Articles

4-(Phenylamino)pyrrolopyrimidines: Potent and Selective, ATP Site Directed Inhibitors of the EGF-Receptor Protein Tyrosine Kinase

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Using a pharmacophore model for ATP-competitive inhibitors interacting with the active site of the EGF-R protein tyrosine kinase (PTK), 4-(phenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidines have been identified as a novel class of potent EGF-R protein tyrosine kinase inhibitors. In an interactive process, this class of compounds was then optimized. **13**, **14**, **28**, **36**, **37**, and **44**, the most potent compounds of this series, inhibited the EGF-R PTK with IC_{50} values in the low nanomolar range. High selectivity toward a panel of nonreceptor tyrosine kinases (*c-Src, v-Abl)* and serine/threonine kinases (PKC α, PKA) was observed. Kinetic analysis revealed competitive type kinetics relative to ATP. In cells, EGF-stimulated cellular tyrosine phosphorylation was inhibited by compounds **13**, **36**, **37**, and **44** at IC_{50} values between 0.1 and 0.4 μM, whereas PDGF-induced tyrosine phosphorylation was not affected by concentrations up to 10 μM. In addition, these compounds were able to selectively inhibit *c-fos* mRNA expression in EGF-dependent cell lines with IC_{50} values between 0.1 and 2 μM, but did not affect *c-fos* mRNA induction in response to PDGF or PMA (IC_{50} >100 μM). Proliferation of the EGF-dependent MK cell line was inhibited with similar IC_{50} values. From SAR studies, a binding mode for 4-(phenylamino)-7*H*-pyrrolo[2,3-d]pyrimidines as well as for the structurally related 4-(phenylamino)quinazolines at the ATP-binding site of the EGF-R tyrosine kinase is proposed. 4-(Phenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidines therefore 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 PTK's can be divided into subgroups which have similar structural organization and amino acid sequence similarity within their kinase domains.[2]

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. 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; etc.)[1,3] and proliferative disorders of the epidermis such as psoriasis.[4]

Inhibitors of the EGF-R PTK could therefore 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.[5−10] Although many of these published compounds exert potent tyrosine kinase inhibition, they often lack selectivity or show weak cellular potency. Few inhibitors demonstrated *in vivo* antitumor efficacy in murine models. Although these inhibitors are without potential for the development as pharmaceuticals, they 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 several examples of structurally different classes have proved to be highly selective ATP-competitive tyrosine kinase inhibitors. This includes benzothiopyranones,[11] lavendustin A,[12] 3-substituted quinoline derivatives,[13,14] and a special group of compounds containing a phenylamino (anilino) moiety in their structure such as dianilinophthalimides (e.g. compound **1**),[15−17] the (phenylamino)pyrimidine CGP 53 716 (**2**),[18] (phenylamino)quinazolines (e.g. compound **3**),[19−25] and

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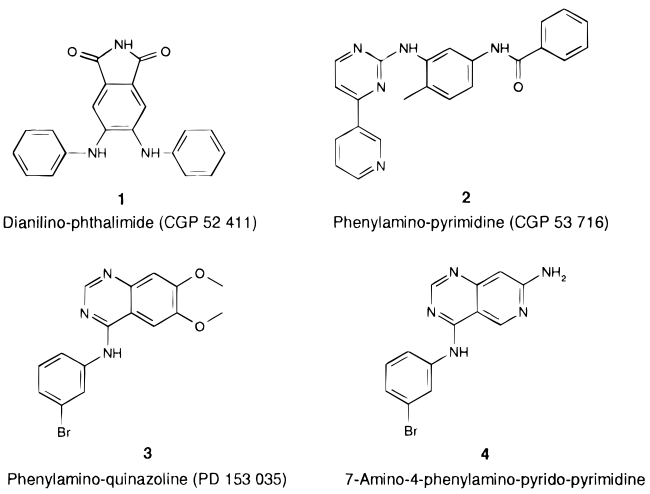


Figure 1. ATP-competitive tyrosine kinase inhibitors.

the very recently published 7-amino-4-(phenylamino)-pyrido[4,3-*d*]pyrimidines **4**[26] (Figure 1).

In the present paper, we describe the rational design, synthesis, biological profile, and structure−activity relationships (SAR) of a novel class of highly potent and selective EGF-R PTK inhibitors containing a (phenylamino)pyrimidine moiety as a structural element in their molecule.

Inhibitor Design

Using a calculated 3-D computer model of the catalytic domain of the EGF-R tyrosine kinase together with the dianilinophthalimide **1** as example of an ATP-competitive inhibitor, we developed a pharmacophore model for ATP-competitive inhibitors in the active site of the EGF-R PTK.[27] On the basis of this model, the 4-(phenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidine **10** was then identified as a potent EGF-R PTK inhibitor (Figure 2). In an interactive process, this class of compounds was further optimized.

This pharmacophore model is based on the following assumptions:

(1) ATP is anchored in 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).

(2) Such a donor−acceptor system is important for binding.

(3) In pyrrolopyrimidines, the pyrrole NH(7) and the N(1) of the pyrimidine ring form a similar bidentate hydrogen bond donor−acceptor system as ATP.

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

(5) A large hydrophobic pocket in the region corresponding to the N7-position of the adenine ring of ATP opposite to the "sugar pocket" exists.

The assumption of a bidendate hydrogen bond donor−acceptor system is supported by the binding mode observed crystallographically for the cyclin-dependent protein kinase 2 (CDK-2) kinase inhibitor W6-(Δ^2-isopentenyl)adenine.[28] The replacement of a sugar moiety by a phenyl ring has already been proposed in the design of inhibitors of the enzyme purine nucleoside phosphorylase.[29] Further optimization of 4-(phenylamino)pyrrolopyrimidines, especially by meta substitutions in the anilino moiety and at positions 5 and 6 of the pyrrole ring, led to the identification of highly potent derivatives which inhibited the EGF-R PTK in the low nanomolar range and in addition showed cellular activity in EGF-dependent cellular systems at IC_{50}'s below 1 μM.

