforded, after evaporation of solvents, 6.6 g (77%) of **7g** as an off-white solid: mp 123−124 °C; ^{1}H NMR (DMSO-d_6) δ 7.89 (s, 2H), 6.80 (s, 2H), 2.44−2.49 (m, 2H), 1.50−1.60 (m, 2H), 1.22−1.34 (m, 2H), 0.84 (t, 3H, J = 7.3 Hz); MS (*m/z*) 343 ($M + H)^+$.

5-Amino-4-cyano-3-phenyl-1-(2,4,6-trichlorophenyl)-pyrazole (7e): yield 68%; mp 193−194 °C; ^{1}H NMR (DMSO-d_6) δ 8.00 (s, 2H), 7.83 (dd, 2H, J = 8.3, 1.7 Hz), 7.42−7.53 (m, 3H), 7.09 (s, 2H); MS (*m/z*) 363 ($M + H)^+$.

5-Amino-4-cyano-3-ethyl-1-(2,4,6-trichlorophenyl)pyrazole (7i): yield 83%; mp 152−153 °C (hemietherate); ^{1}H NMR (DMSO-d_6) δ 7.93 (s, 1H), 6.85 (s, 2H), 2.48−2.60 (m, 2H), 1.18 (t, 3H, J = 7.5 Hz); MS m/z calcd for $C_{12}H_9N_4Cl_3$ (M^+) 313.9893, found 313.9878.

5-Amino-4-cyano-3-methyl-1-(2,4,6-trichlorophenyl)-pyrazole (7j): yield 67%; mp 229−230 °C; ^{1}H NMR ($CDCl_3$) δ 7.52 (s, 2H), 4.32 (s, 2H), 2.34 (s, 3H); MS (*m/z*) 299 ($M - H)^-$.

5-Amino-4-cyano-1-(2,4,6-trichlorophenyl)pyrazole (7k): yield 49%; MS (*m/z*) 287 ($M + H)^+$.

5-Amino-4-cyano-3-(2-hydroxyethyl)-1-(2,4,6-trichlorophenyl)pyrazole (7m). To a stirred solution of 1.87 g (6.0 mmol) of 2-(*tert*-butyldiphenylsilyloxy)propionaldehyde in 30 mL of ethanol was added 1.40 g (6.6 mmol) of 2,4,6-trichlorophenylhydrazine. The solution was stirred for 3 h at reflux, and about half the solvent was evaporated. The solution was cooled, poured into ether, and washed twice with 1 N aqueous HCl. The solution was then washed with aqueous $NaHCO_3$ and then brine, dried ($MgSO_4$), and concentrated under reduced pressure to afford 5.04 g (quant.) of an oil. A 1.52 g (3.0 mmol) portion of the crude hydrazone was dissolved in 10 mL of dry DMF, treated with 0.59 g (3.3 mmol) of *N*-bromosuccinimide, and stirred for 1 h at ambient temperature. The dark solution was poured into half-saturated brine, and this mixture was extracted with ether. The organic extract was washed with water and then brine, dried ($MgSO_4$), and concentrated under reduced pressure to afford a dark oil. Malononitrile anion was generated in ethanol by treatment of 330 mg (5.0 mmol) of malononitrile in 10 mL of ethanol with 2.0 mL of 2.7 M sodium ethoxide in ethanol. This mixture was treated at ambient temperature with the crude bromohydrazone in 10 mL of absolute ethanol, and the reaction was then refluxed for 30 min. The reaction was cooled to room temperature, stirred another 16 h, and quenched with 10% aqueous HOAc. The mixture was extracted with ether, and the organic extract was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel (elution with hexanes, then ether−hexanes) afforded, after removal of solvents, 1.11 g (65%) of 5-amino-4-cyano-3-(2-(*tert*-butyldiphenylsilyloxy)ethyl)-1-(2,4,6-trichlorophenyl)pyrazole as a light brown foam: ^{1}H NMR (DMSO-d_6) δ 7.89 (s, 2H), 7.58 (dd, 4H, J = 7.5, 1.5 Hz), 7.33−7.43 (m, 6H), 6.83 (s, 2H), 3.87 (t, 2H, J = 6.5 Hz), 2.76 (t, 2H, J = 6.2 Hz), 0.92 (s, 9H).

To a stirred solution of 0.36 g (0.63 mmol) of 5-amino-4-cyano-3-(2-(*tert*-butyldiphenylsilyloxy)ethyl)-1-(2,4,6-trichlorophenyl)pyrazole in 10 mL of THF was added 0.73 mL (2.0 mmol) of aqueous 75 wt % solution of TBAF. The solution was stirred for 2 h and poured into water, and the resulting mixture was extracted with EtOAc. The organic extract was washed with brine, dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography on silica gel (elution with 3:1 hexanes−EtOAc, then 1:1 hexanes−EtOAc) afforded, after removal of solvent, 187 mg (89%) of **7m** as an off-white solid: mp 158−160 °C; ^{1}H NMR (DMSO-d_6) δ 7.89 (s, 2H), 6.81 (s, 2H), 4.71 (t, 1H, J = 5.3 Hz), 3.58−3.66 (m, 2H), 2.62 (t, 2H, J = 7.4 Hz).

Representative Procedure for the Preparation of 5-Amino-4-carboxamido-1-(2,4,6-trichlorophenyl)pyrazoles (8) from 5-Amino-4-cyano-1-(2,4,6-trichlorophenyl)-pyrazoles (7): 5-Amino-3-*n*-butyl-4-carboxamido-1-(2,4,6-trichlorophenyl)pyrazole (8g). A 3.43 g (10 mmol) portion of **7g** was added to 20 mL of concentrated sulfuric acid with rapid stirring. After 2.5 h the solution was added dropwise by pipet to a rapidly stirred solution of saturated aqueous $NaHCO_3$. This mixture was stirred overnight, and the larger chunks were broken up with a spatula. The solid was then

filtered and rinsed with water. Vacuum drying at 70 °C for 3 h afforded 3.56 g (98%) of **8g** as an off-white solid: mp 103−105 °C; ^{1}H NMR (DMSO-d_6) δ 7.89 (s, 2H), 6.58 (br s, 2H), 6.32 (d, 2H, J = 4.8 Hz), 2.67 (t, 2H, J = 7.5 Hz), 1.48−1.58 (m, 2H), 1.22−1.34 (m, 2H), 0.83 (t, 3H, J = 7.4 Hz).

5-Amino-4-carboxamido-3-phenyl-1-(2,4,6-trichlorophenyl)pyrazole (8e): yield 89%; ^{1}H NMR ($CDCl_3$) δ 7.59−7.65 (m, 2H), 7.53 (s, 2H), 7.42−7.57 (m, 3H); MS m/z calcd for $C_{16}H_{11}N_4OCl_3$ (M^+) 379.9998, found 379.9999.

5-Amino-4-carboxamido-3-ethyl-1-(2,4,6-trichlorophenyl)pyrazole (8i): yield 99%; mp 186−188 °C; ^{1}H NMR ($CDCl_3$) δ 7.89 (s, 1H), 6.58 (br s, 2H), 6.35 (s, 2H), 2.70 (m, 2H), 1.10 (t, 3H, J = 7.5 Hz); MS m/z calcd for $C_{12}H_{11}N_4OCl_3$ (M^+) 331.9998, found 331.9989.

5-Amino-4-carboxamido-3-methyl-1-(2,4,6-trichlorophenyl)pyrazole (8j): yield 99%; mp 223−224 °C; ^{1}H NMR (DMSO-d_6) δ 7.88 (s, 2H), 6.59 (br s, 2H), 6.36 (d, 2H, J = 4.4 Hz), 2.25 (s, 3H); MS (m/z) 317($M - H$)$^-$.

5-Amino-4-carboxamido-1-(2,4,6-trichlorophenyl)pyrazole (8k): yield 88%; MS (m/z) 305 ($M + H$)$^+$.

5-Amino-4-carboxamido-3-*n*-propyl-1-(2,4,6-trichlorophenyl)pyrazole (8h): To a stirred solution of 480 mg (2.85 mmol) of **6h** in 11 mL of MeOH was added 698 mg (3.30 mmol) of 2,4,6-trichlorophenylhydrazine. The solution was stirred for 3 h at reflux, cooled, and treated with 4 mL of water. The mixture was filtered, and the crude solid was chromatographed on silica gel (elution with 3:1 then 1:1 ether−hexanes). Concentration of the appropriate fractions afforded 800 mg (81%) of **8h** as an off-white solid: mp 177−178 °C. ^{1}H NMR (DMSO-d_6) δ 11.82 (s, 1H), 7.97 (s, 1H), 7.63 (s, 2H), 2.60−2.72 (m, 2H), 1.53−1.69 (m, 2H), 0.97 (t, 3H, J = 7.5 Hz); MS m/z calcd for $C_{13}H_{13}N_4OCl_3$ (M)$^+$ 346.0155, found 346.0163.

5-Amino-4-carboxamido-3-(2-hydroxyethyl)-1-(2,4,6-trichlorophenyl)pyrazole (8m): A solution of 0.090 g (0.27 mmol) of **7m** in 4 mL of concentrated sulfuric acid was stirred for 2 h at ambient temperature. The solution was added dropwise to a rapidly stirred saturated aqueous solution of sodium carbonate. The mixture was extracted twice with ethyl acetate, and the combined organic extracts were washed (brine), dried ($MgSO_4$), and concentrated under reduced pressure to afford 72 mg (76%) of **8m** as a foam. ^{1}H NMR (DMSO-d_6) δ 7.96 (s, 2H), 6.36 (br s, 2H), 4.23 (t, 2H, J = 5.9 Hz), 2.73 (t, 2H, J = 4.7 Hz); MS (m/z) 331 ($M + H - H_2O$)$^+$.

