Tyrosine Kinase Inhibitors. 13. Structure−Activity Relationships for Soluble 7-Substituted 4-[(3-Bromophenyl)amino]pyrido[4,3-*d*]pyrimidines Designed as Inhibitors of the Tyrosine Kinase Activity of the Epidermal Growth Factor Receptor

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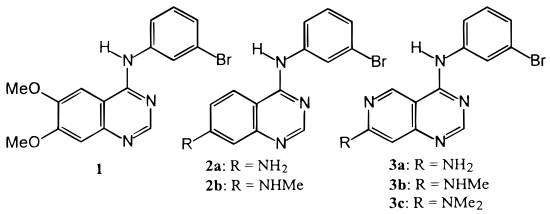
The general class of 4-(phenylamino)quinazolines are potent (some members with IC_{50} values ≪ 1 nM) and selective inhibitors of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR), via competitive binding at the ATP site of the enzyme, but many of the early analogues had poor aqueous solubility (≪1 mM). A series of 7-substituted 4-[(3-bromophenyl)-amino]pyrido[4,3-*d*]pyrimidines, together with selected (3-methylphenyl)amino analogues, were prepared by reaction of the analogous 7-fluoro derivatives with appropriate amine nucleophiles in 2-BuOH or aqueous 1-PrOH. All of the compounds were evaluated for their ability to inhibit the tyrosine-phosphorylating action of EGF-stimulated full-length EGFR enzyme. Selected analogues were also evaluated for their inhibition of autophosphorylation of the EGF receptor in A431 human epidermoid carcinoma cells in culture and against A431 tumor xenografts in mice. Analogues bearing a wide variety of polyol, cationic, and anionic solubilizing substituents retained activity, but the most effective in terms of both increased aqueous solubility (>40 mM) and retention of overall inhibitory activity (IC_{50}'s of 0.5−10 nM against isolated enzyme and 8−40 nM for inhibition of EGFR autophosphorylation in A431 cells) were weakly basic amine derivatives. These results are broadly consistent with a proposed model for the binding of these compounds to EGFR, in which the 6- and 7-positions of the pyridopyrimidine ring are in a largely hydrophobic binding region of considerable steric freedom, at the entrance of the adenine binding cleft. The most active cationic analogues have a weakly basic side chain where the amine moiety is three or more carbon atoms away from the nucleus. Two of the compounds (bearing weakly basic morpholinopropyl and strongly basic (dimethylamino)butyl solubilizing groups) produced in vivo tumor growth delays of 13−21 days against advanced stage A431 epidermoid xenografts in nude mice, when administered ip twice per day on days 7−21 posttumor implant. Treated tumors did not increase in size during therapy and resumed growth at the termination of therapy, indicating an apparent cytostatic effect for these compounds under these treatment conditions. The data suggest that continuous long-term therapy with these compounds may result in substantial tumor growth inhibition.

The 4-(phenylamino)quinazolines (e.g., **1**, **2**) are known to be potent and selective inhibitors of the tyrosine kinase activity of the epidermal growth factor receptor (EGFR), via competitive binding at the ATP site of the enzyme.[1−7] These compounds are of interest as potential anticancer drugs. The EGFR and related members of this family such as *erb*B2 constitute the starting points for many signal transduction pathways in cells, and their overexpression is an indicator of a poor prognosis in a number of human cancers, including mammary,[8,9] ovarian,[10] esophageal,[11] and squamous cell head and neck carcinomas.[12] Structure−activity relationships (SAR) for this class of compounds showed the desirability of a 3-bromo substituent on the phenyl ring and of small electron-donating groups off the long axis (at positions 6 and/or 7) of the quinazoline.[2,3]

We later showed[13] that related 4-[(3-bromophenyl)-amino]pyrido[*d*]pyrimidines, including 7-substituted pyrido[4,3-*d*]pyrimidines (e.g., **3a**−**c**), were also potent inhibitors of the isolated enzyme but with significantly different SAR for enzyme inhibition. In particular, while the 7-aminopyrido[4,3-*d*]pyrimidine (**3a**) was considerably less potent than the corresponding 7-amino-quinazoline (**2a**) against the isolated enzyme, (IC_{50}'s of 10 and 0.1 nM, respectively), the 7-(methylamino)-pyrido[4,3-*d*]pyrimidine (**3b**) was much more effective than the corresponding 7-(methylamino)quinazoline analogue (**2b**) (IC_{50}'s of 0.13 and 4 nM, respectively). The impressive enzyme inhibitory potency of these compounds made them of interest for in vivo evaluation, but their poor aqueous solubility (similar to that of the original quinazolines) has made this difficult.[14]



1 **2a**: R = NH_2 **2b**: R = NHMe **3a**: R = NH_2 **3b**: R = NHMe **3c**: R = NMe_2

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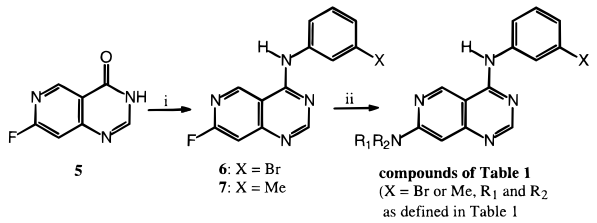
Scheme 1[a]



[a] (i) $SOCl_2$/reflux/3.5 h, then 3-bromoaniline or 3-methylaniline/CH_2Cl_2/2-PrOH/20 °C/16 h; (ii) R_1R_2NH/2-BuOH or *n*-PrOH−water/90−100 °C/16 h−8 days.

Model-building studies[15] suggest that the adenine of ATP binds in a cleft between the N- and C-terminal lobes, with the adenine forming H-bonds between N-1 and the backbone amide of Met-769 and between N-6 and the backbone carbonyl of Gln-767. The 4-(phenylamino)quinazolines and pyridopyrimidines are suggested to bind similarly, placing the bicyclic chromophore in the adenine pocket, with N-1 H-bonding again to Met-769 and N-3 to the side chain of Thr-766 on strand 5 deep in the binding cleft. The high potency and selectivity of these compounds for EGFR may be due to an increased hydrophobic binding of the phenylamino side chain to a unique hydrophobic region in the enzyme, adjacent to the ATP binding pocket and formed in part by three additional sulfur-containing amino acids (Cys-751, Met-769, and Met-742). In this model, the 6- and 7-positions of the quinazoline or pyridopyrimidine ring lie in the entrance of the adenine binding cleft, providing some steric freedom.

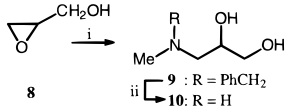
With the above indications for steric tolerance at the 7-position of the 4-[(3-bromophenyl)amino]pyrido[4,3-*d*]pyrimidines, we sought to develop soluble analogues of **3b**,**c** by the attachment of solubilizing functions via an alkylamine at this position and report here the synthesis, aqueous solubilities, and SAR for these compounds, together with in vivo data for two analogues.

Chemistry

The 7-substituted 4-[(3-bromo- and 3-methylphenyl)amino]pyrido[4,3-*d*]pyrimidines (**3** and **4**) of Table 1 were prepared from the known[13] 4-[(3-bromophenyl)amino]-7-fluoropyrido[4,3-*d*]pyrimidine, **6**, or its methyl analogue **7** (made by reaction of the known[13] 7-fluoropyrido[4,3-*d*]pyrimidin-4-one, **5**, with thionyl chloride followed by 3-methylaniline) (Scheme 1). Displacement of the fluorine atom in **6** and **7** with appropriate amine nucleophiles was normally conducted in 2-BuOH at 95 °C for 1−5 days, although for very polar amines (e.g., *N*-methyl-D-glucamine), aqueous 1-PrOH was a more effective solvent. The products from reactions with amino alcohols were generally solids which could be isolated and crystallized directly (method A). Crude products from reactions with diamines were generally oils which required basic workup and chromatography (method B). In the case of amino acid nucleophiles, it was necessary to preform their sodium salts prior to reaction (method C).

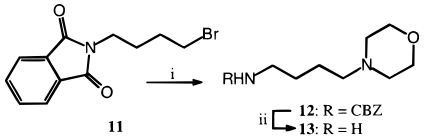
The above conditions were not successful with **6** and hydrazine as the nucleophile, since its greater reactivity led to the formation of a complex mixture of products. However, by allowing the reaction to proceed over several days at 20 °C, the desired product **3x** crystallized out of the reaction mixture in good purity directly. This compound was slightly unstable in solution and partly decomposed during conversion to its hydrochloride salt. The *N*-oxide **3t** was obtained in excellent yield from **3r** by selective oxidation of the side chain aliphatic tertiary nitrogen using Davis' reagent, 2-(phenylsulfonyl)-3-phenyloxaziridine.[16]

Scheme 2[a]



[a] (i) BnNHMe/MeOH/reflux/2 h; (ii) Pd/C/H_2/MeOH/20 °C/18 h.

Scheme 3[a]



[a] (i) Morpholine/EtOH/Et_3N/reflux/19 h, then $NH_2NH_2 \cdot H_2O$/EtOH/reflux/6 h, then BnOCOCl/THF/Et_3N/20 °C/15 h; (ii) Pd/C/H_2/MeOH/20 °C/1 day.

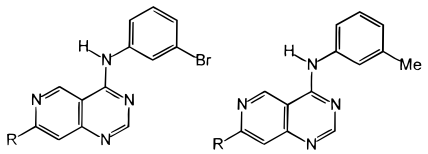
Most of the required amines were commercially available. 3-(Methylamino)propane-1,2-diol (**10**) was prepared by amination of glycidol (**8**) with *N*-benzylmethylamine followed by catalytic hydrogenolysis of the benzyl group (5% Pd/C) (Scheme 2). 4-(4-Aminobutyl)-morpholine[17] (**13**) was prepared by alkylation of morpholine with 4-bromobutylphthalimide (**11**) followed by standard cleavage of the phthalimide group with hydrazine and purification of the resulting amine as its benzyloxycarbonyl derivative **12** (Scheme 3).

Results and Discussion

The structures of the 7-substituted 4-[(3-bromophenyl)amino]pyrido[4,3-*d*]pyrimidines (**3a**−**ee**) prepared are recorded in Table 1. They were evaluated for their ability to inhibit tyrosine phosphorylation of a 14-residue polypeptide (a portion of phospholipase C-γ1) by EGF-stimulated full-length EGFR enzyme isolated from A431 cells.[1] At least two complete dose−response curves were determined for each compound, and averaged IC_{50}'s are listed in Table 1. Selected compounds were also evaluated for their ability to inhibit autophosphorylation of the EGF receptor in A431 human epidermoid carcinoma cells.

Three types of solubilizing 7-substituents were investigated: neutral (alcohols and polyols, **3d**−**k**), cationic (amines, **3l**−**z**), and anionic (acids, **3aa**−**ee**). Because both the methylamino and dimethylamino parent compounds **3b**,**c** proved equally effective, three pairs of compounds with NHR and corresponding N(Me)R neutral side chains were evaluated (**3d**/**3e**, **3f**/**3g**, and **3j**/**3k**). In the first two cases, while the NHR compounds had IC_{50}'s below 1 nM, the N(Me)R derivatives were significantly less effective, and in the third case both compounds were similarly less effective (IC_{50}'s of 3−5 nM). These three pairs explored the use of mono-, di-, and pentols, respectively. The diols **3f**,**g** proved the most soluble, but only 10−15-fold more than the parent compounds. Surprisingly, the pentols **3j**,**k** were slightly less soluble than the parent compounds, possibly due to tight internal H-bonding. The other two examples (**3h**,**i**) possess branched diol side chains of much larger

Table 1. Structural and Biological Properties of Soluble 7-Substituted 4-[(3-Bromophenyl)amino]pyrido[4,3-*d*]pyrimidines and Selected (3-Methylphenyl)amino Analogues