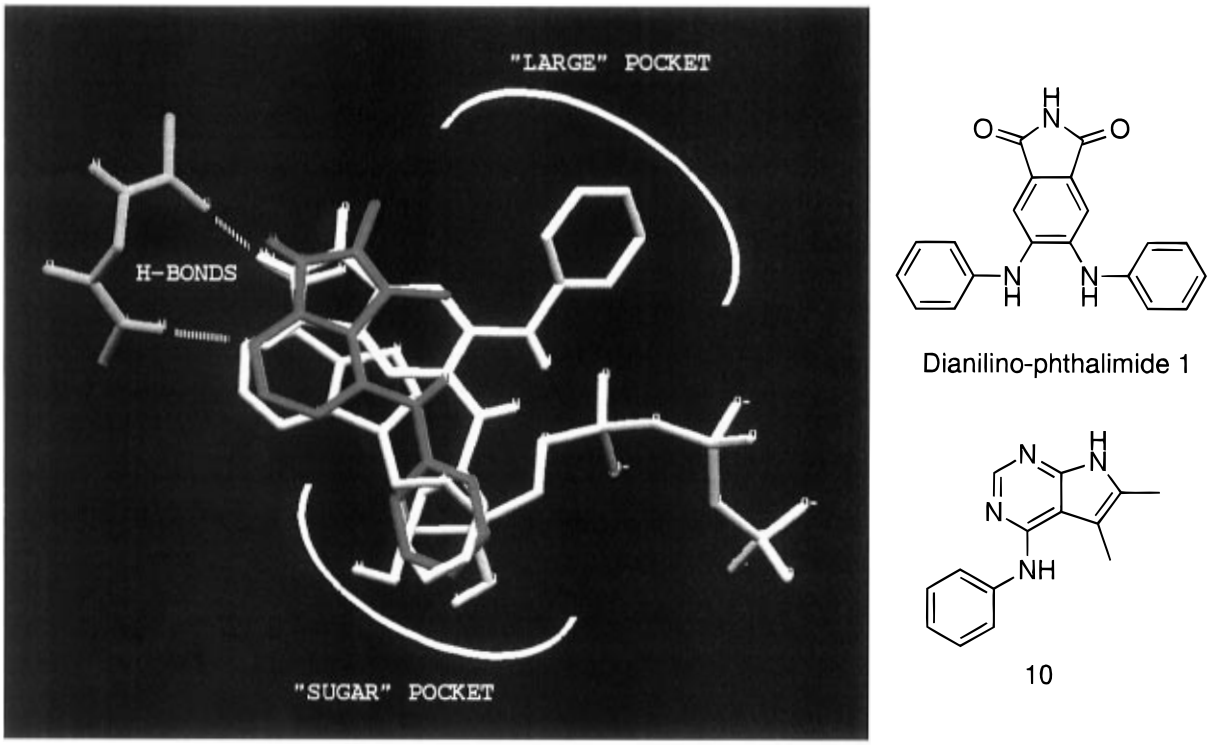
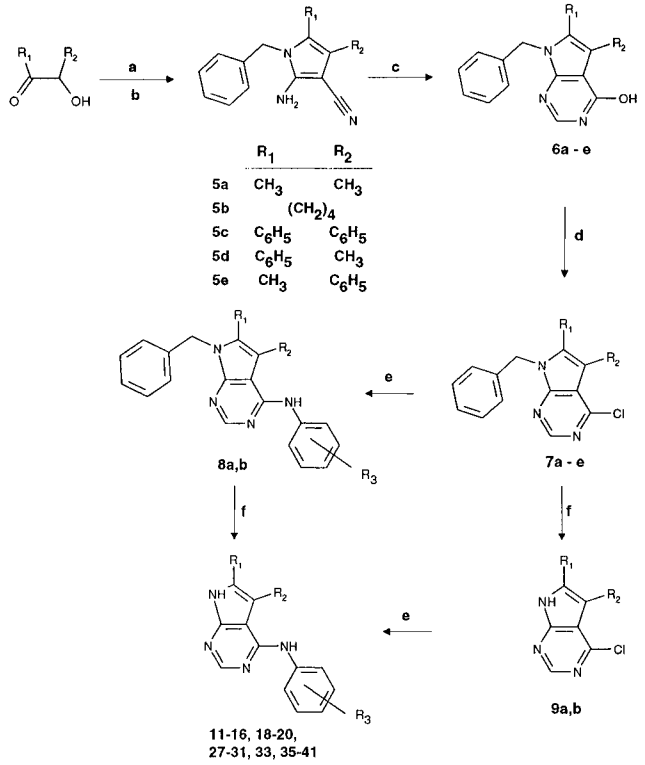


Figure 2. Superposition of dianilinophthalimide (**1**) (white), 4-(phenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidine (**10**) (red), and ATP (yellow).

Scheme 1[a]



[a] Reagents and conditions: (a) benzylamine, toluene, HCl, reflux; (b) malonodinitrile, toluene, reflux; (c) formic acid (85%), 110 °C, 5 h; (d) $POCl_3$, reflux; (e) substituted aniline (phenol), ethanol (1-butanol), reflux; (f) $AlCl_3$, toluene, reflux.

Chemistry

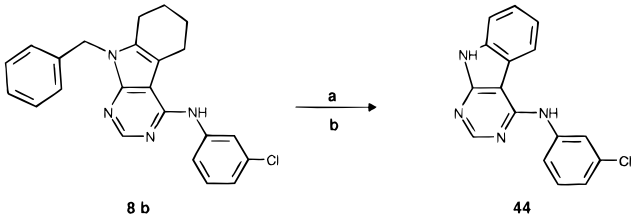
In general, (phenylamino)-7*H*-pyrrolo[2,3-*d*]pyrimidines were synthesized in five or six steps starting from the corresponding substituted α-hydroxy ketones (Scheme 1). Compounds **10**, **17**, **21**−**26**, **32**, **34**, **42**, **43**, and **45** have already been described in the literature.[30]

Substituted 1-benzyl-2-amino-3-cyanopyrroles **5a**−**e** were obtained by reaction of α-hydroxy ketones with benzylamine and malonodinitrile according to a procedure described in the literature.[31] Reflux of cyanoaminopyrroles **5a**−**e** with 85% formic acid afforded the 5,6-substituted 4-hydroxy-7-benzylpyrrolo[2,3-*d*]pyrimidines **6a**−**e** which were then converted to the corresponding chlorides **7a**−**e** by reaction with phosphoroxy chloride. Replacement of the chlorine with the corresponding substituted aniline afforded 4-(phenylamino)-5,6-substituted-7-benzylpyrrolo[2,3-*d*]pyrimidines **(8a,b)**. Finally, the benzyl protecting group was removed with $AlCl_3$ in toluene to give the final products. Compounds **12**−**14**, **20**, **27**−**31**, **36**, **37**, and **40** were prepared by this route. Alternatively, the benzyl group of **7a,b** could be removed to the unprotected chlorides **9a,b** before the anilino moiety was introduced. Compounds **11**, **15**, **16**, **18**, **19**, **35**, **38**, **39**, **41**, and **46** were prepared by this alternative route. The phenoxy compound **33** was prepared by reaction of **7a** with *m*-chlorophenol followed by removal of the benzyl group; compound **46,** by reaction of **7a** with cyclohexylamine. The indolopyrimidine **44** was obtained by oxidation of the N-protected pyrrolopyrimidine **8 b** with DDQ following removal of the benzyl group (Scheme 2).