Representative Procedure for the Preparation of 5-Amino-4-carboxamido-1-(2,4,6-trichlorophenyl)pyrazoles (8) from Aldehydes or Aldehyde Equivalents and 2,4,6-Trichlorophenylhydrazine: 5-Amino-4-carboxamido-1-(2,4,6-trichlorophenyl)-3-trifluoromethylpyrazole (8d). To a stirred solution of 2.88 g (20 mmol) of 1-ethoxy-2,2,2-trifluoroethanol in 15 mL of ethanol was added 3.81 g (18 mmol) of 2,4,6-trichlorophenylhydrazine. The solution was stirred for 16 h at reflux, cooled, and poured into water. A little brine was added, and the mixture was then extracted with ether. The organic extract was washed with brine, dried over $MgSO_4$, and concentrated to give the hydrazone as a chalky, off-white solid. A stirred solution of 583 mg (2.00 mmol) of this hydrazone in 10 mL of dry DMF was treated with 374 mg (2.10 mmol) of *N*-bromosuccinimide, and the solution was stirred for 1 h at ambient temperature. The reaction was diluted with ether and washed three times with water and once with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Cyanoacetamide anion was generated in ethanol by treatment of 127 mg (1.5 mmol) of cyanoacetamide in 5 mL of ethanol with 1.11 mL of 2.7 M sodium ethoxide in ethanol. This mixture was treated at ambient temperature with the crude bromohydrazone in 5 mL of absolute ethanol, and the reaction was then stirred for 16 h at ambient temperature. The reaction was quenched with 10% aqueous HOAc, and the mixture was extracted with ether. The organic extract was washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. Chromatography on silica gel (elution with 3:1 hexanes−EtOAc, then 1:1 hexanes−EtOAc) afforded, after removal of solvent, 230 mg (31%) of **8d** as an amorphous solid: ^{1}H NMR ($CDCl_3$) δ 7.54 (s, 2H), 5.78−6.03 (m, 2H), 5.62−5.76 (m, 2H); ^{19}F NMR ($CDCl_3$) δ −61.5; MS (m/z) 373 ($M + H$)$^+$.

5-Amino-3-benzyl-4-carboxamido-1-(2,4,6-trichlorophenyl)pyrazole (8f): yield 15%; ^{1}H NMR ($CDCl_3$) δ 7.54 (s, 2H), 7.26−7.38 (m, 5H), 5.42 (s, 2H), 5.31 (s, 2H), 4.17 (s, 2H); MS (m/z) 373 ($M + H$)$^+$.

5-Amino-4-carboxamido-3-(hydroxymethyl)-1-(2,4,6-trichlorophenyl)pyrazole (8l): yield 40%. ^{1}H NMR (DMSO-d_6) δ 7.89 (s, 2H), 6.48 (s, 2H), 6.15 (t, 1H, J = 7.3 Hz), 4.01 (d, 2H, J = 7.3 Hz); MS (m/z) 335 ($M + H$)$^+$.

3-Cyano-6-methyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10a). A solution of 10 g (32 mmol) of **7a** in 100 mL of acetic anhydride was refluxed 2 days. The mixture was cooled and concentrated under reduced pressure. The residue was taken up in 200 mL of acetic acid and 10 mL of water and was heated to reflux. After refluxing overnight, the solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5% methanol/CH_2Cl_2) and then triturated in EtOAc to give 2.1 g (18%) of **10a** as a white powder: ^{1}H NMR (DMSO-d_6) δ 12.92 (br s, 1H), 8.06 (s, 2H), 2.33 (s, 3H); MS (m/z) 371 ($M + NH_4$)$^+$; IR (KBr) ν 2242, 1688 cm^{-1}. Anal. [$C_{13}H_6N_5OCl_3 \cdot 0.05CH_2Cl_2$, ($CH_2Cl_2$ detected in NMR)] C, H, N, Cl.

6-Methyl-3-methanesulfonyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10b): A suspension of 150 mg (0.4 mmol) of **4w** in 5 mL of 1:1 MeOH−CH_2Cl_2 was treated with 140 mg (0.8 mmol) of *m*-CPBA. The suspension was stirred for 20 min, becoming clear as the solids dissolve. The reaction was quenched with 0.5 mL of 10% aqueous $NaHSO_3$ and then poured into 10% aqueous Na_2CO_3, and the mixture was extracted with EtOAc. The organic extract was dried ($MgSO_4$), concentrated under reduced pressure, and recrystallized from EtOH to give 95 mg (60%) of **10b** as an amorphous solid: ^{1}HNMR (DMSO-d_6) δ 12.9 (br s, 1 H), 8.1 (s, 2 H), 3.6 (s, 3 H), 2.4 (s, 3 H), HRMS calcd for $C_{13}H_{10}N_4O_3SCl_3$ ($M + H$)$^+$ 406.9539, found 406.9558.

3-Carboxamido-6-methyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10c). A solution of 250 mg (0.71 mmol) of **10a** in 6 mL of concentrated sulfuric acid was stirred overnight. The mixture was poured into ice water and the resulting solid was filtered and washed with water. The solid was triturated in boiling ethanol to give 137 mg (52%) of **10c** as a white powder: ^{1}H NMR (DMSO-d_6) δ 12.95 (br s, 1H), 9.46 (s, 1H), 8.04 (s, 2H), 8.02 (s, 1H), 2.34 (s, 3H); MS (m/z) 372 ($M + H$)$^+$. Anal. ($C_{13}H_8N_5O_2Cl_3$) C, H, N, Cl.

Representative Procedure for the Preparation of 6-Methyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones (10) from 5-Amino-4-carboxamido-1-(2,4,6-trichlorophenyl)pyrazoles (8): 3-*n*-Butyl-6-methyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10g). To a stirred solution of 180 mg (0.50 mmol) of **8g** in 6 mL of EtOH was added 0.46 mL (4.6 mmol) of EtOAc followed by 1.13 mL (3.0 mmol) of a 2.66 M solution of NaOEt in EtOH. The solution was stirred for 16 h at reflux, cooled to ambient temperature, and treated with 10 mL of 10% aqueous HOAc. The resulting solid was filtered and rinsed with 6 mL of 1:1 methanol−water and then 6 mL of 1:1 ether−hexanes. The solid was then briefly air-dried to afford 145 mg (75%) of **10g** as an off-white solid: mp 219−222 °C; ^{1}H NMR (DMSO-d_6) δ 12.25 (br s, 1H), 7.95 (s, 2H), 2.82 (t, 2H, J = 7.3 Hz), 2.26 (s, 3H), 1.81−1.97 (m, 2H), 1.21−1.35 (m, 2H), 0.85 (t, 3H, J = 7.4 Hz); MS m/z calcd for $C_{16}H_{16}N_4OCl_3$($M + H$)$^+$ 385.0390, found 385.0382. Anal. ($C_{16}H_{15}N_4OCl_3$) C, H, N, Cl.

6-Methyl-1-(2,4,6-trichlorophenyl)-3-trifluoromethyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10d): yield 43%; mp >300 °C; ^{1}H NMR ($CDCl_3$) δ 7.55 (s, 2H), 2.55 (s, 3H); MS m/z calcd for $C_{13}H_6N_4OF_3Cl_3$ (M^+) 395.9559, found 395.9559.

6-Methyl-3-phenyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1H-pyrazolo[3,4-*d*]pyrimidin-4-one (10e) was prepared as example **10g** except that precipitation from the reaction with

water gave the sodium salt: yield 64%; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 8.39 (d, 2H, J = 7.9 Hz), 8.00 (s, 2H), 7.39−7.49 (m, 3H), 2.30 (s, 3H); MS m/z calcd for $C_{18}H_{12}N_4OCl_3$ $(M + H)^+$ 405.0077, found 405.0081. Anal. ($C_{18}H_{10}N_4ONaCl_3 \cdot {}^1/_2H_2O$) C, H, N.

3-Benzyl-6-methyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10f): yield 81%; mp 266−270 °C; ^{1}H NMR (DMSO-d_6) δ 12.26 (s, 1H), 8.00 (s, 2H), 7.21−7.60 (m, 4H), 7.12−7.17 (m, 1H), 4.20 (s, 2H), 2.26 (s, 3H); MS (m/z) 417$(M - H)^-$. Anal. ($C_{19}H_{13}N_4OCl_3 \cdot {}^1/_2$cyclohexane) C, H, N, Cl.

6-Methyl-3-*n*-propyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10h): yield 13%; mp 244−246 °C; ^{1}H NMR (DMSO-d_6) δ 12.3 (br s, 1H), 7.99 (s, 2H), 2.80−2.88 (m, 2H), 2.29 (s, 3H), 1.69−1.83 (m, 2H), 0.89 (t, 3H, J = 7.4 Hz); MS m/z calcd for $C_{15}H_{13}N_4OCl_3$ $(M)^+$ 370.0155, found 370.0157.

