Use of a Pharmacophore Model for the Design of EGF-R Tyrosine Kinase Inhibitors: 4-(Phenylamino)pyrazolo[3,4-*d*]pyrimidines

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In the course of the random screening of a pool of CIBA chemicals, the two pyrazolopyrimidines **1** and **2** have been identified as fairly potent inhibitors of the EGF-R tyrosine kinase. Using a pharmacophore model for ATP-competitive inhibitors interacting with the active site of the EGF-R protein tyrosine kinase (PTK), the class of the pyrazolo[3,4-*d*]pyrimidines was then optimized in an interactive process leading to a series of 4-(phenylamino)-1*H*-pyrazolo[3,4-*d*]-pyrimidines as highly potent inhibitors of the EGF-R tyrosine kinase. The most potent compounds **13**, **14**, **15**, **17**, **19**, **22**, **26**, **28**, and **30** of this series inhibited the EGF-R PTK with IC_{50} values below 10 nM. High selectivity toward a panel of nonreceptor tyrosine kinases (*c-Src*, *v-Abl* and serine/threonine kinases (PKC α, CDK1) was observed. In cells, EGF-stimulated cellular tyrosine phosphorylation was inhibited by compounds **13**, **15**, **19**, **22**, and **23** at IC_{50} values below 50 nM, whereas PDGF-induced tyrosine phosphorylation was not affected by concentrations up to 10 μM, thus indicating high selectivity for the inhibition of the ligand-activated EGF-R signal transduction pathway. Compounds **15** and **19** inhibited proliferation of the EGF-dependent MK cell line with IC_{50} values below 0.5 μM. In addition, two compounds, **9** and **11**, showing satisfactory oral bioavailability in mice after oral administration, exhibited good *in vivo* efficacy at doses of 12.5 and 50 mg/kg in a nude mouse tumor model using xenografts of the EGF-R overexpressing A431 cell line. From SAR studies, a binding mode for 4-(phenylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidines, especially for compound **15**, at the ATP-binding site of the EGF-R tyrosine kinase is proposed. 4-(Phenylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidines represent a new class of highly potent tyrosine kinase inhibitors which preferentially inhibit the EGF-mediated signal transduction pathway and have the potential for further evaluation as anticancer agents.

Introduction

Protein tyrosine kinases (PTK) play a fundamental role in signal transduction pathways. Deregulated PTK activity has been observed in many proliferative diseases (e.g. cancer, psoriasis, restenosis, etc.).[1] Tyrosine kinases are therefore attractive targets for the design of new therapeutic agents.

The family of the epidermal growth factor receptor (EGF-R) PTK belongs to the larger class of the transmembrane growth factor receptor PTK's. This EGF-R family contains four members, the EGF-R kinase (*c-erb*B-1 gene product), the p185^{erb}B2 (*c-erb*B-2 gene product), and the recently identified *c-erb*B-3 and *c-erb*B-4 gene products. The EGF-R and its ligands (EGF, TGF-α) have been implicated in numerous tumors of epithelial origin (e.g. squamous cell carcinoma; breast, ovarian, NSC lung cancer)[1,2] and proliferative disorders of the epidermis such as psoriasis.[3]

Inhibitors of the EGF-R PTK are therefore expected to have great therapeutic potential in the treatment of malignant and nonmalignant epithelial diseases. Due to the involvement of tyrosine kinases in many signal transduction pathways, it will be important to develop inhibitors with high selectivity at the enzyme level.

In recent years, a number of different classes of compounds have been reported as tyrosine kinase inhibitors and reviewed in several articles.[4−9] Although many of these published compounds exert potent tyrosine kinase inhibition, they often lack selectivity or show only weak cellular and *in vivo* potency. In our view, these inhibitors appear to have low potential for the development as pharmaceuticals, but can serve as excellent tools for *in vitro* signal transduction studies.

Kinase inhibitors competing with ATP for binding at the catalytic domain of their target enzyme form a separate class of inhibitors. Due to the fact that the catalytic domains of most protein kinases have significant amino acid sequence homology and a conserved core structure, it was believed for a long time that compounds interacting with the ATP-binding site will not result in selective inhibitors. However, in the meantime numerous examples of structurally diverse classes have proved to be highly potent and selective ATP-competitive tyrosine kinase. This includes dianilinophthalimides (e.g. CGP 52 411)[10−12] and a special group of compounds containing a (phenylamino)pyrimidine moiety in their structure such as (phenylamino)-pyrimidines (e.g. CGP 53 716 or CGP 57 148),[13−15] (phenylamino)quinazolines (e.g. PD 153 035),[16−22] 7-amino-4-(phenylamino)pyrido[4,3-*d*]pyrimidines (e.g. PD 158 780),[23,24] and the recently published 4-(phenylamino)pyrrolopyrimidines (e.g. CGP 59 326)[25] (Figure 1).

In the present paper, we describe the rational design, synthesis, biological profile, and structure−activity relationships (SAR) of a further class of compounds containing a (phenylamino)pyrimidine moiety as a

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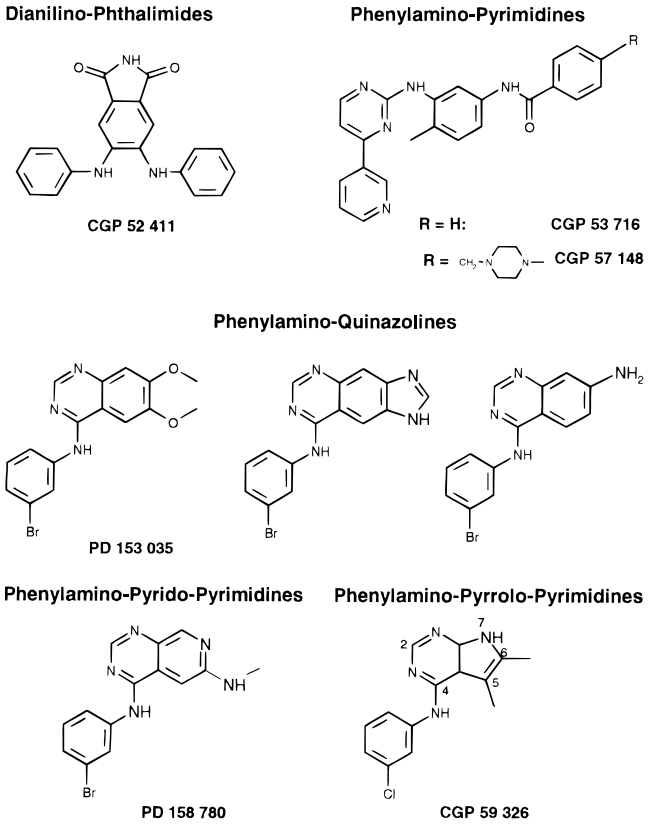


Figure 1. ATP-competitive tyrosine kinase inhibitors.

structural element in their molecule as highly potent and selective EGF-R PTK inhibitors.

Inhibitor Design

In the course of our search for PTK inhibitors via the random screening of a pool of CIBA chemicals, we identified the two pyrazolopyrimidines **1** and **2** as fairly potent inhibitors of the EGF-R PTK with IC_{50} values of 0.80 and 0.22 μM, respectively (Figure 2). Simultaneously, we developed a pharmacophore model for ATP-competitive inhibitors in the active site of the EGF-R PTK.[26] Using a calculated 3D computer model of the catalytic domain of the EGF-R tyrosine kinase,[27] which was based on published data of the X-ray structure of the cyclic-AMP-dependent protein kinase,[29] together with the dianilinophthalimide CGP 52 411 (Figure 1) as an example of a selective ATP-competitive inhibitor of the EGF-R kinase, we arrived at the following assumptions for this pharmacophore model[26] (Figure 3):

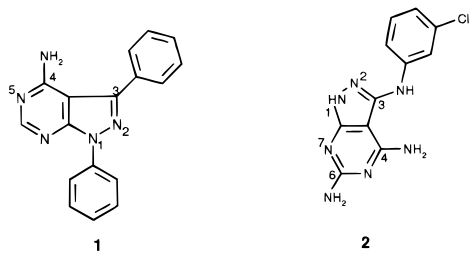


Figure 2.

ATP is anchored to the active site of the enzyme by two key hydrogen bonds involving the amino group and the N(1) pyrimidine nitrogen of the adenine moiety (donor−acceptor system).

Such a donor−acceptor system is important for EGF-R PTK inhibitors binding at the ATP binding site.

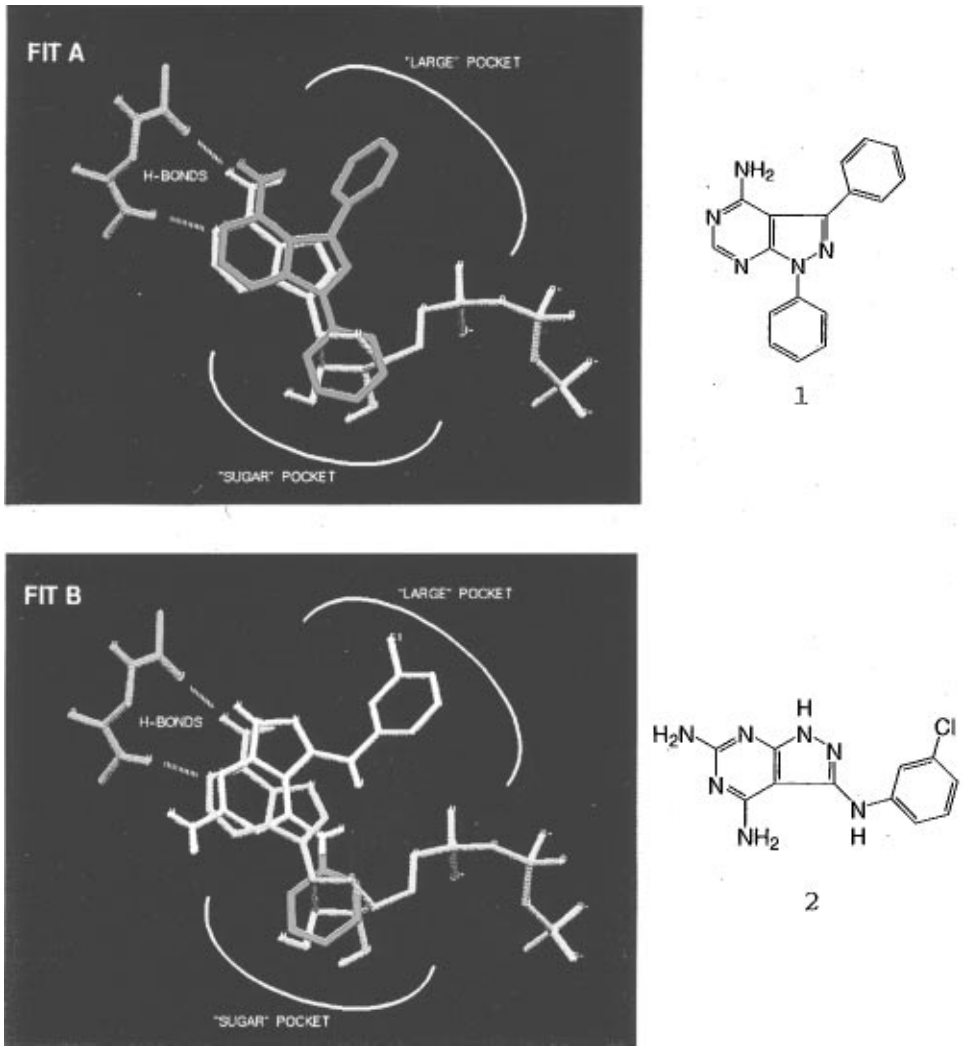
The ribose moiety of ATP can be replaced by a phenyl moiety conferring potency as well as selectivity for the EGF-R PTK ("sugar pocket").

Existence of a large hydrophobic pocket in the cavity of the enzyme not exploited by ATP (opposite to the sugar pocket).

This model has been instrumental in the identification of the 4-(phenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidines as a novel structural class of highly potent and selective EGF-R PTK inhibitors (Figure 1) which were then further optimized in an interactive process.[25] It was postulated that in 4-(phenylamino)pyrrolopyrimidines (e.g. CGP 59326), the pyrrole NH(7) and the N(1) of the pyrimidine ring form a similar bidentate hydrogen bond donor−acceptor system with the EGF-R enzyme as ATP and that a *m*-chlorophenyl moiety replaces ribose in the sugar pocket.

Stimulated by the successful application of our pharmacophore model to the class of the (phenylamino)-pyrrolopyrimidines, we tried to apply it on the pyrazolopyrimidines **1** and **2**. In fact, we found an application of our model for structure **1** as well as for structure **2** (dual fit). Superimposition of structure **1** with ATP suggests the bidentate hydrogen bond donor−acceptor system for the 4-amino group and the N(5) pyrimidine nitrogen. In this binding mode, the phenyl ring attached to the N(1) of the pyrazole ring would replace ribose of ATP in the sugar pocket, whereas the phenyl substituent at the C(3) position of the pyrazole ring points toward the large hydrophobic pocket (fit A, Figure 3). In fact, when we screened our compound library for structural analogues of pyrazole **1**, three compounds exhibiting moderate tyrosine kinase inhibitory activity were found, thus supporting the hypothesis of this binding mode A. 4-Amino-1-phenylpyrazolo[3,4-*d*]pyrimidine **3** as well as 4-amino-7-phenyl-5,6-dimethylpyrrolo[3,4]pyrimidine **4** had an IC_{50} value of 2.7 and 2.8 μM, respectively, whereas 4-amino-9-purine **5** only had slightly lower inhibitory activity (Figure 4). Recently, Pfizer scientists described in a patent[30] and in a publication[31] a series of 4-aminopyrazolo[3,4-*d*]pyrimidines with substituted aromatic rings at the pyrazole nitrogen and at the 3-position of the pyrazole ring corresponding to binding mode A. Compound **1** of this paper was included in this patent. An analogue thereof with a tertiary butyl group on the pyrazole nitrogen was described to be a potent inhibitor of the *Src* family of tyrosine kinases.[31]

Superposition of compound **2** on ATP (fit B, Figure 3) assumes a similar binding mode of pyrazole **2** (or its equipotent 6-desamino derivative **6**, Table 1) as has already been demonstrated with 4-(phenylamino)pyrrolopyrimidines.[25] This mode implies that (1) the NH-(1) of the pyrazole ring and N(7) of the pyrimidine ring form the bidentate hydrogen bond donor−acceptor system, (2) the 4-amino group points toward the sugar pocket not yet filling it, and (3) the anilino substituent at the C(3) position of the pyrazole ring again is filling the large hydrophobic pocket. This binding mode B suggests to add a phenyl or *m*-chlorophenyl moiety, respectively, to the 4-amino group of the pyrimidine ring, thus leading to the pyrazolopyrimidine analogues **7**−**9** as first target structures. In fact, the designed



1

2

Figure 3. Superimposition of ATP (yellow) with compound **1** (red) or compound **2** (white). Fit A: compound **1** (red) superimposed on ATP (yellow). Fit B: compound **2** (white) superimposed on ATP (yellow). According to the pharmacophore hypothesis, the model suggests to add a phenyl moiety (pink) to the 4-amino group of **2** (or **6**, the equipotent 6-desamino analogue of **2**).