Biological Evaluation

Enzymatic Activity. Compounds were tested for inhibition of the tyrosine kinase activity of a recombinant, intracellular domain of the EGF-R (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) (Table 2).

Scheme 2[a]

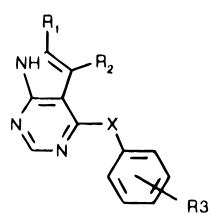


[a] Reagents and conditions: (a) DDQ, toluene, reflux, 30 min; (b) $AlCl_3$, toluene, reflux.

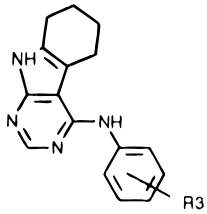
The following SAR were derived from the *in vitro* data: There is a loss of activity with substituents in the ortho position (compounds **25** and **26**) or in the para position (compounds **21**−**24**) of the anilino moiety. As already observed in the (phenylamino)quinazoline series,[19,21,25] there was an increase of activity with small lipophilic electron-withdrawing groups at the 3-position of the aniline (Cl ∼ Br > CN ∼ F ∼ CH_3 > OH ∼ OCH_3 > CF_3 ≫ COOH). With IC_{50} values of 27 and 25 nM, respectively, the 3-chloroanilino and the 3-bromoanilino derivatives **13** and **14** are the most potent compounds of this series. Replacement of the anilino moiety by a benzyl, cyclohexylamino, or phenoxy group showed no benefit on the *in vitro* activity. The benzyl derivative **32** had similar activity as **10**, whereas the cyclohexylamine derivative **46** was slightly less active and the *m*-chlorophenoxy derivative **33** ca. 100-fold less active compared to compound **13**. Methylation at the anilino nitrogen decreased activity 20-fold (compound **31**), whereas a methyl group in position 2 of the pyrimidine ring gave an inactive compound **45**. In a further series, the replacement of either one or both methyl groups in positions 5 and 6 of the pyrrole ring by bulkier substituents was explored as suggested by the pharmacophore model (compounds **28**−**30**). In general, bulky lipophilic groups were well tolerated in both positions. Replacement of the 5-methyl group or of both methyl groups by a phenyl moiety (compounds **29** and **30**) only led to a slight decrease of activity, whereas the 6-phenyl analog **28**, with an IC_{50} of 10 nM, was slightly more active than compound **13**. Replacement of both methyl groups by a cyclohexyl ring led to an interesting series of derivatives (compounds **34**−**43**). With respect to substituents in the anilino part of the molecule, the same SAR as in the dimethyl series was observed. Again, the *m*-chloro derivative **36** was the most active compound of this series (IC_{50} = 29 nM). However, oxidation of the cyclohexyl ring of **36** to the indolopyrimidine derivative **44** further increased the activity against the EGF-R kinase. With an IC_{50} of 6 nM, this compound was as equipotent as the ((3-bromophenyl)-amino)quinazoline **3**,[23,25] which in our assay system had an IC_{50} value of 4 nM.

When tested for selectivity against the *v-Abl* and *c-Src* tyrosine kinases and against the serine/threonine kinase PKC-α, all active compounds showed a high selectivity ratio > 50. With the exception of compound **14**, which showed weak activity against the *v-Abl* kinase, the most potent compounds **13**, **28**−**30**, **36**, **37**, and **44** had ratios

Table 1. EGF-R Tyrosine Kinase Activity of Derivatives



10-33



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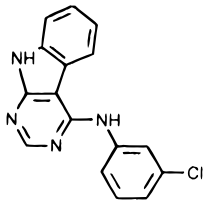

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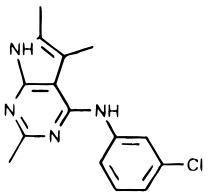
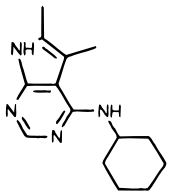


45



46

compd	R_1	R_2	R_3	X	formula	FABMS $(M + H)^+$	anal.	EGF-R IC_{50} (μM)
10[a]	Me	Me	H	NH	$C_{14}H_{14}N_4$	239		1.90
11	Me	Me	3-Me	NH	$C_{15}H_{16}N_4$	253	*b*	0.57
12	Me	Me	3-F	NH	$C_{14}H_{13}FN_4$	257	C,H,N,F	0.55
13	Me	Me	3-Cl	NH	$C_{14}H_{13}ClN_4$	273	C,H,N,Cl	0.027
14	Me	Me	3-Br	NH	$C_{14}H_{13}BrN_4$	317	C,H,N,Br	0.025
15	Me	Me	3-OH	NH	$C_{14}H_{14}N_4O$	255	C,H,N	1.25
16	Me	Me	3-OMe	NH	$C_{15}H_{16}N_4O$	269	C,H,N	1.20
17[a]	Me	Me	3-CF_3	NH	$C_{15}H_{13}F_3N_4$	307		1.90
18	Me	Me	3-COOH	NH	$C_{15}H_{14}N_4O_2$	283	C,H,N	16.3
19	Me	Me	3-COOEt	NH	$C_{17}H_{18}N_4O_2$	311	C,H,N	>100
20	Me	Me	3-CN	NH	$C_{15}H_{13}N_5$	264	C,H,N	0.20
21[a]	Me	Me	4-Me	NH	$C_{15}H_{16}N_4$	253		2.10
22[a]	Me	Me	4-ethyl	NH	$C_{16}H_{18}N_4$	267		77.5
23[a]	Me	Me	4-F	NH	$C_{14}H_{13}FN_4$	257		0.56
24[a]	Me	Me	4-Cl	NH	$C_{14}H_{13}ClN_4$	273		>50
25[a]	Me	Me	2-ethyl	NH	$C_{16}H_{18}N_4$	267		>100
26[a]	Me	Me	2-Cl	NH	$C_{14}H_{13}ClN_4$	273		75.1
27	Me	Me	3,5-Cl	NH	$C_{14}H_{12}Cl_2N_4$	307	*b*	0.17
28	C_6H_5	Me	3-Cl	NH	$C_{19}H_{15}ClN_4$	335	*b*	0.010
29	Me	C_6H_5	3-Cl	NH	$C_{19}H_{15}ClN_4$	335	*b*	0.23
30	C_6H_5	C_6H_5	3-Cl	NH	$C_{24}H_{17}ClN_4$	396	*b*	0.096
31	Me	Me	3-Cl	NMe	$C_{15}H_{15}ClN_4$	287	C,H,N,Cl	0.50
32[a]	Me	Me	H	$NHCH_2$	$C_{15}H_{16}N_4$	253		1.08
33	Me	Me	3-Cl	O	$C_{14}H_{12}ClN_3O$	274	C,H,N	2.50
34[a]			H		$C_{16}H_{16}N_4$	265		0.31
35			3-Me		$C_{17}H_{18}N_4$	279	C,H,N	0.82
36			3-Cl		$C_{16}H_{15}ClN_4$	299	C,H,N,Cl	0.029
37			3-Br		$C_{16}H_{15}BrN_4$	343	C,H,N,Br	0.046
38			3-OH		$C_{16}H_{16}N_4O$	281	C,H,N	0.42
39			3-OMe		$C_{17}H_{18}N_4O$	295	C,H,N	0.86
40			3-CF_3		$C_{17}H_{15}F_3N_4$	333	C,H,N,F	0.36
41			3-COOH		$C_{17}H_{16}N_4O_2$	309	*b*	25.7
42[a]			4-Me		$C_{17}H_{18}N_4$	277		0.11
43[a]			4-ethyl		$C_{18}H_{20}N_4$	291		>50
44					$C_{16}H_{11}ClN_4$	295	C,H,N,Cl	0.006
45[a]					$C_{15}H_{15}ClN_4$	287		>100
46					$C_{14}H_{20}N_4$	245	C,H,N	6.92
3								0.004