3-Ethyl-6-methyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10i): yield 78%; ^{1}H NMR (DMSO-d_6) δ 12.31 (br s, 1H), 7.99 (s, 2H), 2.88(q, 2H, J = 7.3 Hz), 2.50 (s, 3H), 1.27 (t, 3H, J = 7.3 Hz). MS m/z calcd for $C_{14}H_{11}N_4OCl_3$ $(M)^+$ 355.9998, found 355.9988. Anal. ($C_{14}H_{11}N_4OCl_3$) C, H, N, Cl.

3,6-Dimethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10j): yield 81%; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.26 (br s, 1H), 7.96 (s, 2H), 2.45 (s, 3H), 2.26 (s, 3H); MS m/z calcd for $C_{13}H_9N_4OCl_3$ $(M)^+$ 341.9842, found 341.9840. Anal. ($C_{13}H_9N_4OCl_3$) C, H, N, Cl.

6-Methyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10k): yield 47%. ^{1}H NMR ($CDCl_3$) δ 12.2 (bs, 1H), 8.25 (s, 2H), 7.3 (s, 1H), 2.6 (s, 3H); MS m/z calcd for $C_{12}H_8N_4OCl_3$ $(M + H)^+$ 328.9764, found: 328.9778.

3-(Hydroxymethyl)-6-methyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10l): yield 58%. ^{1}H NMR (DMSO-d_6) δ 12.40 (s, 1H), 7.97 (s, 2H), 5.26−5.37 (m, 1H), 4.68 (br s, 2H), 2.79 (t, 2H, J = 7.5 Hz), 2.28 (s, 3H); MS (m/z) 357 $(M - H)^-$.

3-(2-Hydroxyethyl)-6-methyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (10m): yield 66%; ^{1}H NMR (DMSO-d_6) δ 10.54 (s, 1H), 7.97 (s, 2H), 4.81 (t, 1H, J = 5 Hz), 3.64−3.68 (m, 2H), 2.79 (t, 2H, J = 7.5 Hz), 1.96 (s, 3H); MS (m/z) 371 $(M - H)^-$.

5,6-Dimethyl-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (11). To a stirred solution of 250 mg (0.70 mmol) of **10i** in 10 mL of *N,N*-dimethylacetamide were added 130 mg (0.70 mmol) of methyl *p*-toluenesulfonate and 113 mg (0.82 mmol) of potassium carbonate, and the mixture was heated to 100 °C. After stirring overnight, the solvent was removed under reduced pressure and the residue was diluted with water and extracted with EtOAc. The combined organic layers were washed with water and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 2.5% methanol/CH_2Cl_2) to give 115 mg (44%) of **11** as a white powder: ^{1}H NMR($CDCl_3$) δ 7.50 (s, 2H), 3.58 (s, 3H), 3.05(q, J = 7.7 Hz), 2.53 (s, 3H), 1.39 (t, J = 7.7 Hz, 3H); MS (m/z) 371 $(M + H)^+$. Anal. ($C_{15}H_{13}N_4OCl_3$) C, H, N, Cl.

3-Ethyl-4-methoxy-6-methyl-1-(2,4,6-trichlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (12b). A solution of 100 mg (0.28 mmol) of **10i** in 1 mL of $POCl_3$ was stirred for 30 min at reflux. The solution was cooled and poured onto ice, and resulting white solid was then stirred vigorously for a few minutes. The mixture was filtered, and the product was rinsed with water and air-dried. The white solid was suspended in 1:3 ether−hexanes and filtered. The filtrate was concentrated under reduced pressure and suspended in 2 mL of MeOH. This suspension was treated with 0.05 mL of a 4.4 M solution of NaOMe in MeOH, warmed to reflux, and stirred for 30 min. The mixture was cooled to ambient temperature, and 6 mL of water was added. The white solid was filtered, rinsed with water then hexanes, and air-dried to afford 34 mg (35%) of **12b** as a white solid: mp 154−158 °C; ^{1}H NMR (DMSO-d_6) δ 7.98 (s, 2H), 4.09 (s, 3H), 2.93 (q, 2H, J = 7.6 Hz), 2.49 (s, 3H), 1.27 (t, 3H, J = 7.6 Hz); MS m/z calcd for $C_{15}H_{14}N_4OCl_3$ $(M + H)^+$ 371.0233, found 371.0257. Anal. ($C_{15}H_{13}N_4OCl_3 \cdot {}^1/_3H_2O$) C, H, N, Cl.

4-Amino-3-ethyl-6-methyl-1-(2,4,6-trichlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidine (12c). A solution of 548 mg (1.42 mmol) of **13** in 20 mL of saturated ammonia in ethanol was stirred for 2 days. The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 50 to 75% EtOAc/hexanes) to give 122 mg (24%) of **12c** as a white powder: ^{1}H NMR (DMSO-d_6) δ 7.93 (s, 2H), 2.97 (q, 2H, J = 7.7 Hz), 2.27 (s, 3H), 1.20 (t, 3H, J = 7.7 Hz); MS (m/z) 356 $(M + H)^+$. Anal. ($C_{14}H_{12}N_5Cl_3$) C, H, N, Cl.

Ethyl *N*-(3-Ethyl-4-cyano-6-methyl-1-(2,4,6-trichlorophenyl)pyrazol-5-yl)acetimidate (13). A stirred solution of 5.0 g (15.8 mmol) of **7i** in 5.64 g (34.7 mmol) of triethyl orthoacetate and 3.54 g (34.7 mmol) of acetic anhydride was heated to 130 °C. After stirring for 3 h, the solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 25−33% EtOAc/hexanes) to give 5.57 g (91%) of **13** as a white powder: ^{1}H NMR (DMSO-d_6) δ 7.92 (s, 2H), 3.97 (q, 2H, J = 6.9 Hz), 2.67 (q, 2H, J = 7.5 Hz), 2.09 (s, 3H), 1.21 (t, 3H, J = 7.5 Hz), 1.03 (t, 3H, J = 6.9 Hz); MS (m/z) 385 $(M + H)^+$.

5-Amino-3-ethyl-1-(2,4,6-trichlorophenyl)pyrazole (14). To a stirred solution of 10.0 g (47 mmol) of 2,4,6-trichlorophenylhydrazine in 200 mL of ethanol was added 4.6 g (47 mmol) of 3-oxovaleronitrile and the solution was heated to reflux. After refluxing overnight, the solvent was partially removed under reduced pressure and the resulting solid was filtered. The solid was taken up in 250 mL of ethanol and 75 mL of concentrated HCl was slowly added. The solution was refluxed for 4 h and the solution was concentrated under reduced pressure. The residue was diluted with EtOAc and washed with 1 N aqueous NaOH and brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure to afford 6.66 g (48%) of **14** as a white solid: ^{1}H NMR ($CDCl_3$) δ 7.47 (s, 2H), 5.52 (s, 1H), 3.52 (br s, 1H), 2.61 (q, 2H, J = 7.7 Hz), 1.25 (t, 3H, J = 7.7 Hz); MS (m/z) 290 $(M + H)^+$.

5-Amino-3-ethyl-4-sulfonamido-1-(2,4,6-trichlorophenyl)pyrazole (15). Compound **14** (4.45 g, 15.3 mmol) was added to 7.0 mL of chlorosulfonic acid at 0 °C in portions. After stirring for 1 h the solution was heated on a steam bath for 10 min. The mixture was poured into ice/water and the resulting solid was filtered and washed well with water. The solid was taken up in 100 mL of THF at 0 °C and the solution was saturated with ammonia. After stirring for 2 days, the solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 5 to 10% methanol/CH_2Cl_2) to give 4.25 g (75%) of **15** as a solid: ^{1}H NMR ($CDCl_3$) δ 7.51 (s, 2H), 4.80 (br s, 4H), 2.84 (q, J = 7.7 Hz, 2H), 1.33 (t, J = 7.7 Hz, 3H); MS (m/z) 369 $(M + H)^+$.

3-Ethyl-6-methyl-1-(2,4,6-trichlorophenyl)-4-thia-1,2,5,7-tetraazaindene 4,4-dioxide (16). To a solution of 100 mg (0.27 mmol) of **15** in 4 mL of ethanol was added 166 mg (2.0 mmol) of ethyl acetate and 0.5 mL of 21% sodium ethoxide in ethanol. The solution was heated to reflux. After refluxing for 2 days, the mixture was diluted with EtOAc, washed with water and brine, and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was chromatographed (silica gel, 10% methanol/CH_2Cl_2) to give 7 mg (7%) of **16** as a solid: ^{1}H NMR(CD_3OD) δ 7.81 (s, 2H), 2.86 (q, 2H, J = 7.7 Hz), 2.29 (s, 3H), 1.36 (t, 3H, J = 7.7 Hz); HRMS calcd for $C_{13}H_{12}N_4O_2Cl_3S$ $(M + H)^+$ 392.9747, found 392.9766.

Representative Procedures for the Preparation of 3-(Methylthio)-1-(2,4,6-tichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones (17) and 3-Ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones (18 and 19) from 5-amino-4-carboxamido-1-(2,4,6-trichlorophenyl)pyrazoles (3w and 8i): 6-Benzyl-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (18i). To a stirred solution of 167 mg (0.50 mmol) of **8i** in 6 mL of absolute ethanol was

added 0.43 mL (3.0 mmol) of methyl phenylacetate followed by 1.13 mL (3.0 mmol) of 2.7 M NaOEt in ethanol. The solution was stirred for 18 h at reflux, cooled slightly, and quenched with 5 mL of 10% aqueous HOAc. The mixture was cooled, filtered, rinsed with 1:1 MeOH−water and then 3:1 hexanes−ether, and air-dried to afford 192 mg (88%) of **18i** as an off-white solid: mp 240−242 °C; ^{1}H NMR (DMSO-d_6) δ 12.50 (br s, 1H), 7.99 (s, 2H), 7.15−7.30 (m, 5H), 3.85 (s, 2H), 2.83 (q, 2H, J = 7.5 Hz), 1.23 (t, 3H, J = 7.5 Hz); MS (*m*/*z*) 431 (M − H)$^-$. Anal. ($C_{20}H_{15}N_4OCl_3$) C, H, N, Cl.