Compounds **3** Compounds **4**

						IC$_{50}$ (nM)	
no.	R	mp	formula	anal.	solubility[a] (mM)	E[b]	A[c]
			Parent				
3a	NH_2	ref 13				10	110
3b	NHMe	ref 13			0.15	0.13	16
3c	NMe_2	ref 13			0.13	0.09	14
			Alcohols				
3d	$NHCH_2CH_2OH$	218−219	$C_{15}H_{14}BrN_5O$	C,H,N	0.40	0.24	
3e	$N(Me)CH_2CH_2OH$	232−235	$C_{16}H_{16}BrN_5O$	C,H,N	0.06	2.6	
3f	$NHCH_2CH(OH)CH_2OH$	222.5−224.5	$C_{16}H_{16}BrN_5O_2$	C,H,N	1.6	0.92	227
3g	$N(Me)CH_2CH(OH)CH_2OH$	232−233	$C_{17}H_{18}BrN_5O_2$	C,H,N	1.9	3.2	
3h	$NHCH(CH_2OH)_2$	168−171	$C_{16}H_{16}BrN_5O_2$	C,H,N	2.2	14	
3i	$N(CH_2CH_2OH)_2$	199−201	$C_{17}H_{18}BrN_5O_2 \cdot H_2O$	C,H,N	0.96	12	
3j	$NHCH_2(CHOH)_4CH_2OH$	207−208	$C_{19}H_{22}BrN_5O_5 \cdot H_2O$	C,H,N	0.10	4.8	
3k	$N(Me)CH_2(CHOH)_4CH_2OH$	224.5 dec	$C_{20}H_{24}BrN_5O_5$	C,H,N	0.12	3.2	
			Amines				
3l	$NH(CH_2)_2NMe_2$	207−208	$C_{17}H_{19}BrN_6$	C,H,N	>40	45	
3m	$NH(CH_2)_3NMe_2$	196−198	$C_{18}H_{21}BrN_6$	C,H,N	>40	8.8	
3n	$NH(CH_2)_4NMe_2$	171−174	$C_{19}H_{23}BrN_6 \cdot H_2O$	C,H,N	>40	7.4	38
3o	$NH(CH_2)_5NMe_2$	123−126	$C_{20}H_{25}BrN_6$	C,H,N	37	8.4	
3p	$N(Me)(CH_2)_2NMe_2$	195−196	$C_{18}H_{21}BrN_6$	C,H,N	>40	40	
3q	$NH(CH_2)_2$Nmorph[d]	250−253	$C_{19}H_{21}BrN_6O$	H,N;C[g]	>40	3.2	36
3r	$NH(CH_2)_3$Nmorph[d]	173−174	$C_{20}H_{23}BrN_6O$	C,H,N	>40	1.9	8.1
3s	$NH(CH_2)_4$Nmorph[d]	149−151	$C_{21}H_{25}BrN_6O$	C,H,N	>40	5.4	
3t	$NH(CH_2)_3N(O)$morph[d]	189−190	$C_{20}H_{23}BrN_6O_2 \cdot 2H_2O$	C,H,N	33	0.74	>1000
3u	$NH(CH_2)_3$NMepip[e]	111−112.5	$C_{21}H_{26}BrN_7 \cdot H_2O$	C,H,N	>40	4.9	
3v	$NH(CH_2)_2N(CH_2CH_2OH)_2$	121.5−124.5	$C_{19}H_{23}BrN_6O_2 \cdot H_2O$	H,N;C[g]	36	9.2	
3w	$NH(CH_2)_3N(CH_2CH_2OH)_2$	144−146	$C_{20}H_{25}BrN_6O_2$	C,H,N	>40	1.2	
3x	$NHNH_2$	220 dec	$C_{13}H_{11}BrN_6$	C,H,N	<18	7.1	
3y	$NH(CH_2)_3$(1-imid)[f]	231−232.5	$C_{19}H_{18}BrN_7$	C,H,N	>40	0.51	
3z	$NH(CH_2)_2$(4-imid)[f]	246−249	$C_{18}H_{16}BrN_7$	C,H,N	35	0.91	
			Acids				
3aa	$NHCH_2COOH$	230−236 dec	$C_{15}H_{12}BrN_5O_2$	H,N;C[g]	7.4	1.5	
3bb	$NHCH_2CH_2COOH$	241−243	$C_{16}H_{14}BrN_5O_2$	C,H,N	>40	0.61	11400
3cc	$NH(CH_2)_3COOH$	230−233	$C_{17}H_{16}BrN_5O_2 \cdot 1.5H_2O$	C,N;H[h]	32	0.28	
3dd	$N(Me)CH_2COOH$	215 dec	$C_{16}H_{14}BrN_5O_2 \cdot H_2O$	C,H,N	28	16	
3ee	$NHCH_2CH_2SO_3H$	338−345 dec	$C_{15}H_{14}BrN_5O_3S$	C,H	>40	1.4	>1000
			3′-Me Amines				
4b	NHMe	275−277	$C_{15}H_{15}N_5$	C,H,N	1.3	1.4	
4n	$NH(CH_2)_4NMe_2$	178−180	$C_{20}H_{26}N_6 \cdot 0.25H_2O$	C,H,N	>40	5.4	
4r	$NH(CH_2)_3$Nmorph[d]	181.5−182.5	$C_{21}H_{26}N_6O$	C,H,N	>40	9.3	
4u	$NH(CH_2)_3$NMepip[e]	142−145	$C_{22}H_{29}N_7 \cdot 0.25H_2O$	C,H,N	>40	5.6	
4y	$NH(CH_2)_3$(1-imid)[f]	158−159	$C_{20}H_{21}N_7 \cdot 0.25H_2O$	C,N;H[i]	>34	3.5	
4z	$NH(CH_2)_2$(4-imid)[f]	216−216.5	$C_{19}H_{19}N_7$	C,H,N	>39	7.2	

[a] Solubility in water or aqueous buffer at 20 °C, determined by HPLC (see text). Values are for the hydrochloride salt form of amines and the sodium salt form of acids. [b] IC$_{50}$, concentration of drug (nM) to inhibit the phosphorylation of a polyglutamic acid/tyrosine random copolymer by EGFR (prepared from human A431 carcinoma cell vesicles by immunoaffinity chromatography). See Experimental Section for details. Values are the averages from at least two independent dose−response curves; variation was generally ±15%. [c] IC$_{50}$'s for inhibition of autophosphorylation of EGFR in A431 cells in culture. Values are the average of two experiments; see Experimental Section for details. [d] *N*-Morpholine derivative. [e] 4-Methylpiperazin-1-yl. [f] Imidazolyl. [g] C out by 0.5. [h] H out by 0.5. [i] H out by 0.6.

cross-sectional area than the other examples. While the $NHCH(CH_2OH)_2$ derivative **3h** showed the highest solubility of all the polyol side chain analogues (2.2 mM), **3h**,**i** were the least potent of the polyols (IC$_{50}$'s of 14 and 12 nM, respectively), suggesting some steric limitation due to the width of the binding site. Diol **3f**, which was one of the more potent in the isolated enzyme assay, was evaluated in the autophosphorylation assay but was relatively ineffective (IC$_{50}$ = 227 nM), possibly because of poor cellular uptake.

As expected, the cationic derivatives **3l**−**z** showed much greater aqueous solubility (as the hydrochloride salts), with all but five being >40 mM (Table 1). Compounds **3l**−**o** explore a homologous series of strongly basic side chains (pK_a ca. 10),[18] where activity against the isolated enzyme improves steadily as the cationic charge is shifted away from the ring for a certain distance, leveling off only after the chain is four-carbon atoms long. In this case the pair of NHR and N(Me)R compounds **(3l**,**p)** shows similar (albeit relatively low) potency. The more weakly basic morpholide analogues **3q**−**s** (pK_a ca. 7)[18] proved much more effective against the isolated enzyme. Potency against the isolated enzyme again increased as the charge was shifted away

Table 2. In Vivo Anticancer Activity of **3n**,**r** against the A431 Epidermoid Carcinoma and a Mouse Fibroblast Line Transfected with the h-EGF Receptor

tumor system[a]	compd	dose (mg/kg)	schedule[b]	wt loss (g)	T/C[c] (%)	T−C (days)[d]	net log cell kill[e]
A431	**3r**	25	b.i.d. days 7−21	1.7	34	12.8	−0.1
A431	**3n**	25	b.i.d. days 7−21	1.0	11	20.5	−0.1
EGFR	**3r**	25	b.i.d. days 3−17	0.9	85	0	
EGFR	**3n**	25	b.i.d. days 1−15	1.0	69	2.3	−1.1

[a] Tumor fragments of A431 or EGFR were implanted sc into the right axilla of mice on day 0. [b] All treatments were ip on the indicated schedules. The maximum tolerated dose ($\leq LD_{10}$) from a complete dose−response is shown. [c] Ratio of median treated tumor mass (mg) on the last day of therapy divided by the median control tumor mass × 100%. [d] The difference in days for the treated (T) and the control (C) tumors to reach 750 mg. [e] The net reduction in tumor burden, in logs, between the first and last treatments.

from the ring, with the C3 analogue **3r** being the best. The morpholide *N*-oxide derivative **3t** was prepared as an example of a neutral dipolar compound and proved the most potent of the morpholide derivatives against the isolated enzyme (IC_{50} = 0.74 nM). The piperazine derivative **3u** employed a different weak base (pK_a ca. 8)[18] but was less effective than the morpholide of similar chain length. The amine diols **3v**,**w** were prepared to further increase water solubility using a weak base (pK_a ca. 8.5),[18] and the longer side-chain analogue (**3w**) was again the more effective against the isolated enzyme. The hydrazine **3x** was investigated as an example of a nonbulky weak base but had relatively poor solubility and stability and only moderate potency. The imidazoles **3y**,**z** (pK_a's ca. 7.5)[18] were the most potent of all the amine analogues, with IC_{50}'s below 1 nM for inhibition of the isolated enzyme.

Representative analogues were tested in the cellular autophosphorylation assay in A431 cells. The morpholide **3r** was the most potent (IC_{50} = 8 nM; 2 times as effective as the parent compound **3b**, despite the fact that **3b** is 15-fold more potent than **3r** in the isolated enzyme assay). The more weakly basic but dipolar morpholine *N*-oxide **3t** had low activity in this assay (IC_{50} > 1000 nM), again suggesting poor uptake by cells.

Compounds **3aa**−**ee** were prepared to study the utility of anionic side chains. All but the glycine ($NHCH_2COOH$) analogue **3aa** had adequate aqueous solubility as the sodium salts. The $NH(CH_2)_nCOOH$ series (**3aa**−**cc**) showed excellent activity against the isolated enzyme, with **3cc** being one of the most potent 7-NH-substituted compounds (IC_{50} = 0.28 nM). As shown above for the alcohols, the NMe analogue **3dd** was much less effective than the corresponding NH derivative **3aa**. The sulfonic acid **3ee**, made as an example of a fully ionized acid (pK_a ca. 1),[19] maintained high potency in the isolated enzyme assay. However, the low activity of both **3bb** (IC_{50} = 11 400 nM) and **3ee** (IC_{50} > 1000 nM) in the cellular autophosphorylation assay suggests that these acids have difficulty getting into cells. A similar phenomenon was reported earlier for indolinethione acids[20] and lavendustin analogues[21] evaluated as EGFR inhibitors.

Finally, a small series of (3-methylphenyl)amino analogues (**4b**,**n**,**r**,**u**,**y**,**z**) were prepared for comparison with their 3-bromophenyl counterparts. This substituent group is moderately effective in (phenylamino)-quinazoline[5] and (phenylamino)pyrrolopyrimidine EGFR inhibitors,[22] resulting in more soluble compounds, but with 5−20-fold lower potency. Of the six 3′-Me derivatives, two (**4n**,**u**) had IC_{50}'s similar to their bromo counterparts, but four (**4b**,**r**,**y**,**z**) were 5−10-fold less potent. The parent NHMe derivative **4b** was, however,

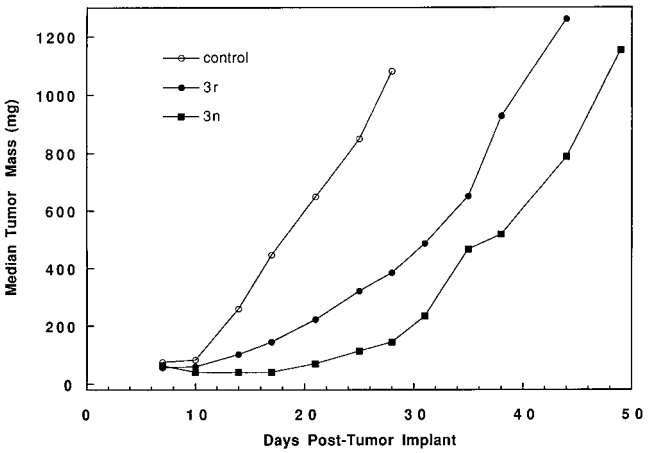


Figure 1. Effect of **3n**,**r** on the growth of the A431 epidermoid xenograft in nude mice. Tumors were implanted sc on day 0. Intraperitoneal treatments were administered b.i.d. on days 7−21. Both compounds were dosed at 25 mg/kg/injection, which was the maximum tolerated dose ($\leq LD_{10}$) obtained from a complete dose−response.

9-fold more soluble than **3b**. A recent study of analogous pyrimido[5,4-*d*]pyrimidines showed similar features.[23]

Overall, the cationic derivatives (**3l**−**z**) were the most effective. Accordingly, two of these (the strongly basic (dimethylamino)butyl analogue **3n** and the weakly basic morpholinopropyl analogue **3r**) were selected for preliminary in vivo studies against two different tumor xenografts in nude mice. The A431 epidermoid xenograft was selected based on its in vitro mitogenic responsiveness to EGF and the inhibition of growth on plastic by anti-EGF receptor monoclonal antibodies. The EGFR cell line was selected due to its expression of the transformed phenotype upon transfection with the human EGF receptor and its EGF requirement for clone formation in soft agar. Both compounds were measurably active against the A431 xenograft, as evidenced by tumor growth delays in the range of 13−21 days (Table 2 and Figure 1). There was, however, no apparent tumor burden reduction against this model, based on the near-zero net kill values. In contrast, both compounds were ineffective against the EGFR tumor model on the basis of tumor growth delay, percent T/C, and net log cell kill (Table 2). Weight loss data indicated that at maximum tolerated doses both compounds induced a similar amount of treatment-related weight loss.