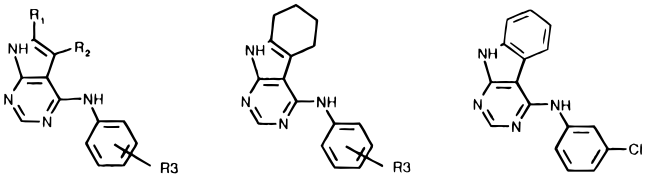
[a] Compound published in the literature.[30] [b] No elemental analysis (only small amount of compound available); puritiy according to HPLC: ≥95%.

> 1000 against these kinases (Table 2), including PKA (data not shown). Compounds **13**, **36**, and **44** showed competitive type inhibition against ATP (data not shown).

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, IL-3, etc.). Monitoring both the modulation of receptor autophosphorylation and the levels of expression of *c-fos*-mRNA offers convenient methods 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.[15] In order to define the specificity of inhibitors, inhibition of PDGF-stimulated tyrosine phosphorylation was assayed in BALB/c 3T3 cells. The effect of drugs on the inhibition of EGF-, PDGF-, or PMA-induced *c-fos* mRNA expression was also tested in BALB/c 3T3 cells. Finally, inhibition of cell proliferation by inhibitors was measured using an EGF-dependent mouse keratinocyte cell line (Balb/MK).

As shown in Table 3, the *in vitro* active compounds **13**, **14**, **28**−**30**, **34**, **36**, **37**, and **44** inhibited EGF-stimulated cellular tyrosine phosphorylation. In this assay, the most potent compounds **13**, **36**, **37**, and **44** had IC_{50} values between 120 and 400 nM. Compounds **28**−**30** with bulky substituents in position 5 and/or 6 of the pyrrole ring only showed IC_{50} values between 3 and 6 μM. PDGF-induced tyrosine phosphorylation was not inhibited by these compounds up to a concentration of 10 μM, thus indicating high selectify. Although highly active *in vitro*, compounds **10**−**12** and **35** were not able to inhibit cellular tyrosine phosphorylation, probably due to lack of penetration into the cells. Cellular activity and specificity of active compounds **13**,

Table 2. *In Vitro* Selectivity



10-14, 27-29 34-37 44

				IC$_{50}$ (μM)			
compd	R_1	R_2	R_3	EGF-R	*v-Abl*	*c-Src*	PKC-α
10	Me	Me	H	1.90	2.0	>100	75
11	Me	Me	3-Me	0.57	5.3	>100	>100
12	Me	Me	3-F	0.55	99	>100	>100
13	Me	Me	3-Cl	0.027	40.5	>100	>100
14	Me	Me	3-Br	0.025	2.3	>100	>100
28	C_6H_5	Me	3-Cl	0.010	>100	>100	>100
29	Me	C_6H_5	3-Cl	0.23	> 100	>100	nt[a]
30	C_6H_5	C_6H_5	3-Cl	0.096	>100	>100	nt
34			H	0.31	>100	>100	>100
35			3-Me	0.82	>100	>100	>100
36			3-Cl	0.026	>100	>100	>100
37			3-Br	0.046	>100	>100	>100
44				0.006	30	>100	>100

[a] nt = not tested.

Table 3. Cellular Activity and Specificity[a]

compd	MK cell	EGF ELISA	PDGF ELISA	*c-fos* EGF	*c-fos* PDGF	*c-fos* PMA
10	24.6	>100	>10	nt[b]	nt	nt
11	7.2	>100	>10	nt	nt	nt
12	29.9	>100	>10	nt	nt	nt
13	1.4	0.3	>10	2	>100	>100
14	1.4	1.5	>10	0.6	>100	>100
28	>50	6	>10	nt	nt	nt
29	38.3	6	nt	nt	nt	nt
30	>50	3	>10	nt	nt	nt
34	12.8	3	>10	nt	nt	nt
35	>50	>100	>10	nt	nt	nt
36	0.98	0.12	>10	0.3	>100	>100
37	1.4	0.4	>10	0.50	>100	>100
44	0.47	0.12	10	0.1	>100	>100

[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/c 3T3 cells (IC$_{50}$, μM). *c-fos* EGF: inhibition of EGF-induced *c-fos* mRNA expression in BALB/c 3T3 cells (IC$_{50}$, μM). *c-fos* PDGF: inhibition of PDGF-induced *c-fos* mRNA expression in BALB/c 3T3 cells (IC$_{50}$, μM). *c-fos* PMA: inhibition of PKC-mediated *c-fos* mRNA expression in BALB/c 3T3 cells (IC$_{50}$, μM). [b] nt = not tested.

14, **36**, **37**, **and 44** were confirmed in the *c-fos* assay. Inhibition of EGF-induced *c-fos* mRNA expression was observed with IC$_{50}$ values between 0.1 and 2 μM. In contrast, PDGF- and PMA-induced *c-fos* mRNA expression was not blocked with these compounds (IC$_{50}$ > 100 μM).

Finally, all compounds, which showed potent inhibition of tyrosine phosphorylation as well as *c-fos* mRNA expression also inhibited proliferation of the EGF-dependent Balb/MK cells. The most potent inhibition was observed with compound **44** (IC$_{50}$ = 0.47 μM). In general, there is a good correlation between the IC$_{50}$ values for inhibition of proliferation, tyrosine phosphorylation, and *c-fos* mRNA expression, thus indicating high selectivity of this compound class for the inhibition of the ligand-activated EGF-R signal transduction pathway.

In addition, compounds **13**, **36**, and **44** showed good *in vivo* efficacy at low doses after oral or subcutaneous administration in nude mouse tumor models using xenografts of the EGF-R overexpressing A431 cell line. The ED$_{50}$ values for **13** and **44** after oral application were 1.5 and 2 mg/kg (data not shown, to be reported elsewhere).