3
EGF-R: 2.7 μM

4
EGF-R: 2.71 μM

5
EGF-R: 15 μM

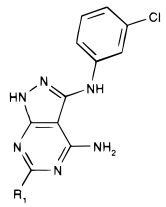
Figure 4. ATP analogues.

compound **9** was almost 1 order of magnitude more potent than the parent compound **2** or 5 times more potent than the desamino compound **6**. Since we already knew from our own experiences with 4-(phenylamino)pyrrolopyrimidines[25] and from experiences with 4-(phenylamino)quinazolines[21] and related structural classes[23] that electron-withdrawing substituents (Cl, Br) in the meta position of the phenylamino moiety are optimal for inhibitory activity, we did only limited SAR studies at this position. Our efforts in this first series of 4-(phenylamino)pyrazolopyrimidines concentrated mainly on derivatives with various substituents at the C(3) position of the pyrazole ring, thereby exploiting the capacity of the large hydrophobic pocket of the target enzyme.

Chemistry

4-(Phenylamino)pyrazolo[3,4-*d*]pyrimidines **2**, **6**−**32** were synthesized according to different procedures (Schemes 1−3). Commercially available 3,3-bis(methylthio)-2-cyanoacrylonitrile was used as starting material for derivatives **2**, **6**−**22** bearing a substituted anilino or benzylamino side chain in position 3 of the pyrazole ring. Displacement reaction of 3,3-bis(methylthio)-2-cyanoacrylonitrile with substituted anilines or substituted benzylamines, respectively, according to published procedure[32,33] afforded the substituted 3-anilino-3-(methylthio)-2-cyanoacrylonitriles **33a**−**k** (Scheme 1). Reflux of **33a**,**b** (R = H or Cl) with benzylhydrazine in ethanol gave the 1-*N*-benzyl-3-anilino-5-amino-4-cyanopyrazoles **34a**,**b**. If hydrazine hydrate was used for the pyrazole ring formation, pyrazoles **35a**−**i** were obtained. Conversion of 3-anilino-5-amino-4-cyanopyrazoles **34a**,**b** or **35a**−**i** to pyrazolo[3,4-*d*]pyrimidines was achieved by two different methods: Ring closure of **34b** (R_2 = *m*-Cl) with guanidine hydrochloride gave the *N*-benzyl-protected 4,6-diaminopyrazolo[3,4-*d*]pyrimidine **36a** and upon removal of the benzyl protecting group with $AlCl_3$ in toluene compound **2** (Scheme 1). If formamide was used for the pyrimidine ring formation, the 4-aminopyrazolopyrimidine **36b** was obtained and

Table 1. EGF- R Tyrosine Kinase Activity and *in Vitro* Selectivity of Derivatives



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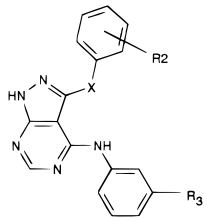

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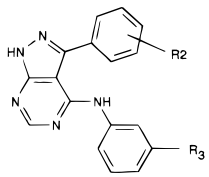

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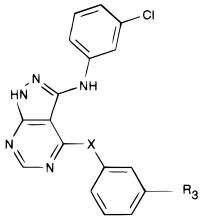


31, 32

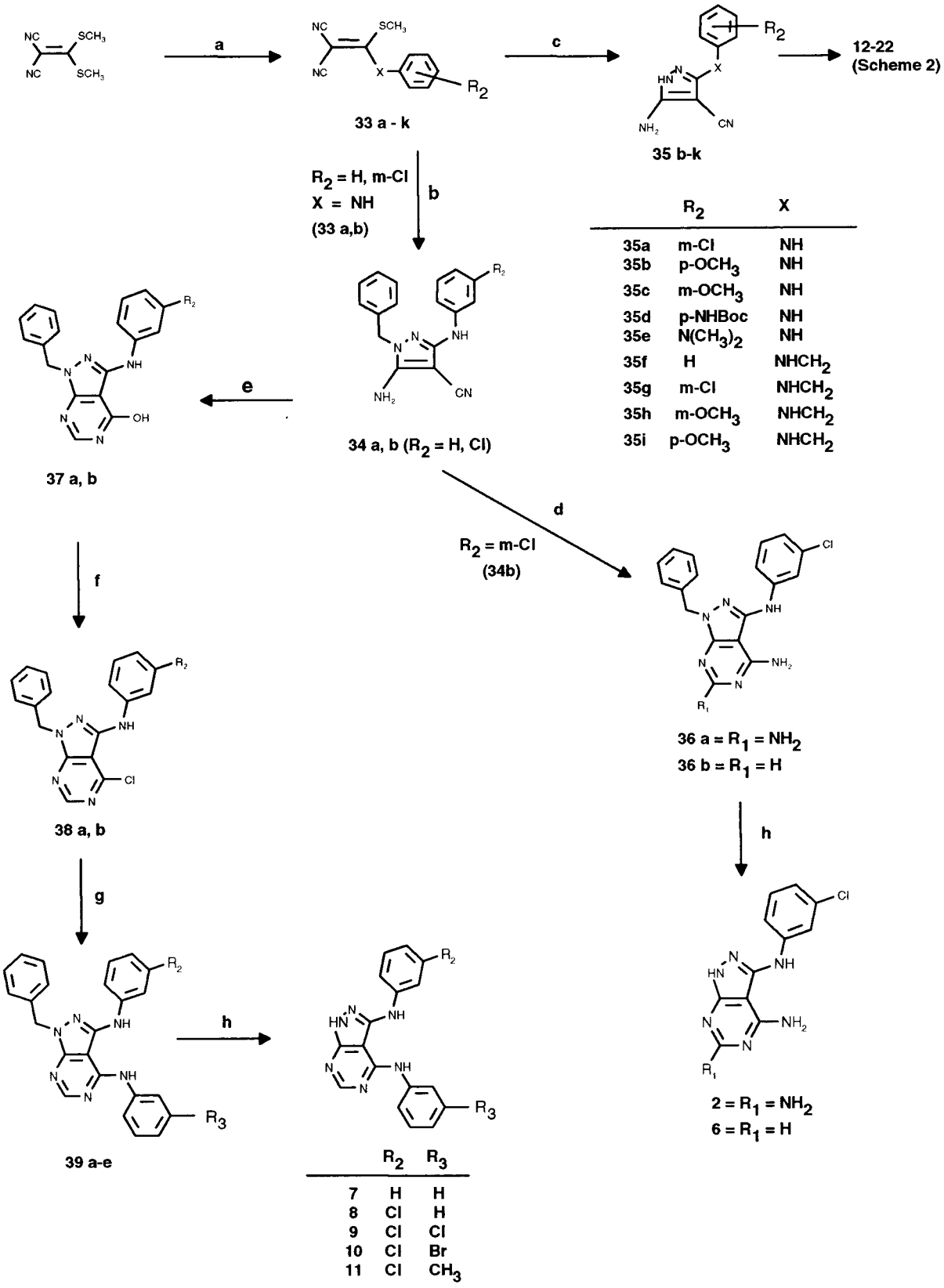
compd	R_1	R_2	R_3	X	formula	EGF-R[a]	*v-Abl*[a]	*c-Src*[a]	PKC-α[a]	CDK1[a]
1					$C_{17}H_{13}N_5$	0.80	>10	>10	>100	nt
2	NH_2				$C_{11}H_{10}ClN_7$	0.22	1.90	>10	>100	nt
6	H				$C_{11}H_9ClN_6$	0.16	0.41	6	24	nt
7		H	H	NH	$C_{17}H_{14}N_6$	0.058	1.96	75	6.9	nt
8		*m*-Cl	H	NH	$C_{17}H_{13}ClN_6$	0.084	1.36	2.25	7.8	nt
9		*m*-Cl	Cl	NH	$C_{17}H_{12}Cl_2N_6$	0.033	1.98	5.3	5.9	nt
10		*m*-Cl	Br	NH	$C_{17}H_{12}BrClN_6$	0.13	1.36	>10	3.8	nt
11		*m*-Cl	CH_3	NH	$C_{18}H_{15}ClN_6$	0.13	1.65	8.08	4.7	nt
12		*p*-OCH_3	Cl	NH	$C_{18}H_{15}ClN_6O$	0.030	>10	>10	19	5.80
13		*p*-OH	Cl	NH	$C_{17}H_{13}ClN_6O$	0.008	0.15	0.4	>1	>1
14		*m*-OCH_3	Cl	NH	$C_{18}H_{15}ClN_6O$	0.008	3.40	7.8	4.70	4.30
15		*m*-OH	Cl	NH	$C_{17}H_{13}ClN_6O$	0.001	0.26	0.22	0.56	3.0
16		*p*-NHBOC	Cl	NH	$C_{22}H_{22}ClN_7O_2$	>10	>10	>10	>10	57
17		*p*-NH_2	Cl	NH	$C_{17}H_{14}ClN_7$	0.005	1.18	2.4	>1	<10
18		*p*-$N(CH_3)_2$	Cl	NH	$C_{19}H_{18}ClN_7$	0.029	>10	>10	2.90	0.35
19		H	Cl	$NHCH_2$	$C_{18}H_{15}ClN_6$	0.007	6.60	>10	10.0	7.40
20		*m*-Cl	Cl	$NHCH_2$	$C_{18}H_{14}Cl_2N_6$	0.026	>10	2.09	>10	16.0
21		*m*-OCH_3	Cl	$NHCH_2$	$C_{19}H_{17}ClN_6O$	0.008	3.50	>10	>100	3.10
22		*p*-OCH_3	Cl	$NHCH_2$	$C_{19}H_{17}ClN_6O$	0.007	>10	>10	>100	5.0
23		H	Cl		$C_{17}H_{12}ClN_5$	0.019	3.76	>10	>10	2.80
24		*m*-OH	Cl		$C_{17}H_{12}ClN_5O$	0.026	0.18	0.002	>10	0.2
25		*p*-OCH_3	Cl		$C_{18}H_{14}ClN_5O$	0.096	16.50	>10	>10	>100
26		*p*-OH	Cl		$C_{17}H_{12}ClN_5O$	0.006	2.4	8.55	1.85	0.26
27		*m*-NO_2	Cl		$C_{17}H_{11}ClN_6O_2$	0.037	>10	>10	>100	>10
28		*m*-NH_2	Cl		$C_{17}H_{13}ClN_5$	0.005	3.09	>10	1.50	0.68
29		*p*-NHBOC	Cl		$C_{22}H_{21}ClN_6O_2$	0.27	>10	9.8	>10	>100
30		*p*-NH_2	Cl		$C_{17}H_{13}ClN_6$	0.002	3.60	4.9	3.6	4.50
31			Cl	O	$C_{17}H_{11}Cl_2N_5O$	24.2	>10	>10	>100	nt
32			H	$NHCH_2$	$C_{18}H_{15}ClN_6$	0.87	>10	>10	4.50	9.0
PD 153 035						0.006	0.03	1.4	>100	~100

[a] Expressed in IC_{50} values (μM).

compound **6** after deprotection. For the synthesis of compounds **7**−**11** (Scheme 1), 5-amino-4-cyanopyrazoles **34a**,**b** were refluxed with 85% formic acid to give the corresponding 3-substituted *N*-benzyl-4-hydroxypyrazolo[2,3-*d*]pyrimidines **37a**,**b** which were then converted to the corresponding chlorides **38a**,**b** by reaction with phosphoryl chloride. Substitution of the chloride with various substituted anilines afforded 1-benzyl-3-substituted-4-(phenylamino)pyrazolo[2,3-*d*]pyrimidines **39a**−**e**, which after removal of the benzyl group gave the final products **7**−**11**. The phenoxy compound **31** was prepared by reaction of the chloride **38b** with *m*-chlorophenol followed by removal of the benzyl group. Using pyrazoles **35a**−**i**, an improved and shorter synthesis, avoiding benzyl protection, was applied for the preparation of derivatives **12**−**22** and **32** (Scheme 2). Reaction of **35a**−**c**,**e**−**i** with *N*,*N*-dimethylformamide diethyl acetal in toluene afforded the amidines **40a**−**h** while by the reaction of **35d** (R_2 = NHBOC) with orthoformic acid triethyl ester **41a** was obtained. The amidines **40a**−**h** as well as **41a** were directly converted to the final products **12**, **14**, **16**, **18**, and **19**−**22** with *m*-chloroaniline in boiling alcohols. The reaction probably proceeds via an imino-type intermediate (only isolated in the conversion of **41a** to **16**) which rearranged to the final products. Compounds **13** and **15** were obtained by cleavage of the corresponding methyl ethers **12** and **14**, respectively, with boron tribromide or aluminum trichloride. The amine **17** was obtained by removal of the BOC protection group from **16**. Compound **32** was obtained by reaction of **40a** (R = *m*-Cl) with benzylamine instead of *m*-chloroaniline.