Conclusions

The above results show that a wide variety of solubilizing substituents are tolerated at the 7-position of the 4-[(3-bromophenyl)amino]pyrido[4,3-*d*]pyrimidine nucleus, as determined by the ability of the compounds

to inhibit tyrosine phosphorylation of a substrate by EGF-stimulated full-length EGFR enzyme. While polyhydroxy (neutral), amino (cationic), and carboxylic acid (anionic) groups were all tolerated, the most effective in terms of aqueous solubility and retention of both enzyme and cellular activity were weakly basic amine derivatives. These results are broadly interpretable in terms of the proposed binding model,[15] which indicates considerable bulk tolerance (in the entrance of the adenine binding cleft of the enzyme) for substituents at the 7-position. The above SAR for cationic derivatives suggests that this is largely a hydrophobic binding region, with preferred activity for analogues where the amine group is either sufficiently far away from the nucleus (e.g., **3n**) or a sufficiently weak base that a significant proportion of neutral form may be present (e.g., **3r**).

The soluble analogues **3n**,**r** gave substantial growth delays in A431 xenografts, but not in the EGFR tumor model, despite the fact that the EGFR cell line has a lower number of EGFR targets per cell than the A431 (200 000−300 000 versus several million). Thus, overall receptor number is probably not responsible for the lack of in vivo effectiveness against the EGFR cell line. It is possible that, while this cell line requires EGF for clone formation in soft agar, it may not absolutely require EGF in vivo, where other growth factors may take over. This is supported by a similar lack of an effect with anti-EGFR monoclonal antibodies against this tumor model in vivo.[24] The treated A431 tumors began to increase in size at the end of therapy, and it is not clear whether longer periods of therapy would produce greater apparent antitumor effects by maintaining tumor growth suppression. The A431 tumors treated with **3n** did not appear to decrease in size during treatment, indicating that these compounds are cytostatic rather than cytotoxic under these conditions. It remains to be determined if prolonged therapy will ultimately result in a cytotoxic effect in vivo (reflected by a positive net cell kill).

Experimental Section

Analyses were performed by the Microchemical Laboratory, University of Otago, Dunedin, NZ. Melting points were determined using an Electrothermal model 9200 digital melting point apparatus and are as read. NMR spectra were measured on Bruker AC-200 or DRX-400 spectrometers and referenced to Me_4Si. HPLC was carried out using a Bondclone 10 C18 reverse-phase silica gel column, with a Phillips PU4100M gradient elution pump and a Phillips PU 4120 diode array detector, eluting with the appropriate ratios of 80% MeCN/20% water (solvent A) and ammonium formate buffer (solvent B; 28 g of ammonium formate + 2.55 mL of formic acid, made up to 1 L in deionized water, pH 4.5).

3-(Methylamino)propane-1,2-diol (10) (Scheme 2). A solution of racemic glycidol (**8**) (5.00 mL, 0.075 mol) and *N*-benzylmethylamine (9.25 mL, 0.072 mol) in MeOH (100 mL) was heated under reflux for 2 h and then cooled. The resulting solution of crude 3-(*N*-benzyl-*N*-methylamino)propane-1,2-diol (**9**) was hydrogenated over 5% Pd/C (2 g) at 50 psi for 18 h and then filtered through Celite, washing with MeOH. Evaporation of the combined filtrates gave **10** as a viscous liquid, which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 3.79 (ddt, J = 7.2, 5.1, 3.9 Hz, 1 H, C*H*OH), 3.70 (dd, J = 11.4, 3.7 Hz, 1 H, C*H*OH), 3.59 (dd, J = 11.4, 5.2 Hz, 1 H, C*H*OH), 2.73 (dd, J = 12.1, 4.0 Hz, 1 H, NCH), 2.66 (dd, J = 12.2, 7.3 Hz, 1 H, NCH), 2.44 (s, 3 H, CH_3).

4-(4-Aminobutyl)morpholine (13) (Scheme 3). A solution of 4-bromobutylphthalimide (**11**) (5.00 g, 17.7 mmol), morpholine (2.00 mL, 23.0 mmol), and Et_3N (5.00 mL, 35.9 mmol) in absolute EtOH (50 mL) was refluxed for 19 h and then cooled. Hydrazine hydrate (2.00 mL, 41.2 mmol) and absolute EtOH (50 mL) were added; then the mixture was refluxed for 6 h, cooled, and filtered, washing the precipitate with absolute EtOH. The solvent was removed from the filtrate and the residue suspended in dry THF (100 mL) and Et_3N (20 mL) and cooled in ice. Benzyl chloroformate (15 mL of a 50% solution in toluene, 52.5 mmol) was added dropwise with stirring; then the mixture stirred at 20 °C for 15 h. Excess reagent was quenched with MeOH; then the solvents were removed under reduced pressure. The residue was diluted with water, and the resulting solution was acidified to pH 1 (dilute HCl), washed with CH_2Cl_2, then treated with excess Na_2CO_3 (to pH 10), and extracted with EtOAc (4 × 100 mL). Removal of the solvent and chromatography of the residue on silica gel, eluting with 1−3% MeOH/CH_2Cl_2, gave *N*-(benzyloxycarbonyl)-4-(4-aminobutyl)morpholine (**12**) (3.04 g, 59% overall): mp (CH_2Cl_2/light petroleum) 45−46.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 7.37−7.28 (m, 5 H, ArH), 5.58 (br s, 1 H, NH), 5.09 (s, 2 H, OCH_2), 3.70 (t, J = 4.6 Hz, 4 H, $(CH_2)_2O$), 3.20 (q, J = 6.0 Hz, 2 H, NHC*H*$_2$), 2.41 (m, 4 H, $(CH_2)_2N$), 2.34 (t, J = 6.7 Hz, 2 H, NCH_2), 1.55 (m, 4 H, $2CH_2$); ^{13}C NMR δ 156.43 (s, OCONH), 136.68 (s, Ar), 128.47 (d, 2 C, Ar), 128.08 (d, Ar), 128.03 (d, 2 C, Ar), 66.82 (t, 2 C, $(CH_2)_2O$), 66.50 (t, OCH_2), 58.46 (t, NCH_2), 53.61 (t, 2 C, $N(CH_2)_2$), 40.95 (t, CH_2N), 27.89, 23.92 (2 t, $2CH_2$). Anal. ($C_{16}H_{24}N_2O_3$) C, H, N.

Hydrogenation of **12** with 5% Pd/C in MeOH at 60 psi for 24 h, followed by workup as above, gave **13** as an oil (lit.[17]), which was used without further purification: ^{1}H NMR [$(CD_3)_2$-SO] δ 3.55 (t, J = 4.7 Hz, 4 H, $(CH_2)_2O$), 2.54 (br t, J = 6.6 Hz, 2 H, NH_2CH_2), 2.31 (br t, J = 4.6 Hz, 4 H, $(CH_2)_2N$), 2.23 (t, J = 7.2 Hz, 2 H, NCH_2), 1.47−1.30 (m, 4 H, $2CH_2$).

7-Fluoro-4-[(3-methylphenyl)amino]pyrido[4,3-*d*]pyrimidine (7) (Scheme 1). A suspension of 7-fluoropyrido[4,3-*d*]pyrimidin-4(*3H*)-one[13] (**5**) (457 mg, 2.77 mmol) in $SOCl_2$ (25 mL) containing 2 drops of DMF was stirred under reflux for 3.5 h and then concentrated under reduced pressure to give crude 4-chloro-7-fluoropyrido[4,3-*d*]pyrimidine as an oil. This was cooled in ice, and a solution of 3-methylaniline (1.0 mL, 9.35 mmol) in CH_2Cl_2 (50 mL) was added followed by dry 2-PrOH (40 mL). The mixture was stirred at 20 °C for 16 h, then solvents were removed under reduced pressure, and the residue was diluted with aqueous Na_2CO_3 (100 mL) and extracted with EtOAc (3 × 100 mL). Chromatography of this extract on silica gel, eluting with 1% MeOH/$CHCl_3$, gave **7** (648 mg, 92%): mp (MeOH/$CHCl_3$/light petroleum) 212−213.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 10.35 (s, 1 H, NH), 9.61 (s, 1 H, H-5), 8.66 (s, 1 H, H-2), 7.60 (m, 2 H, H-2′,6′), 7.36 (s, 1 H, H-8), 7.32 (dd, J = 8.4, 7.8 Hz, 1 H, H-5′), 7.03 (d, J = 7.6 Hz, 1 H, H-4′), 2.35 (s, 3 H, 3′-CH_3); ^{13}C NMR δ 164.72 (d, J_{C-F} = 237 Hz, C-7), 159.35 (d, C-2), 158.01 (s, C-4), 157.32 (d, J_{C-F} = 13 Hz, C-8a), 148.37 (dd, J_{C-F} = 19 Hz, C-5), 137.85, 137.78 (2 s, C-1′,3′), 128.38 (d, C-5′), 125.44 (d, C-4′), 123.47 (d, C-2′), 120.21 (d, C-6′), 110.56 (d, J_{C-F} = 3 Hz, C-4a), 102.87 (dd, J_{C-F} = 35 Hz, C-8), 21.05 (q, CH_3). Anal. ($C_{14}H_{11}FN_4$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[(2,3-dihydroxypropyl)-amino]pyrido[4,3-*d*]pyrimidine (3f). Example of General Method A. A solution of 4-[(3-bromophenyl)amino]-7-fluoropyrido[4,3-*d*]pyrimidine[13] (**6**) (101 mg, 0.32 mmol) and 3-aminopropane-1,2-diol (0.60 mL, 7.74 mmol) in 2-BuOH (10 mL) was stirred at 100 °C for 3 days. The resulting solution was concentrated under reduced pressure and cooled to give a solid, which was collected by filtration and washed well with water. Recrystallization from DMSO/water gave **3f** (100 mg, 81%): mp (DMSO/water) 222.5−224.5 °C; ^{1}H NMR [$(CD_3)_2$-SO] δ 9.89 (br s, 1 H, NH), 9.35 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.19 (s, 1 H, H-2′), 7.84 (d, J = 8.0 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (br d, J = 8.1 Hz, 1 H, H-4′), 7.05 (br t, J = 5.7 Hz, 1 H, 7-N*H*CH_2), 6.50 (s, 1 H, H-8), 4.88 (d, J = 4.9 Hz, 1 H, CHO*H*), 4.64 (t, J = 5.7 Hz, 1 H, CH_2O*H*), 3.67 (m, 1 H, C*H*OH), 3.5−3.15 (m, 4 H, 7-NHCH_2CH(OH)-CH_2OH); ^{13}C NMR δ 160.99 (s, C-7), 157.94 (d, C-2), 157.71 (s, C-4), 154.57 (s, C-8a), 148.23 (d, C-5), 140.72 (s, C-1′), 130.39 (d, C-5′), 126.03 (d, C-4′), 124.27 (d, C-2′), 121.15 (s, C-3′), 120.79 (d, C-6′), 103.76 (s, C-4a), 95.80 (br d, C-8), 70.23 (d,

CHOH), 63.83 (t, CH_2OH), 44.78 (t, NCH_2). Anal. ($C_{16}H_{16}BrN_5O_2$) C, H, N.

The following analogues were prepared similarly.