3-Methylthio-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17a): yield 59%; mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 12.53 (br s, 1H), 8.10 (s, 1H), 8.03 (s, 2H), 2.53 (s, 3H); MS *m*/*z* calcd for $C_{12}H_7N_4SOCl_3$ (M^+) 359.9406, found 359.9399. Anal. ($C_{12}H_7N_4SOCl_3$) C, H, N, Cl, S.

6-Isopropyl-3-(methylthio)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17b): yield 59%; mp 238−240 °C; ^{1}H NMR ($CDCl_3$) δ 10.90 (br s, 1H), 7.51 (s, 2H), 2.88−3.01 (m, 1H), 2.64 (s, 3H), 1.33 (d, 6H, J = 7.2 Hz); MS *m*/*z* calcd for $C_{15}H_{13}N_4SOCl_3$ (M^+) 401.9876, found 401.9894. Anal. ($C_{15}H_{13}N_4OSCl_3$) C, H, N, S, Cl.

3-(Methylthio)-6-propyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17c): yield 52%; mp 236−241°C; ^{1}H NMR ($CDCl_3$) δ ^{1}H NMR ($CDCl_3$) d 12.40 (br s, 1H), 8.02 (s, 2H), 2.47−2.59 (m, 5H), 1.56−1.67 (m, 2H), 0.86 (t, 3H, J = 7.5 Hz); MS *m*/*z* calcd for $C_{15}H_{13}N_4SOCl_3$ (M^+) 408.9876, found 401.9873. Anal. ($C_{15}H_{13}N_4OSCl_3$) C, H, N, S, Cl.

6-Cyclopropyl-3-(methylthio)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17d): yield 35%; mp 250−252 °C; ^{1}H NMR (DMSO-d_6) δ 12.60 (br s, not integrated), 7.99 (s, 2H), 1.97−2.03 (m, 1H), 2.51 (s, integrates with solvent), 0.99−1.08 (m, 2H), 0.84−0.91 (m, 2H); MS *m*/*z* calcd for $C_{13}H_{10}N_4SOCl$ (M^+) 399.9719, found 399.9727. Anal. ($C_{15}H_{11}N_4OSCl_3$) C, H, N, S, Cl.

6-(*N*-(*tert*-Butoxycarbonyl)-*N*-methylaminomethyl)-3-(methylthio)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17e): yield 100%; ^{1}H NMR ($CDCl_3$) δ 10.05 (br s, not integrated), 7.51 (s, 2H), 4.26 (q, 2H), 2.92 (br s, 3H), 2.63 (s, 3H), 1.45 (br s, 9H); MS *m*/*z* calcd for $C_{19}H_{21}N_5SO_3Cl_3$ (M + H)$^+$ 504.0431, found 504.0435. Anal. ($C_{19}H_{20}N_5SO_3Cl_3$) C, H, N, S, Cl.

6-(2-(*N*-(*tert*-Butoxycarbonyl)-*N*-methylamino)ethyl)-3-(methylthio)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17f): yield 69%; mp 201−202 °C; ^{1}H NMR (DMSO-d_6) δ 12.40−12.52 (br s, 1H), 7.96 (s, 2H), 3.40−3.53 (m, 2H), 3.65−3.74 (m, 2H), 2.64 (s, 3H), 2.49 (s, 3H), 1.16−1.32 (m, 9H). Anal. ($C_{20}H_{22}N_5O_3SCl_3$) C, H, N, S, Cl.

(±)-6-(1-Hydroxyethyl)-3-(methylthio)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17g): yield 66%; ^{1}H NMR (DMSO-d_6) δ 12.49 (br s, 1H), 8.02 (s, 2H), 5.76 (d, 1H), 4.47−4.59 (m, 1H), 2.57 (s, 3H), 1.30 (d, 3H); MS *m*/*z* calcd for $C_{14}H_{11}N_4SO_2Cl_3$ (M^+) 403.9668, found 403.9653. Anal. ($C_{14}H_{11}N_4O_2SCl_3$) C, H, N, S, Cl.

3-(Methylthio)-6-methylthiomethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17h): yield 52%; mp 219−222 °C; ^{1}H NMR (DMSO-d_6) δ 12.50 (br s, 1H), 8.02 (s, 2H), 3.50 (s, 2H), 2.54 (s, 3H), 2.05 (s, 3H); MS *m*/*z* calcd for $C_{14}H_{11}N_4S_2OCl_3$ (M^+) 419.9440, found 419.9448. Anal. ($C_{14}H_{11}N_4OS_2Cl_3$) C, H, N, S, Cl.

6-Methoxycarbonyl-3-(methylthio)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17i): yield 20%; mp 214−218 °C; ^{1}H NMR (DMSO-d_6) δ 12.98 (br s, 1H), 8.06 (s, 2H), 3.88 (s, 3H), 2.55 (s, 3H); MS *m*/*z* calcd for $C_{143}H_9N_4SO_3Cl_3$ (M + H)$^+$ 420.9539, found 420.9503. Anal. ($C_{14}H_9N_4O_3SCl_3$) C, H, N, S, Cl.

6-(3-Hydroxypropyl)-3-(methylthio)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17j): yield 81%; mp 219−220 °C; ^{1}H NMR (DMSO-d_6) δ 7.97 (s, 2H), 3.31−3.37 (m, 2H), 2.56 (t, 2H, J = 7.6 Hz), 2.48 (s, 3H), 1.63−1.72 (m, 2H); MS (*m*/*z*) 417 (M − H)$^-$. Anal. ($C_{15}H_{13}N_4O_2SCl_3$) C, H, N, S, Cl.

6-(5-Hydroxypentyl)-3-(methylthio)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17k): yield 80%; mp 193−197 °C; ^{1}H NMR (DMSO-d_6) δ 12.35 (br s, 1H), 7.97 (s, 2H), 4.29 (t, 1H, J = 5.3 Hz), 3.24−3.34 (m, 2H), 2.43−2.54 (m, 2H), 1.49−1.61 (m, 2H), 1.17−1.40 (m, 4H); MS *m*/*z* calcd for $C_{17}H_{18}N_4SO_2Cl_3$ (M + H)$^+$ 447.0216, found 447.0198.

6-Fluoromethyl-3-(methylthio)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17l): yield 81%; mp 248−250 °C; ^{1}H NMR (DMSO-d_6) δ 12.74 (br s, 1H), 7.99 (s, 2H), 5.25 (d, 2H, J = 46.2 Hz), 2.50 (s, 3H); MS (*m*/*z*) 391 (M − H)$^-$. Anal. ($C_{13}H_8N_4OSFCl_3$) C, H, N, S, Cl.

3-(Methylthio)-1-(2,4,6-trichlorophenyl)-6-trifluoromethyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17m): yield 100%; mp 228−230 °C; ^{1}H NMR (DMSO-d_6) δ 8.06 (s, 2H), 2.56 (s, 3H); MS *m*/*z* calcd for $C_{13}H_7N_4SOF_3Cl_3$ (M + H)$^+$ 428.9358, found 428.9354. Anal. ($C_{13}H_6N_4OSF_3Cl_3$) C, H, N, S, Cl.

6-Fur-2-yl-3-(methylthio)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17n): yield 55%. ^{1}H NMR (DMSO-d_6) δ 12.65 (br s, 1H), 8.05 (s, 2H), 7.98 (d, 1H, J = 1 Hz), 7.71 (d, 1H, J = 6 Hz), 6.73−6.77 (m, 1H), 2.57 (s, 3H); MS *m*/*z* calcd for $C_{16}H_{10}N_4SO_2Cl_3$ (M + H)$^+$ 426.9604, found 426.9605.

3-(Methylthio)-6-(thien-2-ylmethyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17o): yield 83%; mp 218−222 °C; ^{1}H NMR (DMSO-d_6) δ 12.69 (br s, 1H), 8.03 (s, 2H), 7.37−7.42 (m, 1H), 6.90−6.99 (m, 2H), 4.10 (s, 2H), 2.52 (s, 3H); MS *m*/*z* calcd for $C_{17}H_{11}N_4OS_2Cl_3$ (M + H)$^+$ 456.9490, found 456.9518. Anal. ($C_{16}H_9N_4OS_2Cl_3$) C, H, N, S, Cl.

3-(Methylthio)-6-benzyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (17p): yield 84%; mp 238−240 °C; ^{1}H NMR (DMSO-d_6) δ 12.63 (br s, 1H), 8.00 (s, 2H), 7.20−7.29 (m, 5H), 3.87 (s, 2H), 2.51 (s, 3H); MS (*m*/*z*) 451 (M + H)$^+$. Anal. ($C_{19}H_{13}N_4SOCl_3$) C, H, N.