The synthesis of compounds **23**−**29**, where an aromatic moiety is directly attached to the pyrazole ring, started from commercially available tetracyanoethylene oxide (Scheme 3). Reaction of substituted phenyl dithioesters with tetracyanoethylene oxide in toluene according to a published procedure[34] gave the corresponding 3-phenyl-2-cyano-3-(methylthio)acrylonitriles **42a**−**e** in good yields. As described before, two different ways for the conversion of **42a**−**e** to the pyrazolopyrimidines **23**−**29** were chosen. Reaction of **42a**,**b** with benzylhydrazine gave the *N*-benzyl-3-aryl-5-amino-4-cyanopyrazoles **43a**,**b**. The conversion of **43a**,**b** to the 3-aryl-4-[(3-chlorophenyl)amino]pyrazolo[3,4-*d*]pyrimidines **23** and **24** followed the same sequence of reactions as described in Scheme 1 for the conversion of **34a**,**b** to compounds **7**−**11**, which is ring closure with formic acid to **44a**,**b**, followed by $POCl_3$ reaction to the chlorides **45a**,**b**, substitution of the chlorides with *m*-chloroaniline to **46a**,**b**, and finally removal of the benzyl protecting group to the final products **23** and **24**. In the course of the removal of the benzyl protecting group from the methoxy derivative **46b** with $AlCl_3$, the ether was also

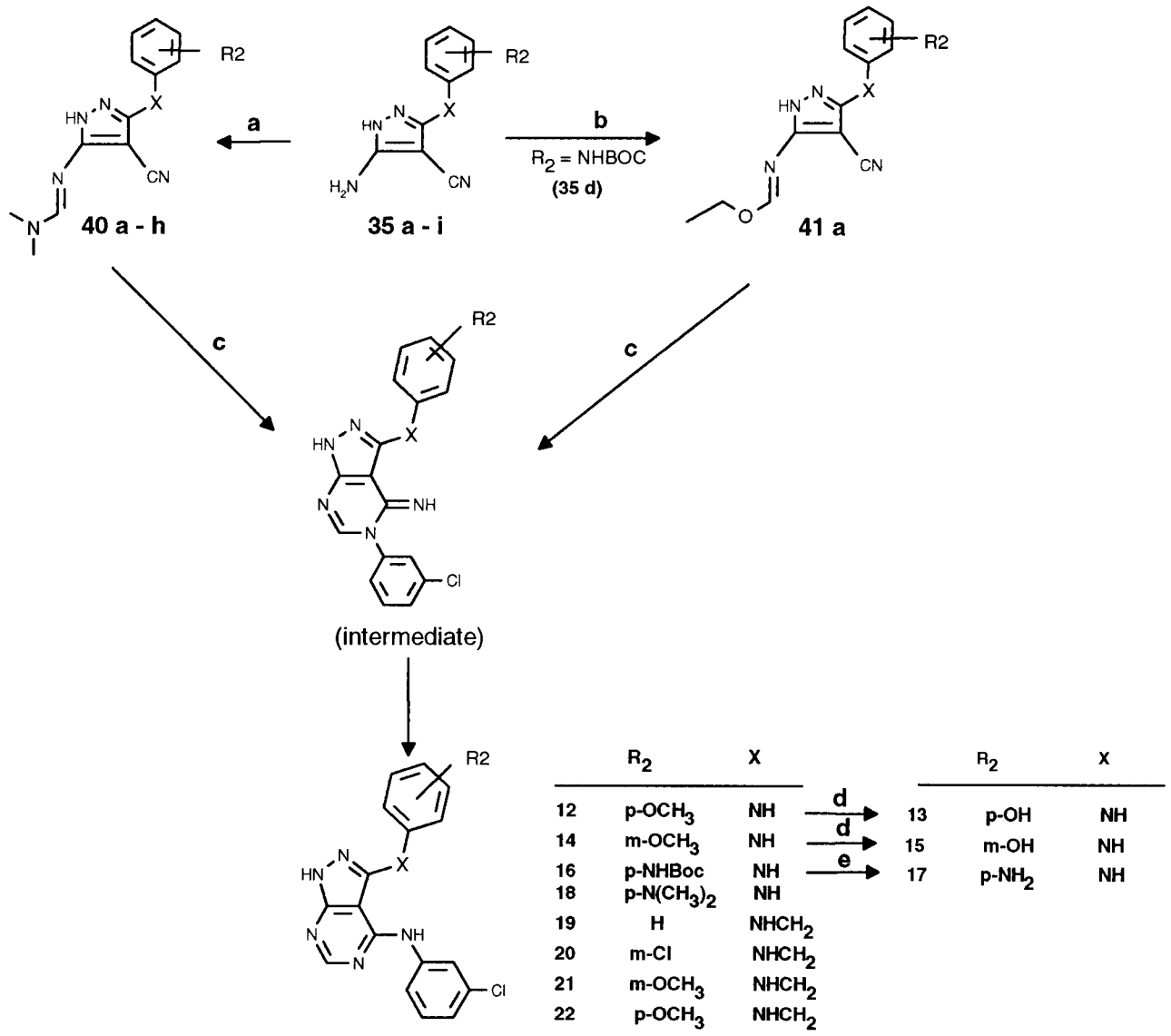
Scheme 1[a]



[a] Reagents and conditions: (a) substituted aniline or benzylamine; (b) benzylhydrazine, sodium, ethanol, reflux; (c) hydrazine hydrate, methanol, reflux; (d) guanidine hydrochloride, sodium, methoxyethanol, 100 °C or formamide; (e) formic acid (85%), 110 °C, 5 h; (f) $POCl_3$, reflux; (g) substituted aniline (phenol), ethanol (1-butanol), reflux; (h) $AlCl_3$, benzene or toulene, reflux.

cleaved to give **24**. Again, a shorter synthesis uses **42c**−**e** which were converted to the corresponding substituted 3-aryl-5-amino-4-cyanopyrazoles **47a**−**c** by reflux with hydrazine hydrate and then to the corresponding amidines **48a**−**c** with dimethylformamide diethyl acetal. Only amidines **48a**,**b** could directly be converted to the final products **25** and **27** in acceptable yields by reaction with *m*-chloroaniline. The *p*-nitro analogue **48c** had to be converted to its *N*-BOC-protected amine **48e** before reaction with *m*-chloroa-

Scheme 2[a]



[a] Reagents and conditions: (a) *N,N'*-dimethylformamide dimethyl acetal, toluene, reflux, 5 h; (b) orthoformic acid triethylester, reflux; (c) *m*-chloroaniline hydrochloride, methanol, reflux, 17 h; (d) BBr_3 or $AlCl_3$; (e) HCl, methanol (dioxane).

niline occurred (reduction of **48c** to the amine **48d**, followed by *N*-BOC protection). Finally, ether cleavage of **25** gave **26**; reduction of the nitro derivative **27** gave the amino derivative **28** and by deprotection of **29** the amine **30** was obtained.

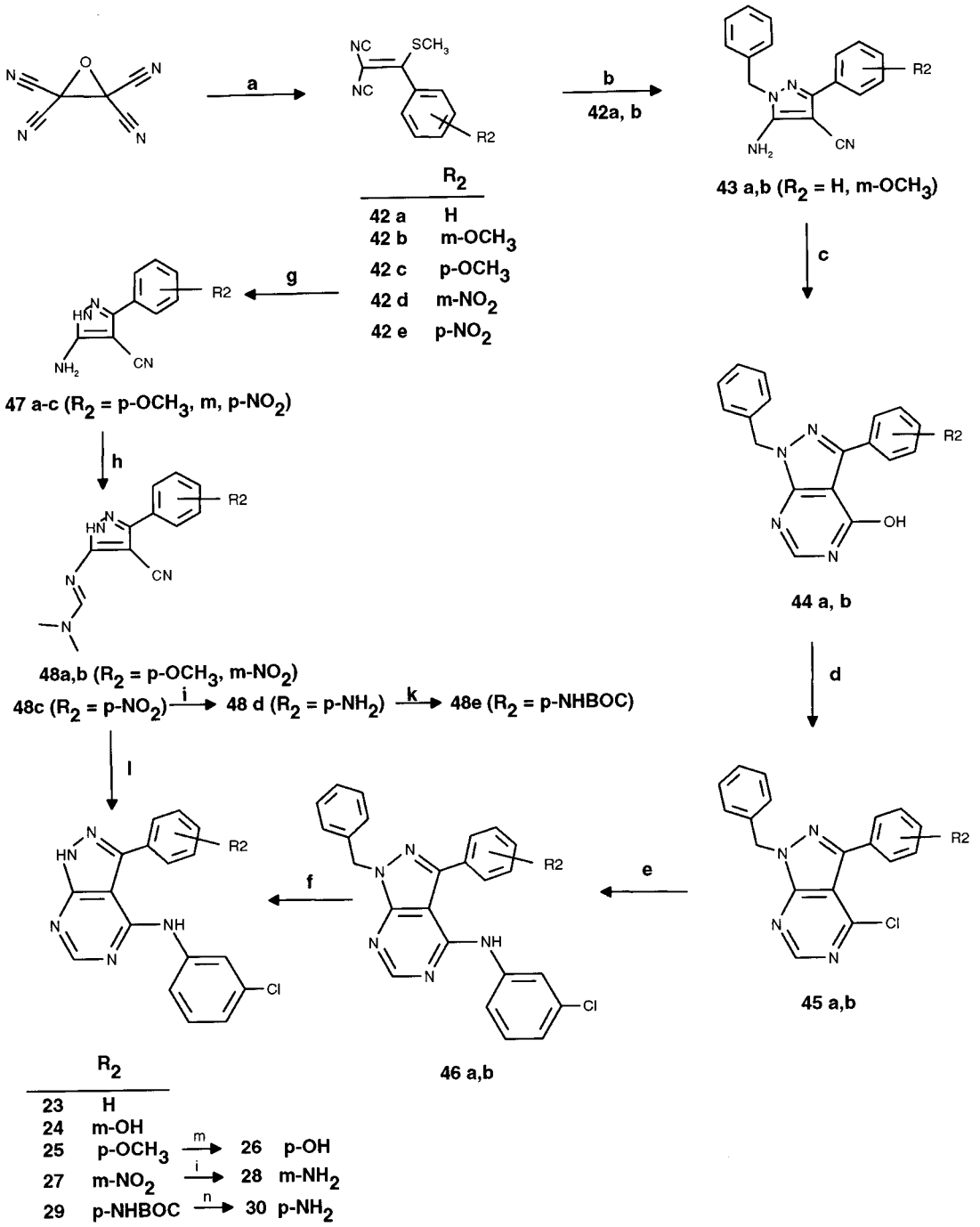
Biological Evaluation

Enzymatic Activity. Compounds were tested for inhibition of the tyrosine kinase activity of a recombinant, intracellular domain of the EGF-R (ICD) using angiotensin II as the phosphate-acceptor substrate (Table 1). Selectivity was assayed against a panel of tyrosine (*c-Src* and *v-Abl*) and serine/threonine kinases (PKC-α and CDK1) (Table 1).

The following structure−activity relationships (SAR) were derived from the *in vitro* data:

The amino group in position 6 of the pyrimidine ring can be removed without loss of activity (compounds **2** and **6**). As predicted from our pharmacophore model, introduction of an anilino, or especially the *m*-chloroanilino moiety in the position 4 of the pyrimidine ring led to compounds with improved activity (compounds **7**−**9**). Compound **9** (IC_{50} = 33 nM) was 5 times more active than compound **6** (IC_{50} = 160 nM) with an amino group in position 4. From our own experiences with 4-(phenylamino)pyrrolopyrimidines[25] and from experiences with 4-(phenylamino)quinazolines and related structural classes we already knew that electron-withdrawing substituents (Cl, Br) in the meta position of the phenylamino moiety are optimal for inhibitory activity. Therefore, only a few substituents were introduced in this position (compounds **9**−**11**). As in the (phenylamino)pyrrolopyrimidine series,[25] the *m*-chloro analogue **9** was superior to the *m*-bromo- or *m*-methyl analogues **10** and **11**. In exploiting the size of the large hydrophobic pocket, we learned that replacement of the anilino moiety in position 3 of the pyrazole ring by a larger substituted benzylamino moiety (compounds **19**−**22**) or by a substituted phenyl ring directly attached to the pyrazole ring (compounds **23**−**30**) also led to highly potent inhibitors in the nanomolar range. With regard to substituents at the aromatic ring, there seems to be

Scheme 3[a]



[a] Reagents and conditions: (a) substituted phenyl dithioester, toluene, room temperature/reflux; (b) benzylhydrazine, methanol, reflux, 7 h; (c) formic acid (85%), 110 °C, 5 h; (d) $POCl_3$, reflux; (e) *m*-chloroaniline, ethanol, reflux; (f) $AlCl_3$, benzene or toulene, reflux; (g) hydrazine hydrate, methanol, reflux; (h) *N*,*N*-dimethylformamidediethyl acetal, reflux; (i) Raney nickel, methanol; (k) di-*tert*-butyl dicarbonate, dioxane, 80 °C, 10 h; (l) *m*-chloroaniline hydrochloride, methanol, reflux; (m) BBr_3 or $AlCl_3$; (n) HCl/dioxane.

no preference in all three series. Electron-withdrawing substituents in the meta or para position as Cl (compounds **9** or **20**), methoxy (compounds **12**, **14**, **21**, **22**, or **25**), or hydroxy (compounds **13**, **15**, **24**, or **26**) as well as electron-donating NH_2 or $N(CH_3)_2$ substituents (compounds **17**, **18**, **28**, and **30**) led to derivatives with IC_{50}s below 30 nM. Cleavage of the methyl ethers **12**, **14**, and **25** to the corresponding hydroxy compounds **13**, **15**, and **26** increased potency: the *m*-hydroxy derivative **15** (IC_{50} = 1 nM), the *p*-hydroxy analogue **13** (IC_{50} = 8 nM), or the *p*-hydroxy analogue **26** (IC_{50} = 6 nM) are 4−16 times more potent than their corresponding methoxy analogues **14**, **12**, or **25**, respectively. The limitation of the size of the hydrophobic pocket is indicated by the two *N*-BOC-protected compounds **16** and **29** which were more than 100 times less active than their free amines **17** and **30**. Interestingly, the *N*-BOC-phenyl compound **29** (IC_{50} = 270 nM) still retained some activity, whereas the *N*-BOC-anilino compound **16** was devoid of any activity (IC_{50} > 10 μM). This can be explained by the fact that in **16** and **29** the substituents point in slightly different directions. In the inactive compound **16**, the boundary of the pocket is probably reached, whereas in **29** the *N*-BOC anilino substituent extends in the direction of the triphosphate of ATP, thereby retaining some moderate activity. The most potent derivatives of this

whole series were the hydroxy compounds **13** (IC_{50} = 8 nM), **15** (IC_{50} = 1 nM), and **26** (IC_{50} = 6 nM) and the three amino compounds **17** (IC_{50} = 5 nM), **28** (IC_{50} = 5 nM), and **30** (IC_{50} = 2 nM), indicating a possible interaction with the enzyme backbone. Compounds **15**, **17**, **19**, **22**, **26**, **28**, and **30** are at least as potent as the 4-anilinoquinazoline PD 153 035,[19] which in our assay system had an IC_{50} value of 6 nM. As already observed in the pyrrolopyrimidine series,[25] replacement of the phenylamino moiety by a conformationally different benzyl (compound **32**) or a *m*-chlorophenoxy group (compound **31**) led to a dramatic loss of activity, thus indicating that there is only limited space available in the sugar pocket.