4-[(3-Bromophenyl)amino]-7-[(2-hydroxyethyl)amino]pyrido[4,3-*d*]pyrimidine (3d): 78%, by reaction of **6** with 2-aminoethanol (20 equiv) in 2-BuOH (95 °C for 4 days), followed by chromatography of the product on silica gel, eluting with 5−7% MeOH in CH_2Cl_2; mp (MeOH/CH_2Cl_2) 218−219 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.89 (br s, 1 H, NH), 9.36 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.19 (br s, 1 H, H-2′), 7.84 (br d, J = 7.9 Hz, 1 H, H-6′), 7.34 (t, J = 7.9 Hz, 1 H, H-5′), 7.29 (dt, J = 7.9, 1.2 Hz, 1 H, H-4′), 7.13 (br t, J = 5.7 Hz, 1 H, N*H*CH_2), 6.46 (s, 1 H, H-8), 4.78 (t, J = 5.5 Hz, 1 H, CH_2OH), 3.59 (q, J = 5.8 Hz, 2 H, C*H*$_2$OH), 3.38 (br q, J = 5.8 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 160.86 (s, C-7), 157.84 (d, C-2), 157.62 (s, C-4), 154.51 (s, C-8a), 148.25 (d, C-5), 140.70 (s, C-1′), 130.30 (d, C-5′), 125.91 (d, C-4′), 124.17 (d, C-2′), 121.08 (s, C-3′), 120.67 (d, C-6′), 103.69 (s, C-4a), 95.85 (br d, C-8), 59.61 (t, CH_2OH), 43.92 (t, NCH_2). Anal. ($C_{15}H_{14}BrN_5O$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[*N*-(2-hydroxyethyl)-*N*-methylamino]pyrido[4,3-*d*]pyrimidine (3e): 99%, by reaction of **6** with 2-(methylamino)ethanol (20 equiv) in 2-BuOH (100 °C for 2 days); mp (2-BuOH) 232−235 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.91 (br s, 1 H, NH), 9.39 (s, 1 H, H-5), 8.47 (s, 1 H, H-2), 8.19 (t, J = 1.9 Hz, 1 H, H-2′), 7.84 (dt, J = 8.1, 1.7 Hz, 1 H, H-6′), 7.34 (t, J = 7.9 Hz, 1 H, H-5′), 7.29 (dt, J = 8.1, 1.4 Hz, 1 H, H-4′), 6.53 (s, 1 H, H-8), 4.75 (t, J = 5.4 Hz, 1 H, CH_2OH), 3.73 (t, J = 6.0 Hz, 2 H, NCH_2), 3.61 (q, J = 5.8 Hz, 2 H, C*H*$_2$OH), 3.14 (s, 3 H, NCH_3); ^{13}C NMR δ 159.81 (s, C-7), 157.89 (d, C-2), 157.63 (s, C-4), 154.74 (s, C-8a), 147.66 (d, C-5), 140.67 (s, C-1′), 130.31 (d, C-5′), 125.94 (d, C-4′), 124.17 (d, C-2′), 121.09 (s, C-3′), 120.56 (d, C-6′), 103.15 (s, C-4a), 95.58 (d, C-8), 58.45 (t, CH_2OH), 51.88 (t, NCH_2), 37.11 (q, NCH_3). Anal. ($C_{16}H_{16}BrN_5O$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[*N*-(2,3-dihydroxypropyl)-*N*-methylamino]pyrido[4,3-*d*]pyrimidine (3g): 61%, by reaction of **6** with 3-(methylamino)propane-1,2-diol (**10**) (15 equiv) in 2-BuOH (97 °C for 2 days); mp (MeOH/CH_2Cl_2) 232−233 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.91 (br s, 1 H, NH), 9.41 (s, 1 H, H-5), 8.48 (s, 1 H, H-2), 8.20 (br s, 1 H, H-2′), 7.85 (br d, J = 7.9 Hz, 1 H, H-6′), 7.35 (t, J = 8.0 Hz, 1 H, H-5′), 7.30 (dt, J = 8.2, 1.4 Hz, 1 H, H-4′), 6.56 (s, 1 H, H-8), 4.85 (d, J = 5.1 Hz, 1 H, CHO*H*), 4.69 (t, J = 5.7 Hz, 1 H, CH_2OH), 3.80 (m, 2 H, 2CH), 3.50 (m, 1 H, CH), 3.38 (t, J = 5.4 Hz, 2 H, C*H*$_2$OH), 3.17 (s, 3 H, NCH_3); ^{13}C NMR δ 159.93 (s, C-7), 157.92 (d, C-2), 157.65 (s, C-4), 154.67 (s, C-8a), 147.59 (d, C-5), 140.70 (s, C-1′), 130.33 (d, C-5′), 125.93 (d, C-4′), 124.13 (d, C-2′), 121.11 (s, C-3′), 120.65 (d, C-6′), 103.15 (s, C-4a), 95.74 (d, C-8), 69.84 (d, CHOH), 63.85 (t, CH_2OH), 53.03 (t, NCH_2), 37.69 (q, NCH_3). Anal. ($C_{17}H_{18}BrN_5O_2$) C, H, N. Chromatography of the mother liquors on silica gel, eluting with 6% MeOH/CH_2Cl_2, gave further pure product (20%).

4-[(3-Bromophenyl)amino]-7-[*N*,*N*-bis(2-hydroxyethyl)amino]pyrido[4,3-*d*]pyrimidine (3i): 24%, by reaction of **6** with diethanolamine (46 equiv) in 2-BuOH (98 °C for 5 days) followed by column chromatography of the product on silica gel (eluting with 1−5% MeOH/EtOAc) and then two rounds of preparative silica gel TLC (development with 10% MeOH/CH_2Cl_2 and 10% MeOH/EtOAc, respectively); mp (MeOH/$CHCl_3$) 199−201 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.99 (br s, 1 H, NH), 9.39 (s, 1 H, H-5), 8.48 (s, 1 H, H-2), 8.18 (br s, 1 H, H-2′), 7.83 (br d, J = 7.8 Hz, 1 H, H-6′), 7.35 (t, J = 7.9 Hz, 1 H, H-5′), 7.30 (dt, J = 8.0, 1.6 Hz, 1 H, H-4′), 6.61 (s, 1 H, H-8), 4.84 (br s, 2 H, 2OH), 3.69 (t, J = 5.4 Hz, 4 H, N(CH_2)$_2$), 3.63 (br q, J = 5.1 Hz, 4 H, 2C*H*$_2$OH); ^{13}C NMR δ 159.41 (s, C-7), 157.69 (d, C-2), 157.64 (s, C-4), 154.22 (s, C-8a), 147.79 (d, C-5), 140.60 (s, C-1′), 130.35 (d, C-5′), 126.07 (d, C-4′), 124.26 (d, C-2′), 121.11 (s, C-3′), 120.78 (d, C-6′), 103.13 (s, C-4a), 95.42 (d, C-8), 58.32 (t, 2 C, 2CH_2OH), 51.62 (t, 2 C, N(CH_2)$_2$). Anal. ($C_{17}H_{18}BrN_5O_2\cdot H_2O$) C, H, N.

4-[(3-Bromophenyl)amino]-7-D-glucaminopyrido[4,3-*d*]pyrimidine (3j): 78%, by reaction of **6** with D-glucamine (31 equiv) in 1-PrOH/water (7:1) (100 °C for 1 day); mp (water) 207−208 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.89 (br s, 1 H, NH), 9.36 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.18 (br s, 1 H, H-2′), 7.84 (br d, J = 7.9 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (br d, J = 8.0 Hz, 1 H, H-4′), 7.01 (br s, 1 H, N*H*CH_2), 6.50 (s, 1 H, H-8), 4.92 (d, J = 4.7 Hz, 1 H, CHO*H*), 4.50 (d, J = 5.1 Hz, 1 H, CHO*H*), 4.46 (d, J = 5.6 Hz, 1 H, CHO*H*), 4.38 (d, J = 6.7 Hz, 1 H, CHO*H*), 4.35 (t, J = 5.6 Hz, 1 H, CH_2OH), 3.82, 3.70, 3.60 (3 m, 3 × 1 H, 3CH), 3.50 (m, 3 H, 3CH), 3.41, 3.27 (2 m, 2 × 1 H, 2CH); ^{13}C NMR δ 160.92 (s, C-7), 157.86 (d, C-2), 157.62 (s, C-4), 154.58 (s, C-8a), 148.19 (d, C-5), 140.72 (s, C-1′), 130.32 (d, C-5′), 125.91 (d, C-4′), 124.16 (d, C-2′), 121.09 (s, C-3′), 120.67 (d, C-6′), 103.69 (s, C-4a), 95.50 (br d, C-8), 72.04 (d, CHOH), 71.42 (d, 2 C, 2CHOH), 69.62 (d, CHOH), 63.29 (t, CH_2OH), 44.45 (t, NCH_2). Anal. ($C_{19}H_{22}BrN_5O_5\cdot H_2O$) C, H, N.

4-[(3-Bromophenyl)amino]-7-(*N*-methyl-D-glucamino)pyrido[4,3-*d*]pyrimidine (3k): 90%, by reaction of **6** with *N*-methyl-D-glucamine (33 equiv) in 1-PrOH/water (3:1) (95 °C for 1 day); mp (water) 224.5 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 9.91 (br s, 1 H, NH), 9.41 (s, 1 H, H-5), 8.47 (s, 1 H, H-2), 8.20 (s, 1 H, H-2′), 7.85 (br d, J = 8.0 Hz, 1 H, H-6′), 7.35 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (br d, J = 8.0 Hz, 1 H, H-4′), 6.59 (s, 1 H, H-8), 4.90 (d, J = 5.1 Hz, 1 H, CHO*H*), 4.52 (d, J = 4.8 Hz, 1 H, CHO*H*), 4.44 (d, J = 5.5 Hz, 2 H, 2CHO*H*), 4.35 (t, J = 5.6 Hz, 1 H, CH_2OH), 3.93 (m, 1 H, CH), 3.82 (dd, J = 14.2, 4.1 Hz, 1 H, NCH), 3.68−3.35 (m, 6 H, 6CH); ^{13}C NMR δ 159.95 (s, C-7), 157.88 (d, C-2), 157.66 (s, C-4), 154.64 (s, C-8a), 147.61 (d, C-5), 140.72 (s, C-1′), 130.34 (d, C-5′), 125.91 (d, C-4′), 124.10 (d, C-2′), 121.11 (s, C-3′), 120.63 (d, C-6′), 103.13 (s, C-4a), 95.81 (d, C-8), 72.32, 71.38, 71.07, 69.52 (4 d, 4CHOH), 63.24 (t, CH_2OH), 52.94 (t, NCH_2), 37.58 (q, NCH_3). Anal. ($C_{20}H_{24}BrN_5O_5$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[[2-(dimethylamino)ethyl]amino]pyrido[4,3-*d*]pyrimidine (3l). Example of General Method B. A solution of **6** (136 mg, 0.43 mmol) and *N*,*N*-dimethylethylenediamine (0.50 mL, 4.58 mmol) in 2-BuOH (25 mL) was stirred at 100 °C for 2 days. The solvent was removed under reduced pressure, and the residue was diluted with aqueous Na_2CO_3 and extracted with EtOAc (3 × 50 mL). Chromatography of this extract on alumina, eluting with 3% EtOH/$CHCl_3$, gave **3l** (120 mg, 73%): mp (CH_2Cl_2/light petroleum) 210−211 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.89 (br s, 1 H, NH), 9.37 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.20 (s, 1 H, H-2′), 7.84 (d, J = 8.1 Hz, 1 H, H-6′), 7.34 (t, J = 7.9 Hz, 1 H, H-5′), 7.29 (br d, J = 8.1 Hz, 1 H, H-4′), 7.00 (br t, J = 5.7 Hz, 1 H, N*H*CH_2), 6.45 (s, 1 H, H-8), 3.39 (q, J = 6.1 Hz, 2 H, NHC*H*$_2$), 2.47 (t, J = 6.6 Hz, 2 H, NCH_2), 2.21 (s, 6 H, N(CH_3)$_2$); ^{13}C NMR δ 160.66 (s, C-7), 157.85 (d, C-2), 157.62 (s, C-4), 154.56 (s, C-8a), 148.30 (d, C-5), 140.72 (s, C-1′), 130.30 (d, C-5′), 125.89 (d, C-4′), 124.12 (d, C-2′), 121.09 (s, C-3′), 120.63 (d, C-6′), 103.70 (s, C-4a), 95.70 (br d, C-8), 57.74 (t, NCH_2), 45.16 (q, 2 C, N(CH_3)$_2$), 39.14 (t, NCH_2). Anal. ($C_{17}H_{19}BrN_6$) C, H, N.

The following analogues were prepared similarly.

4-[(3-Bromophenyl)amino]-7-[[3-(dimethylamino)propyl]amino]pyrido[4,3-*d*]pyrimidine (3m): 67%, by reaction of **6** with 3-(dimethylamino)propylamine (20 equiv) in 2-BuOH (98 °C for 1 day) followed by chromatography of the product on alumina, eluting with 1−2% MeOH/CH_2Cl_2; mp (MeOH/$CHCl_3$/light petroleum) 196−198 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.89 (br s, 1 H, NH), 9.36 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.19 (br s, 1 H, H-2′), 7.84 (br d, J = 8.1 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (br d, J = 8.1 Hz, 1 H, H-4′), 7.23 (br t, J = 5.6 Hz, 1 H, N*H*CH_2), 6.40 (s, 1 H, H-8), 3.30 (br q, J = 6.3 Hz, 2 H, NHC*H*$_2$), 2.31 (t, J = 7.0 Hz, 2 H, NCH_2), 2.15 (s, 6 H, N(CH_3)$_2$), 1.71 (pentet, J = 7.0 Hz, 2 H, NCH_2CH_2); ^{13}C NMR δ 160.85 (s, C-7), 157.87 (d, C-2), 157.62 (s, C-4), 154.63 (s, C-8a), 148.33 (d, C-5), 140.74 (s, C-1′), 130.32 (d, C-5′), 125.91 (d, C-4′), 124.17 (d, C-2′), 121.10 (s, C-3′), 120.68 (d, C-6′), 103.60 (s, C-4a), 95.16 (br d, C-8), 56.76 (t, NCH_2), 45.16 (q, 2 C, N(CH_3)$_2$), 39.55 (t, NCH_2), 29.59 (t, CH_2). Anal. ($C_{18}H_{21}BrN_6$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[[4-(dimethylamino)butyl]amino]pyrido[4,3-*d*]pyrimidine (3n): 86%, by reaction of **6** with 4-(dimethylamino)butylamine (21 equiv) in 2-BuOH (95 °C for 19 h) followed by chromatography of the product on alumina, eluting with 1−2% MeOH/CH_2Cl_2; mp ($CHCl_3$/light petroleum) 171−174 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.87 (br s, 1 H, NH), 9.35 (s, 1 H, H-5), 8.44 (s, 1 H, H-2), 8.19 (br s, 1 H, H-2′),