Discussion and Conclusions

The SAR in a series of 4-(phenylamino)pyrrolopyrimidine derivatives show a preference for halogen substituents at the 3-position of the anilino moiety and for bulky substituents in 5- and/or 6-position of the pyrrole ring. 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. This assumption is supported by the rather low inhibitory activity of compound **46** (IC$_{50}$ = 6.92 μM), where the phenyl ring is replaced by a cyclohexyl ring. In addition, the marked decrease of inhibitory activity with substituents in ortho and para position of the anilino moiety indicates that there is only limited space available for substituents in the "sugar pocket". The tolerance for rather bulky substituents in the pyrrole moiety 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. Furthermore, by comparison of the SAR data at the anilino moiety between 4-(phenylamino)pyrrolopyrimidines and 4-(phenylamino)quinazolines[21,25] or 7-amino-4-(phenylamino)pyridopyrimidines,[26] it is obvious that they follow similar rules, thus indicating a similar binding mode for both structural classes. In their model for the bisubstrate-type binding mode of 4-(phenylamino)quinazolines, the authors hypothesize that the anilino ring matches a tyrosine moiety where the anilino nitrogen corresponds to the oxygen of the tyrosine hydroxyl group and that the quinazoline nitrogens correspond to the γ-phosphate group of ATP.[22] However, this model is not in accordance with the kinetic behavior of 4-(phenylamino)quinazolines (e.g. compound **3**) which show clear competitiveness against ATP and noncompetitiveness against the peptide substrate. On the basis of our data, we propose that the anilino moiety of 4-(phenylamino)quinazolines binds as in the 4-(phenylamino)pyrrolopyrimidine series into the "sugar pocket", thereby replacing the ribose ring of ATP and that the two methoxy groups of the quinazoline moiety point toward the "large hydrophobic pocket". This binding mode would imply that in the context of the active site of the EGF-R PTK the phenyl ring of the (phenylamino)quinazolines could be isosteric to the pyrrole ring of our inhibitor class with its postulated hydrogen-bond interactions. There is precedence in the literature where a chemical group of a ligand, known to form a stable hydrogen bond with its macromolecular receptor, can be replaced by a hydrophobic group without loss of activity.[32] This is a manifestation of the delicate balance that exists between the cost of energy for desolvating the ligand and the energy gained by forming new interactions with the receptor.

The enzymatic and cellular data presented clearly demonstrate that 4-(phenylamino)pyrrolopyrimidines are an interesting class of compounds with high selectivity and specificity for the EGF-mediated signal transduction pathway. This class of molecules represents a

second generation of EGF-R tyrosine kinase inhibitors with high *in vitro* and *in vivo* activity. Further optimization and SAR studies are ongoing.

Experimental Section

Kinase Assays. Purification of protein kinases and *in vitro* enzyme tests were performed as previously described.[11,15,1,18]

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 **(1)** ($IC_{50} = 0.3\ \mu M$)[15,16] 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.[15]

Inhibition of *c-fos* mRNA expression. *c-fos* induction assays were performed as previously described.[16]

Antiproliferative Assays. Assays were performed essentially as previously described.[15]

Compounds. Compounds **10**, **17**, **21**−**26**, **32**, **34**, **42**, and **43** are published in the literature.[30] These compounds together with compound **45** were obtained from Prof. Erik B. Pedersen (Department of Chemistry, Odense University, Denmark).

Synthesis. Elemental analyses were within ±4% of the theoretical value. ^1H NMR and ^{13}C NMR were recorded on a Varian Gemini 200, a Varian Gemini 300, or a Brucker WM-360 spectrometer. The coupling constants are recorded in hertz (Hz), and 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. 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; eluents, $H_2O + CH_3CN +$ 0.1% TFA).

General Procedure for Ring Closure. 4-Hydroxy-5,6-dimethyl-7-benzyl-pyrrolo[2,3-*d*]pyrimidine (6a). A 9.5 g (51.5 mmol) portion of 2-amino-4,5-dimethyl-l-benzyl-3-cyano-pyrrole **(5a)**[31] was boiled in 85% formic acid for 5 h at 110 °C. After cooling, 200 mL of ice water was added to the reaction mixture. Crystals of the product separated which were filtered off and washed with water and hexane to give 4-hydroxy-5,5-dimethyl-7-benzylpyrrolo[2,3-*d*]pyrimidine **(6a)** (yield: 60%) of mp 251−253 °C (dec).

In an analogous way were prepared the following.

4-Hydroxy-5,6-tetramethylene-7-benzylpyrrolo[2,3-*d*]-pyrimidine (6b): colorless crystals of mp 104−105 °C; FABMS *m/z* 280 $(M + H)^+$ ($C_{27}H_{17}N_3O$).

4-Hydroxy-5,6-diphenyl-7-benzylpyrrolo[2,3-*d*]pyrimidine (6c): colorless crystals of mp 225−230 °C; FABMS *m/z* 378 $(M + H)^+$ ($C_{25}H_{19}N_3O$).

4-Hydroxy-5-methyl-6-phenyl-7-benzylpyrrolo[2,3-*d*]-pyrimidine (6d) and 4-Hydroxy-5-phenyl-6-methyl-7-benzylpyrrolo[2,3-*d*]pyrimidine (6e). Using the same procedure, a mixture of **6d** and **6e** was obtained which was separated by column chromatography and used without further purification for chlorination.

6d: amorphous; FABMS *m/z* 378 $(M + H)^+$ ($C_{25}H_{19}N_3O$).

6e: amorphous; FABMS *m/z* 378 $(M + H)^+$ ($C_{25}H_{19}N_3O$).

4-Chloro-5,6-dimethyl-7-benzylpyrrolo[2,3-*d*]pyrimidine (7a): General Procedure. A 2.5 g (9.9 mmol) sample of 4-hydroxy-5,6-dimethyl-7-benzylpyrrolo[2,3-*d*]pyrimidine **(6a)** was refluxed with 20 mL of $POCl_3$ for 2.5 h. Excess of $POCl_3$ was removed under reduced pressure and ice water added to the residue. The crude product was separated, filtered off, and dissolved in ethyl acetate. The organic phase was washed with water, dried, and concentrated. White crystals of **7a** separated which were filtered off (yield 76%): mp 115−116 °C; FABMS *m/z* 272 $(M + H)^+$. Anal. ($C_{15}H_{14}ClN_3$) C, H, N, Cl.

Using the same method as for the preparation of **7a**, the following compounds were prepared and used without further purification for reaction with substituted anilines.

4-Chloro-5,6-tetramethylene-7-benzylpyrrolo[2,3-*d*]pyrimidine (7b): colorless crystals of mp 110−112 °C; FABMS *m/z* 298 $(M + H)^+$($C_{17}H_{16}ClN_3$).