When tested for selectivity against the serine/threonine kinases PKC-α and CDK1, active compounds in general showed a high selectivity ratio against PKC-α and CDK1 (Table 2). A slightly different selectivity picture was observed with regard to selectivity against the *v-Abl* and *c-Src* tyrosine kinases. Decreased selectivity was observed with compounds **13**, **15**, and **24**, which also showed some activity against the *v-Abl* and *c-Src* kinases. Interestingly, compound **24** was even more potent against the *c-Src* kinase (IC_{50} = 2 nM) than against the EGF-R kinase. However, in most cases a selectivity ratio of >50 was achieved.

Cellular Activity. To study the cellular mode of action and specificity, active compounds were tested in a series of cellular assay systems using cell lines responding to EGF or other growth factors (e.g. PDGF) for their growth. Monitoring both the modulation of tyrosine phosphorylation in EGF-dependent and in EGF-independent cell lines offers a convenient method to analyze the cellular mode of action and selectivity of protein kinase inhibitors for EGF-mediated signal transduction in the cell. Inhibition of EGF-stimulated protein phosphorylation was therefore measured in EGF-R overexpressing A431 cells using an ELISA-type assay,[10] whereas inhibition of PDGF-stimulated tyrosine phosphorylation was assayed in BALB/c 3T3 cells. Finally, inhibition of cell proliferation by inhibitors was measured using an EGF-dependent mouse keratinocyte cell line (Balb/MK). Cellular inhibition of phosphorylation or proliferation of other members of the EGF-R family of kinases (c-erb B2, B3, B4) was not assayed with these compounds.

As shown in Table 2, in general, *in vitro* potent compounds with a *m*-chloro-substituted phenylamino moiety in position 4 of the pyrimidine ring were also potent inhibitors of EGF-stimulated cellular tyrosine phosphorylation. In this assay, the most potent compounds **13**, **15**, **19**, **22**, and **23** had IC_{50}s < 50 nM; compounds **15** and **19** even below <5 nM. Compounds **1**, **2**, and **6**, although fairly potent at the enzyme level but lacking a 4-phenylamino moiety, were only weakly active in the cellular assays. Compounds **16**, **31**, and **32** which showed only weak enzyme inhibition were also inactive in cells. Although potent *in vitro*, the nitro derivative **27** was not able to inhibit cellular tyrosine phosphorylation, probably due to lack of penetration into the cells. PDGF-induced phosphorylation was not inhibited by any of the compounds of this series, thus indicating high selectivity for the inhibition of the ligand-activated EGF-R signal transduction pathway.

Table 2. Cellular Activity and Specificity[a]

compd	MK Cell	EGF-ELISA	PDGF-ELISA
1	>50	nt	>10
2	2.87	9.0	>10
6	14.0	35	>10
7	8.4	12	>10
8	2.56	1.0	>10
9	1.40	0.25	10
10	1.43	<0.8	>10
11	2.70	0.15	>10
12	1.03	1.5	10
13	1.06	0.04	>10
14	0.95	0.8	>10
15	0.46	0.004	>10
16	>10	>100	>10
17	>50	0.5	>10
18	1.55	1.5	10
19	0.30	0.003	>10
20	3.19	1.5	>10
21	0.81	0.2	>10
22	0.86	0.02	>10
23	0.82	0.03	>10
24	0.99	1.5	>10
25	1.60	1.50	>10
26	0.56	0.5	>10
27	>50	>100	>10
28	0.94	0.8	>10
29	4.3	1.5	>10
30	0.78	0.80	>10
31	>50	>100	10
32	49.9	1.5	>10
PD 153 035	0.20	0.03	>10

[a] MK cell: inhibition of proliferation of EGF-dependent BALB/MK cells (IC_{50}, μM). EGF ELISA: inhibition of EGF-stimulated tyrosine phosphorylation in A431 cells (IC_{50}, μM). PDGF ELISA: inhibition of PDGF-stimulated tyrosine phosphorylation in BALB/c3T3 cells (IC_{50}, μM). nt, not tested.

All compounds, which showed potent inhibition of tyrosine phosphorylation also inhibited proliferation of the EGF-dependent Balb/MK cells. Compounds **12**−**15**, **19**, **21**−**24**, **26**, **28**, and **30** had IC_{50} values ≤ 1 μM in this assay. The most potent inhibition was observed with compounds **15** (IC_{50} = 0.46 μM) and **19** (IC_{50} = 0.30 μM). In general, there is a good correlation between the IC_{50} values for inhibition of proliferation and inhibition of tyrosine phosphorylation. There is no explanation for the behavior of the amino compound **17** which was highly active at the enzyme level and showed inhibition of tyrosine phosphorylation in cells but did not inhibit cell MK proliferation.

***In Vivo* Activity.** Unfortunately, many of the potent inhibitors at the enzyme as well as the cellular level of this series showed insufficient plasma levels (C_{max} < 1 μM) after oral administration to mice (data not shown) to be of interest for *in vivo* testing. From the whole series, only compounds **9** and **11** fulfilled the criteria of oral bioavailabilty (C_{max} > 3 μM after 2 h) and where therefore tested in a nude mouse tumor model using xenografts of the EGF-R overexpressing A431 cell line. Both compounds showed good *in vivo* efficacy at doses of 12.5 and 50 mg/kg after oral or subcutaneous administration (Table 3). Their T/C (treatment/control) values at 50 mg/kg (po administration) were 31% (compound **9**) and 23% (compound **11)**, respectively.

Discussion and Conclusions

Application of our pharmacophore model for the ATP binding site of the EGF-R PTK led to the identification of 4-(phenylamino)pyrazolo[3,4-*d*]pyrimidines as a novel class of potent and selective EGF-R PTK inhibitors. SAR

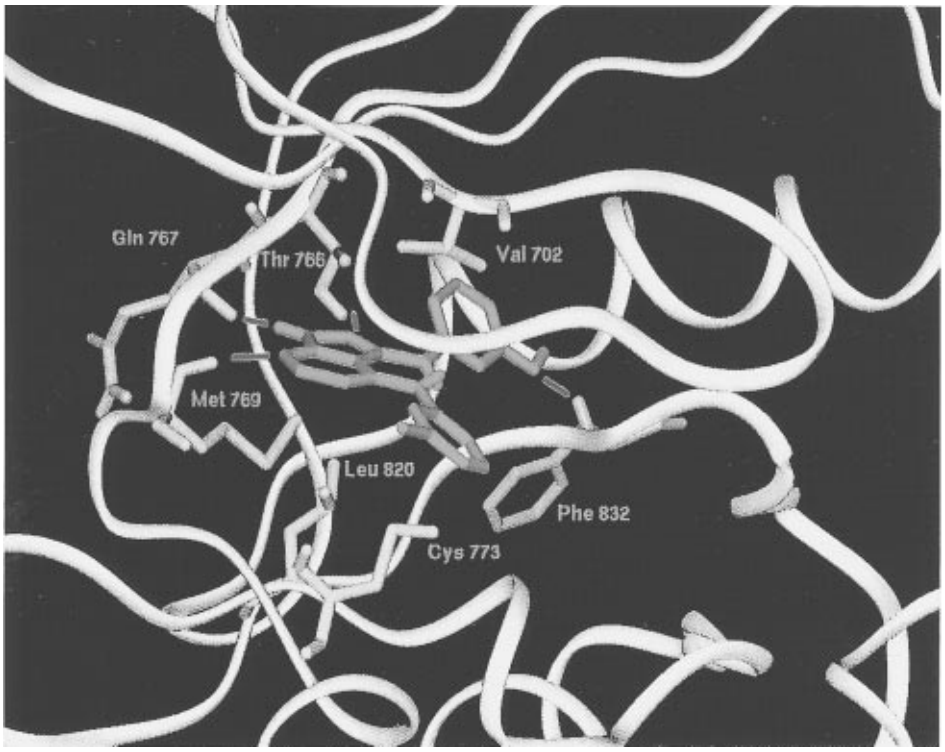


Figure 5. Compound **15** docked in the ATP binding site of the EGF-R PTK model. Compound **15** docked in the ATP binding site of the homology built model of the EGF-R PTK[27] according to the alignment with ATP defined in Figure 2 (fit B). Hydrogen bond interactions are represented as green lines. The bidentate hydrogen bonds of the pharmacophore model are made with amino acids Met 769 and Gln 767 of the protein (residues homologue to Val 123 and Glu 121 of the cyclic-AMP dependent protein kinase[29]). Two additional hydrogen bonds are assumed: one betwen the N(6) of the pyrazole ring and the side chain of Thr 766, the other between the hydroxyl group of the phenol moiety and the backbone of Phe 832. Amino acids that make large hydrophobic contacts with the inhibitor (Leu 820, Val 702, and Cys 773) are also shown. In particular, a sulfur−aromatic interaction between the chlorophenyl ring of **15** and the side chain of Cys 773 is hypothesized.

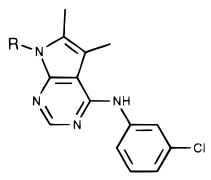
Table 3. *In Vivo* Activity of Compounds **9** and **11**[a]

		T/C (%)	
dose (mg/kg)	application	compd **9**	compd **11**
control		100	100
50	ip	25	21
12.5	ip	46	27
50	po	31	23
12.5	po	41	44

[a] Animals: female Balb/c/nu/nu (Bomholtgaard). Formulation: DMSO/Tween 80/NaCl 0.9%. Start of treatment: day 6 after transplantation. Treatment: once daily for 15 consecutive days (day 6−20).

studies in this series of derivatives show a preference for a chlorine substituent at the meta position of the 4-phenylamino moiety and for bulky substituents in 3-position of the pyrazole ring. As already found for the related class of 4-(phenylamino)pyrrolo[2,3-*d*]pyrimidines,[25] these data are in accordance with our pharmacophore CAMM model of the ATP-binding site, where we postulate the replacement of the ribose of ATP by a phenyl moiety, preferentially by a *m*-chloro-substituted phenyl ring. The marked decrease of inhibitory activity observed with compound **32** (IC_{50} = 0.87 μM) and especially with compound **31** (IC_{50} = 24.2 μM) compared to compound **9** (IC_{50} = 0.033 μM) indicates that there are stringent structural requirements for binding to the sugar pocket. In **32**, the phenyl ring is replaced by a larger benzyl group, and in **31**, the *m*-chloroanilino moiety by a *m*-chlorophenoxy group which both have different conformational properties. In compound **31**, the *m*-chloro phenyl ring adapts a perpendicular orientation with respect to the plane of the pyrimidine ring. Aligning compounds **31** and **32** on ATP according to our hydrogen bond donor−acceptor hypothesis in a homology-built model of the EGF-R PTK[27] shows that, unlike **9**, they are unable to form a favorable sulfur−aromatic interaction with residue Cys 773 of the sugar pocket. We have proposed[26] that this cysteine residue, which is unique for the EGF-R family of tyrosine kinases, plays an important role in the selective recognition of ligands by the EGF-R PTK. The tolerance for rather bulky substituted phenyl, anilino, and benzyl rings in the 3 position of the pyrazole ring confirms the presence of a large hydrophobic pocket in the ATP-binding site of the EGF-R, opening many possibilities for further optimization of this lead class. The fact, that the most potent compounds **13**, **15**, **17**, **26**, **28**, and **30** of this series have a hydroxy or amino function in the meta or para position of the phenylamino or phenyl moiety in the 3 position of the pyrazole ring indicates that an additional interaction to one of the amino acids of the enzyme is formed (Figure 5). Docking analyses of these compounds in the homology-built model of the EGF-R PTK[27] suggest a possible hydrogen bond interaction with the backbone amide function of residue Phe 832 as illustrated in Figure 5 with compound **15**. Phe 185, the residue homologue to Phe 832 in the X-ray structure of the cyclic-AMP-dependent protein kinase,[29] on which the EGF-R PTK model is based, is seen to form a hydrogen bond with a water molecule. Expulsion and replacement of this buried water molecule by an hydroxyl or amino substituent in the inhibitors can provide additional binding affinity.