7.84 (br d, J = 8.0 Hz, 1 H, H-6′), 7.34 (t, J = 7.9 Hz, 1 H, H-5′), 7.29 (br d, J = 8.1 Hz, 1 H, H-4′), 7.24 (br t, J = 5.6 Hz, 1 H, N*H*CH_2), 6.39 (s, 1 H, H-8), 3.26 (br q, J = 6.2 Hz, 2 H, NHC*H*$_2$), 2.23 (t, J = 7.1 Hz, 2 H, NCH_2), 2.12 (s, 6 H, $N(CH_3)_2$), 1.59 (pentet, J = 7.0 Hz, 2 H, CH_2), 1.49 (pentet, J = 6.9 Hz, 2 H, CH_2); ^{13}C NMR δ 160.84 (s, C-7), 157.84 (d, C-2), 157.60 (s, C-4), 154.59 (s, C-8a), 148.32 (d, C-5), 140.73 (s, C-1′), 130.31 (d, C-5′), 125.88 (d, C-4′), 124.13 (d, C-2′), 121.09 (s, C-3′), 120.64 (d, C-6′), 103.55 (s, C-4a), 95.12 (br d, C-8), 58.72 (t, NCH_2), 45.07 (q, 2 C, $N(CH_3)_2$), 41.12 (t, NCH_2), 26.40, 24.52 (2 t, $2CH_2$). Anal. ($C_{19}H_{23}BrN_6 \cdot H_2O$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[[5-(dimethylamino)pentyl]amino]pyrido[4,3-*d*]pyrimidine (3o): 86%, by reaction of **6** with 5-(dimethylamino)pentylamine (20 equiv) in 2-BuOH (97 °C for 21 h) followed by chromatography of the product on alumina, eluting with 1−2% MeOH/CH_2Cl_2; mp (MeOH/$CHCl_3$/light petroleum) 123−126 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.89 (br s, 1 H, NH), 9.36 (s, 1 H, H-5), 8.44 (s, 1 H, H-2), 8.19 (br s, 1 H, H-2′), 7.84 (br d, J = 7.8 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (br d, J = 8.1 Hz, 1 H, H-4′), 7.20 (br t, J = 5.6 Hz, 1 H, N*H*CH_2), 6.39 (s, 1 H, H-8), 3.27 (br q, J = 6.0 Hz, 2 H, NHC*H*$_2$), 2.19 (t, J = 7.1 Hz, 2 H, NCH_2), 2.11 (s, 6 H, $N(CH_3)_2$), 1.59 (pentet, J = 7.2 Hz, 2 H, CH_2), 1.39 (m, 4 H, $2CH_2$); ^{13}C NMR δ 160.85 (s, C-7), 157.85 (d, C-2), 157.63 (s, C-4), 154.61 (s, C-8a), 148.35 (d, C-5), 140.76 (s, C-1′), 130.33 (d, C-5′), 125.90 (d, C-4′), 124.17 (d, C-2′), 121.11 (s, C-3′), 120.68 (d, C-6′), 103.57 (s, C-4a), 95.18 (br d, C-8), 59.03 (t, NCH_2), 45.11 (q, 2 C, $N(CH_3)_2$), 41.19 (t, NCH_2), 28.51, 26.76, 24.38 (3 t, $3CH_2$). Anal. ($C_{20}H_{25}BrN_6$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[*N*-[2-(dimethylamino)ethyl]-*N*-methylamino]pyrido[4,3-*d*]pyrimidine (3p): 93%, by reaction of **6** with *N*,*N*,*N*′-trimethylethylenediamine (8 equiv) in 2-BuOH (100 °C for 5 days) followed by dissolution of the crude product in dilute aqueous HCl, filtration, and basification with Na_2CO_3; mp (water) 195−196 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.91 (br s, 1 H, NH), 9.42 (s, 1 H, H-5), 8.48 (s, 1 H, H-2), 8.21 (s, 1 H, H-2′), 7.85 (d, J = 8.0 Hz, 1 H, H-6′), 7.35 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (br d, J = 8.1 Hz, 1 H, H-4′), 6.49 (s, 1 H, H-8), 3.78 (t, J = 6.8 Hz, 2 H, NCH_2), 3.10 (s, 3 H, NCH_3), 2.45 (t, J = 6.9 Hz, 2 H, NCH_2), 2.20 (s, 6 H, $N(CH_3)_2$); ^{13}C NMR δ 159.70 (s, C-7), 157.92 (d, C-2), 157.63 (s, C-4), 154.79 (s, C-8a), 147.75 (d, C-5), 140.72 (s, C-1′), 130.33 (d, C-5′), 125.92 (d, C-4′), 124.10 (d, C-2′), 121.12 (s, C-3′), 120.61 (d, C-6′), 103.18 (s, C-4a), 95.43 (d, C-8), 56.23, 47.16 (2 t, 2 CH_2N), 45.43 (q, 2 C, $N(CH_3)_2$), 36.36 (q, NCH_3). Anal. ($C_{18}H_{21}BrN_6$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[[2-(4-morpholino)ethyl]amino]pyrido[4,3-*d*]pyrimidine (3q): 86%, by reaction of **6** with 4-(2-aminoethyl)morpholine (10 equiv) in 2-BuOH (100 °C for 4 days) followed by chromatography of the product on silica gel, eluting with 6−7% MeOH/CH_2Cl_2; mp (MeOH/CH_2Cl_2) 250−253 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.89 (br s, 1 H, NH), 9.37 (s, 1 H, H-5), 8.46 (s, 1 H, H-2), 8.20 (t, J = 1.9 Hz, 1 H, H-2′), 7.84 (ddd, J = 8.0, 1.8, 1.3 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (dt, J = 8.0, 1.4 Hz, 1 H, H-4′), 7.02 (br t, J = 5.7 Hz, 1 H, N*H*CH_2), 6.45 (s, 1 H, H-8), 3.59 (t, J = 4.6 Hz, 4 H, $(CH_2)_2O$), 3.42 (q, J = 5.9 Hz, 2 H, NHC*H*$_2$), 2.53 (t, J = 6.7 Hz, 2 H, NCH_2), 2.44 (br t, J = 4.6 Hz, 4 H, $N(CH_2)_2$); ^{13}C NMR δ 160.65 (s, C-7), 157.87 (d, C-2), 157.61 (s, C-4), 154.59 (s, C-8a), 148.30 (d, C-5), 140.71 (s, C-1′), 130.31 (d, C-5′), 125.90 (d, C-4′), 124.13 (d, C-2′), 121.09 (s, C-3′), 120.64 (d, C-6′), 103.72 (s, C-4a), 95.64 (br d, C-8), 66.12 (t, 2 C, $(CH_2)_2O$), 57.00 (t, NCH_2), 53.29 (t, 2 C, $(CH_2)_2N$), 38.32 (t, NCH_2). Anal. ($C_{19}H_{21}BrN_6O$) H, N; C: calcd, 53.2; found, 52.7.

4-[(3-Bromophenyl)amino]-7-[[3-(4-morpholino)propyl]amino]pyrido[4,3-*d*]pyrimidine (3r): 96%, by reaction of **6** with 4-(3-aminopropyl)morpholine (20 equiv) in 2-BuOH (95 °C for 19 h); mp ($CHCl_3$/light petroleum) 173−174 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.86 (br s, 1 H, NH), 9.36 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.19 (br s, 1 H, H-2′), 7.84 (br d, J = 7.9 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (dt, J = 8.0, 1.4 Hz, 1 H, H-4′), 7.24 (br t, J = 5.7 Hz, 1 H, N*H*CH_2), 6.41 (s, 1 H, H-8), 3.59 (t, J = 4.6 Hz, 4 H, $(CH_2)_2O$), 3.33 (br q, J = 6.3 Hz, 2 H, NHC*H*$_2$), 2.37 (m, 6 H, $(CH_2)_2NCH_2$), 1.74 (pentet, J = 6.9 Hz, 2 H, NCH_2CH_2); ^{13}C NMR δ 160.83 (s, C-7), 157.80 (d, C-2), 157.54 (s, C-4), 154.60 (s, C-8a), 148.31 (d, C-5), 140.77 (s, C-1′), 130.29 (d, C-5′), 125.85 (d, C-4′), 124.11 (d, C-2′), 121.08 (s, C-3′), 120.62 (d, C-6′), 103.61 (s, C-4a), 95.24 (br d, C-8), 66.15 (t, 2 C, $(CH_2)_2O$), 55.83 (t, NCH_2), 53.30 (t, 2 C, $N(CH_2)_2$), 39.52 (t, NCH_2), 25.50 (t, CH_2). Anal. ($C_{20}H_{23}BrN_6O$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[[4-(4-morpholino)butyl]amino]pyrido[4,3-*d*]pyrimidine (3s): 90%, by reaction of **6** with 4-(4-aminobutyl)morpholine (**13**) (10 equiv) in 2-BuOH (95 °C for 40 h) followed by chromatography of the product on alumina, eluting with 0.7−0.8% MeOH/CH_2Cl_2; mp (CH_2Cl_2/light petroleum) 149−151 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.87 (br s, 1 H, NH), 9.35 (s, 1 H, H-5), 8.44 (s, 1 H, H-2), 8.19 (t, J = 1.8 Hz, 1 H, H-2′), 7.84 (br d, J = 8.3 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (dt, J = 8.0, 1.4 Hz, 1 H, H-4′), 7.24 (br t, J = 5.6 Hz, 1 H, N*H*CH_2), 6.40 (s, 1 H, H-8), 3.57 (t, J = 4.6 Hz, 4 H, $(CH_2)_2O$), 3.31 (br q, J = 5.4 Hz, 2 H, NHC*H*$_2$), 2.34 (m, 4 H, $(CH_2)_2N$), 2.30 (t, J = 7.0 Hz, 2 H, NCH_2), 1.60, 1.53 (2 m, 2 × 2 H, $2CH_2$); ^{13}C NMR δ 160.83 (s, C-7), 157.84 (d, C-2), 157.59 (s, C-4), 154.63 (s, C-8a), 148.32 (d, C-5), 140.73 (s, C-1′), 130.31 (d, C-5′), 125.89 (d, C-4′), 124.13 (d, C-2′), 121.09 (s, C-3′), 120.64 (d, C-6′), 103.55 (s, C-4a), 95.18 (br d, C-8), 66.12 (t, 2 C, $(CH_2)_2O$), 57.83 (t, NCH_2), 53.27 (t, 2 C, $(CH_2)_2N$), 41.11 (t, NCH_2), 26.36, 23.37 (2 t, $2CH_2$). Anal. ($C_{21}H_{25}BrN_6O$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[[3-(4-methylpiperazin-1-yl)propyl]amino]pyrido[4,3-*d*]pyrimidine (3u): 98%, by reaction of **6** with 1-(3-aminopropyl)-4-methylpiperazine (20 equiv) in 2-BuOH (97 °C for 16 h); mp (MeOH/water) 111−112.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.87 (br s, 1 H, NH), 9.36 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.19 (t, J = 1.8 Hz, 1 H, H-2′), 7.84 (br d, J = 8.1 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (dt, J = 8.0, 1.4 Hz, 1 H, H-4′), 7.24 (br t, J = 5.6 Hz, 1 H, N*H*CH_2), 6.40 (s, 1 H, H-8), 3.30 (br q, J = 6.4 Hz, 2 H, NHC*H*$_2$), 2.6−2.0 (br s, 8 H, $N(CH_2)_4N$), 2.37 (t, J = 7.0 Hz, 2 H, NCH_2), 2.15 (s, 3 H, NCH_3), 1.72 (pentet, J = 6.9 Hz, 2 H, $NHCH_2CH_2$); ^{13}C NMR δ 160.84 (s, C-7), 157.84 (d, C-2), 157.58 (s, C-4), 154.65 (s, C-8a), 148.31 (d, C-5), 140.73 (s, C-1′), 130.30 (d, C-5′), 125.87 (d, C-4′), 124.11 (d, C-2′), 121.08 (s, C-3′), 120.61 (d, C-6′), 103.58 (s, C-4a), 95.30 (br d, C-8), 55.46 (t, NCH_2), 54.72, 52.65 (2 t, 2 × 2 C, $N(CH_2)_4N$), 45.69 (q, NCH_3), 39.66 (t, NCH_2), 25.81 (t, CH_2). Anal. ($C_{21}H_{26}BrN_7 \cdot H_2O$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[[2-[*N*,*N*-bis(2-hydroxyethyl)amino]ethyl]amino]pyrido[4,3-*d*]pyrimidine (3v): 39%, by reaction of **6** (103 mg, 0.32 mmol) with *N*,*N*-bis(2-hydroxyethyl)ethylenediamine dihydrochloride (1.55 g, 7.01 mmol) in 2.1 M aqueous NaOH (5 mL) and 1-PrOH (10 mL) (95 °C for 20 h) followed by evaporation of solvent under reduced pressure and dilution of the residue with aqueous Na_2CO_3: mp (MeOH/water) 121.5−124.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.89 (br s, 1 H, NH), 9.37 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.19 (t, J = 1.9 Hz, 1 H, H-2′), 7.84 (dt, J = 8.0, 1.4 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (dt, J = 8.0, 1.5 Hz, 1 H, H-4′), 7.04 (br t, J = 5.3 Hz, 1 H, N*H*CH_2), 6.44 (s, 1 H, H-8), 4.40 (t, J = 4.9 Hz, 2 H, 2OH), 3.45 (q, J = 5.8 Hz, 4 H, $2CH_2OH$), 3.35 (br q, J = 5.8 Hz, 2 H, NHC*H*$_2$), 2.72 (t, J = 6.3 Hz, 2 H, NCH_2), 2.61 (t, J = 6.0 Hz, 4 H, $N(CH_2)_2$); ^{13}C NMR δ 160.73 (s, C-7), 157.87 (d, C-2), 157.61 (s, C-4), 154.58 (s, C-8a), 148.30 (d, C-5), 140.70 (s, C-1′), 130.31 (d, C-5′), 125.91 (d, C-4′), 124.15 (d, C-2′), 121.08 (s, C-3′), 120.66 (d, C-6′), 103.70 (s, C-4a), 95.77 (br d, C-8), 59.17, 56.82 (2 t, 2 × 2 C, $N(CH_2CH_2OH)_2$), 53.50 (t, NCH_2), 39.56 (t, NCH_2). Anal. ($C_{19}H_{23}BrN_6O_2 \cdot H_2O$) H, N; C: calcd, 49.0; found, 49.5.