3-Ethyl-6-isobutyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (18a): yield 36%; mp 219−222 °C; ^{1}H NMR (DMSO-d_6) δ 12.24 (br s, 1H), 7.96 (s, 2H), 2.84 (q, 2H, J = 7.6 Hz), 2.38 (d, 2H, J = 7.3 Hz), 1.89−2.03 (m, 1H), 1.25 (t, 3H, J = 7.5 Hz), 0.81 (d, 6H, J = 6.6 Hz); MS (*m*/*z*) 399 (M + H)$^+$. Anal. ($C_{17}H_{17}N_4OCl_3$) C, H, N, Cl.

3, 6-Diethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-*1H*-pyrazolo[3,4-*d*]pyrimidin-4-one (18b): yield 84%; mp 202−203 °C; ^{1}H NMR (DMSO-d_6) δ 12.25 (br s, 1H), 7.97 (s, 2H), 2.85 (q, 2H, J = 7.4 Hz), 2.51 (q, 2H, J = 7.4 Hz), 1.24 (t, 3H, J = 7.4 Hz), 1.08 (t, 3H, J = 7.4 Hz); MS *m*/*z* calcd for $C_{15}H_{13}N_4SOCl_3$ (M^+) 370.0155, found 370.0141. Anal. ($C_{15}H_{13}N_4OCl_3$) C, H, N, Cl.

3-Ethyl-6-(2-methylthioethyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (18c): yield 80%; mp 196−198 °C; ^{1}H NMR (DMSO-d_6) δ 12.36 (br s, 1H), 8.00 (s, 2H), 2.82−2.93 (m, 4H), 2.75 (t, 2H), 2.03 (s, 3H), 1.28 (t, 3H, J = 7.5 Hz); MS (*m*/*z*) 415 (M − H)$^-$. Anal. ($C_{16}H_{15}N_4OSCl_3$) C, H, N, S, Cl.

3-Ethyl-6-(4-hydroxybutyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (18d): yield 80%. ^{1}H NMR ($CDCl_3$) δ 11.57 (br s, 1H), 7.51 (s, 2H), 3.73 (q, 2H, J = 6.0 Hz), 3.05 (q, 2H, J = 7.6 Hz), 2.78 (t, 2H, J = 7.5 Hz), 1.82−1.97 (m, 2H), 1.58−1.69 (m, 2H), 1.42 (t, 3H, J = 7.7 Hz); MS *m*/*z* calcd for $C_{17}H_{18}N_4O_2Cl_3$ (M^+) 415.0495, found 415.0519. Anal. ($C_{17}H_{17}N_4O_2Cl_3$) C, H, N, Cl.

6-(3,4-Dimethoxyphenyl)-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (18e): yield 14%; mp 262−263 °C; ^{1}H NMR (DMSO-d_6) δ 12.44 (br s, 1H), 7.99 (s, 2H), 7.63 (br s, 1H), 7.57 (d, 1H), 7.03 (d, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 2.89 (q, 2H, J = 7.5 Hz), 1.28 (t, 3H, J = 7.5 Hz); MS (*m*/*z*) 477 (M − H)$^-$.

3-Ethyl-6-(2-phenylethyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (18g): yield 89%; mp 228−231 °C; ^{1}H NMR (DMSO-d_6) δ 12.30 (br s, <1H), 7.97 (s, 2H), 7.08−7.22 (m, 5H), 2.78−2.88 (m, 6H), 1.24 (s,

3H, J = 7.5 Hz); MS m/z calcd for $C_{21}H_{17}N_4OCl_3$ (M^+) 446.0468, found 446.0466. Anal. ($C_{21}H_{17}N_4OCl_3$) C, H, N, Cl.

3-Ethyl-6-(2-(imidazol-4-yl)ethyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (18h): yield 55%; mp 271−275 °C; ^{1}H NMR (DMSO-d_6) δ 7.92 (s, 2H), 7.46 (s, 1H), 6.65 (s, 1H), 2.76−2.88 (m, 6H), 1.25 (t, 3H, J = 7.5 Hz); MS m/z calcd for $C_{21}H_{17}N_4OCl_3$ (M^+) 436.0373, found 436.0367. Anal. ($C_{18}H_{15}N_6OCl_3 \cdot {}^1/_4H_2O$) C, H, N, Cl.

3-Ethyl-6-(2-methoxybenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19a): yield 91%; mp 188−191 °C; ^{1}H NMR (DMSO-d_6) δ 12.31 (br s, 1H), 7.91 (s, 2H), 7.15−7.22 (m, 1H), 6.89−6.98 (m, 2H), 6.80 (dd, 1H, J = 7.4, 0.9 Hz), 3.82 (s, 2H), 3.67 (s, 3H), 2.85 (q, 2H, J = 7.4 Hz), 1.25 (t, 3H, J = 7.5 Hz); MS (m/z) 461 (M − H)$^-$. Anal. ($C_{21}H_{17}N_4O_2Cl_3$) C, H, N, Cl.

3-Ethyl-6-(2-(hydroxymethyl)benzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19b): yield 88%; mp 220−222 °C; ^{1}H NMR (DMSO-d_6) δ 7.95 (s, 2H), 7.33 (dd, 1H, J = 6.9, 1.8 Hz), 7.06−7.22 (m, 3H), 5.36 (br s, 1H), 4.53 (s, 2H), 3.91 (s, 2H), 2.83 (q, 2H, J = 7.6 Hz), 1.24 (t, 3H, J = 7.5 Hz); MS m/z calcd for $C_{21}H_{17}N_4O_2Cl_3$ (M)$^+$ 462.0417, found 462.0404. Anal. ($C_{21}H_{17}N_4O_2Cl_3$) C, H, N, Cl.

3-Ethyl-6-(pyrid-2-ylmethyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19c): yield 87%; mp 164−169 °C; ^{1}H NMR (DMSO-d_6) δ 12.46 (br s, 1H), 8.40−8.43 (m, 1H), 7.91 (s, 2H), 7.69 (ddd, 1H, J = 7.7, 7.6, 1.8 Hz), 7.26 (d, 1H, J = 8.0 Hz), 7.18−7.24 (m, 1H), 4.08 (s, 2H), 2.85 (q, 2H, J = 7.4 Hz), 1.25 (t, 3H, J = 7.5 Hz); MS m/z calcd for $C_{19}H_{14}N_5OCl_3$ (M^+) 433.0264, found 433.0255. Anal. ($C_{19}H_{14}N_5OCl_3 \cdot {}^1/_4HOAc$) C, H, N, Cl.

6-(3-Amino-2-methylbenzyl)-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19d): yield 89%; mp 242−243 °C; ^{1}H NMR (DMSO-d_6) δ 12.26 (br s, 1H), 7.94 (s, 2H), 6.74 (d, 1H, J = 7.7 Hz), 6.47 (d, 1H, J = 7.7 Hz), 6.30 (d, 1H, J = 7.4 Hz), 4.76 (br s, 1−2H), 3.78 (s, 2H), 2.84 (q, 2H, J = 7.6 Hz), 1.92 (s, 3H), 1.24 (t, 3H, J = 7.5 Hz); MS (m/z) 462 (M + H)$^+$. Anal. ($C_{21}H_{18}N_5OCl_3$) C, H, N, Cl.

3-Ethyl-6-(3-methylbenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19e): yield 95%; mp 238−240 °C; ^{1}H NMR (DMSO-d_6) δ 12.47 (br s, 1H), 7.98 (s, 2H), 7.13 (d, 1H, J = 7.6 Hz), 7.07 (br s, 1H), 6.97−7.04 (m, 2H), 3.80 (s, 2H), 2.83 (q, 2H, J = 7.6 Hz), 2.02 (s, 3H), 1.23 (t, 3H, J = 7.5 Hz, CH_3). Anal. ($C_{21}H_{17}N_4OCl_3$) C, H, N, Cl.

6-(3-(Ethoxycarbonylmethyl)benzyl)-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19f): yield 42%; mp 168−169 °C; ^{1}H NMR ($CDCl_3$) δ 10.41 (br s, 1H), 7.54 (s, 2H), 7.21−7.34 (m, 4H), 4.13 (q, 2H, J = 7.1 Hz), 3.98 (s, 2H), 3.58 (s, 2H), 3.05 (q, 2H, J = 7.4 Hz), 1.42 (t, 3H, J = 7.5 Hz), 1.23 (t, 3H, J = 7.1 Hz); MS (m/z) 517 (M − H)$^-$. Anal. ($C_{24}H_{21}N_4O_3Cl_3$) C, H, N, Cl.

3-Ethyl-6-(3-methoxybenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19g): yield 76%; mp 235−238 °C; ^{1}H NMR (DMSO-d_6) δ 12.47 (br s, 1H), 7.97 (s, 2H), 7.16 (t, 1H, J = 7.9 Hz), 6.73−6.85 (m, 3H), 3.81 (s, 2H), 3.66 (s, 3H), 2.83 (q, 2H, J = 7.5 Hz), 1.23 (t, 3H, J = 7.5 Hz); MS (m/z) 461 (M − H)$^-$. Anal. ($C_{21}H_{17}N_4O_2Cl_3$) C, H, N, Cl.

6-(3-Aminobenzyl)-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19h): yield 69%; mp 236−240 °C; ^{1}H NMR (DMSO-d_6) δ 12.41 (br s, 1H), 7.96 (s, 2H), 6.84−6.89 (m, 1H), 6.33−6.38 (m, 3H), 4.97 (br s, 1H), 3.67 (s, 2H), 2.83 (q, 2H, J = 7.6 Hz), 1.243 (t, 3H, J = 7.5 Hz); MS m/z calcd for $C_{20}H_{16}N_5OCl_3$ (M^+) 447.0420, found 447.0418.