This pharmacophore model has now proven its validity in the optimization of the classes of the 4-anilinopyrrolo-[25] and pyrazolopyrimidines. The structural similarity between these two classes and the 4-anilino quinazolines and 4-anilinopyridopyrimidines would suggest an identical binding mode. However, Parke-Davis scientists recently published a model for the binding mode of their 4-anilinoquinazolines and 4-anilinopyridopyrimidines, respectively, which differs in some aspects from our model.[35] While in both models a nitrogen atom of the pyrimidine ring forms the same hydrogen acceptor bond with the enzyme as the N1 atom of the adenine ring of ATP, in the Parke-Davis model the hydrogen-bond donor is missing, and the anilino moiety attached to the pyrimidine ring does not fill the sugar pocket but rather the large hydrophobic pocket. We think that there is no contradiction between the two models. X-ray crystal structures of the protein kinase CDK2 complexed with different ligands[36] has clearly shown that molecules belonging to the same chemical class (adenine derivatives) can bind to the enzyme in different orientations although utilizing common interaction sites of the protein. In fact, exactly as in the Park-Davis hypothesis concerning the 4-anilinoquinazolines, the *m*-chloroanilino moiety of compound **2** in our model occupies the large hydrophobic pocket and not the sugar pocket (Figure 3). Our strategy was to design compounds that fill both pockets. The basic difference between the 4-anilinoquinazoline and our series of pyrrolo- and pyrazolopyrimidine inhibitors is the presence of a N−H hydrogen bond donor functionality in the latter. It is unlikely that in potent inhibitors, the high desolvation energy cost due to this functionality when the compounds bind to the protein, is not at least compensated by the formation of a good hydrogen bond with the protein. The hydrogen bond formed between N6 of ATP and the kinase that our inhibitors are assumed to mimic occurs in a cavity environment and is not solvent exposed. It is therefore a strong hydrogen bond as also suggested by the presence of a crystallographically determined water molecule at this location in the X-ray structure of the apo form of the CDK2 kinase.[37] If the pyrrolo- and pyrazolopyrimidine inhibitors are docked in the enzyme model with exactly the same orientation of the pyrimidine ring as the quinazoline inhibitors in the Park-Davis model, no good hydrogen bond partner for the N−H functionality is found. The importance, but not absolute necessity, of this free NH function is documented by a recent finding. In the 4-anilinopyrrolopyrimidine series, where we assume a similar binding mode as for the pyrazolopyrimidines, methylation of the pyrrole nitrogen (compound **48**), leads to a approximately 15-fold loss of activity against the EGF-R PTK compared to the parent compound **49** (unpublished results).



	R	IC_{50} (nM)
48	CH_3	450
49	H	27

Thus, we think that as far as the pyrrolo- and pyrazolopyrimidines are concerned, the alignment with ATP we propose is consistent. The predictive value of the resulting model supports this assertion as well as the fact that our postulated binding mode has been observed crystallographically for isopentenyladenine, a compound structurally related to our inhibitors.[36]

So far, only a few tyrosine kinase inhibitors satisfying the desired profile of potent and selective enzymatic and cellular inhibition were found to be active *in vivo*. Dianilinophthalimides (CGP 52 411, CGP 53 353), 4-(phenylamino)pyrrolopyrimidines (CGP 59 326), 2-(phenylamino)pyrimidines (CGP 53 716, CGP 57 148), and certain 4-(phenylamino)quinazolines, 4-(phenylamino)pyridopyrimidines, and tyrphostin derivatives were described in the literature to fulfill these criteria. 4-(Phenylamino)pyrazolopyrimidines, especially compounds **9** and **11**, represent further examples of selective EGF-R tyrosine kinase inhibitors where potent *in vitro* and cellular activity is combined with *in vivo* efficacy. The enzymatic, cellular, and *in vivo* data presented in this paper clearly demonstrate that 4-(phenylamino)-pyrazolopyrimidines are an interesting class of compounds with high selectivity and specificity for the EGF-mediated signal transduction pathway. Further optimization and SAR studies to further improve their cellular and *in vivo* potency are ongoing and will be reported elsewhere.

Experimental Section

Kinase Assays. Purification of protein kinases and *in vitro* enzyme tests were performed as previously described.[10−13]

All compounds were dissolved in DMSO and diluted in buffer, giving a final DMSO concentration of 1% in the assay. IC_{50} values represent averages of at least three determinations. The dianilinophthalimide CGP 52 411 (IC_{50} = 0.3 μM)[10,11] served as an internal standard inhibitor in all EGF-R kinase assays.

Inhibition of Cellular Tyrosine Phosphorylation. Inhibition of EGF- and PDGF-stimulated total cellular tyrosine phosphorylation in A431 cells and BALB/c 3T3 cells, respectively, was measured using a microtiter ELISA assay as previously reported.[10] Under our assay conditions, EGF or PDGF stimulation caused a 3−4-fold increase in tyrosine or substrate phosphorylation.

Antiproliferative Assays. Assays were performed essentially as previously described.[38] Drug (in a final DMSO concentration of 0.5%) was added 24 h after plating, and growth was monitored after 3−5 days of incubation using methyleneblue staining. Cell growth of nonadherent cells was monitored by using the calorimetric MTT assay. IC_{50} was defined as the drug concentration causing a 50% reduction of signal as compared to control cultures which contain indentical solvent concentrations (100%). IC_{50} values represent the mean of at least two independent assays using serial dilutions of the drug diluted with culture medium.

***In Vivo* Antitumor Activity.** *In vivo* antitumor activity of compounds was tested in a dose range of 12.5−50 mg/kg, once daily, either by the oral or intraperitonal route against the human epithelial carcinoma A431 implanted subcutaneously into female BALB/c nude mice. For all *in vivo* experiments, tumors were serially passaged by a minimum of three transplantations prior to use. Tumor fragments (ca. 25 mg) were implanted into the left flank of animals with a 13-gauge trocar needle under Forene (Abbott, Cham, Switzerland) anesthesia. Animals were kept under sterile conditions with free access to food and water. Drug treatment was started 6 days after tumor transplantation when tumors reached a mean volume of approximately 100 mm^3. Tumor growth was followed by measuring perpendicular tumor diameters. Tumor volumes were calculated as previously described using the formula ($p \times L \times D^2/6$) (65). Values are expressed as treatment/control (T/C) percentage values.

Synthesis. Elemental analyses were within ±0.4% of the theoretical value. ^{1}H NMR were recorded on a Varian Gemini

200, a Varian Gemini 300, or a Brucker WM-360 spectrometer. The chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). Mass spectra (MS) and fast-atom-bombardment mass spectra (FABMS) were recorded on a VG Manchester apparatus; electrospray ionization MS (ESIMS) on a VG Platform 2 (Fisons Instruments). Analytical thin-layer chromatography (TLC) was carried out on precoated plates (silica gel, 60 F-254, Merck), and spots were visualized with UV light or iodine. Column chromatography was performed with Kieselgel 60 (230−400 mesh) silica gel (Merck). HPLC was performed on a Kontron MT 450 (column, Nucleosil 5C18L, 4.6×25, eluens: H_2O + CH_3CN + 0.1% TFA).

Substituted 3-(Phenylamino)2-cyano-3-(methylthio)-acrylonitriles 33a−f and Substituted 3-(Benzylamino)-2-cyano-3-(methylthio)acrylonitriles 33g−k were synthesized according to a procedure described in the literature[32,33] by refluxing 3,3-bis(methylthio)-2-cyanoacrylonitrile (Maybridge) together with the corresponding aniline or benzylamine, respectively, in ethanol (methoxyethanol)[32] or ethyl acetate or with DBU in THF[33] for several hours. After evaporation of the solvent, the residue was crystallized from methanol or petroleum ether or diethyl ether/petroleum ether to give the desired orange-colored product in high yields. It was used for the next step without further purification.

3-(Phenylamino)-2-cyano-3-(methylthio)acrylonitrile (33a):[32,34] orange needles of mp 171−174 °C; FABMS *m/z* 216 $(M + H)^+$.

3-((3-Chlorophenyl)amino)-2-cyano-3-(methylthio)acrylonitrile (33b): yellowish crystals of mp 158−161 °C; FABMS *m/z* 250 $(M + H)^+$.

3-((4-Methoxyphenyl)amino)-2-cyano-3-(methylthio)-acrylonitrile (33c):[32] mp 145−146 °C; ESIMS *m/z* 246 $(M + H)^+$.

3-((3-Methoxyphenyl)amino)-2-cyano-3-(methylthio)-acrylonitrile (33d): mp 137−140 °C; ESIMS *m/z* 246 $(M + H)^+$.

3-((4-(*N*-BOC-amino)phenyl)amino)-2-cyano-3-(methylthio)acrylonitrile (33e): mp 190−191 °C; ESIMS *m/z* 331 $(M + H)^+$.

3-((4-(Dimethylamino)phenyl)amino)-2-cyano-3-(methylthio)acrylonitrile (33f): mp 165−166 °C; ESIMS *m/z* 259 $(M + H)^+$.

3-(Benzylamino)-2-cyano-3-(methylthio)acrylonitrile (33g): mp 87−88 °C; FABMS *m/z* 230 $(M + H)^+$.

3-((3-Methoxybenzyl)amino)-2-cyano-3-(methylthio)-acrylonitrile (33i): mp 100−101 °C; ESIMS *m/z* 258 $(M + H)^-$.

3-((4-Methoxybenzyl)amino)-2-cyano-3-(methylthio)-acrylonitrile (33k): amorphous; ESIMS *m/z* 258 $(M + H)^-$.

Substituted 3-Aryl-2-cyano-3-(methylthio)acrylonitriles 42a−e (Scheme 3) were synthesized according to a procedure described in the literature[34] by reaction of tetracyanoethylene oxide (FLUKA) together with the corresponding phenyl dithioester in ethanol (methoxyethanol) or toluene at room temperature/reflux for 15−20 h. After evaporation of the solvent, the crude product was chromatographed or directly crystallized from the appropriate solvent (methanol, benzene, toluene, acetonitrile) and used without further purification for the next step.

3-Phenyl-3-(methylthio)-2-cyanoacrylonitrile (42a):[34] obtained from dithiobenzoate (FLUKA) and tetracyanoethylene oxide (65% yield); mp 95−97 °C; FABMS *m/z* 201 $(M + H)^+$.

3-(3-Methoxyphenyl)-2-cyano-3-(methylthio)acrylonitrile (42b): obtained from 4.88 g (24.6 mmol) of methyl 3-methoxydithiobenzoate and 4.26 g (29.5 mmol) of tetracyanoethylene oxide as oil (62% yield): FABMS *m/z* 231 $(M + H)^+$.

3-(4-Methoxyphenyl)-2-cyano-3-(methylthio)acrylonitrile (42c):[34] obtained from 11.1 g (56 mmol) of methyl 4-methoxydithiobenzoate[39] and 9.6 g (66.5 mmol) of tetracyanoethylene oxide (80% yield); mp 89−91 °C; FABMS *m/z* 231 $(M + H)^+$.

3-(3-Nitrophenyl)-2-cyano-3-(methylthio)acrylonitrile (42d): obtained from 14.93 g (70 mmol) of methyl 3-nitrodithiobenzoate and 20.17 g (140 mmol) of tetracyanoethylene oxide in boiling toluene (23% yield); mp 29−32 °C; MS *m/z* 245 $(M)^+$; IR (KBr) 2222 (CN), 1531, 1514, and 1353 (NO_2).

3-(4-Nitrophenyl)-2-cyano-3-(methylthio)acrylonitrile (42e): obtained from 13.55 g (63.5 mmol) methyl 4-nitrodithiobenzoate[40] and 11 g (36 mmol) of tetracyanoethylene oxide in boiling toluene (43% yield); mp 112−114 °C; MS *m/z* 245 $(M)^+$; IR (CH_2Cl_2) 2228 (CN), 1530 and 1352 (NO_2).

Procedures for the Ring Closure of Substituted 3-[(Phenylamino), (Benzylamino), or Aryl]-2-cyano-3-(methylthio)acrylonitriles to Pyrazoles (Schemes 1 and 3). With Benzylhydrazine. 1-*N*-Benzyl-3-(phenylamino)-5-amino-4-cyanopyrazole (34a). A 15 mL portion of a 5.4 N sodium methanolate solution in 15 mL of methanol (purissimum) were added under ice-cooling to 7.5 g of benzylhydrazine dihydrochloride in 20 mL of absolute methanol. The reaction mixture was stirred for ca. 15 min at room temperature and then introduced into a solution of 3.5 g (16 mmol) of 3-(phenylamino)-2-cyano-3-(methylthio)acrylonitrile (**33a**) in 110 mL of absolute ethanol. The mixture was heated under reflux for ca. 21 h and cooled to room temperature. Insoluble material was filtered off. The filtrate was concentrated by evaporation, and the brown oily residue was chromatographed on 190 g of silica gel, using methylene chloride/ethyl acetate mixtures as eluant. A 1.95 g (42%) yield of colorless crystals of **34a** of mp 139−140 °C was obtained after crystallization from hexane: FABMS *m/z* 290 $(M + H)^+$ (corresponding to $C_{17}H_{15}N_5$); ^1H NMR (DMSO-d_6) δ 8.45 (s, NH), 7.7−7.6 (m, 10 aromat H), 6.68 (s, NH_2), 5.08 (s, benzyl-CH_2).

Using the same procedure, the following were prepared.

1-*N*-Benzyl-3-((3-chlorophenyl)amino)-5-amino-4-cyanopyrazole (34b): colorless crystals (70% yield) of mp 163−164 °C (hexane); FABMS *m/z* 324 $(M + H)^+$ ($C_{17}H_{14}ClN_5$).

1-*N*-Benzyl-3-phenyl-4-cyano-5-aminopyrazole (43a): 75% yield; mp 171−172 °C; FABMS *m/z* 275 $(M + H)^+$ ($C_{17}H_{14}N_4$).

1-*N*-Benzyl-3-(3-methoxyphenyl)-5-amino-4-cyanopyrazole (43b): 67% yield; mp 147−149 °C; FABMS *m/z* $(M + H)^+$ 305 ($C_{18}H_{16}N_4O$).

With Hydrazine (Schemes 1 + 3). 3-Substituted 3-[(phenylamino) or (benzylamino)]-5-amino-4-cyanopyrazoles **35a−i** or 3-substituted 3-aryl-5-amino-4-cyanopyrazoles **47a−c** were synthesized according to a procedure described in the literature[32] by heating the corresponding substituted 3-[(phenylamino) or (benzylamino)]-2-cyano-3-(methylthio)acrylonitriles **33c−k** or substituted 3-aryl-2-cyano-3-(methylthio)acrylonitriles **42c−e** with hydrazine hydrate in boiling methanol for 1−12 h and crystallization of the reaction product from methanol or ethyl acetate/hexane (85−95% yield).

3-((3-Chlorophenyl)amino)-5-amino-4-cyanopyrazole (35a): mp 195−200 °C; ESIMS *m/z* 234 $(M + H)^+$.