4-[(3-Bromophenyl)amino]-7-[[3-[*N*,*N*-bis(2-hydroxyethyl)amino]propyl]amino]pyrido[4,3-*d*]pyrimidine (3w): 93%, by reaction of **6** with 3-[*N*,*N*-bis(2-hydroxyethyl)amino]-propylamine (20 equiv) in 1-PrOH/water (10:1) (95 °C for 17 h); mp (MeOH/water) 144−146 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.87 (br s, 1 H, NH), 9.35 (s, 1 H, H-5), 8.44 (s, 1 H, H-2), 8.18 (br s, 1 H, H-2′), 7.83 (br d, J = 7.8 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (br d, J = 7.8 Hz, 1 H, H-4′), 7.24 (br t, J = 5.4 Hz, 1 H, N*H*CH_2), 6.40 (s, 1 H, H-8), 4.38 (br s, 2 H, 2OH), 3.44 (m, 4 H, $2CH_2OH$), 3.31 (br q, J = 6.0 Hz, 2 H, NHC*H*$_2$), 2.57 (t, J = 6.9 Hz, 2 H, NCH_2), 2.53 (t, J = 6.4 Hz, 4 H, $N(CH_2)_2$), 1.70 (pentet, J = 6.7 Hz, 2 H, $NHCH_2CH_2$); ^{13}C NMR δ 160.84 (s, C-7), 157.78 (d, C-2), 157.55 (s, C-4), 154.57

(s, C-8a), 148.29 (d, C-5), 140.76 (s, C-1′), 130.29 (d, C-5′), 125.85 (d, C-4′), 124.13 (d, C-2′), 121.08 (s, C-3′), 120.64 (d, C-6′), 103.56 (s, C-4a), 95.12 (br d, C-8), 59.20, 56.57 (2 t, 2 × 2 C, $N(CH_2CH_2OH)_2$), 52.54 (t, NCH_2), 39.62 (t, NCH_2), 26.30 (t, CH_2). Anal. ($C_{20}H_{25}BrN_6O_2$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[[3-(imidazol-1-yl)propyl]amino]pyrido[4,3-*d*]pyrimidine (3y): 80%, by reaction of **6** with 1-(3-aminopropyl)imidazole (20 equiv) in 2-BuOH (95 °C for 2 days) followed by chromatography of the product on silica gel, eluting with 7−10% MeOH/CH_2Cl_2, and then on alumina, eluting with 5% MeOH/CH_2Cl_2; mp (MeOH/$CHCl_3$/light petroleum) 231−232.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.90 (br s, 1 H, NH), 9.38 (s, 1 H, H-5), 8.46 (s, 1 H, H-2), 8.19 (br s, 1 H, H-2′), 7.84 (br d, J = 8.0 Hz, 1 H, H-6′), 7.68 (s, 1 H, H-2″), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.32 (br t, J = 5.4 Hz, 1 H, N*H*CH_2), 7.29 (dt, J = 8.0, 1.4 Hz, 1 H, H-4′), 7.23 (s, 1 H, H-4″), 6.92 (s, 1 H, H-5″), 6.41 (s, 1 H, H-8), 4.09 (t, J = 6.9 Hz, 2 H, NCH_2), 3.25 (br q, J = 6.1 Hz, 2 H, NHC*H*$_2$), 2.02 (pentet, J = 6.8 Hz, 2 H, $NHCH_2CH_2$); ^{13}C NMR δ 160.69 (s, C-7), 157.86 (d, C-2), 157.59 (s, C-4), 154.58 (s, C-8a), 148.32 (d, C-5), 140.72 (s, C-1′), 137.24 (d, C-2″), 130.30 (d, C-5′), 128.29 (d, C-4″), 125.90 (d, C-4′), 124.14 (d, C-2′), 121.08 (s, C-3′), 120.65 (d, C-6′), 119.28 (d, C-5″), 103.78 (s, C-4a), 95.57 (br d, C-8), 43.68 (t, NCH_2), 38.30 (t, NCH_2), 30.19 (t, CH_2). Anal. ($C_{19}H_{18}BrN_7$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[[2-(imidazol-4-yl)ethyl]amino]pyrido[4,3-*d*]pyrimidine (3z): 74%, by reaction of **6** (100 mg, 0.31 mmol) with histamine dihydrochloride (0.60 g, 3.26 mmol) in 2.9 M aqueous NaOH (2 mL) and 1-PrOH (8 mL) (96 °C for 2 days); mp (MeOH/water) 246−249 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.90 (br s, 1 H, NH), 9.89 (br s, 1 H, NH), 9.37 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.19 (br s, 1 H, H-2′), 7.84 (br d, J = 8.0 Hz, 1 H, H-6′), 7.58 (s, 1 H, H-2″), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (d, J = 7.9 Hz, 1 H, H-4′), 7.25 (br t, J = 5.6 Hz, 1 H, N*H*CH_2), 6.87 (s, 1 H, H-5″), 6.43 (s, 1 H, H-8), 3.51 (br q, J = 6.2 Hz, 2 H, NHC*H*$_2$), 2.81 (t, J = 7.3 Hz, 2 H, $NHCH_2CH_2$); ^{13}C NMR δ 160.73 (s, C-7), 157.96 (d, C-2), 157.71 (s, C-4), 154.68 (s, C-8a), 148.42 (d, C-5), 140.75 (s, C-1′), 134.73 (d + s, 2 C, C-2″,4″), 130.42 (d, C-5′), 126.06 (d, C-4′), 124.31 (d, C-2′), 121.18 (s, C-3′), 120.83 (d, C-6′), 103.79 (s, C-4a), 95.48 (br d, C-8), 41.54 (t, NCH_2), 26.52 (t, CH_2). Anal. ($C_{18}H_{16}BrN_7$) C, H, N.

7-[[4-(Dimethylamino)butyl]amino]-4-[(3-methylphenyl)amino]pyrido[4,3-*d*]pyrimidine (4n): 76%, by reaction of **7** with 4-(dimethylamino)butylamine (10 equiv) in 2-BuOH (95 °C for 2 days) followed by chromatography of the product on silica gel, eluting with 8−15% MeOH/CH_2Cl_2 containing 0.3% Et_3N, treatment of this crude product with aqueous Na_2CO_3, and extraction with EtOAc (3 × 50 mL); mp (CH_2Cl_2/light petroleum) 178−180 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.73 (br s, 1 H, NH), 9.34 (s, 1 H, H-5), 8.37 (s, 1 H, H-2), 7.61 (m, 2 H, H-2′,6′), 7.25 (dd, J = 8.7, 7.6 Hz, 1 H, H-5′), 7.16 (br t, J = 5.6 Hz, 1 H, N*H*CH_2), 6.94 (d, J = 7.7 Hz, 1 H, H-4′), 6.36 (s, 1 H, H-8), 3.27 (br q, J = 6.3 Hz, 2 H, NHC*H*$_2$), 2.33 (s, 3 H, 3′-CH_3), 2.23 (t, J = 7.1 Hz, 2 H, NCH_2), 2.12 (s, 6 H, $N(CH_3)_2$), 1.58 (pentet, J = 7.0 Hz, 2 H, CH_2), 1.49 (pentet, J = 7.2 Hz, 2 H, CH_2); ^{13}C NMR δ 160.76 (s, C-7), 158.07 (d, C-2), 157.83 (s, C-4), 154.66 (s, C-8a), 148.21 (d, C-5), 138.80, 137.52 (2 s, C-1′,3′), 128.20 (d, C-5′), 124.36 (d, C-4′), 122.88 (d, C-2′), 119.62 (d, C-6′), 103.65 (s, C-4a), 95.36 (br d, C-8), 58.74 (t, NCH_2), 45.08 (q, 2 C, $N(CH_3)_2$), 41.14 (t, NCH_2), 26.44, 24.56 (2 t, $2CH_2$), 21.12 (q, 3′-CH_3). Anal. ($C_{20}H_{26}N_6 \cdot 0.25H_2O$) C, H, N.

4-[(3-Methylphenyl)amino]-7-[[3-(4-morpholino)propyl]amino]pyrido[4,3-*d*]pyrimidine (4r): 86%, by reaction of **7** with 4-(3-aminopropyl)morpholine (10 equiv) in 2-BuOH (93 °C for 26 h) followed by chromatography of the product on silica gel, eluting with 6−8% MeOH/CH_2Cl_2, and then on alumina, eluting with 5% MeOH/$CHCl_3$; mp (MeOH/$CHCl_3$/light petroleum) 181.5−182.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.74 (br s, 1 H, NH), 9.35 (s, 1 H, H-5), 8.38 (s, 1 H, H-2), 7.61 (m, 2 H, H-2′,6′), 7.26 (dd, J = 8.5, 7.8 Hz, 1 H, H-5′), 7.16 (br t, J = 5.6 Hz, 1 H, N*H*CH_2), 6.94 (d, J = 7.4 Hz, 1 H, H-4′), 6.39 (s, 1 H, H-8), 3.59 (t, J = 4.6 Hz, 4 H, $(CH_2)_2O$), 3.31 (br q, J = 6.2 Hz, 2 H, NHC*H*$_2$), 2.38 (t, J = 6.9 Hz, 2 H, NCH_2), 2.35 (br m, 4 H, $(CH_2)_2N$), 2.33 (s, 3 H, 3′-CH_3), 1.74 (pentet, J = 6.9 Hz, 2 H, NCH_2CH_2); ^{13}C NMR δ 160.75 (s, C-7), 158.08 (d, C-2), 157.81 (s, C-4), 154.66 (s, C-8a), 148.20 (d, C-5), 138.78, 137.50 (2 s, C-1′,3′), 128.19 (d, C-5′), 124.35 (d, C-4′), 122.86 (d, C-2′), 119.61 (d, C-6′), 103.68 (s, C-4a), 95.29 (br d, C-8), 66.15 (t, 2 C, $(CH_2)_2O$), 55.85 (t, NCH_2), 53.30 (t, 2 C, $(CH_2)_2N$), 39.51 (t, NCH_2), 25.51 (t, CH_2), 21.10 (q, CH_3). Anal. ($C_{21}H_{26}N_6O$) C, H, N.

4-[(3-Methylphenyl)amino]-7-[[3-(4-methylpiperazin-1-yl)propyl]amino]pyrido[4,3-*d*]pyrimidine (4u): 77%, by reaction of **7** with 1-(3-aminopropyl)-4-methylpiperazine (10 equiv) in 2-BuOH (94 °C for 2 days) followed by chromatography of the product on silica gel, eluting with 15−20% MeOH/CH_2Cl_2, treatment of this crude product with aqueous Na_2CO_3, and extraction with EtOAc (3 × 50 mL); mp (CH_2Cl_2/light petroleum) 142−145 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.73 (br s, 1 H, NH), 9.34 (s, 1 H, H-5), 8.37 (s, 1 H, H-2), 7.61 (m, 2 H, H-2′,6′), 7.25 (dd, J = 8.6, 7.6 Hz, 1 H, H-5′), 7.16 (br t, J = 5.6 Hz, 1 H, N*H*CH_2), 6.94 (d, J = 7.6 Hz, 1 H, H-4′), 6.36 (s, 1 H, H-8), 3.29 (br q, J = 6.6 Hz, 2 H, NHC*H*$_2$), 2.6−2.0 (br s, 8 H, $N(CH_2)_4N$), 2.37 (t, J = 7.0 Hz, 2 H, NCH_2), 2.33 (s, 3 H, 3′-CH_3), 2.15 (s, 3 H, NCH_3), 1.72 (pentet, J = 6.9 Hz, 2 H, $NHCH_2CH_2$); ^{13}C NMR δ 160.76 (s, C-7), 158.09 (d, C-2), 157.82 (s, C-4), 154.67 (s, C-8a), 148.22 (d, C-5), 138.79, 137.53 (2 s, C-1′,3′), 128.21 (d, C-5′), 124.37 (d, C-4′), 122.88 (d, C-2′), 119.62 (d, C-6′), 103.68 (s, C-4a), 95.32 (br d, C-8), 55.49 (t, NCH_2), 54.72, 52.66 (2 t, 2 × 2 C, $N(CH_2)_4N$), 45.69 (q, NCH_3), 39.66 (t, NCH_2), 25.84 (t, CH_2), 21.12 (q, 3′-CH_3). Anal. ($C_{22}H_{29}N_7 \cdot 0.25H_2O$) C, H, N.