4-Chloro-5,6-diphenyl-7-benzylpyrrolo[2,3-*d*]pyrimidine (7c): colorless crystals of mp 272−274 °C; FABMS *m/z* 396 $(M + H)^+$ ($C_{25}H_{18}ClN_3$).

4-Chloro-5-methyl-6-phenyl-7-benzylpyrrolo[2,3-*d*]pyrimidine (7d): amorphous; FABMS *m/z* 334 $(M + H)^+$ ($C_{20}H_{16}ClN_3$).

4-Chloro-5-phenyl-6-methyl-7-benzylpyrrolo[2,3-*d*]pyrimidine (7e): amorphous; FABMS *m/z* 334 $(M + H)^+$ ($C_{20}H_{16}ClN_3$).

General Method of Coupling Procedure with Substituted Anilines. 4-((3-Chlorophenyl)amino)-5,6-dimethyl-7-benzyl-pyrrolo[2,3-*d*]pyrimidine (8a). A 6.29 g (23 mmol) sample of 4-chloro-5,6-dimethyl-7-benzyl-pyrrolo[2,3-*d*]pyrimidine **(7a)** in 100 mL of ethanol and 2.92 mL (28 mmol) of 3-chloroaniline were refluxed for 17 h. The solution was evaporated and the residue dissolved in ethyl acetate. The organic phase was washed with sodium bicarbonate solution and water, dried, and evaporated. **8a** (6.02 g, yield 94%) crystallized from ethyl acetate/hexane as colorless crystals of mp 132−133 °C. FABMS *m/z* 363 $(M + H)^+$; ^1H NMR ($CDCl_3$) δ 8.42 (s, pyrimidine H), 7.89 (m, aromat H), 7.58 (m, aromat H), 7.2−7.35 (m, 4 aromat H), 7.0 (m, 3 aromat H + NH), 5.43 (s, benzyl CH_2), 2.49 (s, CH_3), 2.22 (s, CH_3). Anal. ($C_{21}H_{19}ClN_4$) C, H, N, Cl.

Using the same method as for the preparation of **8a** was prepared the following.

4-((3-Chlorophenyl)amino)-5,6-tetramethylene-7-benzylpyrrolo[2,3-*d*]pyrimidine (8b): colorless crystals from ethyl acetate/hexane of mp 145−147 °C (yield 80%); FABMS *m/z* 389 $(M + H)^+$; NMR ($CDC1_3$) δ 8.43 (s, pyrimidine H), 7.88 (m, aromat H), 7.58 (m, aromat H), 7.2−7.35 (m, 3 aromat H), 7.10 (m, 2 aromat H), 7.04 (d, aromat H), 6.80 (s, NH), 5.38 (s, benzyl CH_2), 2.93 (m, 2 CH_2), 2.56 (m, 2 CH_2). Anal. ($C_{23}H_{21}ClN_4$) C, H, N, Cl.

General Method for Removal of the Benzyl Protecting Group. 4-((3-Chlorophenyl)amino)-5,6-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (13). A 1 g (2.76 mmol) portion of 4-((3-chlorophenyl)amino)-5,6-dimethyl-7-benzylpyrrolo[2,3-*d*]-pyrimidine **(8a)** and 2.57 g (19.32 mmol) of $AlCl_3$ in 20 mL of toluene were refluxed for 2 h. After the mixture was cooled to room temperature, ice−water was added. The mixture was stirred at 0 °C for 2 h and the precipitate filtered off. The residue was dissolved in hot ethyl acetate. The organic phase was washed with sodium bicarbonate solution (5%) and water, dried, and evaporated. The residue was crystallized from ethyl acetate/hexane to give colorless crystals of **13** (yield: 94%) of mp 239−240 °C: FABMS *m/z* 273 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 11.46 (s, NH), 8.18 (s, pyrimidine H), 8.10 (s, NH), 7.93 (s, aromat H), 7.68 (d, aromat H), 7.31 (tr, aromat H), 7.02 (d, aromat H), 2.38 (s, CH_3), 2.25 (s, CH_3). Anal. ($C_{14}H_{13}ClN_4$) C, H, N, Cl.

By removal of the N-benzyl protecting group from the corresponding substituted 4-(phenylamino)-5,6-substituted-7-benzylpyrrolo[2,3-*d*]pyrimidines in a similar way as described for **13,** the following final products were obtained.

4-((3-Fluorophenyl)amino)-5,6-dimethyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine (12): colorless crystals of mp 245−255 °C; FABMS *m/z* 257 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 11.46 (s, NH), 8.21 (s, pyrimidine H), 8.13 (s, NH), 7.73 (d, aromat H), 7.50 (d, aromat H), 7.34 (m, aromat H), 6.78 (tr, aromat H), 2.41 (s, CH_3), 2.28 (s, CH_3). Anal. ($C_{14}H_{13}FN_4$) C, H, N, F.

4-((3-Bromophenyl)amino)-5,6-dimethyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine (14): colorless crystals of mp 243−244 °C; FABMS *m/z* 317 $(M + H)^+$; ^1H NMR (DMSO-d_6) δ 11.50 (s, NH), 8.20 (s, pyrimidine H), 8.15 (s, NH), 8.08 (d, aromat H), 7.78 (d, aromat H), 7.48 (tr, aromat H), 7.16 (d, aromat H), 2.41 (s, CH_3), 2.29 (s, CH_3). Anal. ($C_{14}H_{13}BrN_4$) C, H, N, Br.

4-((3-Cyanophenyl)amino)-5,6-dimethyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine (20): FABMS *m*/*z* 264 $(M + H)^+$ ($C_{15}H_{13}N_5$); ^{1}H NMR (DMSO-d_6) δ 11.50 (s, NH), 8.26 (d, NH), 8.21 (s, pyrimidine H), 8.25 (s, aromat H), 8.06 (d, aromat H), 7.48 (tr, aromat H), 7.41 (d, aromat H), 2.42 (s, CH_3), 2.27 (s, CH_3).

4-((3,5-Dichlorophenyl)amino)-5,6-dimethyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine (27): colorless crystals of mp >250 °C; FABMS *m*/*z* 307 $(M + H)^+$ ($C_{14}H_{12}Cl_2N_4$); ^{1}H NMR (DMSO-d_6) δ 11.54 (s, pyrrole NH), 8.26 (s, NH), 8.23 (s, pyrimidine H), 7.91 (s, 2 aromat H), 7.12 (m, aromat H), 2.39 (s, CH_3), 2.19 (s, CH_3).