3-((4-Methoxyphenyl)amino)-5-amino-4-cyanopyrazole (35b): colorless crystals of mp 183−184 °C; ESIMS *m/z* 230 $(M + H)^+$.

3-((3-Methoxyphenyl)amino)-5-amino-4-cyanopyrazole (35d): colorless crystals of mp 177−180 °C; ESIMS *m/z* 230 $(M + H)^+$.

3-((4-(*N*-BOC-amino)phenyl)amino)-5-amino-4-cyanopyrazole (35e): mp 166−168 °C; ESIMS *m/z* 315 $(M + H)^+$.

3-((4-(Dimethylamino)phenyl)amino)-5-amino-4-cyanopyrazole (35e): mp 172−173 °C; ESIMS *m/z* 243 $(M + H)^+$.

3-(Benzylamino)-5-amino-4-cyanopyrazole (35f): mp 150−152 °C; FABMS *m/z* 214 $(M + H)^+$ ($C_{11}H_{11}N_5$).

3-((3-Methoxybenzyl)amino)-5-amino-4-cyanopyrazole (35h): mp 151−153 °C; ESIMS *m/z* 242 $(M + H)^+$.

3-((4-Methoxybenzyl)amino)-5-amino-4-cyanopyrazole (35i): mp 148−149 °C; ESIMS *m/z* 242 $(M + H)^+$.

3-(4-Methoxyphenyl)-5-amino-4-cyanopyrazole (47a):[32] mp 183−186 °C; MS *m/z* 214 $(M)^+$ ($C_{11}H_9N_4O$).

3-(3-Nitrophenyl)-5-amino-4-cyanopyrazole (47b): MS *m/z* 229 $(M)^+$. Anal. ($C_{10}H_7N_5O_2$) C, H, N.

3-(4-Nitrophenyl)-5-amino-4-cyanopyrazole (47c): MS *m/z* 229 $(M)^+$. Anal. ($C_{10}H_7N_5O_2$) C, H, N.

Procedures for the Ring Closure of 5-Amino-4-cyanopyrazoles to Pyrazolo[3,4-*d*]pyrimidines. With Guani-

dine (Scheme 1). 1-*N*-Benzyl-3-((3-chlorophenyl)amino)-4,6-diaminopyrazolo[3,4-*d*]pyrimidine (36a). A 2.42 g (25.4 mmol) portion of guanidine hydrochloride was added to 670 mg (29.1 mmol) of sodium in 70 mL of 2-methoxyethanol at 0 °C and stirred for 10 min. Next 2.9 g (6.1 mmol) of **34b** was added and the mixture heated at 105−110 °C for 40 h. The dark-brown solution was cooled to room temperature and insoluble filtered off. The filtrate was evaporated to dryness and the crude product chromatographed on 200 g of silica gel, using methylene chloride/methanol mixtures as eluant. Fractions with crude **36a** were collected and crystallized from methanol/hexane to yield 1.68 g (51% yield) light-yellow-colored amorphous **36a**: FABMS *m/z* 366 $(M + H)^+$ ($C_{18}H_{17}ClN_7$); ^{1}H NMR (DMSO-d_6) δ 8.45 (s, NH), 7.75 (m, aromat H), 7.50 m, aromat H), 7.33 (m, 2 aromat H), 7.15−7.30 (m, 4 aromat H), 7.05 (s, NH_2), 6.83 (m, aromat H), 6.15 (s, NH_2), 5.20 (s, benzyl CH_2).

With Formamide (Scheme 1). 1-*N*-Benzyl-3-((4-chlorophenyl)amino)-4-aminopyrazolo[2,3-*d*]pyrimidine (36b). Ring closure of the pyrazole **34b** by heating in formamide according to ref 34 and crystallization of the crude solid gave yellowish crystals of **36b**: FABMS *m/z* 351 $(M + H)^+$ ($C_{18}H_{15}ClN_6$).

With Formic Acid (Schemes 1 and 3). 1-*N*-Benzyl-3-(phenylamino)-4-hydroxypyrazolo[3,4-*d*]pyrimidine (37a). A mixture of 1 g (3.46 mmol) of 1-benzyl-3-(phenylamino)-5-amino-4-cyanopyrazole (**34a**) and 6 mL of 85% aqueous formic acid was heated under reflux for 12 h and then cooled to room temperature. The suspension was stirred into ice−water, and the crude product was filtered off and washed with water. Recrystallization from tetrahydrofuran/cyclohexane yielded 0.61 g (61% yield) of colorless crystals of **37a**: mp 246−247 °C; FABMS *m/z* 318 $(M + H)^+$ ($C_{18}H_{15}N_5O$); ^{1}H NMR (DMSO-d_6) δ 12.10 (s, OH), 8.08 (s, pyrimidine H), 7.95 (s, NH), 6.8−7.7 (m, 10 aromat H), 5.40 (s, benzyl CH_2).

Using the same procedure, the following were prepared.

1-*N*-Benzyl-3-((3-chlorophenyl)amino)-4-hydroxypyrazolo[3,4-*d*]pyrimidine (37b): from 5.6 g (17.3 mmol) of **34b** was obtained 5.9 g (97% yield) of **37b** as colorless crystals of mp 234−236 °C; FABMS *m/z* 352 $(M + H)^+$ ($C_{18}H_{14}ClN_5O$).

1-*N*-Benzyl-3-phenyl-4-hydroxypyrazolo[3,4-*d*]pyrimidine (44a): 92% yield; mp 228−230 °C; FABMS *m/z* 303 $(M)^+$ ($C_{18}H_{13}N_4O$).

1-*N*-Benzyl-3-(3-methoxyphenyl)-4-hydroxypyrazolo[3,4-*d*]pyrimidine (44b): 92% yield; mp 183−185 °C; FABMS *m/z* $(M + H)^+$ 333 ($C_{19}H_{16}N_4O_2$).

Reaction with Phosphoroxy Chloride. 4-Chloro-1-*N*-benzyl-3-((3-chlorophenyl)amino)pyrazolo[3,4-*d*]pyrimidine (38b). A 5.8 g (15.6 mmol) portion of 1-*N*-benzyl-3-((3-chlorophenyl)amino)-4-hydroxypyrazolo[3,4-*d*]pyrimidine (**37b**) was heated under reflux for 7 h with 78 mL of $POCl_3$, during which time the suspension slowly became a solution. The light-brown solution was cooled to room temperature, concentrated in vacuum, and stirred with ice−water. The crude product was filtered off and recrystallized from ethanol/water, yielding 5.01 g (82% yield) of product **38b** as fine needles of mp 148−150 °C: FABMS *m/z* 370 $(M + H)^+$ ($C_{18}H_{13}Cl_2N_5$); ^{1}H NMR (DMSO-d_6) δ 8.78 (s, NH), 8.29 (s, pyrimidine H), 6.8−7.7 (m, 9 aromat H), 5.55 (s, benzyl CH_2).

Using the same procedure, the following were prepared.

4-Chloro-1-*N*-benzyl-3-(phenylamino)pyrazolo[3,4-*d*]-pyrimidine (38a): from 690 mg (2.17 mmol) of **37a** was obtained 523 mg (75% yield) of **38a** as fine needles of mp 135−137 °C; FABMS *m/z* 336 $(M + H)^+$ ($C_{18}H_{14}ClN_5$).

4-Chloro-1-*N*-benzyl-3-phenylpyrazolo[3,4-*d*]pyrimidine (45a): 70% yield; mp 120−121 °C; FABMS *m/z* 321 $(M + H)^+$ ($C_{18}H_{13}ClN_4$).

4-Chloro-1-*N*-benzyl-3-(3-methoxyphenyl)pyrazolo[3,4-*d*]pyrimidine (45b): 50% yield; mp 99−101 °C; FABMS *m/z* $(M + H)^+$ 351 ($C_{19}H_{15}ClN_4O$).

Procedure for the Coupling of 38a,b and 45a,b with Substituted Anilines (Phenols). 1-*N*-Benzyl-3,4-bis(phenylamino)pyrazolo[3,4-*d*]pyrimidine (39a). A 1 g (2.55 mmol) portion of 4-chloro-1-benzyl-3-(phenylamino)-pyrazolo[3,4-*d*]pyrimidine (**38a**), suspended in 80 mL of ethanol and 0.27 mL of aniline, was refluxed for 2.5 h until all starting material had disappeared in the TLC. The reaction mixture was concentrated to dryness. The residue was suspended in water and the pH adjusted to 8.5−9 with 0.1 N NaOH. The aqueous phase was extracted with ethyl acetate and the ethyl acetate phase washed, dried, and concentrated to dryness. If needed, the crude product was chromatographed on silica gel or directly crystallized from ethyl acetate/cyclohexane (hexane). A 600 mg (52%) yield of colorless crystals of **39a** of mp 135−137 °C was obtained: FABMS *m/z* 393 $(M + H)^+$ ($C_{24}H_{20}N_6$); ^{1}H NMR (DMSO-d_6) δ 9.03 (s, pyrimidine H), 8.75 (s, NH), 8.35 (s, NH), 6.8−7.7 (m, 15 aromat H), 5.45 (s, benzyl CH_2).

Using the same procedure, the following were prepared.

1-*N*-Benzyl-3-((3-chlorophenyl)amino)-4-(phenylamino)-pyrazolo[3,4-*d*]pyrimidine (39b): from 500 mg (1.35 mmol) of **38b** with aniline was obtained 507 mg (88% yield) of **39b** as colorless crystals of mp 64−65 °C; FABMS *m/z* 427 $(M + H)^+$ ($C_{24}H_{19}ClN_6$)

1-*N*-Benzyl-3,4-bis((3-chlorophenyl)amino)pyrazolo-[3,4-*d*]pyrimidine (39c): from 500 mg (1.35 mmol) of **39b** with 0.143 mL (1.35 mmol) of 3-chloroaniline was obtained 515 mg (82% yield) of **39c** as colorless crystals of mp 131−133 °C; FABMS *m/z* 461 $(M + H)^+$ ($C_{24}H_{18}Cl_2N_6$); ^{1}H NMR (DMSO-d_6) δ 9.18 (s, pyrimidine H), 8.95 (s, NH), 8.40 (s, NH), 6.9−7.9 (m, 14 aromat H), 5.45 (s, benzyl CH_2).

1-*N*-Benzyl-3-((3-chlorophenyl)amino)-4-((3-bromophenyl)amino)pyrazolo[3,4-*d*]pyrimidine (39d): from 300 mg (0.81 mmol) of **38b** with 0.093 mL (0.85 mmol) of 3-bromoaniline was obtained 377 mg (82% yield) of **39d** as colorless crystals of mp > 200 °C; FABMS *m/z* 506 $(M + H)^+$ ($C_{24}H_{18}BrClN_6$).

1-*N*-Benzyl-3-((3-chlorophenyl)amino)-4-((3-methylphenyl)amino)pyrazolo[3,4-*d*]pyrimidine (39e): from 250 mg (0.67 mmol) of **38b** with 0.077 mL (0.71 mmol) of *m*-toluidine was obtained 213 mg (71% yield) of **39e** as colorless crystals; FABMS *m/z* 441 $(M + H)^+$ ($C_{25}H_{21}ClN_6$).

1-*N*-Benzyl-3-((3-chlorophenyl)amino)-4-(3-chlorophenoxy)pyrazolo[3,4-*d*]pyrimidine (39f): from 600 mg (1.62 mmol) of **38b** with 290 mg (2.25 mmol) of *m*-chlorophenol and 360 mg (2.6 mmol) of kaliumcarbonate in 6 mL of DMSO (90 °C/7 h) was obtained 660 mg (88% yield) of **39f** as yellowish crystals; FABMS *m/z* 462 $(M + H)^+$ ($C_{24}H_{17}Cl_2N_5O$); ^{1}H NMR (DMSO-d_6) δ 8.72 (s, NH), 8.48 (s, pyrimidine H), 6.8−7.8 (m, 13 aromat H), 5.55 (s, benzyl CH_2).

1-*N*-Benzyl-3-phenyl-4-((3-chlorophenyl)amino)pyrazolo[3,4-*d*]pyrimidine (46a): from 1.14 g (3.6 mmol) of **45a** and 0.5 mL (4.42 mmol) of *m*-chloroaniline; colorless crystals (56% yield, 0.83 g) of mp 159−160 °C; FABMS *m/z* 412 $(M + H)^+$ ($C_{24}H_{18}ClN_5$); ^{1}H NMR (DMSO-d_6) δ 8.63 (s, NH), 8.57 (s, pyrimidine H), 7.1−7.9 (m, 14 aromat H), 5.67 (s, benzyl CH_2).

1-*N*-Benzyl-3-(3-methoxyphenyl)-4-((3-chlorophenyl)-amino)pyrazolo[3,4-*d*]pyrimidine (46b): from 1.2 g (3.4 mmol) of **45b** and 0.9 mL (8.5 mmol) of *m*-chloroaniline was obtained 1.19 g (79% yield) of **46b**; mp 118−119 °C; FABMS *m/z* 442 $(M + H)^+$ ($C_{25}H_{20}ClN_5O$); ^{1}H NMR (DMSO-d_6) δ 8.65 (s, 1H), 8.55 (s, 1H), 7.87 (s, aromat H), 7.45 (m, 2 aromat H), 7.37−7.25 (m, 8 aromat H), 7.11 (d, aromat H), 7.04 (d, aromat H), 5.64 (s, CH_2), 3.77 (s, CH_3).

Procedure for the Removal of the Benzyl Protecting Group from 36a,b, 39a−f, and 46a,b. 3-((3-Chlorophenyl)amino)-4,6-diamino-1*H*-pyrazolo[3,4-*d*]pyrimidine (2). To a suspension of 896 mg (6.72 mmol) of $AlCl_3$ in 5 mL of absolute benzene was added under exclusion of air and moisture a solution of 410 mg (1.12 mmol) of 1-benzyl-3-((3-chlorophenyl)amino)-4,6-diaminopyrazolo[3,4-*d*]pyrimidine (**36a**) in 20 mL of absolute benzene. The reaction mixture was stirred for 2−4 h at 50 °C until no starting material was detected in the TLC. The reaction mixture was then stirred in ca. 30 mL of water. The precipitate was filtered off and dissolved in hot ethyl acetate. The ethyl acetate phase was washed several times with 5% aqueous sodium hydrogen carbonate solution and finally with saturated sodium chloride solution, dried, and evaporated to dryness. The residue was chromatographed or directly crystallized from ethyl acetate/hexane or chromatographed on silica gel, yielding 180 mg (60% yield) of colorless crystals of **2** of mp 241−242 °C: FABMS

m/z 276 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 8.35 (s, NH), 7.93 (m, aromat H), 7.43 (m, aromat H), 7.22 (m, aromat H), 6.89 (s, NH_2), 6.85 (m, aromat H), 5.87 (s, NH_2). Anal. ($C_{11}H_{10}ClN_7$) C, H, N, Cl.