7-[[3-(Imidazol-1-yl)propyl]amino]-4-[(3-methylphenyl)amino]pyrido[4,3-*d*]pyrimidine (4y): 88%, by reaction of **7** with 1-(3-aminopropyl)imidazole (10 equiv) in 2-BuOH (95 °C for 4 days) followed by chromatography of the product on silica gel, eluting with 6−8% MeOH/CH_2Cl_2, and then on alumina, eluting with 5% MeOH/$CHCl_3$; mp ($CHCl_3$/light petroleum) 158−159 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.77 (br s, 1 H, NH), 9.37 (s, 1 H, H-5), 8.39 (s, 1 H, H-2), 7.67 (s, 1 H, H-2″), 7.61 (m, 2 H, H-2′,6′), 7.26 (dd, J = 8.8, 7.5 Hz, 1 H, H-5′), 7.25 (br t, J = 5.6 Hz, 1 H, N*H*CH_2), 7.22 (s, 1 H, H-4″), 6.95 (d, J = 7.4 Hz, 1 H, H-4′), 6.91 (s, 1 H, H-5″), 6.38 (s, 1 H, H-8), 4.09 (t, J = 6.9 Hz, 2 H, NCH_2), 3.25 (br q, J = 6.2 Hz, 2 H, NHC*H*$_2$), 2.33 (s, 3 H, 3′-CH_3), 2.02 (pentet, J = 6.9 Hz, 2 H, $NHCH_2CH_2$); ^{13}C NMR δ 160.61 (s, C-7), 158.12 (d, C-2), 157.82 (s, C-4), 154.66 (s, C-8a), 148.22 (d, C-5), 138.76, 137.52 (2 s, C-1′,3′), 137.25 (d, C-2″), 128.32, 128.21 (2 d, C-5′,4″), 124.39 (d, C-4′), 122.88 (d, C-2′), 119.62 (d, C-6′), 119.27 (d, C-5″), 103.87 (s, C-4a), 95.64 (br d, C-8), 43.68 (t, NCH_2), 38.30 (t, NCH_2), 30.23 (t, CH_2), 21.11 (q, CH_3). Anal. ($C_{20}H_{21}N_7 \cdot 0.25H_2O$) C, N; H: calcd, 5.9; found, 6.5.

7-[[2-(Imidazol-4-yl)ethyl]amino]-4-[(3-methylphenyl)amino]pyrido[4,3-*d*]pyrimidine (4z): 65%, by reaction of **7** (101 mg, 0.40 mmol) with histamine dihydrochloride (0.74 g, 4.02 mmol) and $NaHCO_3$ (83 mg, 0.99 mmol) in 3.5 M aqueous NaOH (2 mL) and 1-PrOH (8 mL) (94 °C for 19 h) followed by chromatography of the product on silica gel, eluting with 8−10% MeOH/CH_2Cl_2, and then on alumina, eluting with 10−20% MeOH/CH_2Cl_2; mp (MeOH/$CHCl_3$/light petroleum) 216−216.5 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 11.85 (br s, 1 H, NH), 9.76 (br s, 1 H, NH), 9.37 (s, 1 H, H-5), 8.38 (s, 1 H, H-2), 7.61 (m, 2 H, H-2′,6′), 7.56 (d, J = 0.9 Hz, 1 H, H-2″), 7.26 (dd, J = 8.6, 7.7 Hz, 1 H, H-5′), 7.18 (br t, J = 5.7 Hz, 1 H, N*H*CH_2), 6.95 (d, J = 7.6 Hz, 1 H, H-4′), 6.86 (s, 1 H, H-5″), 6.41 (s, 1 H, H-8), 3.51 (br q, J = 6.6 Hz, 2 H, NHC*H*$_2$), 2.82 (t, J = 7.3 Hz, 2 H, $NHCH_2CH_2$), 2.33 (s, 3 H, 3′-CH_3); ^{13}C NMR δ 160.57 (s, C-7), 158.09 (d, C-2), 157.82 (s, C-4), 154.69 (s, C-8a), 148.23 (d, C-5), 138.77, 137.51 (2 s, C-1′,3′), 134.60 (d + s, 2 C, C-2″,4″), 128.20 (d, C-5′), 124.38 (d, C-4′), 122.90 (d, C-2′), 119.65 (d, C-6′), 116.70 (br d, C-5″), 103.78 (s, C-4a), 95.41 (br d, C-8), 41.48 (t, NCH_2), 26.51 (t, CH_2), 21.10 (q, CH_3). Anal. ($C_{19}H_{19}N_7$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[(1,3-dihydroxy-2-propyl)amino]pyrido[4,3-*d*]pyrimidine (3h). Example of General Method C. Serinol oxalate (1.30 g, 9.56 mmol) was added to a solution of sodium (0.19 g, 8.26 mmol) dissolved in 2-BuOH (12 mL). The mixture was stirred until the reaction was complete, then **6** (122 mg, 0.38 mmol) was added, and the mixture was stirred at 100 °C for 8 days. The solvent was

removed under reduced pressure; then the residue was diluted with water (50 mL) and extracted with EtOAc (3 × 40 mL). Chromatography of this extract on silica gel, eluting with 6−10% MeOH/CH_2Cl_2, gave **3h** (120 mg, 80%): mp (MeOH/CH_2Cl_2) 168−171 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.88 (br s, 1 H, NH), 9.35 (s, 1 H, H-5), 8.44 (s, 1 H, H-2), 8.19 (t, J = 1.9 Hz, 1 H, H-2′), 7.84 (dt, J = 7.9, 1.5 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (dt, J = 8.0, 1.5 Hz, 1 H, H-4′), 6.80 (br d, J = 7.5 Hz, 1 H, N*H*CH), 6.55 (s, 1 H, H-8), 4.73 (t, J = 5.6 Hz, 2 H, 2CH_2OH), 3.88 (br s, 1 H, NHC*H*), 3.55 (t, J = 5.5 Hz, 4 H, 2CH_2OH); ^{13}C NMR δ 160.62 (s, C-7), 157.82 (d, C-2), 157.60 (s, C-4), 154.48 (s, C-8a), 148.16 (d, C-5), 140.71 (s, C-1′), 130.31 (d, C-5′), 125.91 (d, C-4′), 124.15 (d, C-2′), 121.09 (s, C-3′), 120.66 (d, C-6′), 103.70 (s, C-4a), 96.50 (br d, C-8), 60.22 (t, 2 C, 2CH_2OH), 54.88 (d, NCH). Anal. ($C_{16}H_{16}BrN_5O_2$) C, H, N.

The following analogues were prepared similarly.

4-[(3-Bromophenyl)amino]-7-[(carboxymethyl)amino]-pyrido[4,3-*d*]pyrimidine (3aa). Glycine (1.35 g, 18.0 mmol) was added to a solution of sodium (0.23 g, 10.0 mmol) dissolved in MeOH (50 mL), and the mixture was stirred until the reaction was complete. The solvent was removed, **6** (203 mg, 0.64 mmol) and 2-BuOH (20 mL) were added, and the mixture was stirred at 98 °C for 5 days. The solvent was removed under reduced pressure, and the residue was dissolved in MeOH/water and treated with dilute HCl to pH ca. 4. Concentration under reduced pressure and cooling gave a solid (215 mg). This was redissolved in MeOH and aqueous Na_2CO_3; then the resulting solution was concentrated under reduced pressure and extracted with EtOAc (2 × 100 mL). Dilution of the aqueous suspension with DMSO (5 mL), then neutralization with excess AcOH, concentration under reduced pressure, and cooling gave **3aa**: mp (DMSO/AcOH) 230−236 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 12.50 (br s, 1 H, COOH), 9.93 (br s, 1 H, NH), 9.37 (s, 1 H, H-5), 8.47 (s, 1 H, H-2), 8.18 (br s, 1 H, H-2′), 7.83 (br d, J = 7.7 Hz, 1 H, H-6′), 7.46 (br t, J = 6.0 Hz, 1 H, N*H*CH_2), 7.35 (t, J = 7.9 Hz, 1 H, H-5′), 7.30 (br d, J = 8.1 Hz, 1 H, H-4′), 6.53 (s, 1 H, H-8), 4.07 (d, J = 6.0 Hz, 2 H, NHC*H*$_2$); ^{13}C NMR δ 172.04 (s, COOH), 160.42 (s, C-7), 157.71 (d + s, C-2,4), 153.83 (s, C-8a), 148.12 (d, C-5), 140.55 (s, C-1′), 130.35 (d, C-5′), 126.13 (d, C-4′), 124.35 (d, C-2′), 121.10 (s, C-3′), 120.86 (d, C-6′), 104.05 (s, C-4a), 96.85 (br d, C-8), 42.87 (t, NCH_2). Anal. ($C_{15}H_{12}BrN_5O_2$) H, N; C: calcd, 48.2; found, 47.7.

4-[(3-Bromophenyl)amino]-7-[(2-carboxyethyl)amino]-pyrido[4,3-*d*]pyrimidine (3bb). β-Alanine (0.89 g, 10.0 mmol) was added to a solution of sodium (0.19 g, 8.26 mmol) dissolved in 2-BuOH (20 mL), and the mixture was stirred until the reaction was complete (monitored by pH). The solution was treated with **6** (145 mg, 0.46 mmol) at 100 °C for 1 day, then the solvent was removed under reduced pressure, and the residue was treated with methanolic HCl to pH ca. 4, evaporated, and chromatographed on silica gel, eluting with 10% MeOH/CH_2Cl_2 containing 0.5% AcOH, to give **3bb** (147 mg, 83%): mp (MeOH/AcOH) 241−243 °C; ^{1}H NMR [$(CD_3)_2$-SO] δ 12.26 (br s, 1 H, COOH), 9.90 (br s, 1 H, NH), 9.38 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.19 (t, J = 1.8 Hz, 1 H, H-2′), 7.84 (br d, J = 8.1 Hz, 1 H, H-6′), 7.34 (t, J = 7.9 Hz, 1 H, H-5′), 7.29 (dt, J = 8.0, 1.4 Hz, 1 H, H-4′), 7.23 (br t, J = 5.6 Hz, 1 H, N*H*CH_2), 6.45 (s, 1 H, H-8), 3.52 (q, J = 6.3 Hz, 2 H, NHC*H*$_2$), 2.56 (t, J = 6.8 Hz, 2 H, C*H*$_2$COOH); ^{13}C NMR δ 173.02 (s, COOH), 160.52 (s, C-7), 157.80 (d, C-2), 157.64 (s, C-4), 154.38 (s, C-8a), 148.32 (d, C-5), 140.68 (s, C-1′), 130.32 (d, C-5′), 125.98 (d, C-4′), 124.23 (d, C-2′), 121.10 (s, C-3′), 120.74 (d, C-6′), 103.80 (s, C-4a), 95.95 (br d, C-8), 37.22, 33.65 (2 t, NCH_2CH_2). Anal. ($C_{16}H_{14}BrN_5O_2$) C, H, N.

4-[(3-Bromophenyl)amino]-7-[(3-carboxypropyl)amino]-pyrido[4,3-*d*]pyrimidine (3cc). 4-Aminobutyric acid (1.28 g, 12.4 mmol) was added to a solution of sodium (0.24 g, 10.4 mmol) dissolved in 2-BuOH (20 mL), and the mixture was stirred until the reaction was complete. The solution was treated with **6** (102 mg, 0.32 mmol) at 100 °C for 1 day; then the solvent was removed under reduced pressure. The residue was dissolved in dilute aqueous Na_2CO_3 (50 mL), washed with EtOAc (2 × 50 mL), diluted with MeOH, filtered, then treated with concentrated HCl to pH ca. 4, and cooled to give a solid (103 mg). This was redissolved in MeOH/aqueous Na_2CO_3, and the solution was filtered, acidified with AcOH, and cooled to give **3cc** (95 mg, 74%): mp (MeOH/water) 230−233 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.92 (br s, 1 H, NH), 9.36 (s, 1 H, H-5), 8.45 (s, 1 H, H-2), 8.18 (br s, 1 H, H-2′), 7.83 (br d, J = 7.9 Hz, 1 H, H-6′), 7.34 (t, J = 7.9 Hz, 1 H, H-5′), 7.30 (dt, J = 8.0, 1.5 Hz, 1 H, H-4′), 7.28 (br t, J = 5.7 Hz, 1 H, N*H*CH_2), 6.41 (s, 1 H, H-8), 3.30 (br q, J = 6.0 Hz, 2 H, NHC*H*$_2$), 2.34 (t, J = 7.4 Hz, 2 H, C*H*$_2$COOH), 1.81 (pentet, J = 7.2 Hz, 2 H, $NHCH_2CH_2$); ^{13}C NMR δ 174.24 (s, COOH), 160.80 (s, C-7), 157.72 (d, C-2), 157.63 (s, C-4), 154.32 (s, C-8a), 148.38 (d, C-5), 140.68 (s, C-1′), 130.33 (d, C-5′), 125.98 (d, C-4′), 124.24 (d, C-2′), 121.09 (s, C-3′), 120.75 (d, C-6′), 103.59 (s, C-4a), 95.18 (br d, C-8), 40.55 (t, $NHCH_2$), 31.05, 24.02 (2 t, 2CH_2). Anal. ($C_{17}H_{16}BrN_5O_2$·1.5H_2O) C, N; H: calcd, 4.4; found, 3.9.