3-Ethyl-6-(pyrid-3-ylmethyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19i): yield 97%; mp 257−260 °C; ^{1}H NMR (DMSO-d_6) δ 12.56 (br s, 1H), 8.46 (d, 1H, J = 1.5 Hz), 8.40 (dd, 1H, J = 4.8, 1.5 Hz), 7.96 (s, 2H), 7.62(dt, 1H, J = 8.0, 2.0 Hz), 7.28 (dd, 1H, J = 7.7, 4.7 Hz), 3.90 (s, 2H), 2.83 (q, 2H, J = 7.4 Hz), 1.23 (t, 3H, J = 7.5 Hz); MS (m/z) 432 (M − H)$^-$. Anal. ($C_{19}H_{14}N_5OCl_3$) C, H, N, Cl.

3-Ethyl-6-(pyrid-4-ylmethyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19j): yield 92%; mp 277−280 °C; ^{1}H NMR (DMSO-d_6) δ 12.58 (br s, 1H), 8.43 (d, 2H, J = 4.0 Hz), 7.96 (s, 2H), 7.22 (d, 2H, J = 5.8 Hz), 3.91 (s, 2H), 2.84 (q, 2H, J = 7.4 Hz), 1.24 (t, 3H, J = 7.5 Hz); MS (m/z) 432 (M − H)$^-$.

6-(4-Aminobenzyl)-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19k): yield 89%; mp 256−259 °C; ^{1}H NMR (DMSO-d_6) δ 12.34 (br s, 1H), 7.97 (s, 2H), 6.89 (d, 2H, J = 8.4 Hz), 6.41 (d, 2H, J = 8.4 Hz), 4.94 (s, 2H), 3.61 (s, 2H), 2.82 (q, 2H, J = 7.6 Hz), 1.23 (t, 3H, J = 7.5 Hz); MS (m/z) 446 (M − H)$^-$. Anal. ($C_{20}H_{16}N_5OCl_3 \cdot {}^1/_4THF$) C, H, N, Cl.

3-Ethyl-6-(4-methoxybenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19l): yield 81%; mp 261−262 °C; ^{1}H NMR (DMSO-d_6) δ 12.43 (br s, 1H), 7.97 (s, 2H), 7.17 (d, 2H, J = 8.7 Hz), 6.81 (d, 2H, J = 8.8 Hz), 3.76 (s, 2H), 3.66 (s, 3H), 2.83 (q, 2H, J = 7.4 Hz), 1.23 (t, 3H, J = 7.5 Hz); MS (m/z) 463 (M + H)$^+$. Anal. ($C_{21}H_{17}N_4O_2Cl_3$) C, H, N, Cl.

3-Ethyl-6-(4-hydroxybenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19m): yield 58%; mp 288−291 °C; ^{1}H NMR (DMSO-d_6) δ 12.40 (br s, 1H), 9.29 (s, 1H), 7.97 (s, 2H), 7.04 (d, 2H, J = 8.4 Hz), 6.62 (d, 2H, J = 8.4 Hz), 3.70 (s, 2H), 2.83 (q, 2H, J = 7.5 Hz), 1.23 (t, 3H, J = 7.5 Hz); MS m/z calcd for $C_{20}H_{15}N_4O_2Cl_3$ (M)$^+$ 448.0261, found 448.0248. Anal. ($C_{20}H_{15}N_4O_2Cl_3$) C, H, N, Cl.

6-(4-Bromobenzyl)-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19n): yield 96%; mp 296−299 °C; ^{1}H NMR (DMSO-d_6) δ 12.52 (br s, 1H), 7.96 (s, 2H), 7.45 (d, 2H, J = 8.4 Hz), 7.19 (d, 2H, J = 8.5 Hz), 3.83 (s, 2H), 2.85 (q, 2H, J = 7.6 Hz), 1.23 (t, 3H, J = 7.5 Hz). Anal. ($C_{20}H_{14}N_4OBr\,Cl_3$) C, H, N; MS (m/z) 509 (M − H)$^-$.

6-(4-(*N*,*N*-Dimethylamino)benzyl)-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19o): yield 80%; mp 238−241 °C; ^{1}H NMR (DMSO-d_6) δ 12.38 (br s, 1H), 7.98 (s, 2H), 7.07 (d, 2H, J = 8.8 Hz), 6.59 (d, 2H, J = 8.8 Hz), 3.68 (s, 2H), 2.82 (q, 2H, J = 7.6 Hz), 2.79 (s, 6H), 1.23 (t, 3H, J = 7.5 Hz); MS m/z calcd for $C_{17}H_{18}N_4O_2Cl_3$ (M + H)$^+$ 476.0771, found 476.0812. Anal. ($C_{22}H_{20}N_5OCl_3$) C, H, N, Cl.

3-Ethyl-6-(4-methoxy-3-methylbenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19p): yield 89%; mp 245−247 °C; ^{1}H NMR (DMSO-d_6) δ 12.40 (br s, 1H), 7.98 (s, 2H), 6.99−7.08 (m, 2H), 6.80 (d, 2H, J = 9.2 Hz), 3.72 (s, 2H), 3.69 (s, 3H), 2.82 (q, 2H, J = 7.4 Hz), 2.04 (s, 3H), 1.23 (t, 3H, J = 7.5 Hz); MS (m/z) 477 (M + H)$^+$. Anal. ($C_{22}H_{19}N_4O_2Cl_3$) C, H, N, Cl.

3-Ethyl-6-(3-methoxy-4-methylbenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19q): yield 97%; mp 263−265 °C; ^{1}H NMR (DMSO-d_6) δ 12.44 (br s, 1H), 7.97 (s, 2H), 6.99 (d, 1H, J = 7.6 Hz), 6.85 (s, 1H), 6.71 (d, 1H, J = 7.3 Hz), 3.78 (s, 2H), 3.68 (s, 3H), 2.83 (q, 2H, J = 7.6 Hz), 2.03 (s, 3H), 1.23 (t, 3H, J = 7.5 Hz); MS (m/z) 477 (M + H)$^+$.

3-Ethyl-6-(4-hydroxy-3-methoxybenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19r): yield 88%; mp 260−280 °C; ^{1}H NMR (DMSO-d_6) δ 12.44 (br s, 1H), 8.87 (s, 1H), 7.97 (s, 2H), 6.82 (s, 1H), 6.59−6.65 (m, 2H), 3.70 (s, 2H), 3.65 (s, 3H), 2.82 (q, 2H, J = 7.4 Hz), 1.23 (t, 3H, J = 7.5 Hz). Anal. ($C_{21}H_{17}N_4O_3Cl_3$) C, H, N, Cl.

6-(3,4-Dimethoxybenzyl)-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19s): yield 57%; mp 220−222 °C; ^{1}H NMR (DMSO-d_6) δ 12.43 (br s, 1H), 7.97 (s, 2H), 6.88 (d, 1H, J = 1.5 Hz), 6.74−6.85 (m, 2H), 3.74 (s, 2H), 3.66 (s, 3H), 3.64 (s, 3H), 2.82 (q, 2H, J = 7.4 Hz), 1.23 (t, 3H, J = 7.5 Hz). Anal. ($C_{22}H_{19}N_4O_3Cl_3$) C, H, N, Cl.

6-(3,5-Dihydroxybenzyl)-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19t): ^{1}H NMR (DMSO-d_6) δ 12.41 (br s, 1H), 9.15 (br s), 7.96 (s, 2H), 5.99−6.08 (m, 3H), 3.64 (s, 2H), 2.84 (q, 2H, J = 7.4 Hz), 1.24 (t, 3H, J = 7.5 Hz). Anal. ($C_{20}H_{15}N_4O_3Cl_3$) C, H, N, Cl.

3-Ethyl-6-(3-hydroxy-4-methoxybenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (19u): yield 65%; mp 241−244 °C; ^{1}H NMR (DMSO-d_6) δ 12.40 (br s), 8.89 (s, 1H), 7.97 (s, 2H), 6.78 (d, 1H, J = 8.0 Hz), 6.66 (br s, 1H), 6.02 (br d, 1H, J = 8.0 Hz), 3.67 (s, 5H), 2.83 (q, 2H, J = 7.4 Hz), 1.23 (t, 3H, J = 7.5 Hz); MS (*m/z*) 479 (M + H)$^+$. Anal. ($C_{21}H_{17}N_4O_3Cl_3$) C, H, N, Cl.

6-(*N*-Methylaminomethyl)-3-(methylthio)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one hydrochloride (20e). To a solution of 5 mL of 4 N HCl in dioxane was added 100 mg (0.20 mmol) of **17e**. The solution was stirred for 1 h and frozen in a dry ice/acetone bath. Lyophilization afforded 87 mg (100%) of **20e** as a white powder: ^{1}H NMR (DMSO-d_6) δ 12.80−12.88 (br s, 1H), 8.96−9.06 (br s, 2H), 8.01 (s, 2H), 4.12−4.20 (m, 2H), 2.53−2.60 (m, 3H), 2.51 (s, 3H); MS *m/z* calcd for $C_{14}H_{12}N_5SO_3Cl_3$ (M)$^+$ 402.9828, found 402.9799.