3-((3-Chlorophenyl)amino)-4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine (6): from 3.3 g (9.5 mmol) of **36b** was obtained 1.2 g of **6** as colorless crystals of mp 262−263 °C; FABMS *m/z* 261 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 8.58 (s, NH), 8.09 (s, pyrimidine H), 7.85 (m, aromat H), 7.50 (m, 2 aromat H + NH_2), 7.30 (m, aromat H), 6.89 (s, aromat H). Anal. ($C_{11}H_9ClN_6$) C, H, N, Cl.

3,4-Di-(phenylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (7): from 100 mg (0.255 mmol) of **39a** was obtained 57 mg (74% yield) of **7** as colorless crystals of mp 263−264 °C; FABMS *m/z* 303 $(M + H)^+$ ($C_{17}H_{14}N_6$); ^1H NMR (DMSO-d_6) δ 12.83 (s, pyrazole-NH), 8.91 (s, NH), 8.65 (s, NH), 8.27 (s, pyrimidine H), 7.66 (m, 2 aromat H), 7.51 (m, 2 aromat H), 7.36 (m, 2 aromat H), 7.28 (m, 2 aromat H), 7.13 (m, aromat H), 6.86 (s, aromat H).

3-((3-Chlorophenyl)amino)-4-(phenylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (8). From 400 mg (0.94 mmol) of **39b** was obtained 211 mg (67% yield) of colorless product **8** of mp 230−235 °C; FABMS *m/z* 337 $(M + H)^+$. Anal. ($C_{17}H_{13}ClN_6$) C, H, N, Cl.

3,4-Bis((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (9): from 390 mg (0.85 mmol) of **39c** was obtained 210 mg (67% yield) of **9** as white crystals of mp 175−178 °C; FABMS *m/z* 371 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 13.0 (s, pyrazole-NH), 9.13 (s, NH) 8.90 (s, NH), 8.25 (s, pyrimidine H), 6.85−7.95 (m, 8 aromat H). Anal. ($C_{17}H_{12}Cl_2N_6$) C, H, N, Cl.

3-((3-Chlorophenyl)amino)-4-((3-bromophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (10): from 330 mg (0.65 mmol) of **39d** was obtained 105 mg (39% yield) of **10** as colorless crystals of mp 179−181 °C; FABMS *m/z* 415 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 13.2 (s, pyrazole-NH), 9.03 (m, 2 NH), 8.45 (s, pyrimidine H), 6.85−8.4 (m, 8 aromat H). Anal. ($C_{17}H_{12}BrClN_6$) C, H, N, Cl.

3-((3-Chlorophenyl)amino)-4-((3-methylphenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (11): from 180 mg (0.41 mmol) of **39e** was obtained 118 mg (82% yield) of **11** as colorless crystals of mp 192−194 °C; FABMS *m/z* 351 $(M + H)^+$ ($C_{18}H_{15}N_6Cl$); ^1H NMR (DMSO-d_6) δ 13.05 (s, pyrazole-NH), 8.92 (d, 2 NH), 8.28 (s, pyrimidine H), 6.80−7.8 (m, 8 aromat H), 2.31 (s, aromat CH_3). Anal. ($C_{18}H_{15}ClN_6$) C, H, N, Cl.

3-Phenyl-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (23): from 0.7 g (1.70 mmol) of **46a** was obtained 0.43 g (78% yield) of **23** as colorless crystals of mp 238−239 °C; FABMS *m/z* 322 $(M + H)^+$; ^1H NMR (DMSO-d_6, 80 °C) δ 13.85 (sb, HN), 8.5 (sb, 1H), 8.49 (s, pyrimidine H), 7.87 (s, aromat H), 7.72 (d, aromat H), 7.1−7.6 (m, 8 aromat H). Anal. ($C_{17}H_{12}ClN_5$) C, H, N, Cl.

3-(3-Hydroxyphenyl)-4-(3-chloroanilino)-1*H*-pyrazolo-[3,4-*d*]pyrimidine (24): from 442 mg (1.00 mmol) of **46b** was obtained 40 mg (12% yield) of **24**; mp >220 °C; FABMS *m/z* 338 $(M + H)^+$ ($C_{17}H_{12}ClN_5O$); ^1H NMR (DMSO-d_6, 80 °C) δ 13.6 (sb, HN-1), 9.5 (sb, 1H), 8.47 (s, pyrimidine H), 8.1 (sb, 1H), 7.89 (s, aromat H), 7.45 (d, aromat H), 7.38 (t, aromat H), 7.34 (t, aromat H), 7.19 (d, aromat H), 7.18 (s, aromat H), 7.10 (d, aromat H), 6.92 (d, aromat H).

3-((3-Chlorophenyl)amino)-4-(3-chlorophenoxy)-1*H*-pyrazolo[3,4-*d*]pyrimidine (31): from 350 mg (0.75 mmol) of **39f** and 3-chlorophenol was obtained 170 mg (55% yield) of **31** as light colored crystals (from ethyl acetate/hexane) of mp 243−244 °C; FABMS *m/z* 372 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 13.35 (s, pyrazole-NH), 8.50 (s, NH), 8.41 (s, pyrimidine H), 6.80−7.8 (m, 8 aromat H). Anal. ($C_{17}H_{11}Cl_2N_5O$) C, H, N, Cl.

General Method for the Reaction of Pyrazoles 35a−i and 47a−c to Amidines (Schemes 2 and 3). With *N,N*-Dimethylformamide Diethyl Acetal. 4-Cyano-5-(((dimethylamino)methylene)amino)-3-((4-methoxyphenyl)amino)pyrazole (40b). A suspension of 5.41 g (23.6 mmol) of 3-((4-methoxyphenyl)amino)-5-amino-4-cyanopyrazole (**35b**) in 4.8 mL (27.2 mmol) of *N,N*-dimethylformamide diethyl acetal (97%) and 100 mL of toluene was heated under reflux for 5 h. The reaction mixture was then cooled to room temperature and filtered. The residue was washed with toluene. Recrystallization of the crude product from methanol/water gave **40b** as colorless crystals of mp 232−234 °C dec which was used without further purification: ESIMS *m/z* 285 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 11.90 (s, pyrazole-NH), 8.27 (s, NH), 8.13 (s, 1H), 7.42 (d, 2 aromat H), 6.81 (d, 2 aromat H), 3.69 (s, OCH_3), 3.08 (s, NCH_3), 2.98 (s, NCH_3).

Using the same method, the following were prepared.

3-((3-Chlorophenyl)amino)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (40a): from 11.7 g (50.07 mmol) of 3-((3-chlorophenyl)amino)-5-amino-4-cyanopyrazole (**35a**) and 9.43 mL (55.07 mmol) of *N,N*-dimethylformamide diethyl acetal (toluene, reflux for 3.5 h) was obtained 14.0 g (96.8% yield) of **40a** as colorless crystals of mp >260 °C; ESIMS *m/z* 289 $(M + H)^+$.

3-((3-Methoxyphenyl)amino)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (40c): from 1.25 g (5.45 mmol) of 3-((3-methoxyphenyl)amino)-5-amino-4-cyanopyrazole (**35c**) and 1.228 mL (7.17 mmol) of *N,N*-dimethylformamide diethyl acetal (toluene, reflux for 4.5 h) was obtained 1.42 g (91.7% yield) of **40c** as colorless crystals of mp 227−231 °C; ESIMS *m/z* 285 $(M + H)^+$.

3-((4-(Dimethylamino)phenyl)amino)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (40d): from 10 g (41.3 mmol) of 3-((4-(dimethylamino)phenyl)amino)-4-cyano-5-aminopyrazole (**35e**) and 8.75 mL (49.5 mmol) of *N,N*-dimethylformamide diethyl acetal (toluene, reflux for 1 h) was obtained 12.27 g (100% yield) of **40c** as colorless crystals (from methanol/water) of mp 281−282 °C dec; ESIMS *m/z* 298 $(M + H)^+$.

3-(Benzylamino)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (40e): from **35f** (98% yield); mp 203−204 °C; FABMS *m/z* $(M + H)^+$ 269 ($C_{14}H_{16}N_6$).

3-((3-Chlorobenzyl)amino)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (40f): from **35g** (75% yield); FABMS *m/z* 303 $(M + H)^+$ ($C_{14}H_{15}ClN_6$).

3-((3-Methoxybenzyl)amino)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (40g): from **35h** (85% yield); mp 147−148 °C; ESIMS *m/z* 299 $(M + H)^+$.

3-((4-Methoxybenzyl)amino)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (40h): from **35i** (90% yield); mp 136−137 °C; ESIMS *m/z* 299 $(M + H)^+$.

3-(4-Methoxyphenyl)-4-cyano-5-(((dimethylamino)-methylene)amino)pyrazole (48a): from **47c** (94% yield); mp 169−171 °C; MS *m/z* 269 $(M)^+$. Anal. ($C_{14}H_{15}N_5O$) C, H, N.

3-(3-Nitrophenyl)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (48b): from **47d** (reflux/15 h, 90% yield); mp 219−221 °C; MS *m/z* 284 $(M)^+$ ($C_{13}H_{12}N_6O_2$).

3-(4-Nitrophenyl)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (48c): from **47e** (reflux/15 h, 94% yield); MS *m/z* 284 $(M)^+$. Anal. ($C_{13}H_{12}N_6O_2$) C, H, N.

3-(4-Aminophenyl)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (48d). In the presence of 1 g of Pd/C (5%), 5.79 g (20.4 mmol) of **48c** was hydrogenated in 900 mL of THF. Filtering, concentration by evaporation, and crystallization from ethanol/ethyl acetate gave **48d** (85% yield): mp 209−221 °C; FABMS *m/z* 255 $(M + H)^+$ ($C_{13}H_{14}N_6$); ^1H NMR (DMSO-d_6) δ 12.7 (sb, NH), 8.17 (s, amidine H), 7.52 and 6.63 (2d, 2 × CH_2), 5.5 (sb, NH_2), 3.08 and 2.98 (2s, 2 CH_3).

3-(4-(*N*-BOC-amino)phenyl)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (48e). A mixture of 3.81 g (15 mmol) of **48d** and 6.54 g (30 mmol) of di-*tert*-butyl dicarbonate in 60 mL dioxane was stirred for 10 h at 80 °C. Partial concentration of the suspension, filtration, and washing with diethyl ether/hexane gave **48e** (95% yield): mp 230−233 °C dec; MS *m/z* 354 $(M)^+$ ($C_{18}H_{22}N_6O_2$).

Procedures for the Conversion of Amidines 40a−h and 48a,b,e to Final Products. 3-((4-Methoxyphenyl)-amino)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (12). A suspension of 1 g (3.52 mmol) of 3-((4-methoxyphenyl)amino)-4-cyano-5-(((dimethylamino)-methylene)amino)pyrazole (**40b**) and 0.9 g (5.48 mmol) of 3-chloroaniline hydrochloride in 7 mL of methanol was heated under reflux for 17 h. The reaction mixture was then cooled to room temperature, poured onto ice−water, rendered alka-

line (pH 10), and filtered. The crude product was washed with methanol/water (1:1). Recrystallization from methanol/water gave 0.9 g (68% yield) of the title compound **12** as colorless crystals of mp 223−224 °C dec; ESIMS *m/z* 367 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 12.75 (s, pyrazole-NH), 9.04 (s, 1H), 8.49 (s, NH), 8.33 (s, pyrimidine H), 7.93 (m, aromat H), 7.66 (d, aromat H), 7.51 (d, 2 aromat H), 7.41 (t, aromat H), 7.18 (d, aromat H), 6.90 (d, 2 aromat H), 3.73 (s, OCH_3). Anal. ($C_{18}H_{15}ClN_6O \cdot 0.35H_2O$) C, H, N, H_2O.

3-((4-Hydroxyphenyl)amino)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (13). The methyl ether **12** was cleaved by heating a mixture of 0.5 g (1.36 mmol) of **12** and 0.87 g (6.55 mmol) of anhydrous aluminum chloride in 15 mL of benzene with the exclusion of air and moisture at 80 °C for 9 h. The benzene phase was removed and the residue partitioned between ethyl acetate and water. The organic phase was washed with water and saturated sodium hydrogen carbonate solution, dried, and evaporated to dryness. The residue was purified by flash chromatography on silica gel using methylene chloride/methanol mixtures (50:1 and 20:1). The product-containing fractions were combined and concentrated to a volume of ca. 10 mL, whereas the product crystallized. The product was filtered off and washed with diethyl ether, yielding colorless crystals of **13**: mp >260 °C; ESIMS *m/z* 353 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 12.68 (br s, pyrazole-NH), 8.94 (br s, NH and OH), 8.36 (s, NH), 8.32 (s, pyrimidine H), 7.93 (s, aromat H), 7.62 (d, aromat H), 7.34−7.42 (m, 3 aromat H), 7.16 (d, aromat H), 6.70 (d, 2 aromat H). Anal. ($C_{17}H_{13}Cl\ N_6O$) C, H, N.

Using the same method, the following were prepared.