4-((3-Chlorophenyl)amino)-5-methyl-6-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (28): crystals of mp 275−280 °C (HCl salt); FABMS *m*/*z* 335 $(M + H)^+$ ($C_{19}H_{15}ClN_4$); ^{1}H NMR (DMSO-d_6) δ 12.27 (s, pyrrole NH), 9.05 (s, NH), 8.31 (s, pyrimidine H), 7.85 (m, aromat H), 7.62 (m, 3 aromat H), 7.56 (m, 2 aromat H), 7.43 (m, 2 aromat H), 7.23 (d, aromat H), 2.61 (s, CH_3).

4-((3-Chlorophenyl)amino)-5-phenyl-6-methyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (29): colorless crystals of mp 257−261 °C; FABMS *m*/*z* 335 $(M + H)^+$ ($C_{19}H_{15}ClN_4$); ^{1}H NMR (DMSO-d_6) δ 10.49 (s, pyrrole NH), 8.50 (s, pyrimidine H), 7.78 (s, aromat H), 7.53 (m, 2 aromat H), 7.50 (m, 3 aromat H), 7.16 (m, 2 aromat H), 6.97 (d, aromat H), 6.81 (s, NH), 2.56 (s, CH_3).

4-((3-Chlorophenyl)amino)-5,6-diphenyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine (30): White amorphous powder; FABMS *m*/*z* 396 $(M + H)^+$ ($C_{24}H_{17}ClN_4$).

4-((3-Chlorophenyl)-*N*-methylamino)-5,6-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (31): orange-colored crystals of mp 191−196 °C; FABMS *m*/*z* 287 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 11.68 (s, pyrrole NH), 8.41 (s, pyrimidine H), 7.28 (m, aromat H), 7.04 (m, 2 aromat H), 6.86 (m, aromat H), 3.48 (s, NCH_3), 2.30 (s, CH_3),1.48 (s, CH_3). Anal. ($C_{15}H_{15}ClN_4$) C, H, N, Cl.

4-(3-Chlorophenoxy)-5,6-dimethyl-7*H*-pyrrolo[2,3-*d*]-pyrimidine (33): colorless crystals of mp 214−216 °C; FABMS *m*/*z* 274 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 12.62 (s, pyrrole NH), 8.97 (s, pyrimidine H), 8.27 (tr, aromat H), 8.18 (m, aromat H), 8.13 (m, aromat H), 8.03 (dd, aromat H), 3.12 (s, 2 CH_3). Anal. ($C_{14}H_{12}ClN_3O$) C, H, N, Cl.

4-((3-Chlorophenyl)amino)-5,6-tetramethylene-7*H*-pyrrolo[2,3-*d*]pyrimidine (36): colorless crystals of mp 246−249 °C; FABMS *m*/*z* 299 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 11.47 (s, pyrrole NH), 8.20 (s, pyrimidine H), 8.00 (s, NH), 7.93 (m, aromat H), 7.68 (m, aromat H), 7.30 (tr, aromat H), 7.02 (m, aromat H), 2.92 (s, 2 cyclohexyl H), 2.67 (s, 2 cyclohexyl H), 1.81 (s, 4 cyclohexyl H). Anal. ($C_{16}H_{15}ClN_4$) C, H, N, Cl.

4-((3-Bromophenyl)amino)-5,6-tetramethylene-7*H*-pyrrolo[2,3-*d*]pyrimidine (37): colorless crystals of mp 240−245 °C; FABMS *m*/*z* 343 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 11.35 (s, pyrrole NH), 8.18 (s, pyrimidine H), 8.06 (m, aromat H), 7.93 (s, NH), 7.72 (m, aromat H), 7.23 (tr, aromat H), 7.12 (m, aromat H), 2.91 (s, 2 cyclohexyl H), 2.67 (s, 2 cyclohexyl H), 1.80 (s, 4 cyclohexyl H). Anal. ($C_{16}H_{15}BrN_4$) C, H, N, Br.

4-((3-(Trifluoromethyl)phenyl)amino)-5,6-tetramethylene-7*H*-pyrrolo[2,3]pyrimidine (40): pale-yellow crystals of mp 259−261 °C; FABMS *m*/*z* 333 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 11.50 (s, pyrrole NH), 8.21 (s, pyrimidine H), 8.18 (m, NH + aromat H), 8.06 (m, aromat H), 7.53 (tr, aromat H), 7.31 (d, aromat H), 2.96 (s, 2 cyclohexyl H), 2.68 (s, 2 cyclohexyl H), 1.82 (s, 4 cyclohexyl H). Anal. ($C_{17}H_{15}F_3N_4$) C, H, N, F.

Removal of the *N*-benzyl protecting group from the chlorides **7a** and **7b** in a similar way gave the deprotected chlorides **9a** and **9b** which were used without further purification:

4-Chloro-5,6-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (9a): mp 247−250 °C; FABMS *m*/*z* 182 $(M + H)^+$ ($C_8H_8ClN_3$).[33]

4-Chloro-5,6-tetramethylene-7*H*-pyrrolo[2,3-*d*]pyrimidine (9b): mp >220 °C dec; FABMS *m*/*z* 208 $(M + H)^+$ ($C_{10}H_{10}ClN_3$).

Reaction of 4-chloro-5,6-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine **(9a)** with the corresponding substituted aniline gave the following products.

4-((3-Methylphenyl)amino)-5,6-dimethyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine (11): colorless crystals of mp 230−234 °C; FABMS *m*/*z* 253 $(M + H)^+$ ($C_{15}H_{16}N_4$); ^{1}H NMR (DMSO-d_6) δ 11.38 (s, NH), 8.12 (s, pyrimidine H), 7.83 (s, NH), 7.57 (d, aromat H), 7.50 (s, aromat H), 7.18 (tr, aromat H), 6.81 (d, aromat H), 2.38 (s, CH_3), 2.27 (s, CH_3).

4-((3-Hydroxyphenyl)amino)-5,6-dimethyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine (15): colorless crystals of mp 230−234 °C; FABMS *m*/*z* 255 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 11.38 (s, NH), 9.25 (s, NH), 8.14 (s, pyrimidine H), 7.80 (s, OH), 7.30 (s, aromat H), 7.08 (d, 2 aromat H), 6.42 (m, aromat H), 2.36 (s, CH_3),2.25 (s, CH_3). Anal. ($C_{14}H_{14}N_4O$) C, H, N.

4-((3-Methoxyphenyl)amino)-5,6-dimethyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine (16): colorless crystals of mp 211−214 °C; FABMS *m*/*z* 269 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 11.46 (s, NH), 8.17 (s, pyrimidine H), 7.93 (s, NH), 7.44 (d, aromat H), 7.33 (d, aromat H), 7.21 (tr, aromat H), 6.61 (dd, aromat H), 2.40 (s, CH_3), 2.28 (s, CH_3). Anal. ($C_{15}H_{16}N_4O$) C, H, N.