6-(2-(*N*-Methylamino)ethyl)-3-(methylthio)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one hydrochloride (20f) was prepared from **17f** by the same procedure used to prepare **21e**: yield 100%; mp 232−233 °C; ^{1}H NMR (DMSO-d_6) δ 12.55 (br s, 1H), 8.60−8.72 (m, 2), 7.99 (s, 2H), 3.37 (s, 3H), 3.08−3.21 (m, 2H), 2.92−3.01 (m, 2H), 2.50 (s, 3H). Anal. ($C_{15}H_{15}N_5OSCl_4$) C, H, N, S, Cl.

1-(2,4,6-Trichlorophenyl)-3-ethyl-6-vinyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (21). To a stirred suspension of 1.1 g (2.6 mmol) of **18d** in 25 mL of warm CH_2Cl_2 was added 4 mL of DMF followed by 582 mg (3.38 mmol) of *m*-CPBA in three portions over 1 h. The solution was stirred for 1 h longer, poured into EtOAc, and washed twice with aqueous $NaHCO_3$ and once with brine. The solution was dried ($MgSO_4$) and concentrated to afford the crude sulfoxide as a chalky solid. This material was suspended in 80 mL of toluene and heated to reflux for 4 h. Solvent was removed under reduced pressure, and the crude olefin was chromatographed on silica gel (elution with 1:1 EtOAc−hexanes) to afford, after concentration, 620 mg (67%) of **21** as a white solid: mp 202−203 °C; ^{1}H NMR (DMSO-d_6) δ 12.39 (br s, 1H), 7.97 (s, 2H), 6.36−6.56 (m, 2H), 5.79 (dd, 1H, J = 10, 1 Hz), 2.85 (q, 2H, J = 7.4 Hz), 1.24 (t, 3H, J = 7.4 Hz); MS (*m/z*) 367 (M − H)$^-$. Anal. ($C_{15}H_{11}N_4OCl_3$) C, H, N, Cl.

Representative Procedure for the Preaparation of 6-(Hydroxybenzyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones (22) from 6-(Methoxybenzyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones (19): 6-(3,4-Dihydroxybenzyl)-3-ethyl-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (22s). To a stirred suspension of 500 mg (1.01 mmol) of **19s** in 20 mL of CH_2Cl_2 was added 10 mL of a 1 M solution of BBr_3 in CH_2Cl_2. The solution was stirred for 1 h at room temperature and then cooled to 0 °C. The reaction was quenched with 1 N aqueous HCl, poured into water, and extracted with 2:1 EtOAc−THF. (Addition of a little of brine and/or MeOH may be necessary to produce two clear phases.) The organic extract was washed with half-saturated brine and then brine. The solution was dried ($MgSO_4$), concentrated under reduced pressure, and chromatographed on silica gel (elution with 1:1 hexanes−THF and then THF). Concentration of the appropriate fractions afforded 40 mg (93%) of **22s** as an off-white, amorphous solid: ^{1}H NMR (DMSO-d_6) δ 12.38 (br s, 1H), 8.79 (t, 1H, J = 3.3 Hz) 7.97 (s, 2H), 6.56−6.61 (m, 2H), 6.46−6.50 (m, 1H), 3.64 (s, 2H), 2.83 (q, 2H, J = 7.6 Hz), 1.23 (t, 3H, J = 7.5 Hz); MS *m/z* calcd for $C_{20}H_{15}N_4O_3Cl_3$ (M)$^+$ 464.0210, found 464.0218.

3-Ethyl-6-(2-hydroxybenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (22a): yield 100%; mp 285−289 °C; ^{1}H NMR (DMSO-d_6) δ 12.28 (br s, 1H), 9.62 (s, 1H), 7.96 (s, 2H), 7.02−7.07 (m, 1H), 6.91 (d, 1H, J = 6.3 Hz), 6.78 (dd, 1H, J − 6.7, 0.7 Hz), 6.69 (dd, J = 7.7, 7.3 Hz), 3.83 (s, 2H), 2.88 (q, 2H, J = 7.6 Hz), 1.29 (t, 3H, J = 7.5 Hz); MS (*m/z*) 449 (M + H)$^+$. Anal. ($C_{20}H_{15}N_4O_2Cl_3 \cdot {}^1/_3H_2O$) C, H, N, Cl.

3-Ethyl-6-(3-hydroxybenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (22g): yield 91%; ^{1}H NMR (DMSO-d_6) δ 12.45 (br s, 1H), 9.33 (s, 1H), 7.96 (s, 2H), 7.03 (t, 1H, J = 7.7 Hz), 6.56−6.65 (m, 3H), 3.75 (s, 2H), 2.83 (q, 2H, J = 7.5 Hz), 1.24 (t, 3H, J = 7.5 Hz); MS (*m/z*) 447 (M − H)$^-$. Anal. ($C_{20}H_{15}N_4O_2Cl_3 \cdot {}^1/_2H_2O$) C, H, N, Cl.

3-Ethyl-6-(4-hydroxy-3-methylbenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (22p): yield 91%; mp 260−263 °C; ^{1}H NMR (DMSO-d_6) δ 12.37 (br s, 1H), 9.17 (s, 1H), 7.98 (s, 2H), 6.96 (br s, 1H), 6.85 (d, 1H, J = 8.0 Hz), 6.62 (d, 1H, J = 8.1 Hz), 3.67 (s, 2H), 2.82 (q, 2H, J = 7.5 Hz), 2.01 (s, 3H), 1.23 (t, 3H, J = 7.5 Hz); MS (*m/z*) 461 (M − H)$^-$. Anal. ($C_{21}H_{17}N_4O_2Cl_3$) C, H, N, Cl.

3-Ethyl-6-(3-hydroxy-4-methylbenzyl)-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (22q): yield 90%. ^{1}H NMR (DMSO-d_6) δ 12.49 (br s, 1H), 9.23 (s, 1H), 8.00 (s, 2H), 6.94 (d, 1H, J = 7.7 Hz), 6.64 (s, 1H), 6.60 (d, 1H, J = 7.7 Hz), 3.75 (s, 2H), 2.87 (q, 2H, J = 7.4 Hz), 2.01 (s, 3H), 1.28 (t, 3H, J = 7.5 Hz); MS *m/z* calcd for $C_{21}H_{17}N_4O_2Cl_3$ (M)$^+$ 462.0417, found 462.0404.

***N*-(3-{[3-Ethyl-4-oxo-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl]methyl}phenyl)-methanesulfonamide (23).** To a stirred susension of 47 mg (0.10 mmol) of **19h** in 2.5 mL of 2:2:1 CH_2Cl_2−ether−pyridine was added 0.02 mL (0.26 mmol) of methanesulfonyl chloride. The mixture was stirred for 16 h at ambient temperature and diluted with ether. The solution was washed twice with 1 N aqueous HCl and once with brine, dried ($MgSO_4$), and concentrated under reduced pressure to afford an off-white solid. Crystallization from EtOAc−hexanes afforded 44 mg (83%) of **23** as a white solid: mp 246−248 °C; ^{1}H NMR (DMSO-d_6) δ 7.96 (s, 2H), 7.18 (t, 1H, J = 7.9 Hz), 7.08 (s, 1H), 7.02 (d, 1H, J = 8.1 Hz), 6.96 (d, 1H, J = 7.7 Hz), 3.83 (s, 2H), 2.90 (s, 3H), 2.83 (q, 2H, J = 7.4 Hz), 1.24 (t, 3H, J = 7.5 Hz); MS *m/z* calcd for $C_{21}H_{19}N_5SO_3Cl_3$ (M + H)$^+$ 526.0274, found 526.0256.

Representative Procedure for the Preparation of Anilide Derivatives 24 from Aniline 19k: 2-(Dimethylamino)-*N*-(4-{[3-ethyl-4-oxo-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl]methyl}-phenyl)acetamide Hydrochloride (24d). To a stirred susension of 70 mg (0.16 mmol) of **19k** in 2 mL of CH_2Cl_2 was added 0.07 mL (1.32 mmol) of Et_3N followed by 52 mg (0.50 mmol) of *N,N*-dimethylglycine and 96 mg (0.50 mmol) of EDC. The mixture was stirred for 24 h at ambient temperature and filtered through a slug of silica gel (elution with 1:1 THF−hexanes). The appropriate fractions were concentrated under reduced pressure, redissolved in 5 mL of benzene and 0.1 mL of 4 N HCl in dioxane, and frozen. Lyophilization afforded 70 mg (80%) of **24d** as an off-white solid: mp 288−290 °C (dec); ^{1}H NMR (DMSO-d_6) δ 12.50 (br s, 1H), 10.41 (s, 1H), 10.69 (br s, 1H), 7.97 (s, 2H), 7.48 (d, 2H, J = 8.8 Hz), 7.23 (d, 2H, J = 8.4 Hz), 4.09 (s, 2H), 3.82 (s, 2H), 2.83 (q, 2H, J = 7.5 Hz), 1.23 (t, 3H, J = 7.5 Hz); MS (*m/z*) 533 (M + H)$^+$. Anal. ($C_{24}H_{24}N_6O_2Cl_4$) C, H, N, Cl.