3-((3-Methoxyphenyl)amino)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (14). A suspension of 1.38 g (4.85 mmol) of 3-((3-methoxyphenyl)amino)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (**40c**) and 0.915 g (5.58 mmol) of 3-chloroaniline hydrochloride in 20 mL of methanol was heated under reflux for 17 h. The resultant solution was evaporated to dryness and the residue crystallized from methanol/water and from methanol to give 0.91 g (51.1% yield) of **14**: mp 202−203 °C; ESIMS *m/z* 367 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 12.94 (s, pyrazole-NH), 9.07 (broad s, NH), 8.72 (s, NH), 8.36 (s, pyrimidine H), 7.95 (m, aromat H), 7.65 (d, aromat H), 7.43 (t, aromat H), 7.07−7.23 (m, 4 aromat H), 6.45−6.51 (m, aromat H), 3.76 (s, OCH_3). Anal. ($C_{18}H_{15}Cl\ N_6O$) C, H, N.

3-((3-Hydroxyphenyl)amino)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (15). Cleavage of the methyl ether **14** as described above gave colorless crystals of **15**: mp 265−266 °C; ESIMS *m/z* 353 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 12.92 (s, pyrazole-NH), 9.24 (s, OH), 8.99 (broad s, NH), 8.60 (s, NH), 8.36 (s, pyrimidine H), 7.93 (m, aromat H), 7.62 (d, aromat H), 7.41 (t, aromat H), 7.18, (d, aromat H), 7.02−7.10 (m, 2 aromat H), 6.89 (d, aromat H) 6.27−6.35 (m, aromat H). Anal. ($C_{17}H_{13}ClN_6O$) C, H, N.

3-(((4-(Dimethylamino)phenyl)amino)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (18): from 5 g (16.8 mmol) of 3-((4-(dimethylamino)phenyl)amino)-4-cyano-5-(((dimethylamino)methylene)amino)pyrazole (**40d**), 3.3 g (20.1 mmol) of 3-chloroaniline hydrochloride, and 40 mL of DMF (heating for 8 h at 130 °C). The reaction mixture was then cooled to room temperature, water was added dropwise, and the reaction mixture was filtered. The residue was dissolved in ca. 20 mL of DMF. After precipitation with water, followed by recrystallization from DMF/water, the resulting crystals were suspended in 10 mL of water. Then 6 mL of 1 N hydrochloric acid was added, and the mixture was heated briefly to reflux and filtered. A 3 mL portion of 2 N sodium hydroxide solution was added to the filtrate. The crystalline precipitate was filtered off, washed with water, and dried, yielding the title compound **18** (1.87 g, 28.6% yield) as colorless crystals of mp 250−255 °C dec: ESIMS *m/z* 380 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 12.69 (s, pyrazole-NH), 8.90 (s, NH), 8.33 (s, NH), 8.31 (s, pyrimidine H), 7.87 (m, aromat H), 7.62 (d, aromat H), 7.37−7.43 (m, 3 aromat H), 7.16 (d, aromat H), 6.74 (d, 2 aromat H), 2.82 (s, $N(CH_3)_2$). Anal. ($C_{19}H_{18}ClN_7 \cdot 0.5H_2O$) C, H, N, H_2O.

3-(Benzylamino)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (19): from 79.2 g (295 mmol) of **40e** and 60.6 g (369 mmol) of 3-chloroaniline hydrochloride in 700 mL of methanol (reflux/22 h) was obtained crystalline **19** (73% yield): mp 216−217 °C; ^{1}H NMR (DMSO-d_6) δ 8.94 (s, NH), 8.25 (s, pyrimidine H), 7.91 (m, aromat H), 7.65 (d, aromat H), 7.5−7.3 (m, 5 aromat H), 7.24 (m, aromat H), 7.15 (d, aromat H), 6.91 (t, NH), 4.50 (d, CH_2). Anal. ($C_{18}H_{15}ClN_6$) C, H, N,Cl.

3-((3-Chlorobenzyl)amino)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (20): from **40f**; mp 161−163 °C; FABMS *m/z* 386 $(M + H)^+$ ($C_{18}H_{14}Cl_2N_6$); ^{1}H NMR (DMSO-d_6, 80 °C) δ 12.38 (s, HN-1), 8.92 (s, NH), 8.27 (s, pyrimidine H), 7.94 (t, aromat H), 7.68 (m, aromat H), 7.50 (s, aromat H), 7.45−7.25 (m, 4 aromat H), 7.17 (m, aromat H), 6.98 (t, NH), 4.53 (d, CH_2).

3-((3-Methoxybenzyl)amino)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (21): from **40g** and 3-chloroaniline hydrochloride (ethanol, reflux/36 h, 70% yield); colorless crystals of mp 194−195 °C; ESIMS *m/z* 381 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 12.38 (s, pyrazole-NH), 8.95 (s, NH), 8.26 (s, pyrimidine H), 7.92 (m, aromat H), 7.67 (d, aromat H), 7.41 (t, aromat H), 6.80−7.44 (m, 5 aromat H, NH), 4.49 (d, CH_2), 3.75 (s, OCH_3). Anal. ($C_{19}H_{17}ClN_6O$) C, H, N.

3-((4-Methoxybenzyl)amino)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (22): from **40h** and 3-chloroaniline hydrochloride (ethanol, reflux/16 h, 75% yield); colorless crystals of mp 222−223 °C; ESIMS *m/z* 381 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 12.37 (s, pyrazole-NH), 8.94 (s, NH), 8.26 (s, pyrimidine H), 7.90 (m, aromat H), 7.66 (d, aromat H), 7.34−7.44 (m, 3 aromat H), 7.16 (d, aromat H), 6.90 (d, 2 aromat H), 6.82 (t, NH), 4.43 (d, CH_2), 3.73 (s, OCH_3). Anal. ($C_{19}H_{17}ClN_6O$) C, H, N.

3-(4-Methoxyphenyl)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (25): from **48a** and 3-chloroaniline hydrochloride (methanol, reflux/18 h, 77% yield); colorless crystals of mp 268−269 °C; FABMS *m/z* 352 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 8.50 (s, 1H), 8.44 (s, 1H), 7.93 (s, aromat H), 7.73 (d, 2H), 7.47 (m, aromat H), 7.36 (t, aromat H), 7.13 (m, 3H), 3.85 (s, CH_3). Anal. ($C_{18}H_{14}ClN_5O$) C, H, N,Cl.

3-(4-Hydroxyphenyl)-4-((3-chlorophenyl)amino)pyrazolo[3,4-*d*]pyrimidine (26): from ether cleavage of **25**; FABMS *m/z* 336 $(M - H)^+$ ($C_{17}H_{12}ClN_5O$); ^{1}H NMR (DMSO-d_6, 80 °C) δ 13.7 (sb, HN), 9.8 (sb, OH), 8.46 (s, pyrimidine H), 8.38 (sb, NH), 7.90 (s, aromat H), 7.57 (d, 2 aromat H), 7.44 (d, aromat H), 7.33 (t, aromat H), 7.190 (d, aromat H), 6.93 (d, 2 aromat H).

3-(3-Nitrophenyl)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (27): from **48b** and 3-chloroaniline hydrochloride (methanol, reflux/17 h, 85% yield); colorless crystals of mp 331−333 °C; FABMS *m/z* 367 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 14.15 (s, NH), 9.00 (s, NH), 8.52 (s, pyrimidine H), 8.52 (m, aromat H), 8.29 (ddd, aromat H), 8.19 (ddd, aromat H), 7.80 (t, aromat H), 7.78 (t, aromat H), 7.46 (ddd, aromat H), 7.29 (t, aromat H), 7.07 (ddd, aromat H). Anal. ($C_{17}H_{11}N_6ClO_2$) C, H, N,Cl.

3-(3-Aminophenyl)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (28). Hydrogenation of 3.44 g (9.38 mmol) of **27** in 300 mL of THF and 10 mL of methanol in the presence of 3 g of Raney nickel, separation of the catalyst, partial concentration, and precipitation with diethyl ether gave amorphous **28** (78% yield) as colorless crystals of mp 264−266 °C: ^{1}H NMR (DMSO-d_6) δ 8.51 (s, pyrimidine H), 8.22 (s, NH), 8.00 (m, aromat H), 7.44 (d, aromat H), 7.37 (t, aromat H), 7.24 (t, aromat H), 7.13 (d, aromat H), 6.98 (s, aromat H), 6.87 (d, aromat H), 6.71 (d, aromat H), 5.43 (s, NH_2); MS *m/z* 336 $(M)^+$ ($C_{17}H_{13}ClN_6$).

3-(4-(BOC-amino)phenyl)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (29): from **48e** and 3-chloroaniline hydrochloride (methanol, reflux/17 h, 63% yield); mp 165−168 °C dec; ^{1}H NMR (DMSO-d_6) δ 9.6 (s, NH), 8.50 (s, pyrimidine H), 8.42 (s, NH), 7.93 (sb, aromat H), 7.67 (AB, 4 aromat H), 7.50 (d, aromat H), 7.37 (t, aromat H), 7.13 (d, aromat H), 1.51 (s, *t*-Bu); MS *m/z* 436 $(M)^+$ ($C_{22}H_{21}ClN_6O_2$).

3-(4-Aminophenyl)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine Hydrochloride (30). In 100 mL of dioxane and 30 mL of HCl/dioxane (4 N) was stirred 3.8 g (8.7 mmol) of **29** for 3 d at room temperature. Filtration of the precipitate and washing with boiling 2-propanol afforded **30** (76% yield): MS *m/z* 336 $(M)^+$ ($C_{17}H_{13}ClN_6$); ^{1}H NMR (DMSO-d_6) δ 8.65 (sb, NH), 8.51 (s, pyrimidine H), 7.86 (s, aromat H), 7.70 (d, 2 aromat H), 7.50 (d, aromat H), 7.37 (t, aromat H), 7.17 (m, 3 aromat H), (ddd, aromat H), 7.80 (t, aromat H), 7.78 (t, aromat H), 7.46 (ddd, aromat H), 7.29 (t, aromat H), 7.07 (ddd, aromat H).

3-((3-Chlorophenyl)amino)-4-(benzylamino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (32). Under a nitrogen atmosphere, a mixture of 1 g (3.46 mmol) of 4-cyano-5-(((dimethylamino)-methylene)amino)-3-((3-chlorophenyl)amino)pyrazole (**40a**) and 10 mL of benzylamine was stirred for 3 h at 120 °C and then evaporated *in vacuo*. The crystalline residue was digested in 10 mL of ice-cold acetonitrile and filtered and the filter residue recrystallized from acetonitrile, yielding 0.536 g (44.2% yield) of the title compound **32** of mp 216−218 °C: ESIMS *m/z* 351 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 12.79 (s, pyrazole-NH), 8.68 (s, NH), 8.19 (s, pyrimidine H), 8.08 (t, NH), 7.79 (m, aromat H), 7.22−7.46 (m, 7 aromat H), 6.90 (d, aromat H), 4.83 (d, benzyl-CH_2). Anal. ($C_{18}H_{15}ClN_6$) C, H, N.

Reaction of Pyrazole 35d with Orthoformic Acid Triethyl Ester (Scheme 2). 3-((4-(*N*-BOC-amino)phenyl)-amino)-4-cyano-5-((ethoxymethylene)amino)pyrazole (41a). A mixture of 13.8 g (43.9 mmol) of 3-((4-(*N*-BOC-amino)phenyl)amino)-5-amino-4-cyanopyrazole (**35c**) and 138 mL of orthoformic acid triethyl ester was heated with stirring, at 120 °C for 3 h. Ethanol, formed in the course of the reaction, was distilled off. After cooling to room temperature, the precipitate was filtered off and the residue washed with ethanol to yield **41a** (44.2% yield) of mp 180−182 °C: ESIMS *m/z* 371 $(M + H)^+$.

Reaction of Pyrazole 41a with 3-Chloroaniline. 3-((4-(*N*-BOC-amino)phenyl)amino)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (16). A mixture of 7 g (18.9 mmol) of 4-cyano-5-((ethoxymethylene)amino)-3-((4-(BOC-amino)phenyl)amino)pyrazole (**41a**), 3.97 mL (37.78 mmol) of 3-chloroaniline, and 150 mL of ethanol was heated under reflux for 9 h and stirred at room temperature for a further 15 h. Filtering and washing the filter residue with cold ethanol afforded 1,5-dihydro-3-((4-(*N*-BOC-amino)phenyl)-amino)-4-imino-5-(3-chlorophenyl)-4*H*-pyrazolo[3,4-*d*]pyrimidine as intermediate (71.1% yield): mp 222 °C; FABMS *m/z* 452 $(M + H)^+$. It was converted into the final product **16** by Dimroth rearrangement (heating in boiling dioxane and water for 22 h). Cooling, filtering, and washing the filter residue with dioxane gave the title compound **16** (67.5% yield) of mp 256−258 °C: ESIMS *m/z* 450 $(M - H)^-$; ^{1}H NMR (DMSO-d_6) δ 12.76 (br s, pyrazole-NH), 9.13 (br s, NH), 9.09 (br s, NH), 8.58 (br s, NH), 8.31 (s, pyrimidine H), 7.91 (s, aromat H), 7.64 (d, aromat H), 7.33−7.47 (m, 5 aromat H), 7.18 (d, aromat H), 1.47 (s, 9 H). Anal. ($C_{22}H_{22}ClN_7O_2l$) C, H, N.

Removal of the BOC Protecting Group from 16. 3-((4-Aminophenyl)amino)-4-((3-chlorophenyl)amino)-1*H*-pyrazolo[3,4-*d*]pyrimidine (17). A mixture of 0.352 g (0.779 mmol) of **16** and 7 mL of a 3 N solution of hydrochloric acid in methanol was stirred at room temperature for 16 h. Then about 10 mL of diethyl ether was added to the reaction mixture which was cooled in an ice bath. The precipitate was filtered off, and the residue recrystallized from hot methanol, yielding compound **17** in form of its hydrochloride (dihydrate), 53.6% yield of mp >260 °C: ESIMS *m/z* 352 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ ~12.70 to 13.20 (br, pyrazole-NH), 10.98 (br s), 10.10 (br s, 3 H), 8.37 (s, pyrimidine H), 7.95 (m, aromat H), 7.87 (d, 2 aromat H), 7.76 (d, aromat H), 7.47 (t, aromat H), 7.28−7.33 (m, 3 aromat H), ~4.80−6.50 (br). Anal. ($C_{17}H_{14}ClN_7 \cdot 1.85HCl \cdot 2H_2O$) C, H, N, Cl, H_2O.

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