4-((3-Carboxyphenyl)amino)-5,6-dimethyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine (18): pale-yellow crystals of mp >260 °C; FABMS *m*/*z* 283 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 12.47 (s, NH), 9.55 (s, NH), 8.25 (s, pyrimidine H), 8.11 (s, aromat H), 7.87 (d, aromat H), 7.82 (d, aromat H), 7.60 (tr, aromat H), 2.41 (s, CH_3), 2.33 (s, CH_3). Anal. ($C_{15}H_{14}N_4O_2$) C, H, N.

4-((3-Ethoxycarbonyl)amino)-5,6-dimethyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine (19): beige crystals of mp 186−190 °C dec; FABMS *m*/*z* 311 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 11.42 (s, pyrrole NH), 8.29 (m, aromat H), 8.23 (s, NH), 8.14 (s, pyrimidine H), 8.02 (m, aromat H), 7.58 (d, aromat H), 7.43 (tr, aromat H), 4.33 (ester CH_2), 2.40 (s, CH_3), 2.17 (s, CH_3), 1.17 (ester CH_3). Anal. ($C_{17}H_{18}N_4O_2$) C, H, N.

4-(Cyclohexylamino)-5,6-dimethyl-7*H*-pyrrolo[2,3-*d*]-pyrimidine (46). Reaction of **9a** with cyclohexylamine gave **46** as colorless crystals of mp >260 °C: FABMS *m*/*z* 245 $(M + H)^+$ ($C_{14}H_{20}N_4$); ^{1}H NMR (DMSO-d_6) δ 12.35 (s, pyrrole NH), 8.18 (s, pyrimidine H), 7.19 (d, NH), 3.95 (m, cyclohexyl H), 2.34 (s, CH_3), 2.26 (s, CH_3), 1.93 (d, 2 cyclohexyl H), 1.75 (m, 2 cyclohexyl H), 1.60 (m, 3 cyclohexyl H), 1.42 (m, 2 cyclohexyl H), 1.17 (m, cyclohexyl H). Anal. ($C_{14}H_{20}N_4$) C, H, N.

Reaction of 4-chloro-5,6-tetramethylene-7*H*-pyrrolo[2,3-*d*]-pyrimidine **(9b)** with the corresponding substituted aniline as described for **7a** gave the following products.

4-((3-Methylphenyl)amino)-5,6-tetramethylene-7*H*-pyrrolo[2,3-*d*]pyrimidine (35): colorless crystals of mp 258−261 °C; FABMS *m*/*z* 279 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 11.37 (s, pyrrole NH), 8.12 (s, pyrimidine H), 7.71 (s, NH), 7.57 (d, aromat H), 7.50 (s, aromat H), 7.17 (tr, aromat H), 6.81 (d, aromat H), 2.92 (s, 2 cyclohexyl H), 2.68 (s, 2 cyclohexyl H), 1.81 (s, 4 cyclohexyl H). Anal. ($C_{17}H_{18}N_4$) C, H, N.

4-((3-Hydroxyphenyl)amino)-5,6-tetramethylene-7*H*-pyrrolo[2,3-*d*]pyrimidine (38): colorless crystals of mp 231−235 °C dec; FABMS *m*/*z* 281 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 11.50 (s, pyrrole NH), 9.51 (s, NH), 8.49 (s, pyrimidine H), 7.87 (m, aromat H), 7.44 (s, OH), 7.35 (m, aromat H), 7.12 (tr, aromat H), 6.75 (m, aromat H), 2.77 (s, 2 cyclohexyl H), 2.60 (s, 2 cyclohexyl H), 1.62 (s, 4 cyclohexyl H). Anal. ($C_{16}H_{16}N_4O$) C, H, N.

4-((3-Methoxyphenyl)amino)-5,6-tetramethylene-7*H*-pyrrolo[2,3-*d*]pyrimidine (39): colorless crystals of mp 239−241 °C; FABMS *m*/*z* 295 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 11.46 (s, pyrrole NH), 8.18 (s, pyrimidine H), 7.81 (s, NH), 7.42 (m, aromat H), 7.34 (d, aromat H), 7.20 (tr, aromat H), 6.58 (m, aromat H), 3.76 (s, OCH_3), 2.94 (s, 2 cyclohexyl H), 2.68 (s, 2 cyclohexyl H), 1.81 (s, 4 cyclohexyl H). Anal. ($C_{17}H_{18}N_4O$) C, H, N.

4-((3-Carboxyphenyl)amino)-5,6-tetramethylene-7*H*-pyrrolo[2,3-*d*]pyrimidine (41): colorless amorphous compound of mp >260 °C; FABMS *m*/*z* 309 $(M + H)^+$ ($C_{17}H_{16}N_2O_2$); ^{1}H NMR (DMSO-d_6) δ 12.85 (s, COOH), 11.44 (s, pyrrole NH), 8.30 (m, aromat H), 8.16 (s, pyrimidine H), 8.10 (s, NH), 8.00 (m, aromat H), 7.57 (m, aromat H), 7.42 (tr, aromat H), 2.95 (s, 2 cyclohexyl H), 2.67 (s, 2 cyclohexyl H), 1.82 (s, 4 cyclohexyl H).

4-((3-Chlorophenyl)amino)pyrimido[4,5-*b*]indole (44). A solution of 12.1 g (31 mmol) of 4-((3-chlorophenyl)amino)-5,6-tetramethylene-7-benzylpyrrolo[2,3-*d*]pyrimidine **(8b)** and 14.1 g (62 mmol) of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in 260 mL of toluene was refluxed for 30 min. A

precipitate was filtered off and the filtrate evaporated to dryness. Chromatography on silicagel gave colorless crystals of 4-((3-chlorophenyl)amino)-*N*-benzylpyrimido[4,5-*b*]indole (yield 55%) of mp 174−176 °C: FABMS *m/z* 385 $(M + H)^+$.

The benzyl group in 4-((3-chlorophenyl)amino)-*N*-benzylpyrimido[4,5]indole was removed with $AlCl_3$/toluene as described for the preparation of compound **13.** Compound **44** was obtained as colorless (yield 80%) crystals of mp > 260 °C (HCl salt 279−286 °C): FABMS *m/z* 295 $(M + H)^+$; ^{1}H NMR (DMSO-d_6) δ 12.02 (s, pyrrole NH), 8.93 (s, NH), 8.50 (s, pyrimidine H), 8.43 (d, aromat H), 7.97 (m, aromat H), 7.52 (d, aromat H), 7.68 (m, aromat H), 7.45 (tr, aromat H), 7.38 (tr, aromat H), 7.32 (tr, aromat H), 7.13 (m, aromat H). Anal. ($C_{16}H_{11}ClN_4$) C, H, N,Cl.

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