***N*-(4-{[3-Ethyl-4-oxo-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl]methyl}phenyl)-acetamide (24a):** yield 86%; ^{1}H NMR (DMSO-d_6) δ 12.50 (s, 1H), 9.90 (s, 1H), 8.01 (s, 1H), 7.47 (d, 2H, J = 8.4 Hz), 7.19 (d, 2H, J = 8.5 Hz), 3.81 (s, 2H), 2.87 (q, 2H, J = 7.6 Hz), 2.01 (s, 3H), 1.27 (t, 3H, J = 7.5 Hz); MS *m/z* calcd for $C_{22}H_{18}N_5O_2Cl_3$ (M)$^+$ 489.0526, found 489.0498.

2-(*tert*-Butoxycarbonylamino)-*N*-(4-{[3-ethyl-4-oxo-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]-pyrimidin-6-yl]methyl}phenyl)acetamide (24b): yield 65%; mp 243−244 °C; ^{1}H NMR (DMSO-d_6) δ 12.48 (br s, 1H), 9.87 (br s, 1H), 7.97 (s, 2H), 7.44 (d, 2H, J = 8.4 Hz), 7.17 (d, 2H, J = 8.4 Hz), 6.99 (t, 1H, J = 5.9 Hz), 3.78 (s, 2H), 3.64 (d, 2H, J = 6.2 Hz), 2.83 (q, 2H, J = 7.5 Hz), 1.35 (s, 9H), 1.23 (t, 3H, J = 7.5 Hz); MS *m/z* calcd for $C_{27}H_{28}N_6O_4Cl_3$ (M + H)$^+$ 605.1238, found 605.1256.

2-Amino-*N*-(4-{[3-ethyl-4-oxo-1-(2,4,6-trichlorophenyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-yl]methyl}-phenyl)acetamide (24c) was prepared from **24b** in the same manner used to prepare **20e** from **17e**: yield 100%; mp 294−296 °C; ^{1}H NMR (DMSO-d_6) δ 12.50 (br s, 1H), 10.41 (br s, 1H), 7.99−8.16 (m, 3H), 7.97 (s, 2H), 7.46 (d, 2H, J = 8.5 Hz), 7.22 (d, 2H, J = 8.4 Hz), 3.81 (s, 2H), 3.68−3.77 (m, 2H), 2.83 (q, 2H, J = 7.6 Hz), 1.23 (t, 3H, J = 7.5 Hz); MS (*m/z*) 505 $(M + H)^+$. Anal. ($C_{22}H_{20}N_6O_2Cl_4$) C, H, N, Cl.

Enzyme IC_{50} Determinations. The in vitro assays employ cell lysates from insect cells expressing either of the kinases and subsequently their corresponding regulatory units. The CDK/cyclin lysate is combined in a microtiter-type plate along with a kinase compatible buffer, ^{32}P-labeled ATP at a concentration of 50 μM, a GST-Rb fusion protein, and the test compound at varying concentrations. The kinase reaction is allowed to proceed with the radiolabeled ATP and then effectively stopped by the addition of a large excess of EDTA and unlabeled ATP. The GST-Rb labeled protein is sequestered on a GSH−Sepharose bead suspension, washed, and resuspended in scintillant, and the ^{32}P activity is detected in a scintillation counter. The compound concentration that inhibits 50% of the kinase activity was calculated for each compound.

Cellular Growth Inhibition Assay. Effects of compounds on the growth of the following transformed cell lines were evaluated in a colorimetric assay using sulforhodamine B (SRB):[28] HCT-116 (human colon carcinoma cell line, ATCC), MDA-MB-468 (human mammary gland adenocarcinoma, ATCC), NCI-H460 (human lung carcinoma, ATCC), A-498 (human kidney carcinoma, ATCC), T-47D (human mammary duct carcinoma, ATCC), MCF 7 (human mammary gland adenocarcinoma), DU 145 (human prostate carcinoma, ATCC), COLO 205 (human colon adenocarcinoma, ATCC). Briefly, exponentially growing cells were seeded in wells of a 96-well microtiter plate at a concentration to allow for 3−5 doublings before obtaining 85% confluence. Eighteen hours later, graded concentrations of test compounds were added to the cell plates. Plates were incubated for 5−6 days at 37 °C in 5% CO_2 or atmospheric CO_2 (MDA-MB-468). Fifty microliters of cold 50% TCA was gently added to each well, and plates were placed at 4 °C for 1 h. Plates were decanted, rinsed five times with cold tap water, and allowed to air-dry. Fifty microliters of 0.4% SRB in 1% acetic acid was added, and the plates were incubated at room temperature for 15 min. Plates were washed four times with 1% acetic acid and again allowed to air-dry. Finally, 150 μL of 10 mM Tris base was added, and plates were agitated on a plate shaker for 5 min before reading optical densities at 570 nm using a BIORAD 3550 plate reader.

Purification and Crystallization of Human CDK2. CDK2 protein was prepared and purified as described,[29] except for the addition of 10% (v/v) glycerol during the SP-sepharose and ATP-agarose column steps. Protein was concentrated to 6 mg/mL using a Collodion concentrator against 10 mM HEPES (pH 7.4), 15 mM NaCl. Crystals were grown at 18 °C from sitting drops containing premixed and filtered solutions of 3.0 mg/mL CDK2, 32.5mM HEPES (pH 7.4), 11.3 mM sodium chloride, 12.5 mM ammonium acetate, 2 mM DTT, 2−4% PEG 4000 against 100 mM HEPES (pH 7.4), 50 mM ammonium acetate, 2 mM DTT, 4−14% PEG 4000. Crystals appeared in 1 day and grew for 1 week to approximately 0.25 mm × 0.25 mm in size.

Crystal Preparation and Data Collection and Processing. The crystals were soaked overnight in inhibitor solution (0.5 mM **22s**, 0.5% DMSO, 10 mM HEPES (pH 7.4), 15 mM sodium chloride) and reflection data were collected on an RAXIS-II imaging plate mounted on a Rigaku RU-200 rotating anode X-ray source, operating at 50 kV and 100 mA. Data were processed, scaled, and merged with the program HKL.[30]

Structure Solution and Refinement. Molecular replacement was used to generate an initial phasing model from a previously determined in-house structure of CDK2. After five cycles of refinement (positional refinement, simulated annealing, and B-factor refinement) using the program X-PLOR,[31] the electron density maps clearly showed the location of the inhibitor inside the ATP binding site. Iterative cycles of refinement and model building were performed, resulting in a final model, which includes residues 1−298, **22s**, and 106 water molecules. Data and refinement statistics are presented in Table 9.

Table 9. Crystallographic Statistics for CDK2/**22s**

space group	$P2_12_12_1$	refinement resoln (Å)	10.0−1.85
cell dimensions (Å)	a = 72.97 b = 73.16 c = 54.26	R factor[b]	0.169
max resolution (Å)	1.85	R_{free}[c]	0.279
total observations	36 372	av protein temp factor (Å)	32.7
unique reflections	20 060	av inhibitor temp factor (Å)	35.2
completeness (%)	78	rms bonds (Å)	0.016
R_{merge}[a]	0.081	rms angles (deg)	3.5

[a] $R_{merge} = \sum_h\sum_j|I_{hj} - I_h|\sum_h\sum_j|I_{hj}|$, where I_{hj} is the jth observation of reflection h. [b] R factor $= \sum_h|F_{obs}| - |F_{calc}|\sum_h|F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes, respectively, for reflection h. [c] R_{free} = R factor for a 9.2% subset of reflections not used in the refinement.

Mouse Xenograft Procedure. HCT116 human colon carcinoma cells or NCI-H460 cells were implanted subcutaneously at a concentration of 1×10^7 cells (0.1 mL) per animal into the inguinal region of female nude mice. The test drugs were administered intraperitoneally once a day, every day, for 14 days beginning 7−10 days following cell injection. On the fifteenth day after initial drug administration, tumors were removed and weighed, and tumor growth inhibition index (%) was determined according to the following formula: tumor growth inhibition index (%) = (1 − mean net tumor weight of experimental group/mean net tumor weight of control group) × 100.

Acknowledgment. We thank Jim Krywko for generating models of the cyclin-bound conformation of CDK4 and J. Gerry Everlof for performing log P measurements.

Supporting Information Available: Table of combustion and HPLC purity values for the final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(19) **Western Blot Analysis**. Asynchronous subconfluent HCT116 or NCI H460 cells were assayed for CDK/cyclin protein expression. Cells were processed by lysing an SDS-based buffer containing phosphatase and proteinase cocktails and subjected to analysis by SDS gel electrophoresis on 10% polyacrylamide gels. Gel loading was normalized for total protein as determined by BioRad's DC protein analysis kit, and 5 μg of protein was loaded per lane. Gels were blotted onto nitrocellulose membranes. Membranes were probed with the following antibodies: anti-himan CDK-4 (#06−139, Upstate Biotechnology), anti-cyclin D1 (#sc-753, Santa Cruz Biotechnology), 0Rb14001A (Pharmingen), anti-CDK2 (#sc-163, Santa Cruz Biotechnology), anti-cyclin E (#sc0247, Santa Cruz Biotechnology). The presence of bound antibody was detected using the following enhanced chemiluminescence procedures as follows: a species-specific secondary antibody conjugated to biotin was allowed to react with blots previously probed with protein-specific primary antibodies. After extensive washes, blots were incubated with biotinylated horseradish peroxidase (HRP) and avidin (Vector Laboratories Vectastain ABC Kit). Following extensive washes, the HRP substrate luminal (NEN Renaissance reagent) was added and allowed to react for 1 min. Membranes were lightly hand-blotted dry, exposed to Kodak X-OMAT Blue autoradiography film, and developed.

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JM020455U