4-[(3-Bromophenyl)amino]-7-[*N*-(carboxymethyl)-*N*-methylamino]pyrido[4,3-*d*]pyrimidine (3dd). Sarcosine (0.92 g, 10.3 mmol) was added to a solution of sodium (0.20 g, 8.70 mmol) dissolved in 2-BuOH (20 mL), and the mixture was stirred until the reaction was complete. The solution was treated with **6** (125 mg, 0.39 mmol) at 95 °C for 3 days, then the solvent was removed under reduced pressure, and the residue was treated with methanolic HCl to pH ca. 3 and cooled to give **3dd** (138 mg, 91%): mp (MeOH) 215 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 12.60 (br s, 1 H, COOH), 10.00 (br s, 1 H, NH), 9.41 (s, 1 H, H-5), 8.52 (s, 1 H, H-2), 8.18 (br s, 1 H, H-2′), 7.84 (br d, J = 7.8 Hz, 1 H, H-6′), 7.36 (t, J = 7.9 Hz, 1 H, H-5′), 7.31 (dt, J = 7.9, 1.4 Hz, 1 H, H-4′), 6.60 (s, 1 H, H-8), 4.43 (s, 2 H, NCH_2), 3.15 (s, 3 H, NCH_3); ^{13}C NMR δ 171.54 (s, COOH), 159.94 (s, C-7), 157.89 (d, C-2), 157.71 (s, C-4), 154.52 (s, C-8a), 147.60 (d, C-5), 140.56 (s, C-1′), 130.36 (d, C-5′), 126.13 (d, C-4′), 124.30 (d, C-2′), 121.12 (s, C-3′), 120.82 (d, C-6′), 103.66 (s, C-4a), 96.00 (d, C-8), 51.25 (t, NCH_2), 37.38 (q, NCH_3). Anal. ($C_{16}H_{14}BrN_5O_2$·H_2O) C, H, N.

4-[(3-Bromophenyl)amino]-7-(sulfoethylamino)pyrido-[4,3-*d*]pyrimidine (3ee). Reaction of **6** (102 mg, 0.32 mmol) with taurine (1.56 g, 12.5 mmol) in 1.1 M aqueous NaOH (10 mL) and 1-PrOH (10 mL) at 95 °C for 2 days followed by removal of solvent under reduced pressure, dilution of the residue with hot water (50 mL), filtration, and neutralization with AcOH gave a solid (108 mg) after cooling. This was dissolved in aqueous Na_2CO_3, washed with EtOAc, filtered, acidified with AcOH, and cooled to give **3ee** (99 mg, 73%): mp 338−345 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 11.11 (br s, 1 H, NH), 9.43 (s, 1 H, H-5), 8.69 (s, 1 H, H-2), 7.99 (s, 1 H, H-2′), 7.91 (br s, 1 H, N*H*CH_2), 7.69 (br d, J = 8.0 Hz, 1 H, H-6′), 7.49 (br d, J = 8.1 Hz, 1 H, H-4′), 7.43 (t, J = 8.0 Hz, 1 H, H-5′), 6.39 (s, 1 H, H-8), 3.58 (br s, 2 H, NHC*H*$_2$), 2.73 (t, J = 7.2 Hz, 2 H, CH_2S). Anal. ($C_{15}H_{14}BrN_5O_3S$) C, H.

4-[(3-Bromophenyl)amino]-7-[[3-(4-morpholino)propyl]amino]pyrido[4,3-*d*]pyrimidine $N^{1'}$-Oxide (3t). A solution of 4-[(3-bromophenyl)amino]-7-[[3-(4-morpholino)propyl]-amino]pyrido[4,3-*d*]pyrimidine (**3r**) (122 mg, 0.28 mmol) and 2-(phenylsulfonyl)-3-phenyloxaziridine (81 mg, 0.31 mmol) in CH_2Cl_2 (30 mL) was stirred at 20 °C for 16 h. Evaporation and chromatography of the residue on alumina, eluting with 3−8% MeOH/$CHCl_3$, gave **3t** (106 mg, 84%): mp (MeOH/$CHCl_3$/light petroleum) 189−190 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.94 (br s, 1 H, NH), 9.37 (s, 1 H, H-5), 8.44 (s, 1 H, H-2), 8.18 (br s, 1 H, H-2′), 8.04 (br s, 1 H, N*H*CH_2), 7.85 (br d, J = 8.0 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (br d, J = 8.0 Hz, 1 H, H-4′), 6.38 (s, 1 H, H-8), 4.14 (dd, J = 11.5, 10.3 Hz, 2 H, 2OCHa), 3.67 (dd, J = 11.7, 2.5 Hz, 2 H, 2OCHe), 3.35 (m, 6 H, NHC*H*$_2$, N(O)CH_2, 2N(O)CHa), 2.89 (br d, J = 11.2 Hz, 2 H, 2N(O)CHe), 2.14 (pentet, J = 6.8 Hz, 2 H, $NHCH_2CH_2$); ^{13}C NMR δ 160.72 (s, C-7), 157.89 (d, C-2), 157.65 (s, C-4), 154.68 (s, C-8a), 148.38 (d, C-5), 140.75 (s, C-1′), 130.34 (d, C-5′), 125.95 (d, C-4′), 124.24 (d, C-2′), 121.11 (s, C-3′), 120.76 (d, C-6′), 103.63 (s, C-4a), 95.58 (br d, C-8), 68.71 (t, N(O)CH_2), 63.52, 60.99 (2 t, 2 × 2 C, N(O)$(CH_2)_4$O), 36.57 (t, NCH_2), 21.04 (t, CH_2). Anal. ($C_{20}H_{23}BrN_6O_2$·2H_2O) C, H, N.

4-[(3-Bromophenyl)amino]-7-hydrazinopyrido[4,3-*d*]-pyrimidine (3x). A solution of **6** (100 mg, 0.31 mmol) and anhydrous hydrazine (0.20 mL, 6.37 mmol) in 2-BuOH (10 mL) was stirred at 20 °C for 3 days. The resulting precipitate was filtered and washed with CH_2Cl_2/light petroleum to give **3x**

(64 mg, 62%): mp (2-BuOH) 220 °C dec; ^{1}H NMR [$(CD_3)_2SO$] δ 9.89 (br s, 1 H, NH), 9.33 (s, 1 H, H-5), 8.47 (s, 1 H, H-2), 8.18 (t, J = 1.9 Hz, 1 H, H-2′), 8.12 (br s, 1 H, NH), 7.84 (dt, J = 7.9, 1.5 Hz, 1 H, H-6′), 7.34 (t, J = 8.0 Hz, 1 H, H-5′), 7.29 (dt, J = 8.0, 1.5 Hz, 1 H, H-4′), 6.73 (s, 1 H, H-8), 4.40 (br s, 2 H, NH_2); ^{13}C NMR δ 164.22 (s, C-7), 157.96 (d, C-2), 157.63 (s, C-4), 155.01 (s, C-8a), 148.14 (d, C-5), 140.75 (s, C-1′), 130.39 (d, C-5′), 126.02 (d, C-4′), 124.29 (d, C-2′), 121.16 (s, C-3′), 120.80 (d, C-6′), 104.05 (s, C-4a), 94.47 (d, C-8). Anal. ($C_{13}H_{11}BrN_6$) C, H, N.

7-(Methylamino)-4-[(3-methylphenyl)amino]pyrido-[4,3-*d*]pyrimidine (4b). A mixture of **7** (100 mg, 0.39 mmol) and 40% aqueous methylamine (5.0 mL, 58 mmol) in 2-PrOH (30 mL) was stirred in a pressure vessel at 65 °C for 3 h. The solvent was removed under reduced pressure; then the residue was diluted with aqueous Na_2CO_3 and extracted with EtOAc (4 × 50 mL). Chromatography of this extract on silica gel, eluting with 3−4% MeOH/CH_2Cl_2, gave **4b** (94 mg, 90%): mp (MeOH/$CHCl_3$/light petroleum) 275−277 °C; ^{1}H NMR [$(CD_3)_2SO$] δ 9.76 (br s, 1 H, NH), 9.36 (s, 1 H, H-5), 8.39 (s, 1 H, H-2), 7.61 (m, 2 H, H-2′,6′), 7.26 (dd, J = 8.7, 7.7 Hz, 1 H, H-5′), 7.10 (br q, J = 4.9 Hz, 1 H, N*H*CH_3), 6.95 (d, J = 7.5 Hz, 1 H, H-4′), 6.33 (s, 1 H, H-8), 2.85 (d, J = 5.0 Hz, 3 H, NHC*H*$_3$), 2.33 (s, 3 H, 3′-CH_3); ^{13}C NMR δ 161.39 (s, C-7), 158.10 (d, C-2), 157.83 (s, C-4), 154.80 (s, C-8a), 148.20 (d, C-5), 138.77, 137.51 (2 s, C-1′,3′), 128.19 (d, C-5′), 124.37 (d, C-4′), 122.90 (d, C-2′), 119.64 (d, C-6′), 103.68 (s, C-4a), 94.71 (br d, C-8), 28.33 (q, NCH_3), 21.10 (q, 3′-CH_3). Anal. ($C_{15}H_{15}N_5$) C, H, N.

Determination of Aqueous Solubility. Stock solutions of drugs were made up in MeOH or DMSO and used to calibrate the HPLC (peak area in nanomoles, assuming a linear response). Accurately weighed amounts (to give approximately a 50 mM solution) were then sonicated for 30 min in 0.05 M sodium lactate buffer (neutral compounds, hydrochloride salts of amines) or in water (sodium salts of acids). After standing for an additional 30 min, the samples were centrifuged at 13 000 rpm for 3 min, and the concentration of drug in the supernatant was determined by HPLC, using the calibration determined previously.

Enzyme Assay. Epidermal growth factor receptor was prepared from human A431 carcinoma cell shed membrane vesicles by immunoaffinity chromatography as previously described.[25] The enzyme assay was performed in 96-well filter plates (Millipore, MADV6550). The total volume was 0.1 mL, containing 20 mM HEPES, pH 7.4, 50 μM sodium vanadate, 40 mM $MgCl_2$, 10 μM ATP containing 5 μCi of [^{32}P]ATP, 20 μg of polyglutamic acid/tyrosine random copolymer (Sigma Chemical Co., Saint Louis, MO), 1 ng of EGFR tyrosine kinase, and appropriate dilutions of inhibitor and/or ATP. All components except the ATP were added to the well, and the plate was incubated with shaking for 10 min at 25 °C. The reaction was started by adding [^{32}P]ATP, the plate was then incubated with shaking for a further 10 min at 25 °C, and the reaction was terminated by addition of 0.1 mL of 20% trichloroacetic acid (TCA). The plate was then kept at 4 °C for at least 15 min to allow the substrate to precipitate, and the wells were then washed five times with 0.125 mL of 10% TCA. [^{32}P]ATP incorporation was determined with a Wallac betaplate counter. Control activity (no drug) gave a count of approximately 100 000 cpm. At least two independent dose−response curves were done and the IC_{50} values computed. The reported values are averages; variation was generally ±15%.

EGFR Autophosphorylation in A431 Human Epidermoid Carcinoma Cells. Cells were grown to confluence in 6-well plates (35 mm diameter) and exposed to serum-free medium for 18 h. The cells were treated with compound for 2 h and then with 100 ng/mL of EGF for 5 min. The monolayers were lysed in 0.2 mL of boiling Laemmli buffer (2% sodium dodecyl sulfate, 5% β-mercaptoethanol, 10% glycerol, and 50 mM Tris, pH 6.8), and the lysates were heated to 100 °C for 5 min. Proteins in the lysate were separated by polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose. The membrane was washed once in a mixture of 10 mM Tris, pH 7.2, 150 mM NaCl, and 0.01% azide (TNA) and blocked overnight in TNA containing 5% bovine serum albumin and 1% ovalbumin. The membrane was blotted for 2 h with antiphosphotyrosine antibody (UBI, 1 μg/mL in blocking buffer) and then washed twice in TNA, once in TNA containing 0.05% Tween-20 and 0.05% nonidet P-40, and twice in TNA. The membranes were then incubated for 2 h in blocking buffer containing 0.1 μCi/mL [^{125}I]protein A and washed again as above. After the blots were dry they were loaded into a film cassette and exposed to X-AR X-ray film for 1−7 days. Band intensities were determined with a Molecular Dynamics laser densitometer.

In Vivo Chemotherapy. Immune-deficient mice were housed in microisolator cages within a barrier facility on a 12-h light/dark cycle and received food and water ad libitum. Animal housing was in accord with AAALAC guidelines. All experimental protocols involving animals were approved by the Institutional Animal Care and Use Committee. The A431 epidermoid carcinoma and the NIH 3T3 fibroblast transfected with the human EGF receptor (EGFR) were maintained by serial passage in nude mice (NCr *nu*/*nu*). Nude mice were also used as tumor hosts for anticancer agent evaluations against these tumor models. In each experiment for anticancer activity evaluation, test mice weighing 18−22 g were randomized and implanted with tumor fragments in the region of the right axilla on day 0. Animals were treated with **3n** or **3r** on the basis of average cage weight on the days indicated in Table 1. The compounds were administered as solutions of the isethionate salts in water and were prepared for 5 days at a time. Host body weight change data are reported as the maximum treatment-related weight loss in these studies. Calculation of tumor growth inhibition (% T/C), tumor growth delay (T − C), and net logs of tumor cell kill was performed as described previously.[26−29] A positive net cell kill indicates that the tumor burden at the end of therapy was less than at the beginning of therapy. A negative net log cell kill indicates that the tumor grew during treatment. Net cell kills near 0 indicate no tumor growth during therapy.

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